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Photolithotrophy, Photoheterotrophy, and Chemoheterotrophy: Patterns of Resource Utilization on an Annual and a Diurnal Basis within a Pelagic Microbial Community

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Abstract. An annual investigation of rates of photolithotrophy, photoheterotrophy, and chemoheterotrophy utilizing glucose and bicarbonate was made within the pelagic zone of a small, hardwater, southwestern Michigan lake. Sampling proceeded on a monthly, diurnal, and depth-wise basis. Annual mean photoheterotrophic uptake was estimated at 2.6 μg C $m^{-3}h^{-1}$. Two periods of relatively high activity were observed: one during spring overturn and the second during the late summer period. In general, greatest contributions to overall carbon cycling occurred during morning to midday incubation periods and at intermediate depths within the water column. Rates of chemoheterotrophy averaged 6.9 μg C $m^{-3}h^{-1}$ and were relatively uniform throughout the annual period. Greatest overall chemoheterotrophic activity was associated with periods of overturn. In general, this activity increased throughout the day and with increasing depth within the water column. The annual mean for photolithotrophic fixation was 1.33 mg C $m^{-3}h^{-1}$. Greatest contributions to rates of photosynthesis were associated with epilimnetic waters during early morning and midday incubations. Relatively minor contributions to inorganic fixation were made by waters below the 6-meter contour. Spring overturn and late summer represented periods of particularly great photolithotrophic activity. Quantitative comparisons among carbon pathways indicate that rates of pelagic heterotrophy, both photo- and chemoheterotrophy combined, contribute small quantities of carbon to overall carbon metabolism in this oligotrophic system. Qualitative comparisons among pathways indicate strong spatial and temporal separation. The late summer period showed greatest seasonal separation of the three pathways. Spring values represented a period of relatively high activity for all three pathways. On a depth-wise basis, photolithotrophic activity was greatest near the surface and chemolithotrophic activity greatest near the bottom. Photoheterotrophy took an intermediate position between the two. Diurnally, photoheterotrophy and photolithotrophy showed greatest activity during midday and early morning periods, whereas chemoheterotrophy increased

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throughout the daylight period and reached maximal values in sunset incubations.

Introduction

A fairly complete description of carbon cycling is now available for a few aquatic systems (cf. 6,16,20). These studies, having carried Lindeman's (9) concept of trophic dynamics to its logical conclusion, permit not only an examination of ecosystem structure, but also the examination of rates of exchange between trophic compartments. From this work it is clear that the most important organisms, in terms of either biomass or rates of carbon flow, are to be found in the lower trophic levels. Within the pelagic zone these organisms are represented by the phytoplankton and the bacteria. Theoretical considerations by Harte and Levy (4) suggest that shifts of organisms within these lower trophic levels may lead to more severe problems of ecosystem instability than similar shifts at higher trophic levels.

Characterizing a system and attempting to explain how or why that system persists are two different tasks. Ecologists are often hard pressed even to define "persistence" or "stability," let alone to be able to quantify those perturbations which may result in instability at a particular trophic level. For this type of information one must look at organism function. There is a fundamental difference in the way in which organisms and systems are viewed. Systems are often delimited and generally compared in terms of maximum rates of exchange between compartments or rates of production (e.g., what are the maximum or annual rates of photosynthesis; can this system be characterized as eutrophic?). Organisms, although they may be characterized by maximal contributions to system-wide production, are constrained by minimum rates for the flow of materials. An organism with the potential to produce nearly 100% of a particular system's photosynthesis will not be present to make that contribution unless mechanisms are present to permit survival during or reintroduction after periods of suboptimal growth.

Questions related to the persistence of organisms within systems must be concerned not only with maximum rates or contributions to total energy flow, but also with the timing and distribution of those events during periods when less than maximal contributions are made. An objective of this study was to quantify the carbon flow within microbial components of the pelagic zone. However, the major objective was to characterize the pathways for carbon flow within the microbial community on an annual, diurnal, and spatial basis to permit further examination of the *qualitative* interrelationships among these various pathways.

Carbon flow within an aerobic pelagic microbial community may be characterized by following rates of photolithotrophy, photoheterotrophy, and chemoheterotrophy. Photolithotrophic fixation of CO_2 in aerobic waters is primarily the result of algal activity since photosynthetic bacteria are generally restricted to highly reduced zones. Chemolithotrophy also makes a minor contribution in aerobic systems, generally being restricted to interfaces between aerobic and anaerobic areas. "Heterotrophy" has generally been used synonymously with "chemoheterotrophy" or "chemoorganotrophy." The majority of investigations

of carbon flow have considered heterotrophic activity as occurring only in the dark. Photoheterotrophy, however, requires both light energy and organic materials for the efficient utilization of organic carbon sources. This pathway has not been studied extensively within an ecological context, but it is now clear that a number of algae possess this pathway and are capable of the utilization of at least a limited number of simple organic materials in the light (2,3,10,11). Photoheterotrophy is also observed in the purple nonsulfur bacteria (13), but these organisms are restricted in their distribution, and their contributions to aerobic photoheterotrophic processes are necessarily minor.

Examination of these three pathways permits the characterization of carbon flow in aerobic waters, and the simultaneous assessment of these pathways also allows comparisons among pathways under potentially competitive conditions. Both photolithotrophy and photoheterotrophy involve algal species and require light energy. Competition for light energy could occur among or within various algal taxa. Competition between biochemical pathways could occur even within a single algal cell. Because photoheterotrophy functions at lower light intensities than photolithotrophy (7,12), one might predict that these two pathways would be separated in space within the pelagic zone along a light gradient, or on a diurnal basis with respect to light availability and intensity.

Chemoheterotrophic and photoheterotrophic activities involve bacteria and algae, both potentially competing for the same organic substrates. Since photoheterotrophic algal species are restricted by light availability, it might be predicted that a spatial separation of bacteria and algae would be evident along a light gradient. Additionally, because bacteria are capable of utilizing a greater variety of organic compounds than are photoheterotrophic algal forms, a spatial separation based on substrate availability might also be predicted for these two potentially competing groups of organisms.

The actual mechanisms by which these potential conflicts are resolved are beyond the scope of this investigation. However, the qualitative patterns described as a result of these investigations may permit us to ask questions about competition for resources within pelagic zones in the near future.

Study Site

Lawrence Lake, a small, oligotrophic, hardwater lake in southwestern Michigan (85°21′W, 42°27′N), was selected as the study site. Lawrence Lake has been described in detail elsewhere (1,14,20). The total surface area of the lake is 5.0 hectares; the maximum depth is 12.6 meters with a mean depth of 5.9 meters.

Lawrence Lake is a typical temperate, dimictic lake. Periods of temporary meromixis are experienced approximately 1 year in every 4 during the spring. The lake is strongly stratified throughout the summer with a maximum temperature during 1974 of 25°C. Minimum temperatures of < 1°C were observed during winter under ice cover. Complete mixing occurred in 1974 following ice loss in March. Stratification began during April. Maximal thermal gradient was achieved during the July–August period at depths between 4 and 8 meters. Autumnal overturn began in early November and continued until ice cover was established in December.

The oxygen distribution for 1974 reflected the low to moderate productivity of this temperate system. Oxygen concentrations ranged from > 13 to <1 mg liter^{-1} with maxima under ice in winter, at all depths during spring mixing and within the metalimnion during July. The latter peak in oxygen concentration was associated with high values of photosynthetic oxygen production within that layer. Although reduced oxygen concentrations were observed in the deepest portions of the hypolimnion during late summer stratification, the hypolimnion did not become anaerobic during 1974. In other years the relatively small (<15% of total) volume of water below the 12-meter contour interval has occasionally had no detectable oxygen.

Because of the great buffering capacity of the bicarbonate system in these hardwater lakes, little change in pH was observed over an annual period. pH ranged from 8.0 to 8.2 in epi- and metalimnetic waters. Only at depth and just above the sediments did values approach a pH of <7.6 near the end of summer stratification. Alkalinity values also varied little ranging from 4.2 to 4.4 mEq liter^{-1} in epi- and metalimnetic waters during the ice-free period. Values increased with depth under ice to 4.8 mEq liter^{-1} and approached 5.0 mEq liter^{-1} during summer stratification. Accompanying the increase at depth in late summer was the phenomenon of epilimnetic decalcification resulting in minimum epilimnetic values, < 4 mEq liter^{-1} (see the discussion by White and Wetzel, 21).

Methods

An annual study was initiated in January 1974, with monthly sampling proceeding throughout the year and including all major periods of annual circulation and stratification. Sampling was performed within the constraints of a three-way factorial split-plot design. All water samples for this study were drawn from the central depression in the lake. For each monthly analysis three depths within the water column were sampled (i.e., 2, 6, and 10 meters). At each of these three depths, four separate samples were drawn, each one consisting of a pair of light and dark bottles (125 ml Pyrex glass-stoppered). Three independent incubations were performed during the daylight period (i.e., sunrise, SR; midday, MD; and pre-sunset, SS). *In situ* incubations ranged from 2.4 to 3.5 hours in duration.

Inorganic carbon fixation was monitored through use of tracer quantities of ^{14}C-bicarbonate (4.6 or 5.1 μCi per bottle). The use of light and dark bottle pairs followed closely methods described in Strickland and Parsons (18) and Saunders et al. (17).

Simultaneous to the addition of ^{14}C-bicarbonate, 1 ml of D-glucose-2-^{3}H (specific activity 500 mCi mmole^{-1}) solution was added for an assessment of heterotrophic activity. The quantity of glucose added was in the range of 4 to 5 μg glucose liter^{-1}. This concentration was achieved by dilution of the radioactive substrate without the addition of any nonradioactive carrier. The quantity was not significantly depleted during the incubations (i.e., generally less than 2% of the material was utilized). The concentration of the glucose added was also well below the range where diffusion mechanisms generally operate.

Following incubation, samples were returned to the laboratory and 50-ml aliquots from each bottle were filtered onto 0.22-μm Millipore filters @ < 51 kPa. Filters were stored under desiccation until acid fumed (19) to remove residual $Ca^{14}CO_3$ which may have precipitated during the incubation period. Filters were combusted in an oxygen atmosphere in a Packard Tri-Carb oxidizer (Model 305). The combustion products were isotopically separated as $^{14}CO_2$ and $^{3}H_2O$, collected in liquid scintillation vials, and radioassayed in a liquid scintillation system (Beckman LS-150).

Rates of inorganic fixation as mg C m^{-3}h^{-1} were calculated as the difference between light and dark bottle estimates correcting for available inorganic carbon, bottle volume, quench, recovery efficiency, and isotopic discrimination.

Organic uptake as μg C $m^{-3}h^{-1}$ was calculated from activity observed correcting for radioactive decay, quench, oxidizer recovery efficiency, and bottle volume variation. It was assumed that there was negligible isotopic discrimination against ^{3}H-glucose. Attempts at measuring the *in situ* concentration of glucose during incubations in this oligotrophic system yielded results not significantly greater than background (i.e., approximately 5–10 μg $liter^{-1}$ for the fluorometric assay used). It was therefore assumed that *in situ* values contributed little to the overall dilution of the glucose added. Rates of uptake were calculated based on the concentration of glucose added only, yielding a conservative underestimate of total microbial activity. Although this procedure is likely acceptable in oligotrophic systems because the quantities of naturally occurring glucose are minimal, see Wright's (22) discussion and caution concerning this assumption in other than oligotrophic systems. This method may be contrasted with the kinetic uptake approach which yields maximum rates of uptake at saturation for a sample (5). Actual *in situ* rates of uptake lie between the extremes represented by the maximum values at saturation for the kinetic uptake approach and calculated *in situ* rates assuming no dilution by naturally occurring glucose concentrations.

Photoheterotrophic uptake of glucose was estimated as the difference between light and dark bottle uptake, the following assumptions having been made [see McKinley (10) for a more detailed discussion]:

Light bottle uptake = Photoheterotrophic activity + Chemoheterotrophic activity + Background

Dark bottle uptake = Chemoheterotrophic activity + Background

Chemoheterotrophic activity was estimated as dark bottle uptake less background. "Background" consisted of counter and oxidizer background and not background from a "killed" control sample. The lack of correction for absorption and adsorption results in artificially high estimates for chemoheterotrophic activity as compared to rates of photoheterotrophy. Care also must be exercised in selecting proper time corrections for incubation periods. Light-related activities proceed only while bottles are *in situ*. Dark-related organic utilization proceeds from the time of injection to the time of filtration. A lack of attention to detail will also result in artificially high estimates of bacterial activity as compared to rates of photoheterotrophy.

No attempt was made to correct heterotrophic estimates for rates of microbial respiration. It is probable that this results in an underestimate of total heterotrophic activity, a point that will be returned to in the discussion section.

Results

Figure 1 shows the pattern of photoheterotrophic activity observed over an annual period. Each bar represents the mean of four replicate samples. Individual measures were highly variable ranging from 0 to 27 μg C $m^{-3}h^{-1}$. An annual mean based on all samples (N = 360) was 2.6 μg C $m^{-3}h^{-1}$. Two periods of relatively high activity were observed, the first corresponding to spring overturn during March and just after ice loss. The water column was a uniform 4.3°C and the plankton dominated by diatom species. The second, a metalimnetic maximum in August and the maxima in September generally corresponded to a period of dominance by nonheterocystous blue-green algae (typically a *Chroococcus-Gomphosphaeria-Aphanocapsa* association). Photoheterotrophic activity was usually greater in metalimnetic or hypolimnetic waters except during winter when greatest activity was observed near the surface under ice cover. Highest activity was also generally observed during morning and midday incubations with pre-sunset incubations contributing least to overall rates of fixation.

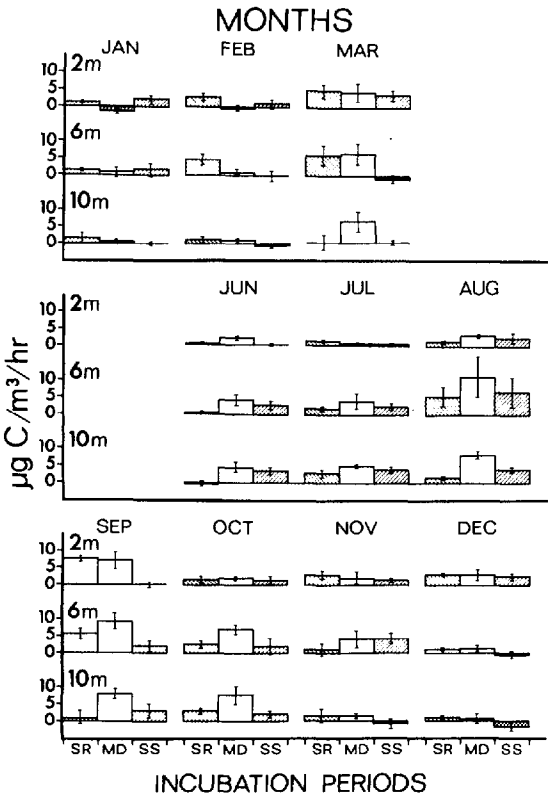

Fig. 1. Estimated values for photoheterotrophic uptake of glucose as $\mu g\ C\ m^{-3}h^{-1}$. Histograms represent means of four replicate samples. Uptake values for each depth interval (2, 6, and 10 meters) and each incubation period [sunrise, midday, and pre-sunset (SR, MD, SS)] are indicated for each month. Bars denote ± SE about the mean. Rare negative mean values are indicated by stippling.

The pattern of chemoheterotrophic activity observed over the annual period is given in Fig. 2. Again each bar represents the mean of replicate samples. Individual measures were less variable than measures of photoheterotrophic activity and ranged from 1 to 18 $\mu g\ C\ m^{-3}h^{-1}$. An annual mean for chemoheterotrophic activity was 6.9 $\mu g\ C\ m^{-3}h^{-1}$ ($N = 360$). The most obvious pattern visible in Fig. 2 is the fairly high, but generally uniform, rates of uptake throughout the entire annual period. Values under ice cover and at 2° to 4°C do not differ substantially from rates observed during the summer or autumn. Greatest overall activity was observed during periods of circulation in March and November. In general, activity was observed to increase throughout the daylight period reaching a maximum during pre-sunset incubations. Activity also increased with increasing depth within the water column.

In the annual pattern of photolithotrophic activity depicted in Fig. 3, it is evident from the error bars that values for inorganic carbon fixation were much less variable than either of the heterotrophic measurements. Mean values ranged from 0 to 9.1 mg $C\ m^{-3}h^{-1}$ with an overall mean based on these values of 1.33 mg $C\ m^{-3}h^{-1}$. Greatest rates of fixation were associated with the metalimnetic nonheterocystous blue-green algal association during August. High epilimnetic values were also observed during March, October, and under ice cover in January. Contributions to total inorganic carbon uptake were dominated by

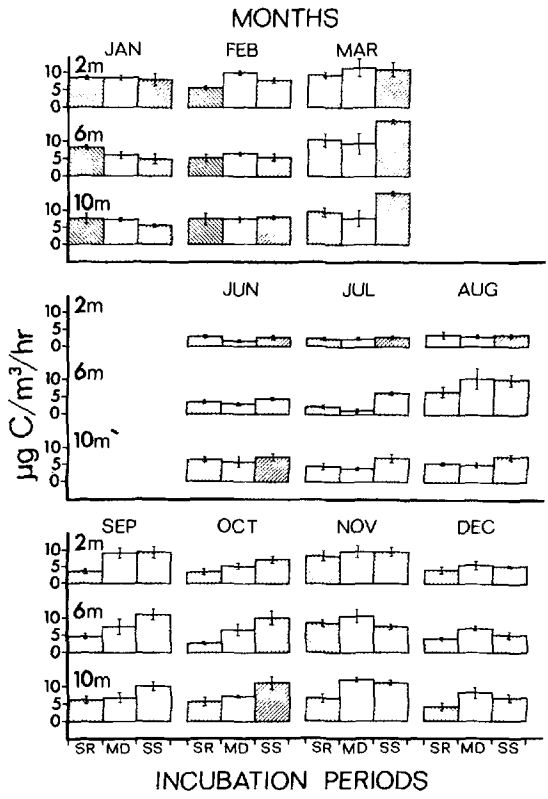

Fig. 2. Estimated values for chemoheterotrophic uptake of glucose as μg C $m^{-3}h^{-1}$. Histograms represent means of four replicate samples. Uptake values for each depth interval (2, 6, and 10 meters) and each incubation period (SR, MD, SS) are indicated for each month. Bars denote ± SE about the mean.

epilimnetic values with insignificant contributions being made by populations at the 10-meter depth interval. Activity was also greater during midday and early morning incubations as compared to pre-sunset incubations.

Discussion

Quantitative Comparisons

It is difficult to obtain good estimates of the rates of carbon transfer in nature. This difficulty is the result of the patchy distribution of organisms, the high variability associated with organismal response, and difficulties in estimating carbon pool sizes. This latter aspect is particularly true with regard to estimates of organotrophy.

The mean estimate derived from this investigation, 1.33 ± 0.12 (SE) mg C $m^{-3}h^{-1}$ (N = 252), for photolithotrophic fixation agrees favorably with a mean from four previous annual estimates, 1.68 ± 0.14 (SD) mg C $m^{-3}h^{-1}$.[1]

[1] This value was calculated from mean estimates reported by Wetzel et al. (20) given a 12-hour daylight period and a mean depth of 5.9 meters for the lake.

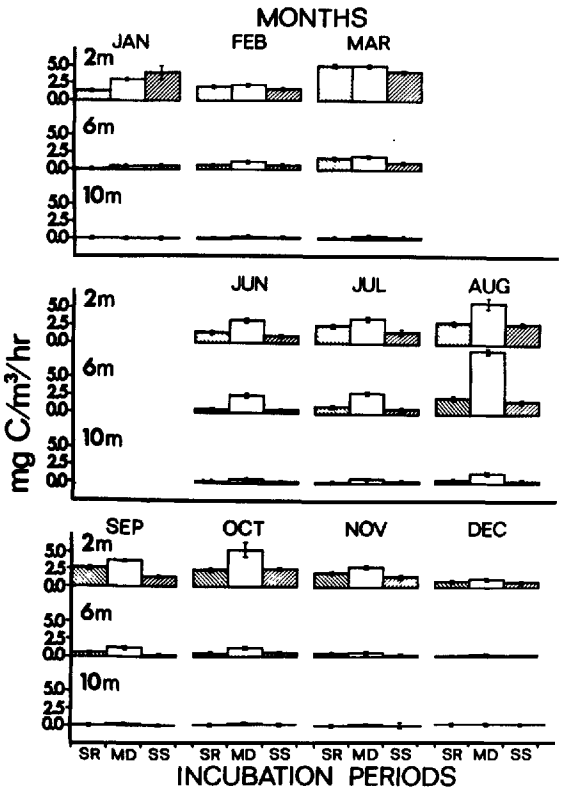

Fig. 3. Estimated values for photolithotrophic uptake of inorganic carbon as mg C $m^{-3}h^{-1}$. Histograms represent means of four replicate samples. Uptake values for each depth interval (2, 6, and 10 meters) and incubation period (SR, MD, SS) are indicated for each month. Bars denote ± SE about the mean.

The design of the *in situ* experiments reported here placed major emphasis on questions of interrelationships in space and time within the microbial community. The carbon pathways of interest were photolithotrophy, photoheterotrophy, and chemoheterotrophy. Less emphasis was placed on absolute measures of rates of heterotrophic activity. The numbers of samples necessary to examine these spatial and temporal relationships precluded any efforts using the kinetic uptake approach or any corrections for microbial respiration. These considerations, however, were of primary importance in the selection of an oligotrophic system in which to examine these pathways [referring again to Wright's discussion (22)].

A more serious fault associated with this study lies in the extrapolation of the data derived from incubations with a single organic compound toward an estimate of total microbial activity rather than their use as relative measures of microbial activity. Surely a number of organic compounds are readily utilized by microorganisms. The germane question therefore is not whether this set of experiments yields an underestimate of microbial activity, but whether the estimates of *in situ* heterotrophic activity generated here can be used as a guide toward a further understanding of actual *in situ* rates of microbial activity. Just what error is likely associated with the estimates derived here? In an attempt to answer this question, we found a comparison to an independent data set for this

system useful. Mean annual rates of chemoheterotrophy and photoheterotrophy obtained as a result of this investigation were 6.9 and 2.6 μg C $m^{-3}h^{-1}$, respectively. Comparisons of these estimates to rates derived from other studies of this system (20) show the following:

The total input to the dissolved organic carbon (DOC) pool on an annual basis was estimated as 41.2 g C $m^{-2}y^{-1}$. This total consists of 14.7 g C from pelagic algal excretion, 5.5 g C from littoral photosynthetic input, and 21 g C as allochthonous carbon entering the lake from the surrounding drainage basin.

Losses to the dissolved organic carbon pool were 35.8 g C $m^{-2}y^{-1}$ due to outflow plus 2.0 g C as a result of co-precipitation with $CaCO_3$ for a total of 37.8 g C $m^{-2}y^{-1}$.

The difference between these estimates (i.e., 41.2 − 37.8 = 3.4 g C $m^{-2}y^{-1}$) might be attributable to heterotrophic metabolism. Assuming that all of this loss is from the autochthonously produced DOC and none is from the more refractory allochthonous fraction, and further that the utilization of the more labile pool is proportional to its production, the pelagic heterotrophic activity may be calculated as follows:[2]

$$(3.4 \text{ g C } m^{-2}y^{-1})(14.7 \text{ g})/(14.7 \text{ g} + 5.5 \text{ g}) = 2.47 \text{ g } m^{-2}y^{-1}$$

The rates of heterotrophic activity obtained experimentally (i.e., 6.9 μg C $m^{-3}h^{-1}$ and 2.6 μg C $m^{-3}h^{-1}$) may be converted to comparable units. Given a 24-hour day for chemoheterotrophic activity, a 12-hour day for photoheterotrophy, and a mean depth of 5.9 meters:

$$\begin{aligned}\text{Photoheterotrophy} &= 67.2 \text{ mg C } m^{-2}y^{-1}\\ \text{Chemoheterotrophy} &= 356.6 \text{ mg C } m^{-2}y^{-1}\\ \text{Total heterotrophy} &= 423.8 \text{ mg C } m^{-2}y^{-1}\end{aligned}$$

Assuming a 50% efficiency in the conversion of carbon to biomass was operative throughout these experiments, not an unreasonable estimate as discussed by Kuznetsov (8), these rates represent a minimum potential loss to the DOC pool of 847.6 mg C $m^{-2}y^{-1}$. Had there been as little as 10 μg glucose or other readily utilizable organic compound present in the system as a result of natural processes in addition to the 4.3 μg glucose added for the experimental determination, estimates obtained here would be in error in proportion to the unaccounted for dilution. The estimate of total heterotrophy would then rise to 2.82 g C $m^{-2}y^{-1}$.

Too much can be made of estimates obtained by calculation. What is clear is that the rates obtained experimentally represent reasonable approximations of pelagic heterotrophic activity for this system. The range 0.85 to 2.82 g C $m^{-2}y^{-1}$ is in remarkable agreement with the independent value obtained from Wetzel et al. (20), representing from 34% to 114% of that value. Whatever the actual rates of heterotrophy, the agreement of these two independent estimates would support the conclusion that rates determined by this investigation are probably within a factor of 3× of actual *in situ* rates of microbial activity.

If the rates determined here are representative of actual rates of heterotrophy

[2] The assumption that none of the loss is due to utilization of refractory allochthonous material is conservative, leading, if anything, to an overestimate of pelagic heterotrophic activity.

within the pelagic zone, there remains one further consideration. However one chooses to express rates of pelagic heterotrophy, both photoheterotrophy and chemoheterotrophy combined appear to contribute small quantities of carbon to the flow of materials within the pelagic zone as compared to rates of photolithotrophic production. For the estimated range obtained above, total heterotrophy is equal to from 2% to a maximum of 8% of photolithotrophic carbon flow. Whether this statement is true of aquatic systems in general, only of oligotrophic systems, or more specifically hardwater, oligotrophic systems must be clarified in future work. Kuznetsov (8) reached a similar conclusion with regard to pelagic heterotrophy based on Hobbie's work on eutrophic Lake Erken. If this pattern holds, we may have to look to littoral and/or benthic areas for a proper assessment of heterotrophic contributions to material flow in aquatic systems.

Qualitative Comparisons

Although it appears that the quantity of carbon cycled by pelagic organotrophic organisms in at least one system is meager, few biologists would argue that the importance of heterotrophic species can always be expressed in quantitative terms. The *in situ* regeneration of nutrients and the production of various organic by-products can have far-reaching importance, even when the quantities of carbon utilized by these organisms remains insignificant. The *in situ* regeneration of materials is likely particularly important in those environments which possess a stratified water column, providing little opportunity for exchange with sediments for much of the year.

Even if the quantitative importance of heterotrophic microorganisms is small, the value of these contributions might be found in the timing—the when and where—that those contributions are made. It is therefore useful to look at the qualitative aspects of the information generated in this investigation. What interactions between or among major carbon pathways occur in aquatic systems? Is competition for resources suggested by the patterns observed, or is partitioning along gradients and within space more prevalent?

Figure 4 shows the annual patterns for Lawrence Lake averaged across depths and times of day for photolithotrophy (mg C $m^{-3}h^{-1}$), photoheterotrophy (μg C $m^{-3}h^{-1}$), and chemoheterotrophy (μg C $m^{-3}h^{-1}$). Each line is based on the mean of 36 samples at 10 monthly intervals ($N = 360$). The best smooth curve reflecting these data was drawn by eye. All three pathways were considerably reduced in activity during winter under ice cover. The lowest rates for chemoheterotrophy were observed during late spring and early summer. Reduced rates for all three processes were observed during early summer. As summer progressed a peak in photosynthetic activity was observed. This maximum was followed in time by broader peaks first in photoheterotrophy, during the declining phase of photolithotrophic fixation, and then by a peak in chemoheterotrophic activity. The drop in algal production in late summer was followed by a slight rebound in October and then by a steady decline into winter. Photoheterotrophic uptake declined steadily from the summer maximum toward winter, but chemoheterotrophic activity continued to increase into November. This increase may have been associated with autumnal overturn and "die-off" of some aquatic macro-

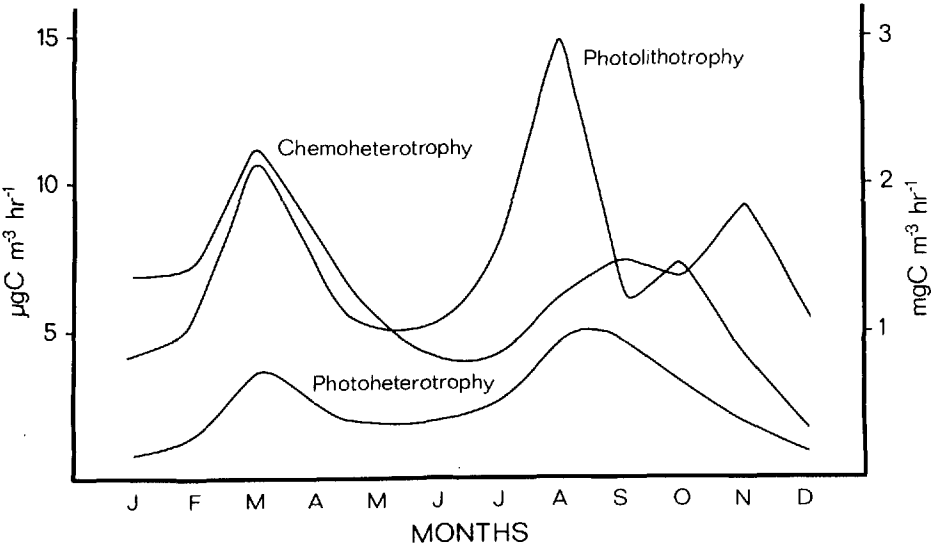

Fig. 4. Annual patterns of activity for photolithotrophy (mg C $m^{-3}h^{-1}$), photoheterotrophy (μg C $m^{-3}h^{-1}$), and chemoheterotrophy (μg C $m^{-3}h^{-1}$) averaged across all depths and times of day. Each line is based on the mean of 36 samples at 10 monthly intervals (N = 360). Best smooth curves reflecting the data were fitted by eye.

phytes and their associated increased concentrations of dissolved organic materials. After "ice-off" in the spring, increased activity occurred in all three pathways.

Assuming that the patterns generated are representative of small, moderately productive lakes, is the apparent synchronization of activities in the spring the result of overriding physical factors (e.g., an increasing availability of light, increasing temperatures, and the recirculation and resuspension of nutrients)? Others (1,20) have suggested that temperature may be a more important factor in regulating heterotrophic activity than substrate availability under aerobic conditions. Although temperature alone cannot account for the patterns observed, some combination of physical factors is likely responsible for the spring pulse.

It is equally obvious that physical factors alone cannot account for the skewed distributions observed in late summer. It may be that we must view the plankton community in terms of its response to major perturbations and yet allow for differential changes under the more stable conditions (e.g., with respect to stratification or nutrient availability). Late summer may represent a period when biogenic mechanisms and physiological capabilities are expressed over time. No such opportunity for differential responses may be available to organisms during the spring.

Figure 5 represents the patterns for the three pathways with increasing depth within the water column averaged across the annual period (N = 252) in this aerobic system. Partitioning is again evident. Whereas maximal values for photolithotrophy were sometimes associated with greater depths (see Fig. 3), maximal activity was generally associated with near surface waters. Chemohet-

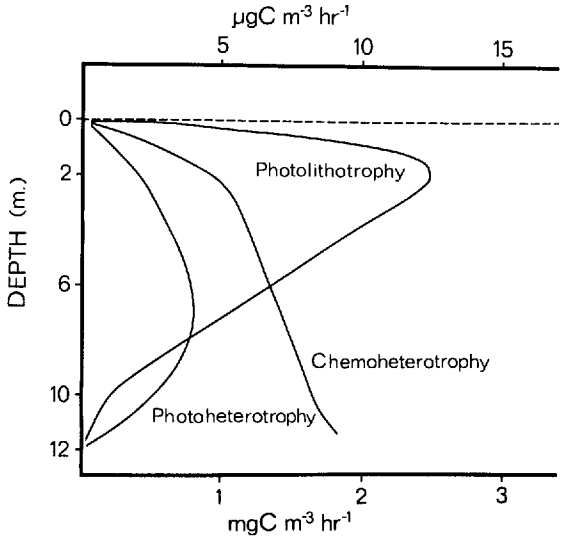

Fig. 5. Patterns of activity for photolithotrophy (mg C $m^{-3}h^{-1}$), photoheterotrophy (μg C $m^{-3}h^{-1}$), and chemoheterotrophy (μg C $m^{-3}h^{-1}$) with increasing depth within the water column. Each line is based on means of 84 samples at the 2-, 6-, and 10-meter intervals (N = 252). Best smooth curves reflecting the data were fitted by eye. All curves were forced through zero at the surface. Curves for photolithotrophy and photoheterotrophy were forced through zero at the sediment/water interface.

erotrophic activity increased steadily with increasing depth. Maximal values were generally associated with the greatest depth interval. There is no evidence of a strong association between rates of photolithotrophy and bacterial heterotrophy. Greatest heterotrophic activity appeared to be associated with hypolimnetic waters, perhaps the result of accumulated quantities of particulate organic carbon in that region.

Photoheterotrophy showed maximal development in intermediate layers. This response was not unexpected because the pathway is capable of operation at light intensities lower than that required for inorganic carbon fixation (7,12). Greatest photoheterotrophic activity was not always associated with intermediate waters and was often closely related to peaks in photolithotrophic activity (compare Figs. 1 and 3). In general, the greatest contributions toward increased total (organic plus inorganic) carbon uptake occurred at greater depth or under conditions of light limitation (e.g., under ice cover). Under these conditions photoheterotrophy contributed from near zero to greater than 250% of the quantities of carbon acquired through photolithotrophy. Based on mean values, however, photoheterotrophy probably contributes only from 2% to 5% of total carbon uptake.

These numbers must be interpreted with caution for two reasons. First, as rates of photolithotrophy approach the limits of sensitivity for the ^{14}C assay (i.e., $\pm$ 75 μg C $m^{-3}h^{-1}$), small changes in the rates of either photoheterotrophy or photolithotrophy are reflected in relatively large departures from unity. Second, it is not likely that all species of algae are photoheterotrophic. Saks et al. (15), working with 12 axenic species of epiphytic algae, found 8 species which were photoheterotrophic. Of those 8 species, 7 showed greater total carbon uptake in the presence of glucose as a result of organic supplementation. Individual responses were highly specific ranging from 103% to 469% ($\bar{x}$ = 180%) of rates of inorganic carbon fixation alone. Thus the benefit of carbon supplementation is

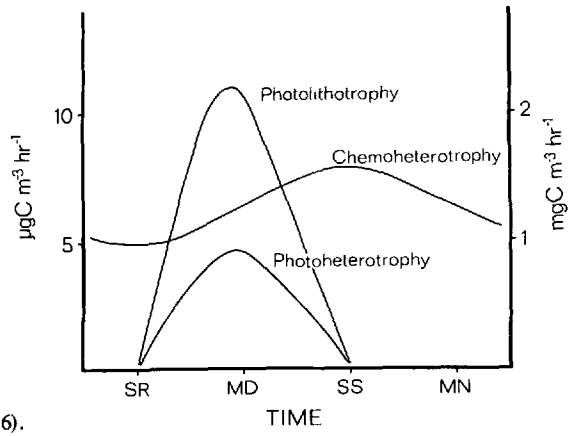

Fig. 6. Diurnal patterns of activity for photolithotrophy (mg C $m^{-3}h^{-1}$), photoheterotrophy (μg C $m^{-3}h^{-1}$), and chemoheterotrophy (μg C $m^{-3}h^{-1}$) averaged across months and depths. Each line is based on means of 84 samples during sunrise (SR), midday (MD), and pre-sunset (SS) incubation intervals with total $N = 252$. Best smooth curves reflecting the data were fitted by eye. Curves for photolithotrophy and photoheterotrophy were forced through zero at sunrise and sunset. The curve for chemoheterotrophy was extrapolated into the dark period based on data published by Saunders (16).

probably realized by only a limited number of species in any algal assemblage. Community uptake values have little meaning in this context.

A summary of the diurnal pattern observed, averaged across months and depths, is represented by Fig. 6. Again lines represent the best fit for the data which produced smooth curves. Values for chemoheterotrophy were extrapolated into the dark period based on general patterns of bacterial activity reported by Saunders (16). Both light phenomena are skewed toward the morning and midday period. Average values for morning incubations are greater than in the late afternoon. Chemoheterotrophic activity is skewed toward the late afternoon with each incubation having produced an increasingly greater value as the day progressed.

Again there is no evidence of a strong relationship between rates of algal photosynthesis and bacterial activity. Rates of photoheterotrophy and chemoheterotrophy are likewise not related on a diurnal basis. One might have predicted a closer relationship between bacterial uptake and photosynthesis with its accompanying production of labile excreted organic materials. The lack of correlation may reflect relatively long lag periods for induction or the fact that bacteria may in general utilize organic materials from other, perhaps less labile, pools.

Conclusions

Many details have necessarily been omitted from this overview of carbon metabolism in the pelagic zone of a hardwater oligotrophic system. Some patterns do emerge, however. First, it appears that heterotrophic pathways within the pelagic account for only minimal cycling of carbon. Second, by examining the patterns of photolithotrophy, photoheterotrophy, and chemoheterotrophy annually, diurnally, and spatially within the water column, we find strong evidence that these three pathways are clearly and distinctly separated in space and time. Whether or not this separation is the result of competition for resources is impossible to say at this time. However, this separation does appear

to result in the maximum utilization of materials within the system, avoiding overt competition for resources, and lending structure via physiological differences to what has been considered a poorly structured environment.

Many questions raised here remain unanswered. But precisely this type of inquiry based on population differences and differing physiological capabilities within the microbial community will allow questions of community structure to be addressed at this particularly important level of ecosystem organization.

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