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Evidence for algal heterotrophy in Lake Tahoe, California–Nevada[1]

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Abstract

Significant differences in the uptake of ^{14}C organic compounds between light and dark bottle incubations of water were recorded from Lake Tahoe. The response to light did not occur in situ below the maximum depth of inorganic carbon photoassimilation and was completely inhibited by a photosynthetic inhibitor. Dark acetate uptake in the deep euphotic zone (ca. 90–150 m) was strongly inhibited by a eucaryotic inhibitor. Microautoradiographic analysis showed that two species of green algae in Tahoe were capable of acetate transport at labeled substrate additions within previously determined ambient limits; in axenic culture these two species grew heterotrophically on acetate. Activities of key enzymes of the major inducible pathway for acetate assimilation were high per unit ATP in the region of the water column where acetate uptake was light stimulated. These data strongly support the hypothesis of heterotrophically active phytoplankton populations at the bottom of the euphotic zone.

The ability of many planktonic algae to use diverse organic nutrients in laboratory culture is well documented (see Neilson and Lewin 1974; Droop 1974). However, the kinetics of heterotrophic uptake by these species often appear inadequate for the low concentrations of organic compounds in natural waters. The high K_s values for organic nutrient assimilation by phytoplankton in general have led to the conclusion that in most planktonic communities it is unlikely that algae can effectively compete with bacteria for the dilute organic substrates present (Wright and Hobbie 1966). Algal heterotrophy has for the most part been considered a laboratory artifact of axenic conditions of culture and artificially high concentrations of organic compounds.

Most attempts to examine algal heterotrophy in the field have relied solely upon short term incubations with radiolabeled organic substrates. By size-fractionating the plankton from such an experiment, Allen (1971) showed that ultraplanktonic flagellates in Star Lake have active transport systems for glucose and acetate which permit them to be competitive with bacteria. Saunders (1972) examined deep-living populations of *Oscillatoria agardhii* var. *isothrix* by microautoradiography and found them capable of organic nutrient transport at ambient substrate concentrations. Other workers have interpreted this type of transport data less cautiously. Maeda and Ichimura (1973) demonstrated passive uptake of labeled organic compounds in samples from an ice-covered lake. They attributed it to algae and concluded that the high algal densities under the ice were sustained by a heterotrophic mode of nutrition. Such studies suffer both from restricted methodological approaches and from the spurious assumption that uptake of isotopically labeled organic nutrients can be equated with organic nutrient utilization, hence with heterotrophy. Organic molecules may enter a cell but only support the synthesis of a very limited range of biochemical compounds (e.g. acetate metabolism without the operation of the glyoxylate pathway: Kornberg and Elsden 1961) or may fail to be metabolized at all (e.g. Palmer and Togasaki 1971).

Final proof of algal heterotrophy in situ must ultimately include the following. 1. Analytical description of the ambient concentrations and rate of supply of utilizable substrates. 2. In situ evidence that the algae have high-affinity transport systems which enable them to take up or-

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ganic compounds effectively at the ambient concentrations. 3. Evidence that the algae are physiologically equipped to use these substrates for growth. 4. An assessment of the relative contribution in situ of dissolved organic substances versus light and CO_2 as carbon and energy sources for phytoplankton growth.

We present here several lines of evidence of types 2 and 3 which strongly support but do not prove the hypothesis that heterotrophically active phytoplankton exist in Lake Tahoe. After preliminary uptake experiments with several low molecular weight organic compounds, we focused on acetate assimilation; previous investigations of type 1 have shown that this substrate is present in relatively high concentrations in the lake throughout the year (up to 4 μg acetate-C·liter^{-1}: Paerl 1973; cf. total biomass carbon, generally 5–10 μg C·liter^{-1}: Holm-Hansen et al. 1976). Radioisotopic data from in situ assays of heterotrophic uptake potential were supplemented with laboratory data assessing the ability of three ultraplanktonic chlorophycean isolates from Lake Tahoe to use organic compounds for growth. As a further guide to the depth distribution of heterotrophically active populations, we examined the water column activities and kinetics of two inducible enzymes of the glyoxylate shunt—the major assimilation pathway for certain C-2 compounds in algae (Neilson and Lewin 1974) and many bacteria (Kornberg and Elsden 1961). Finally, from in situ double-label experiments, microautoradiographic analysis, and previous analytical data we make a preliminary attempt to assess type 4: the relative contribution of heterotrophic and autotrophic modes of metabolism for individual ultraplanktonic algae in Lake Tahoe.

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Methods

Field experiments and assays were done on 12 dates between 17 July 1975 and 23 November 1976 (specific dates in table and figure captions). Photosynthetic rates were determined by the ^{14}C technique of Steemann Nielsen (1952) as modified by Goldman (1963). Heterotrophic uptake was examined by addition of ^{3}H- and ^{14}C-labeled organic compounds to lake water dispensed into 125-ml transparent or darkened Pyrex bottles. Radioactively labeled microorganisms were filtered onto moistened 30-mm-diameter 0.45-μm pore size membrane filters (Millipore) which were rinsed, air-dried, and counted in a Nuclear Chicago gas flow GM counter or, for the double-label experiments, in a basic toluene scintillation cocktail (Lind 1974) with a Beckman LS-100 liquid scintillation spectrometer. In every organic nutrient radioisotope experiment a $HgCl_2$-killed control (0.1 ml of a saturated solution per 125-ml sample) for each depth of sampling was incubated in parallel to correct for adsorption. Microautoradiography was performed by the fission track technique (Knoechel and Kalff 1976) on filters which had been cleared by brief exposure to boiling acetone fumes.

Cells were concentrated for enzymatic analysis by shipboard filtration of 6–10 liters of lake water through 4.25-cm Whatman GF/C filters, after prefiltration through a 75-μm net to remove zooplankton. In the malate synthetase (EC 4.1.3.2) assay, filters were stored frozen for 2 h during transport to the laboratory and then homogenized in ice-cold 50 mM phosphate buffer, pH 8.0, with a Teflon tissue grinder. Homogenates were cleared by centrifugation and used immediately for assay of malate synthetase (Hock and Beevers 1966). Isocitrate lyase (EC 4.1.3.1) activity was measured by Syrett's (1966) assay on cells concentrated by GF/C filtration, rapidly frozen on Dry Ice, and slowly thawed at 4°–5°C. Cellular carbon was estimated by the ATP technique (Holm-Hansen and Booth 1966).

Ultraplanktonic algae from the deep (450 m) aphotic zone of Tahoe were isolated into axenic culture by enrichment, streak plating, and antibiotic treatment (details in Vincent 1977). These isolates

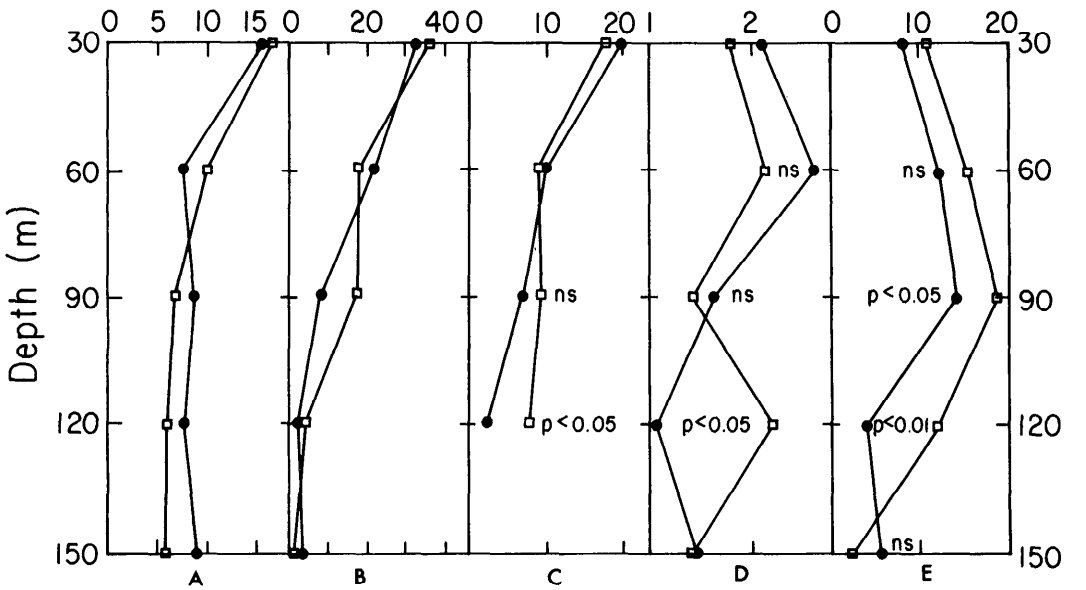

Fig. 1. Uptake of labeled organic compounds (10^2 counts·min^{-1}) in euphotic zone. In situ incubation in light (□) and dark (●) bottles for 4 h: A—[^{14}C]urea (8 August 1975); B—[2-^{14}C]glycine (16 August 1976); C—[2-^{14}C]acetate (17 July 1975); D—[U-^{14}C]glucose (14 August 1975); E—[1-^{14}C]glycolate (9 August 1976). Each point is mean of triplicate samples.

were tested for their ability to grow in the light and dark on dilute inorganic nutrient media ($^1/_{10}$ strength Chu No. 10: Chu 1942) in the presence of glucose, acetate, and glycolate. The organic compounds were added to 50-ml cultures at a rate of 5 μg of carbon (in 0.2 ml) every 2 days; an equivalent volume of water was added to control cultures at the same time. Cells were counted by hemacytometer in small samples removed from the cultures when organic nutrients were added. Each growth experiment was done in duplicate over 10 days. Initial algal densities (10^5 cells·ml^{-1}) were two orders of magnitude higher than in the lake to facilitate counting and to reduce sampling error.

Results

Preliminary in situ incubations—Water samples collected from 30 to 150 m below the surface were incubated for 4 h at the depth of collection with one of five labeled organic substrates at the following activities (per bottle) and final concentrations: 1.0 μCi of [^{14}C]urea, 0.2 μg C·liter^{-1}; 0.5 μCi of [1-^{14}C]glycolate, 8.4 μg C·liter^{-1}; 0.5 μCi of [2-^{14}C]glycine, 1.7 μg C·liter^{-1}; 0.5 μCi of [U-^{14}C]glucose, 11.9 μg C·liter^{-1}; and 0.9 μCi of [2-^{14}C]acetate, 2.9 μg C·liter^{-1}. *F*-test comparisons were made for each depth between final radioisotope levels in the cells of the light and dark bottles. No significant differences were recorded for urea or glycine between light and dark uptake at any depth (Fig. 1A, B). A significant response to light with acetate (Fig. 1C) was recorded at 120 m but not at shallower depths (depths >120 m were not tested). Similarly, differences between light and dark treatments were only significant at 120 m for glucose (Fig. 1D) and at 90 and 120 m for glycolate (Fig. 1E).

The response of cells from the aphotic zone to light and organic compounds was examined in a displacement experiment. Samples from aphotic depths of 150, 250, and 400 m were incubated in light and dark bottles at one depth (105 m) in the euphotic zone with the same ^{14}C-labeled organic compounds. For comparative purposes 50-m water samples were also

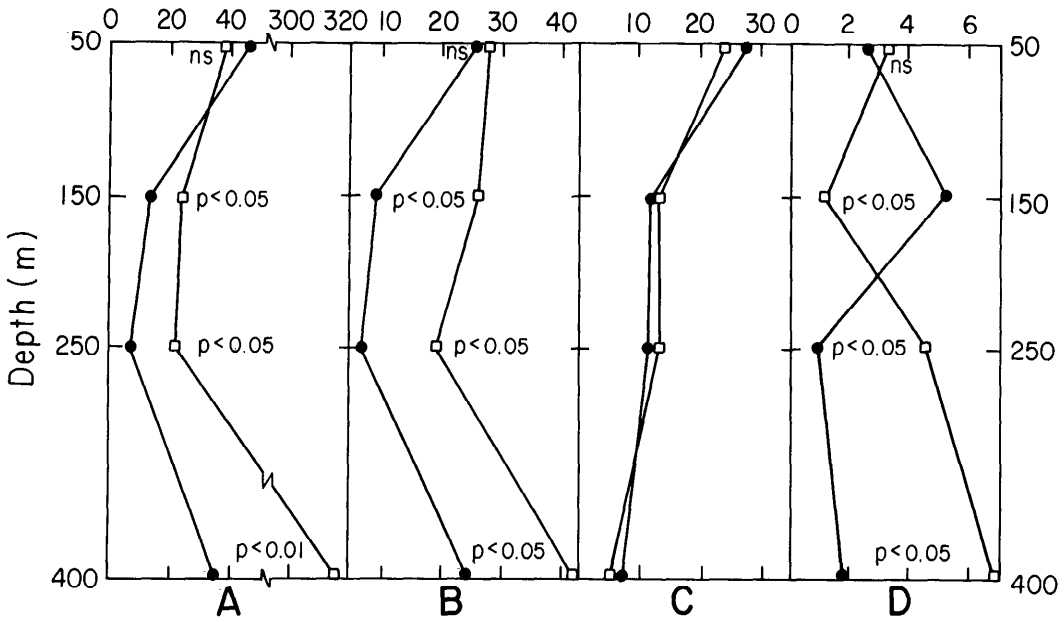

Fig. 2. Displacement experiment. Uptake of labeled organic compounds by aphotic and euphotic samples all incubated at 105 m in light (□) and dark (●) bottles for 4 h on 7 August 1976: A—[^{14}C]glucose; B—[^{14}C]acetate; C—[^{14}C]glycine; D—[^{14}C]glycolate. Each point is mean of duplicate samples.

incubated at 105 m. There were no statistical differences between light and dark bottle uptake by 50-m water for any of the four organic substrates tested (Fig. 2). However, differences were significant in water from all three depths of the aphotic zone with acetate, glucose, and glycolate. The glycine-uptake response was the same as with euphotic samples, with no significant effect of light on samples from any depth.

Response to light in relation to depth of incubation—Water from 30, 120, and 150 m was incubated with [2-^{14}C]acetate (levels as above) at each of the three depths of collection in light and dark bottles. On this occasion a response to light by 30- and 120-m samples could not be induced by incubation at any of the three depths. From an overall analysis of variance for all three depths of incubation, F-values were 0.95 ($P > 0.25$) for 30-m water and 0.02 ($P > 0.5$) for 120-m water. The overall F-value for 150-m water was 7.09 ($P < 0.05$) and a detailed analysis showed that the effect of light on the 150-m microbial populations was statistically significant at all three depths of incubation (Table 1). Significant differences between light and dark acetate-C incorporation at the bottom of the euphotic zone would therefore appear to result from a distinct population of pigmented microorganisms capable of heterotrophic uptake rather than being an effect of the specific light and temperature regime at this depth on heterotrophic species distributed throughout the water column.

Response to light in relation to photosynthesis—To localize the region of acetate transport in relation to inorganic carbon fixation down the water column we did an in situ double-label experiment to measure acetate uptake and photosynthesis simultaneously. [^{3}H]acetate was added at levels of 1.0 μCi per 125-ml bottle, 0.29 μg acetate-C · liter^{-1} final concentration, and NaH$^{14}CO_3^-$ at 10 μCi per bottle. There was no effect of light on acetate uptake below 125 m—the lowest depth of inorganic carbon photofixation (Fig. 3). Since ambient acetate-C concentrations and respiratory losses are not known, no conclusion can be drawn from

Table 1. Light-stimulated [2-^{14}C]acetate uptake by 150-m microbial populations in relation to depth of incubation. Water sampled 11 August 1976 and incubated in duplicate light and dark bottles (counts·min^{-1} corrected for background and adsorption).

Incubation depth (m)	[2-^{14}C]acetate uptake (counts·min^{-1})		F, P for light vs. dark
	Light	Dark	
30	685	459	7.47, $P < 0.05$
120	550	345	6.23, $P < 0.05$
150	765	474	12.40, $P < 0.05$

the variation in magnitude of [^{3}H] acetate uptake with depth.

In a second double-label experiment, samples from 150 m were incubated in light and dark bottles with $H^{14}CO_3^-$ and [^{3}H]acetate with and without 1 h of prior incubation at 5°C in the dark with 10^{-5} M DCMU. Bottles were incubated in situ at 105 m to ensure adequate light for photoassimilation because the photosynthesis experiment done 2 weeks earlier had suggested that 150-m populations at this time of year (midautumn) were light-limited. The inhibitor DCMU completely eliminated the effect of light on uptake of both inorganic-C and acetate-C but had no statistically significant effects on dark uptake of either (Table 2). Uptake and retention of acetate-C by this microbial community is therefore dependent upon full operation of photosystem II and noncyclic electron flow. This is a general requirement for light stimulation of acetate uptake and metabolism by algae (e.g. Neilson and Lewin 1974).

Effect of eucaryotic and procaryotic inhibitors on dark acetate uptake—Water from 30, 90, and 150 m was preincubated for 6 h in the laboratory at 5°C on 8 July 1976 with the eucaryotic antibiotic cycloheximide or the procaryotic antibiotic chloramphenicol, each at a final concentration of 10 mg·liter^{-1}. We have found these concentrations generally successful to inhibit growth selectively. Preliminary experiments had established 6 h (3 h of light, 3 h of dark) as the necessary preincubation time for these compounds to affect the metabolism of the Lake Tahoe microbial flora. After this antibiotic pre-

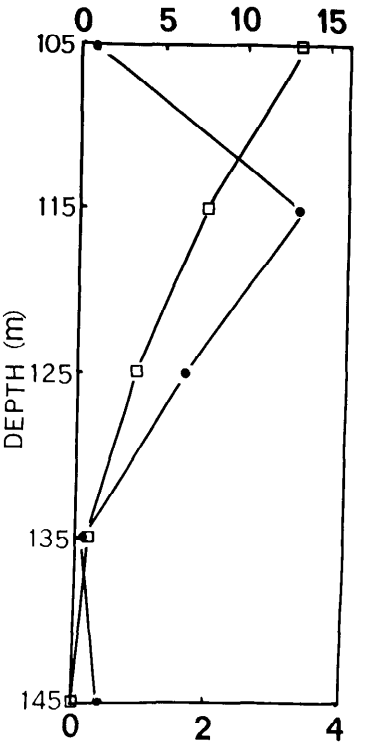

Fig. 3. Upper axis—photofixation (10^{-2} mg C·m^{-3}·h^{-1}) of inorganic carbon (□); lower axis—apparent photoassimilation (10^3 counts·min^{-1}) of [^{3}H]acetate (●); 5 November 1976. Each point represents difference between means of triplicate light and dark bottles incubated 2 h in situ.

treatment [^{14}C]acetate was added (0.9 μCi per bottle, 2.9 μg C·liter^{-1}) and uptake determined for a 2-h incubation in the dark.

Dark uptake of acetate by 30-m samples was little affected by the eucaryotic inhibitor (Table 3) but was almost completely inhibited by the procaryotic an-

Table 2. Effect of 10^{-5} M DCMU on photoassimilation of acetate (dis·min^{-1}) and iorganic carbon (mg C·m^{-3}·h^{-1}). Water collected 23 November 1976 from 150 m. Inhibitor added 1 h before 2-h incubation with [^{3}H]acetate and $H^{14}CO_3^-$ at 105 m. Each figure is mean of triplicate samples.

	[^{3}H]acetate		Inorg-C	
	Light	Dark	Light	Dark
No inhibitor	1,397	747	0.141	0.037
10^{-5} DCMU	633	719	0.042	0.053

Table 3. Effect of eucaryotic (cyloheximide) and procaryotic (chloramphenicol) inhibitors on dark assimilation of [2-^{14}C]acetate (counts·min^{-1}) at 10°C. Antibiotics added 6 h before incubation. Each figure is mean of duplicates. In parentheses are percentage values for decrease from control (no antibiotic addition).

Depth (m)	Dark [2-^{14}C]acetate uptake		
	No addition	Eucaryotic inhibitor	Procaryotic inhibitor
30	5,137	4,471 (13)	88 (98)
90	594	487 (18)	209 (65)
150	5,295	1,222 (77)	4,016 (24)

tibiotic. At 90 m only the procaryotic antibiotic had a statistically significant effect. At 150 m, both antibiotics depressed acetate uptake, with the most pronounced inhibition by the eucaryotic inhibitor.

Species-specific eucaryotic acetate transport—Water collected the same day as the inhibitor experiment above from 90, 120, and 150 m was incubated in light and dark bottles in situ with [^{14}C]acetate. Filters from this experiment were counted by a Geiger-Müller counter and then prepared for fission track microautoradiographic analysis. Statistically significant photoassimilation of acetate was recorded at 120 and 150 m but not at 90 m (Table 4). Phytoplankton counts from samples throughout the water column on this day showed at least six species which achieved peak biomass in the deep euphotic zone. The microautoradiographic analysis of these filters showed only two species, *Monoraphidium contortum* and a 2-μm-diameter chlorosphaeralean, *Friedmannia* sp., capable of acetate transport (Table 4). For both species acetate uptake was higher in the light at all depths except that *M. contortum* at 150 m had very high dark uptake rates that did not differ statistically from rates in the light. Both of these species were usually rare in the surface euphotic zone (*M. contortum* has never been detected above 75 m except during winter mixing) but achieved peak cell densities at the bottom of the euphotic zone and were well distributed throughout the aphotic region (Vincent 1977).

Water column glyoxylate shunt activity—Malate synthetase activity at a midlake station on 5 October 1976 showed two peaks: one at 60 m, the other at 150 m (Fig. 4). The activity of this enzyme per unit biomass was highest at 30 m, decreased by a factor of 4 at 90 m, and then rose again to relatively high values throughout the aphotic zone. Isocitrate lyase activity per unit biomass assayed 3 weeks later at 50, 100, and 150 m showed a similar pattern. The kinetic properties of isocitrate lyase at 50 and 150 m were compared on this date of sampling. V_{max} and K_m values were derived from Woolf-

Table 4. Photoassimilation of [2-^{14}C]acetate, 8 July 1976. Total uptake rates are means for triplicate light and dark bottle counts·min^{-1}, incubated in situ for 4 h. Counts corrected for background and adsorption. Acetate uptake in these samples by *M. contortum* and *Friedmannia* sp. determined by microautoradiography. Each figure is mean number of fission tracks per cell for 15–30 cells ± 95% confidence limits.

		[2-^{14}C]acetate uptake		
		90 m	120 m	150 m
Total uptake				
Light		1,537	3,482	1,740
Dark		1,454	1,970	996
Light vs. dark		NS	$F = 16.32$, $P < 0.01$	$F = 5.29$, $P < 0.05$
Species-specific uptake (tracks/cell)				
Monoraphidium contortum	Light	1.70±0.47	2.00±0.47	2.12±0.50
	Dark	0.93±0.46	1.17±0.16	2.25±0.66
Friedmannia sp.	Light	1.10±0.42	1.50±0.40	1.20±0.18
	Dark	0.52±0.22	0.44±0.26	0.64±0.30

Augustinsson-Hoftsee plots (Segel 1976) of enzyme reaction velocity versus velocity over isocitrate concentration at four concentrations of isocitrate. V_{max} values, which reflect the magnitude of glyoxylate shunt activity at each depth, were similar (50 m: 818 pkatals·liter^{-1}; 150 m: 625 pkatals·liter^{-1}). K_m values, which relate specifically to the isozyme characteristics of each community, differed by almost an order of magnitude (50 m: 2.28 mM D-isocitrate; 150 m: 0.37 mM D-isocitrate). However, since we used only four concentrations of substrate, the error in the K_m estimates is very large and the slopes of the kinetic plots ($-1/K_s$) were not statistically different ($F = 3.95$, $P = 0.1$–0.25).

Ability of Tahoe isolates to grow heterotrophically—Growth of *Chlorella saccharophila* var. *saccharophila* was dramatically stimulated in the light by glucose (F for the difference between control and glucose growth rates is 43.36, $P < 0.005$) but not by acetate or glycolate (Table 5). In the dark no growth was recorded on any compound tested. Acetate greatly stimulated growth of *Friedmannia* sp. in the light (F for control vs. acetate growth rate is 376.4, $P < 0.005$), whereas glycolate and glucose had no effect. In the dark, the cells grew slowly on acetate (F for control vs. acetate is 17.91, $P < 0.005$). *Monoraphidium contortum* grew very slowly under all conditions, and growth rates for this isolate were therefore estimated from day 0 and day 10 cell counts only (Table 5). There was a marked stimulation of growth of *M. contortum* in the light by glycolate (F for day-10 cell densities, control vs. glycolate, is 500.2, $P < 0.005$) and to a lesser extent by acetate (F for day-10 cell densities, control vs. acetate, is 112.5, $P < 0.005$). In the dark only acetate produced final cell concentrations significantly higher than the initial density ($F = 53.26$, $P < 0.025$).

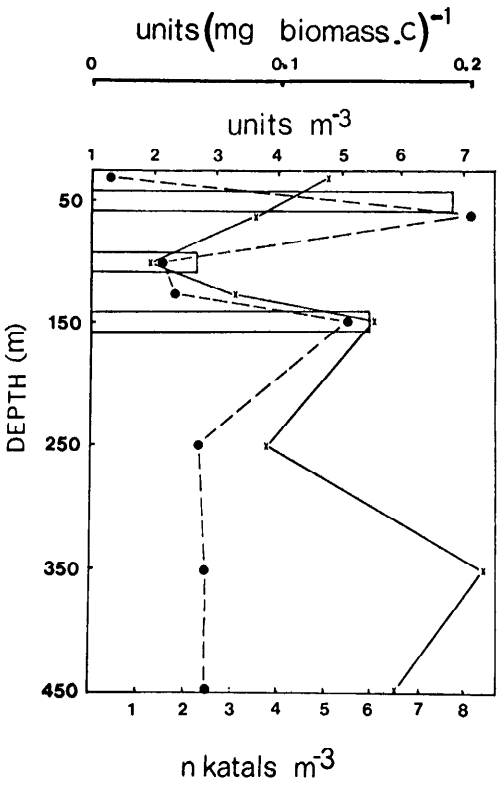

Fig. 4. Activity of key enzymes of glyoxylate pathway throughout water column. Upper axes—relative malate synthetase activity (dashed line—per liter; solid line—per unit biomass carbon) assayed on 5 October 1976 with cell-free homogenates at 30°C. Lower axis—isocitrate lyase activity per liter (bars) assayed at three depths on 25 October 1976 (1 nkatal is conversion of 10^{-9} mol of substrate per second).

Discussion

The effect of light, DCMU, and a eucaryotic inhibitor on organic carbon uptake, retention, or both strongly suggests an algal component to the heterotrophic community of Lake Tahoe. However, several alternative hypotheses must be considered. One possible explanation of the effect of light on uptake is that the labeled organics are metabolized by bacterial populations to $^{14}CO_2$ which is then refixed by algae photosynthetically. This seems highly unlikely, since both bacterial activity and photosynthesis are most intense in the uppermost 60 m of Tahoe where the response to light was never recorded. Such an explanation also requires impossibly high photosynthetic rates and unlikely rates of conversion of [2-^{14}C]acetate to $^{14}CO_2$.

Table 5. Heterotrophic growth rates for ultraplanktonic isolates in axenic culture. Species isolated from Lake Tahoe and grown on 1/10 concentration Chu 10 inorganic nutrient media supplemented with glycolate, acetate, or glucose (5-μg org-C additions to 50-ml cultures every 2 days). Incubation was at 5°C in either complete darkness or under 2,500 lux "cool-white" fluorescent lighting (— no significant difference between final day cell density and initial cell density).

		Growth rate (doublings per day)			
		Control (no addition)	Glycolate	Acetate	Glucose
Chlorella saccharophila var. *saccharophila*	Light	0.31	0.31	0.26	0.50
	Dark	—	—	—	—
Monoraphidium contortum	Light	0.14	0.24	0.17	0.13
	Dark	—	—	0.01	—
Friedmannia sp.	Light	0.33	0.32	0.49	0.31
	Dark	—	—	0.12	—

An alternative explanation for the increased ^{14}C labeling of cells in the light is that the organic molecules are metabolized by algae to $^{14}CO_2$ which is then photosynthetically refixed while still within the cell. This mechanism was suggested by Bennett and Hobbie (1972) who found decreasing $^{14}CO_2$ evolution at increasing light intensities when *Chlamydomonas* was incubated with [^{14}C] glucose. Thus, apparent light stimulation may represent increased retention of label in the light, not photoheterotrophy. Although this explanation remains a possibility for the [^{14}C]glucose and glycolate experiments it seems highly unlikely for the [^{14}C]acetate incubations. Apparent photoassimilation of acetate by this mechanism would require extreme respiratory losses in the dark. For example on 17 July 1975 at 120 m at least 77% of the [^{14}C]acetate which entered the algal cells must have been respired to CO_2 if the increased labeling in the light is to be explained by refixation. Even this percentage loss may be a severe underestimate since it assumes total refixation of respired CO_2. However, the ^{14}C label on the 2-carbon of acetate is lost as $^{14}CO_2$ at very slow (if not insignificant) rates when compared with the catabolism of other labeled substrates including [1-^{14}C]acetate (e.g. Cooksey 1972). At least one substrate, therefore, was taken up heterotrophically in the light, and this light-stimulated uptake was greater than that by bacteria at the depths tested in the deep euphotic zone.

In most of the radioisotope assays the effect of light was to increase the labeling of the microbial communities relative to that in the dark. In the glycolate experiments, however, labeling in the light was sometimes lower than in the dark. This may reflect increased respiratory losses of glycolate carbon in the light since this compound is rapidly converted to CO_2 by both algae and bacteria; respiratory losses by bacterial communities can account for up to 69% of total glycolic acid uptake (Wright 1975). In algae the major metabolic pathway utilizing glycolate is photorespiration, a process which operates only in the light (Tolbert 1974). With the in situ glycolate experiments a separate dissimilation mechanism may have operated in the dark, resulting in lower rates of metabolism of glycolate to CO_2, or alternatively this organic acid might have been taken up in the dark and not metabolized. Fogg (1976) noted that although glycolate photoassimilation has been demonstrated for several algal species, heterotrophic growth of algae on this compound is rare.

Our radioisotope field data show that certain algal species in Lake Tahoe could take up at least one organic compound, acetate, at labeled concentrations within the ambient limits measured by Paerl (1973). However, uptake need not imply utilization (e.g. Palmer and Togasaki 1971). We examined this second aspect of the problem by the culture studies on phytoplankton isolates and by assay throughout the water column for key en-

zymes of the main biochemical pathway for acetate utilization. The culture experiments showed that the two species capable of acetate transport in the field were physiologically equipped to use acetate for growth in both light and dark. The distribution of malate synthetase and isocitrate lyase activities in the water column is also consistent with the hypothesis of heterotrophically active algae at certain depths in Lake Tahoe. The deeper malate synthetase peak corresponds roughly to the zone of light-stimulated, labeled organic nutrient uptake near the bottom of the euphotic zone. The high glyoxylate pathway enzyme activity per unit biomass throughout the aphotic zone correlates well with the distribution of ultraplanktonic green algae and the aphotic community response to light in the radioisotope experiments.

No statistically significant light stimulation of organic uptake has ever been recorded in the region of the upper glyoxylate enzyme peak and this suggests that the upper heterotrophic maximum might be predominantly due to bacteria. This view is in accordance with Paerl's (1973) conclusion that bacterial activity in Tahoe is at a maximum in the upper euphotic waters and is further supported by the insensitivity of acetate uptake by this upper community to the eucaryote inhibitor, cycloheximide.

In the absence of a reliable estimate of sampling error, our enzyme data must be interpreted with caution. Although the differences in enzyme activity down the water column are considerable (up to a 7-fold variation in activity per unit biomass with depth) and the sampling site is well away from shoreline influences (8 km to nearest shore), the scale of horizontal variation in these biochemical parameters remains potentially large. The antibiotic data must also be viewed cautiously. Chloramphenicol will eventually affect eucaryotes by inhibiting mitochondrial and chloroplast protein synthesis. Furthermore, the specificity of cycloheximide as an inhibitor of eucaryotic protein synthesis has been questioned (McMahon 1975). The dramatically different effects of these inhibitors on the upper and lower euphotic populations, however, is strong evidence that two physiologically distinct, vertically separated heterotrophic communities exist in this region of the lake. The order of magnitude (though not statistically proven) difference between community K_m values for the 50- and 150-m isocitrate lyase isozymes is also consistent with a shallow water procaryotic maximum in heterotrophic activity and a deep euphotic, eucaryote-dominated, heterotrophic community.

Previous data concerning algal heterotrophy in Lake Tahoe are limited and contradictory. In a study of bacterial metabolism in Tahoe, Paerl and Goldman (1972) concluded from microautoradiographic analysis that all uptake of ^{14}C-labeled organic compounds was by bacteria since no label was ever associated with the algae. Later, however, Tilzer et al. (1977) noted that Tahoe microautoradiographs revealed slight but significant algal uptake of organic substrates. Our own data suggest that this inconsistency may have arisen from differences in depth of sampling, since throughout most of the euphotic zone the ultraplanktonic algae that can assimilate organic compounds at low substrate concentrations are an inconspicuous component of the phytoplankton. It is only at the bottom of the euphotic zone, and throughout the aphotic zone, that these cells represent a significant proportion of the total planktonic population.

Supplemental organic nutrition must obviously confer a biological advantage to populations living at the bottom of a euphotic zone relative to a simple photoautotrophic mode of existence. In this region of the water column the energy input from solar radiation is extremely low but inorganic nutrients are in relatively plentiful supply. One biochemical strategy for overcoming the potential energy limitation at this depth is a cellular increase in pigment concentrations to improve light-capturing ability, and this has been described for the Tahoe phytoplankton community by Paerl et al.

(1976). A second strategy in Tahoe may involve light-stimulated organic nutrient uptake and, perhaps for some species such as *M. contortum*, true heterotrophy. Tahoe's deep hypolimnetic euphotic zone is well below the region of maximum bacterial activity (Paerl 1973) and therefore algal heterotrophy may be further favored by reduced bacterial competition for the organic substrates available. The potential success of this mechanism of deep euphotic survival has been discussed by Fogg (1976) in relation to glycolate photoassimilation at great depth and by Saunders (1972) in relation to populations of *Oscillatoria agardhii* var. *isothrix* which grow at threshold light intensities just above the aphotic zone.

In terms of total carbon flow the contribution of acetate-C to overall algal nutrition in Lake Tahoe cannot be significant. Light-stimulated organic nutrient uptake was only recorded in a narrow region near the bottom of the euphotic zone where *M. contortum* and *Friedmannia* sp. (but also several other species) achieved maximum population densities. Even in this region acetate-C uptake was low by comparison with photosynthetic rates for the assemblage as a whole. For example, from the data of Fig. 3, maximum light minus dark uptake of labeled acetate was recorded at 115 m. From Paerl's (1973) published range of acetate levels in Lake Tahoe this converts to light-stimulated rates of 1.07–10.7 μg acetate-C $\cdot m^{-3} \cdot h^{-1}$, which amount to only 1.5–15% of the photosynthetic carbon uptake rates at this depth. Organic carbon uptake does seem important however when compared to estimated photosynthetic rates for the two ultraplanktonic species responsible for acetate photoassimilation. On 5 November ATP biomass at 115 m amounted to 8.5 mg C $\cdot m^{-3}$. *Monoraphidium contortum* achieved a biomass of ca. 0.3 mg $\cdot m^{-3}$ (from counts: Vincent 1977). If this species is as photosynthetically active per unit biomass as the community as a whole then photosynthetic rates on this date were 2.6 μg C $\cdot m^{-3} \cdot h^{-1}$. Similarly, a photosynthetic rate of 6.9 μg C $\cdot m^{-3} \cdot h^{-1}$ can be calculated for *Friedmannia* sp.—a total of 9.5 μg C $\cdot m^{-3} \cdot h^{-1}$. Total acetate uptake in the light by these species amounted to about 220% of that of light minus dark (Table 4). Total acetate uptake for the two populations was therefore 2.3–23.5 μg C $\cdot m^{-3} \cdot h^{-1}$, or 25–247% of the photosynthetic carbon uptake by these cells. The operation of dark uptake throughout the night (cf. photosynthesis) and the possibility that other low molecular weight organic compounds are also used suggest that a heterotrophic mode of nutrition is particularly important for these species. These conclusions, however, remain speculative in the absence of reliable analytical data for acetate during the period of sampling and without microautoradiographic data to compare the relative photosynthetic performance of the different algal species at this depth in Lake Tahoe.

Several workers have recognized the potential significance of organic nutrient utilization for aphotic survival of phytoplankton in Lake Tahoe (Kiefer et al. 1972; Tilzer et al. 1977). Certain algae in the deep aphotic zone remain viable and retain a fully operational photosynthetic apparatus for periods in excess of 12 months (Vincent 1978). In culture, algal growth in the dark on acetate was extremely slow, despite relatively high substrate concentrations. It is therefore highly unlikely that Tahoe's aphotic zone supports substantial growth of deep-living populations of phytoplankton. However, our study has shown that several species within this community can actively transport and metabolize low molecular weight organic compounds. It is therefore probable that under prolonged aphotic conditions the biochemical costs of cellular maintenance are at least partially met by heterotrophic nutrition.

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