Autotrophic processes in meromictic Big Soda Lake, Nevada

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Abstract

Daily rates of oxygenic photosynthesis (OP) by phytoplankton, anoxygenic photosynthesis (AP) by purple sulfur bacteria. and chemoautotrophic productivity (CP - dark CO_2 assimilation) were measured once each season in saline. mrromictic Big Soda Lake Total daily productivity and the relative importance of each autotrophic process varied with seasonal changes in vrrtical mixing, light availability. and the biomass of phototrophs. Daily productivity was highest (2,830 mg $C \cdot m^{-2}$) and was dominated by OP in winter when the mixolimnion was isothermal, the biomass of phytoplankton was high, and the biomass of purple sulfur bacteria was low During the summer–fall period of thermal stratification, phytoplankton biomass decreased, a plate of purple sulfur bacteria formed below the oxycline, and daily rates of dark CO_2 assimilation (CP = 390–680 mg $C \cdot m^{-2}$) exceeded phototrophic productivity (OP + AP – 200–370 mg $C \cdot m^{-2}$) Total annual productivity was about 500 g $C \cdot m^{-2}$, of which 60% was produced by phi-toplankton (mostly in winter), 30% by chrmoautotrophs (nitrifying and sulfur-oxiclizing bacteria', and only 10% by photosynthetic bacteria.

411 aspects of aquatic ecology relate, at least indirectly, to processes associated with the production, consumption, or decomposition of organic matter In aerobic pelagic systems, the sole process of autotrophic production is oxygenic photosynthesis by phytoplankton or macrophytes However, in anaerobic waters or in the redox gradient of sediments or stratified waters, two other processes of autotrophic production can be important, anouygenic phototrophy by photosynthetic bacteria (or cyanobacteria), and chemoautoirophy. In some pelagic waters, these two processes are significant For example, in highly stratified lakes anoxygenic photosynthesis can approach or exceed annual primary productivity by phytoplankton (Culver and Brunskill 1969, Cohen et al 1977; Lawrence et al 1978) Seki (1968) found that dark CO_2 assimilation by chemoautotrophs nearly equalled photos) nthetic production during spring in Aburatsubo

Inlet, and Sorokin (1970) and Takahashi et al. (1970) made similar observations in Lake Gek Gel and Lake Suigeta. High rates of dark CO, assimilation have also been measured in the Black Sea (Sorokin 1964), the Saelenvann estuary (Indrebø et al 1979*b*), and the Cariaco 'l'rench ('l'uttle and Jannasch 1979). Kepkay el al. (1979) found that chernoautoirophy may provide an appreciable source of organic matter in marine sediments, and bacterial chemoautotrophy may be the major source of primary productivity for deep-sea thermal vent communities (Jannasch and Wirsen 1979).

Organic matter produced by photosynthetic sulfur bacteria and chemoautotrophs can be used by organisms at higher trophic levels. Autotrophic bacteria are ingested by cladocerans and copepods (Sorokin 1958; Takahashi and Ichimura 1968), and ingestion by zooplankton is enhanced when chemoautotrophy is stimu-

lated (Sorokin 1965). Dense accumulations of zooplankton are often seen in or near bacterial plates (Sorokin and Donato 1973; Matsuyama and Shirouzu 1978), further suggesting that autotrophic bacteria are food for zooplankton. Moreover, autotrophic bacteria play major roles in the cycling of nutrients, the mineralization of organic matter, and in controlling the distribution of oxygen.

Much of our knowledge concerning the production of biomass by photosynthetic sulfur bacteria and chemoautotrophs has come from studies of meromictic lakes. These lakes are useful natural systems for studying all three production processes because they have permanent redox gradients in the water column and often have the overlapping photic and anoxic zones required to sustain anoxygenic photosynthesis. We present here the results of a seasonal study of autotrophic productivity in rneromictic Rig Soda Lake, Nevada. Photosynthetic productivity by phytoplankton and sulfur bacteria and chemoautotrophic productivity (dark assimilation of CO, near the oxycline) were measured with ^{14}C during each of the four seasons. We found that the magnitude and relative importance of each process varies seasonally in response to changes in vertical mixing, light availability, and biomass of autotrophs.

Big Soda Lake is a small ($1.6 km^2$) crater lake that has become meromictic in this century (Kimmel et al. 1978). A sharp chemocline at 34.3 m separates the mixolimnion (total dissolved solids = 26 g·liter^{-1}) from the monimolirnnion (TDS = 88 g.liter^{-1}). Ionic composition is dominated by sodium and chloride-sulfate-bicarbonate (Kharaka et al. 1981); the pH is 9.7. The monimolimnion has a constant temperature of 12°C, is permanently anoxic, and has high concentrations of reduced sulfur compounds (310 mg·liter^{-1} as H_2S), NH, (43 mg·liter^{-1}), dissolved organic carbon (60 mg·liter^{-1}: Kharaka et al. 1981), and CH_4 (50 μM: Oremland et al. 1981). In winter the mixolimnion is nearly isothermal (4°–5°C) and phytoplankton biomass is maximal; the winter bloom is dominated by *Nitzschia palea* and *Chaetoceros*

sp (Cloern et al 1983) During summer and fall the mixolimnion is thermally stratified, algal biomass is low, the phytoplankton comprises several species of chlorophytes, and a plate of purple sulfur photosynthetic bacteria (*Ectothiorhodospira vacuolata*: H Triiper pers. comm) forms below the depth of oxygen disappearance. Axler et al (1978) and Priscu et al (1982) measured primary productivity by phytoplankton and photosynthetic bacteria during spring; total daily photographic productivity was 1,035 mg C·m^{-2} in April 1977 and 235 in May 1980, and the photosynthetic bacteria accounted for 25–30% of the total. The zooplankton includes the cladoceran *Moina hutchinsoni*, the copepod *Dzaptomus sicilis,* and several species of rotifers and ciliates *Diaptomus* is most abundant in spring, *Moina* is most abundant in summer, and zooplankton abundance is greatly reduced in winter (Cloern et al 1983).

Materials and methods

Sampling was done quarterly (November 1981; February, May, July 1982) over the deepest (65 m) part of the lake; results presented here are from the mixolimnion only Temperature and dissolved oxygen (DO) were measured with an Orbisphere Laboratories (model 2714) thermistor and polarographic sensor; temperature was also measured monthly with a thermistor. Vertical profiles of irradiance were measured with a LiCor 192S submersible quantum sensor; daily surface irradiance was measured with a LiCor 190S quantum sensor and integrator. Water samples for analysis of photosynthetic pigments, dissolved nutrients, and ΣCO_2 were collected at discrete depths with a pump and garden hose. Pigments were collected onto Gelman type A/E glass-fiber filters, frozen, then later

ground and extracted in 90% acetone. Chlorophyll a and bacteriochlorophyll a (Bchl) concentrations were determined spectrophotometrically using the equations of Lorenzen (1967) and Takahashi and Ichimura (1970) Dissolved inorganic nutrients (P, Si, NO_2^-, NO_3^-, NH,) were determined colorimetricallp with an AutoAnalyzer using samples that were filtered through 0.4-μm Nuclepore filters and frozen until analyzed. Nutrient standards were prepared in artificial lake water (40 meq·liter^{-1} NaHCO$_3$); analysis of spiked samples (100 μM NO_2^- or N O , in artificial lake water) showed a recovery efficiency of 98 5% for nitrate and 100% for nitrite. Total carbon dioxide ($\Sigma CO_2 = CO,$ + HCO_3^- | CO_3^{2-}) was measured by injecting 2 ml of filter-sterilized (0.45-μm Millex) lake water into sterile, 18- X 150-mm culture tubes sealed with butyl rubber recessed stoppers. Two milliliters of 6 N HCl was injected into the tubes 24 h before analysis. Carbon dioxide was quantified by gas chromatography (Culbertson et al. 1981) using a gastight Pressure-Lok syringe (Precision Sampling Co.) and a 250-μl headspace sample. Replicate zooplankton samples were collected with 20-m vertical tows of a 12.5-cm-diam net (80-μm mesh).

Productivity was measured with in ritu incubations using 5 or 10 μCi NaH^{14}CO$_3$ in 150-ml bottles. Samples were collected from incubation depths by pump, prescreened through a 200-μm mesh net to remove macrozooplankton, then incubated for 8 h beginning at 0800 hours. Replicate light and dark bottles were used at each of 10–13 depths; we tried to expose phytoplankton to a range of irradiances from about 1 to 80% of surface irradiance and to bracket the plate of purple sulfur bacteria (location determined with a light transrnissometer). After incubation, the contents of each bottle were filtered onto Gelman type A/E filters which were then rinsed with NaCl (20 g·liter^{-1}). Filters were placed in scintillation vials and fumed in a desiccator for 12 h with 1 N HCl. Radiocarbon activity was determined with a scintillation spectrometer after adding 20 ml of Aquasol. Counting

efficiencies (external standard method) were 68–78%. Correction was made for abiotic uptake of H^{14}CO$_3^-$ by filtering initial samples immediately after adding ^{14}C; this activity was usually a small fraction of the final activity of light bottles.

Areal productivity was calculated by numerically integrating (trapezoidal quadrature) measured rates of carbon assimilation with respect to depth. Oxygenic photosynthesis (OP) by phytoplankton was calculated as the integrated value of photosynthetic assimilation (light bottles rninus dark bottles) from the surface to the compensation depth (Z,)for algal photosynthesis. Z_1 was estimated from the linear portion of productivity vs. irradiance curves generated from the incubation experiments; the mean value of Z_1 corresponded to the depth of 1.2% surface irradiance, and net algal productivity was assumed to equal zero at depth Z,. Anoxygenic photosyrithesis (AP)was calculated as integral photosynthetic productivity from Z, to the estimated compensation depth (Z_2) of photosynthesis by purple sulfur bacteria. Z_2 was taken as the depth corresponding to a midmorning irradiance of 2 μEinst·m^{-2}·s^{-1}, which is the calculated compensation irradiance of *Lamprocystis roseopersicina* in culture experiments (R. D. Parker pers. comm.). Total daily photosynthetic. productivity was estimated by multiplying the measured productivity by the ratio total daily irradiance:irradiance during the incubation period. Chemoautotrophic productivity (CP) was assumed to be the integrated value of dark CO, assimilation between depths Z, and Z_2; mean hourly rates were multiplied by 24 to give daily rates.

Experiments were conducted to determine the processes responsible for dark ^{14}CO$_2$ fixation near the oxycline. Water was collected from the depth of maximum turbidity just beneath the oxycline (corresponding to the photosynthetic bacterial plate) during February 1982, July 1982, and October 1982. Samples were dispensed to entirely fill dark bottles (120 or 150 ml) and a headspace was generated by removing *3* or 10 ml of the liquid phase. The bottles were sealed with serum stop-

Cloern et al.
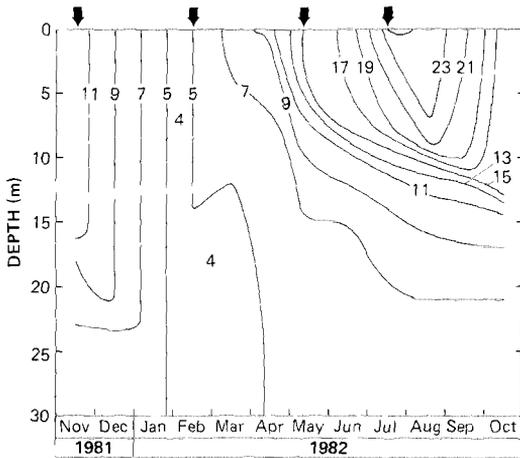
Fig. 1. Annual temperature distribution in the upper 30 m of Rig Soda Lake. Arrows indicate times of productivity measurements.

pers and flushed with either He (≈ 50 cc· min^{-1} for 30 s) or N$_2$ (≈ 50 cc·min^{-1} for ≈ 10 s) to establish microaerophilic conditions For incubations running longer than 5 h (October), a small volume of air (0 5 cc) was injected into the headspace to avoid oxygen limitation of activity (Jørgensen et al 1979). Inhibitors of bacterial chemoautotrophy (N-serve and acetylene) and methane oxidation (CH$_3$F), or substrates (NH$_4$Cl; Na$_2$S$_2$O$_3$; CH$_4$) were added to selected flasks by syringe In some experiments, filter-sterilized lake water was used as a control N-serve (2-chloro-6-trichloromethylpyridine) was added (10 µl) from a 4.4% stock solution in acetone. Addition of a similar volume of acetone to samples did not affect activity (dark ^{14}CO$_2$ fixation) After adding NaH^{14}CO$_3$ (4 5 or 5 µCi), the bottles were placed in a large cooler for constant-temperature incubation (12°C during July; 6°–8°C all other times)

During October 1982 a second experiment was performed using a suspension of washed cells (to remove any substrates) recovered from 21 m. Water (560 ml) was centrifuged (500 rpm) and the resulting pellets were pooled and resuspended in 100 ml of substrate-free artificial Soda Lake surface water of the following composition (g·liter^{-1}): NaCl, 13; Na$_2$SO$_4$, 7.4; MgSO$_4$·7H$_2$O, 1.5; Na$_2$CO$_3$, 0.8; KCl, 0.6; NaBr, 0.0021; CaCl$_2$·2H$_2$O, 0.0017; KI, 0.00026; H$_3$BO$_3$, 0.0237 (final pH adjusted to 9.7). The resuspended cells were dispensed (10 ml) into dark 30-rill serum vials, capped with serum stoppers, and flushed with N$_2$ (≈ 50 ml·min^{-1} for 1 min). Air (0.2 cc) and substrates (NH$_4$Cl, Na$_2$S$_2$O$_3$, or glucose) were injected after gassing, followed by addition of 4 5 µCi NaH^{14}CO$_3$. The vials were then incubated in the dark at 6°–8°C for 4 h. Filtration, acid-fuming, and liquid scintillation counting procedures for the chemoautotrophy experiments were the same as those used for measuring in situ productivity.

Results

Seasonal changes in the temperature of the mixolimnion were comparable to those in a warm monomictic lake (Fig. 1) The upper 30 m were nearly isothermal (4°–5°C) during winter; surface warming established a shallow thermocline by April that descended to about 19 m by November. Maximum temperature of the epilimnion was about 24°C in August, and the temperature of the hypolimnion never exceeded 6°C. Productivity was measured during four distinct periods of thermal (density) structure: the thermocline was located at about 6, 10, 19, and 33 m during these measurements

Seasonal changes in temperature distribution were accompanied by changes in the distribution of dissolved substances (CH$_4$, Si, inorganic nitrogen, oxygen) and plankton biomass (Cloern et al. 1983). For example, during winter circulation (February, Fig. 2) DO mixed down to 28 m, phytoplankton biomass was high (Chl a > 40 mg m^{-3}), transparency was low (Secchi depth < 3 m), light was absent from the

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Fig 2 Vertical distributions of temperature dissolved oxygen bacteriochlorophyll *a*, chlorophyll a, and dissolved inorganic nitrogen in the mixolimnion of Big Soda Lake during February and July
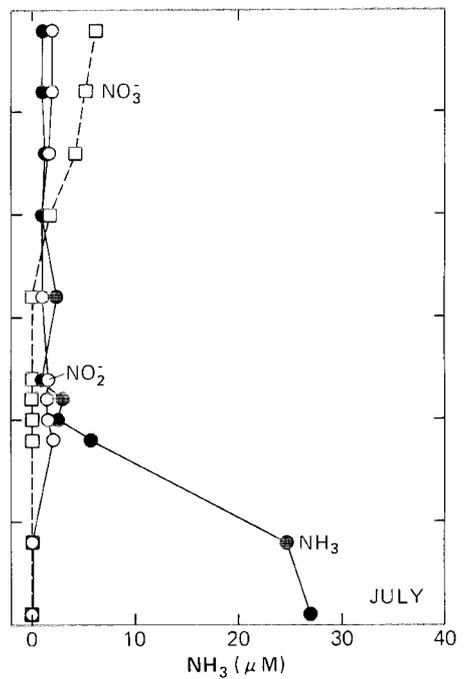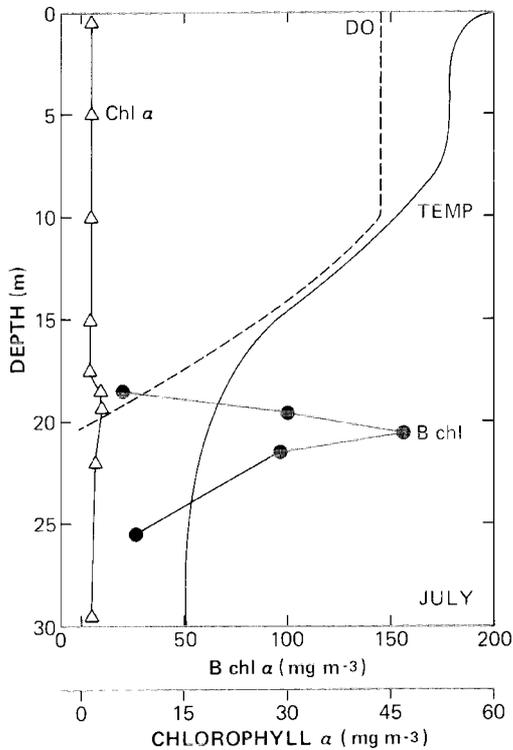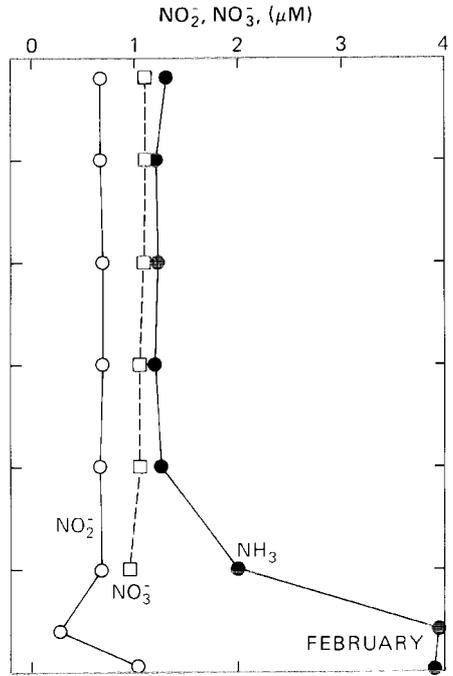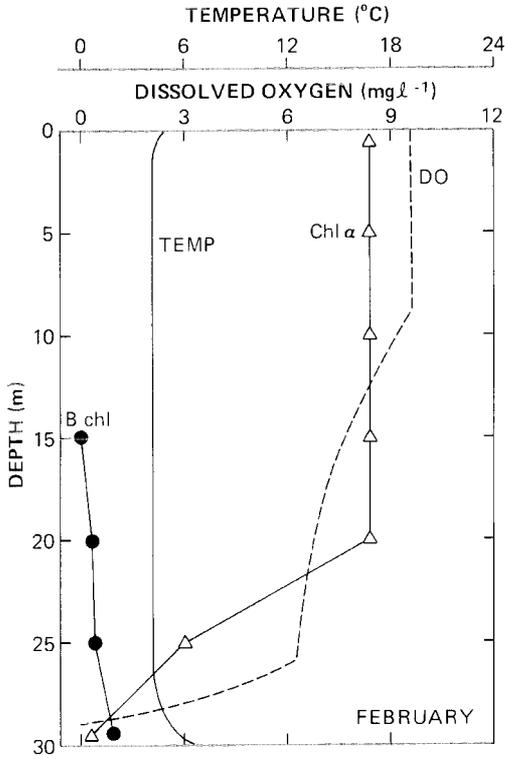
Table 1   Seasonal changes in thermocline depth, oxygen and light penetration, phototroph biomass, primary productivity specific growth rates of phytoplankton ($\hat{\mu}_p$) and phototrophic bacteria ($\hat{\mu}_b$), zooplankton abundance, and estimated zooplankton grazing rate G

	Nov	Feb	May	Jul
Z_T, thermocline depth (m)	19	33	6	10
Z_o, depth of O_2 disappearance (m)	21	28	19.5	21
Attenuation coefficient (m^{-1})	0 17	0.51	0.28	0.21
Z_1 (m)	19	6	17	19
Z_2 (m)	23 5	—	23	22.5
Daily insolation (Einst m^{-2})	3 4	22.9	35.8	56.9
$\int_0^{Z_1}$ Chl dZ (mg·m^{-2})	25	260	27	14
$\int_{Z_0}^{Z_2}$ B chl dZ (mg·m^{-2})	480	0	10	300
OP (mg C·m^{-2}·d^{-1})	90	2,800	160	90
AP (mg C·m^{-2}·d^{-1})	180	0	110	210
CP (mg C·m^{-2}·d^{-1})	390	30	680	410
Total productivity	660	2,830	950	710
$\hat{\mu}_p$ (d^{-1})	0.03–0.14	0.11–0.42	0.06–0.24	0.06–0.26
$\hat{\mu}_b$ (d^{-1})	0.02	0.0	0.56	0.03
Zooplankton abundance (No.·$liter^{-1}$)				
Moina adults	17.0	0.0	0.3	20.2
Diaptomus adults	0.4	0.3	0.8	2.2
Grazing rate G (d^{-1})	0 02	0.0	0.0	0.02

anoxic zone, and there was no well defined plate of photosynthetic bacteria Concentrations of Si, CH,, and YH, showed seasonal maxima in the epilimnion during winter, reflecting the mixing of nutrients from the anoxic hypolimnion to the surface Total dissolved inorganic nitrogen (DIN) was at least 15 μM in the epilimnion and was about 90% NH, (Fig. 2). In contrast, during the summer–fall period of thermal stratification (e.g July, Fig 2) DO disappeared at 20 m (the compensation depth for OP), phytoplankton biomass was low (Chl a < 1 mg·m^{-3} at the surface), the epilimnion was more transparent (Secchi depth > 13 m), light penetrated to the anoxic hypolimnion, and a plate of purple sulfur photosynthetic bacteria had developed below the depth (Z_0) of oxygen disappearance. During stratification, DIN was greatly reduced (<1 μM) in the epilimnion but increased below the oxycline (Fig. 2) Phosphate concentrations were always high (>100 μM) in the epilimnion and Si distributions were similar to those of NH_3 (Cloern et al 1983). Table 1 includes a summary of thermo-

cline depth, light penetration (attenuation coefficients for the upper 20 m and calculated values of Z_1 and Z_2), and biomass of phototrophs in the photic zone during the four sampling periods. The macrozooplankton community (Table 1) was characterized by low abundance in winter, seasonally high abundance of Moina adults in summer–fall, and a smaller peak abundance of *Diaptomus* adults in spring–summer. *Uiaptomus* copepodites and nauplii were most abundant (2.9 and 8.2 per liter) in May.

Rates of oxygenic photosynthesis were always low (<3 mg C·m^{-3}·h^{-1}) during the stratified period (Fig. 3); subsurface peaks of algal productivity coincided with subsurface Chl a maxima observed in May and July. During winter circulation, OP was very high (maximum = 100 mg C·m^{-3}·h^{-1}) in the upper 6 m. The seasonality of anoxygenic photosynthesis followed seasonal changes in the biomass of purple sulfur bacteria. AP was highest (up to 11.4 mg C·m^{-3}·h^{-1}) during July and November when the plate of photosynthetic bacteria was well defined; AP was lower in
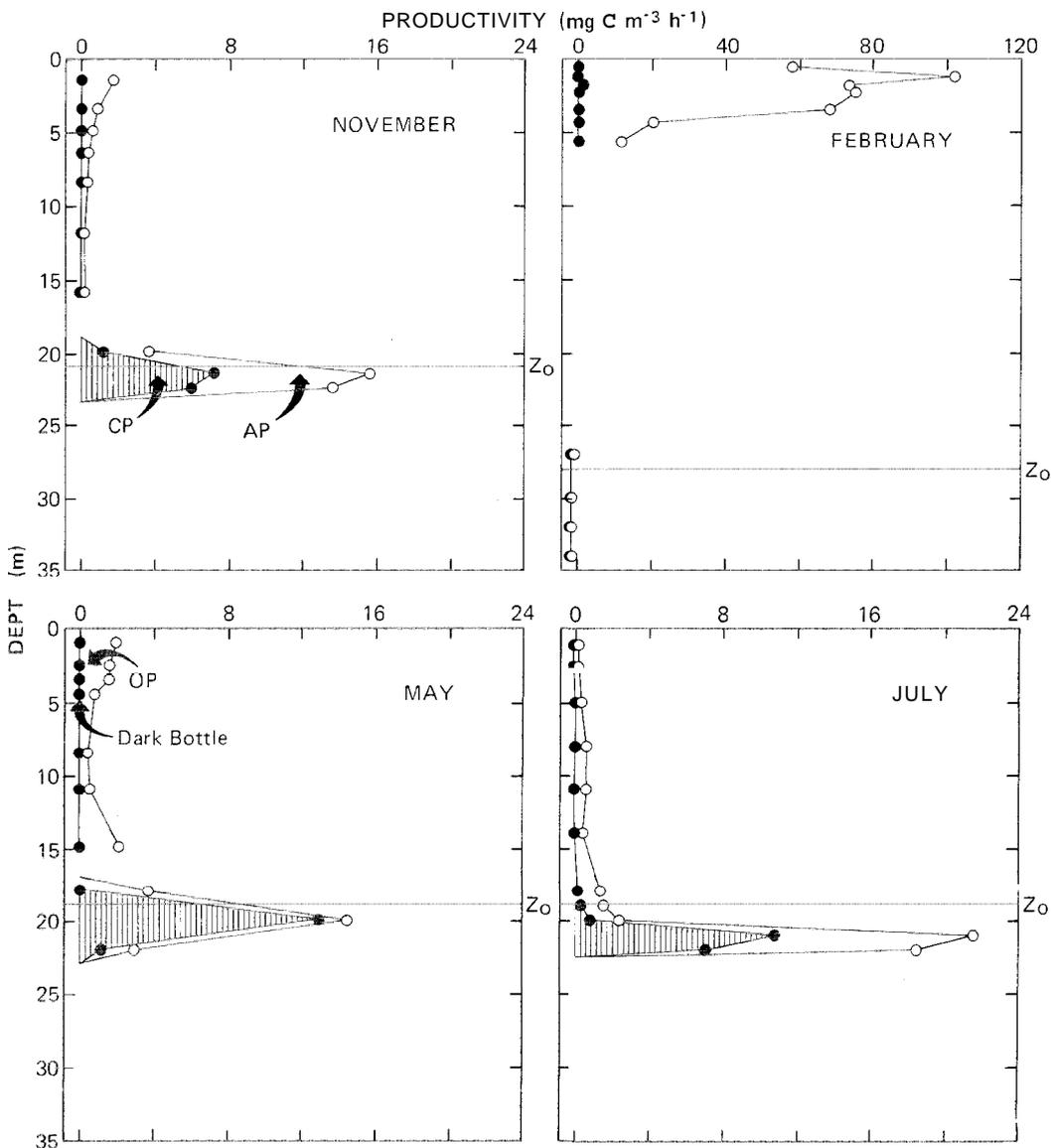
Fig 3 Vertical profiles of carbon assimilation rate in the mixolimnion of Big Soda Lake during the four seasons ● — Rates in dark bottles, O — rates in light bottles Chemoautotrophic productivity (CP) and anoxygenic photosynthesis (AP) were associated with the bacterial plate near the depth of oxygen disappearance (Z_0) Oxygenic photosynthesis (OP) occurred above Z_0 Note scale change for February

May and zero in February when light was absent from the anoxic zone (Fig. 3). Rates of dark assimilation of CO, were usually very low (<0.1 mg $C \cdot m^{-3} \cdot h^{-1}$) in the aerobic epilimnion and were <1.5 mg $C \cdot m^{-3} \cdot h^{-1}$ below the plate of photosynthetic bacteria (Fig. **3**). During the stratified period, high rates of dark assimilation (up to 10.9 mg $C \cdot m^{-3} \cdot h^{-1}$) were measured in a narrow (3–4 m) zone below the oxycline. In February, dark fixation rates were low throughout the water column, but they were higher in the epilimnion (up to 1.7 mg $C \cdot m^{-3} \cdot h^{-1}$) than during periods of stratification.

Dark CO_2 fixation was observed in all

Table 2. ^{14}C activity of dark bottles used in the chemoautotrophy experiments. Values given are mean dpm of three replicates and their standard deviation; only duplicate bottles were incubated in February. Inhibitors of chemosynthesis were added in February, July, and October 1982.

	Addition	dpm	Change (%)
February			
29-m sample, 5-h incubation	None	184; 214	—
	N-serve (22 mhl)	115; 112	−43
	Filtered	13; —	−93
July			
21-m sample, 5-h incubation	None	1,241(135)	—
	N-serve (22 mM)	673(75)	−46
	Acetylene (10 cc)	748(115)	−40
	Filtered	79	−94
October			
21-m sample, 3-h incubation	hone	470(22)	—
	N-servr *(22 mhl)*	107(25)	−77
	CH_3F	527(115)	+12*
21-m sample, 7-h incubation	hone	1,086(116)	—
	N-serve *(22 mM)*	212(190)	−80
	CH_3F	1,017(209)	−6*

* Not statistically different from control

chemoautotrophy experiments (Table 2), but rates of CP were higher (about 10-fold) in July and October than in February, which agrees with in situ measurements (Fig. 3). Time-course experiments in October indicated a constant rate of dark CO, fixation ($\approx 156 \, dpm \cdot h^{-1}$). CP was inhibited by both filter-sterilization (93–94% inhibition during February and July) and by acetylene (40% inhibition in July). N-serve inhibited CP at all times tested; however inhibition was considerably greater in October ($\approx 77–80\%$) than in either February ($\approx 43\%$) or July ($\approx 46\%$). No significant inhibition was achieved with CH_3F during the October experiments, and addition of CH, did not stimulate CP. Results of the mashed-cell experiment are shown in Table 3 A large error was associated with the unsupplemented samples; however average: of the CP rates indicated stimulation by the addition of NH, and $S_2O_3{}^{2-}$. No stimulation was achieved by adding glucose.

Discussion

Our results suggest that organic matter is produced in the mixolimnion of Big Soda Lake at a different rate and by different microbial communities during the winter period of holomixis than in the spring–fall period of stratification. In February, areal productivity was high (2,830 mg $C \cdot m^{-2} \cdot d^{-1}$) and resulted almost exclusively (99%) from phytoplankton photosynthesis (Fig. 4). During the other three seasons, however, total daily productivity was lower (660–950 mg $C \cdot m^{-2} \cdot d^{-1}$) and all three autotrophic processes contributed to community production. Chemoautotrophic productivity exceeded the total photosynthetic productivity by phytoplankton and photosynthetic sulfur bacteria during the periods of stratification (Fig. 4). If quarterly measurements are representative of seasonal productivity, then the annual autotrophic productivity in the rnixolimnion of Big Soda Lake is about 500 g $C \cdot m^{-2}$, of which 60% is produced by phytoplankton (mostly in winter), 30% by chemoautotrophs, and 10% by photosynthetic bacteria

We recognize that these general conclusions may be biased by errors inherent in the measurement of in situ carbon assimilation and the calculation of integral productivity However, these errors are not of sufficient magnitude to invalidate our

Tahle 3 ^{14}C activity of dark-bottle incubation of washed cells after addition of substrates, October 1982 Sample from 21 m, incubation time was 4 h, results from duplicate bottles

Addition	dpm	Mean	Stimulation (%)
None	366; 723	545	—
NH₄Cl (1 mM)	788; 609	699	28
Na₂S₂O₃ (1 mM)	704; 704	704	29
Glucose (1 mM)	360; 386	373	None

concepts of autotrophic production in Rig Soda Lake. Eor example, the calculation of integral photosynthetic productivity depends on estimates of Z_1 and Z_2. Although we selected these depths somewhat arbitrarily, a doubling of Z_1 leads to only about a 10% increase in calculated integral OP Anoxygenic phototrophy may be overestimated because bottles from the bacterial plate were exposed to high irradiance during deployment of the incubation line; however, short term measurements simulating deployment of an incubation wries indicated that production during deployment may approach on v about 25% of the daily total. Finally. oxygenic phototrophy measured in November may be an underestimate because daily insolation was low during that experiment (Table 1). However, measured rat s of the three production processes did not vary greatly during the spring–fall period — stratification (Fig. 4), and the proti ictivity pattern seen in February was sufficiently different from that in the other three seasons to suggest that (at least qualitatively) the difference is real.

Phototrophy—Seasonal variations in the relative importance of OP and AP are clearly related to seasonal variations in the biomass of the two phototrophic communities: OP wa highest in winter when phytoplankton biomass was maximal, and AP was highest in summer and fall when the biomass of photosynthetic bacteria was maximal Table 1). Hence, the seasonal variation of phototrophic productivity in Big Sod t T ake is a response to processes that control the biomass of the photo-
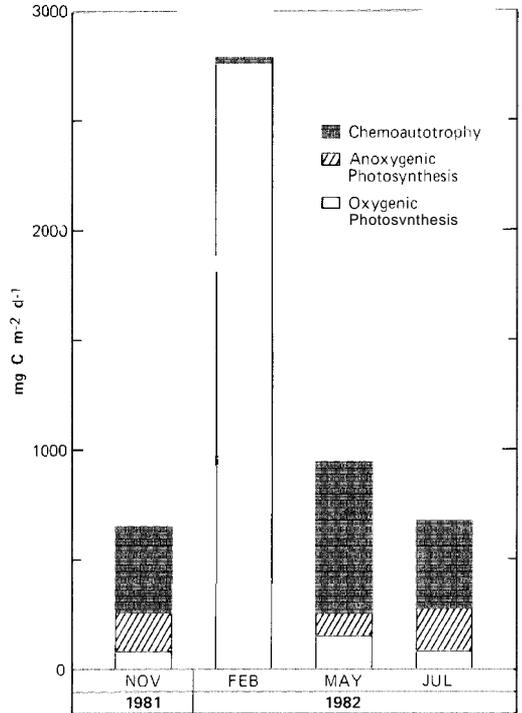

Fig. 4. Seasonal changes in depth-integrated rates of CP, AP, OP. and total autotrophic productivity in the mixolimnion of Big Soda Lake.

trophs. We presume that sinking, zooplankton grazing, and specific growth rate are major controls of phytoplankton biomass, and that specific growth rate varies with seasonal changes in nuirient (DIN or trace element) availability. We estimated specific growth rate of phytoplankton (Table 1) $\hat{\mu}_p$ (d⁻¹) by dividing integral OP (mg C·m⁻²·d⁻¹) by integral biomass B_p (mg C·m⁻²) which was calculated from $\int_0^{Z_1}$ Chl dZ, assuming that the C Chl *a* ratio ranges from 25 to 100 (this method overestimates the true specific growth rate because it ignores respiratory losses, but it does give an index of growth rate in the photic zone for seasonal comparisori) Total phytoplankton biomass and $\hat{\mu}_p$ were both highest during winter circulation (Table 1) when DIN was most abundant in the surface layer Furthermore, our ob-

ter-sterilization of samples and by the fact that Formalin-killed (10% v/v) replicate controls had equivalent counts to zero time samples (69.3 and 78.5 dpm with Formalin vs. 74.9 and 75.5 dpm at zero time).

The inhibitor experiments (Table 2) were designed to identify the specific processes responsible for the observed CP. CP was inhibited by N-serve and acetylene, both of which block the nitrification of ammonia (Goring 1962; Belser and Schmidt 1981; Hynes and Knowles 1978; Walter et al. 1979; Mosier 1980). However, both N-serve (l'opp and Knowles 1982) and acetylene (Dalton and Whittenbury 1976) also inhibit bacterial oxidation of methane. Because methane-oxidizing bacteria that utilize the "serine pathway" of carbon fixation derive about 50% of their cell carbon from CO_2 (Ribbons et al. 1970; Anthony 1975), the possibility existed that this type of methylotroph was responsible for much of the observed CP (Sorokin 1957; Naguib 1978). The presence of a pronounced methane gradient across the oxycline of Big Soda Lake (Cloern et al. 1983) implies the bacterial oxidation of methane in that region, and this has been confirmed by in situ experiments with $^{14}CH_4$ (N. Iverson pers. comm.). However, the addition of CH_3F (a potent inhibitor of methane oxidation: Meyers 1982) to samples did not inhibit CP ('l'able 2), and furthermore, the inclusion of methane in the gas phases of the samples did not enhance CP. Therefore, methylotrophic bacteria did not contribute to CP and the observed CP inhibition achieved with N-serve and acetylene was due to the elimination of chemoautotrophic CO_2 fixation by nitrifying bacteria. Thus, nitrifiers contribute about 45–80% of CP, depending on season (activity is highest in autumn). These values are comparable to the 10–50% estimates of nitrification-derived CP found in the Saelenvann estuary (Indreba et al. 1979*b*).

A strong sulfide gradient present across the bacterial plate in Big Soda Lake during October 1982 (S^{2-} = 0, 31, 156, and 344 mg·liter^{-1} at 20. 21. 22. and 24 m: R.

L. Smith pers. comm.) indicates that microbial oxidation of sulfides, or other reduced sulfur compounds such as thiosulfate (Tuttle and Jannasch 1977), may also contribute to CP. Unfortunately, an inhibitor of microbial sulfide oxidation suitable for ecological studies has yet to be identified. We attempted to inhibit CP by adding molybdate, an analogue of sulfate that effectively blocks sulfate reduction (Peck 1959; Taylor and Orernland 1979), but our results were generally unsuccessful. However, the addition of $Na_2S_2O_3$ (1 mM) to samples during October did produce a 43% stimulation of CP after *3* h.

Because both sulfide and ammonia (Fig. 2) were present within the bacterial plate, the cells oxidizing these compounds may not have been limited by substrate availability. We therefore experimented with washed cells to determine if additions of ammonia or thiosulfate could stimulate CP (Table 3). Although the two unamended samples had a broad range of values (366 and 723 dpm), the addition of NH_4Cl and $Na_2S_2O_3$ to samples consistently yielded high counts (>600 dpm). These results reinforce our hypothesis that the observed CP was due to substantial contributions made by nitrifying and sulfur-oxidizing bacteria. However, some of the CP may have been derived from heterotrophic CO_2 fixation, and to test this we added glucose to washed cell suspensions. Glucose-supplemented samples gave consistently low counts (360 and 386 dpm), clearly indicating that this organic substrate did not stimulate CP. Therefore, if glucose is a representative organic substrate, the observed dark CO_2 fixation near the oxycline of Big Soda Lake appears to be due to chemoautotrophic processes (e.g. nitrification, sulfide oxidation) rather than heterotrophic CO_2 fixation. Nitrification accounts for 45–80% of this activity (depending on season) and presumably the oxidation of reduced sulfur compounds accounts for most of the remainder. Oxidation of reduced metals (e.g. Fe, Mn, etc.) is probably not an important part of CP because of the low concentrations of these

substances in the water (Y. K. Kharaka pers. comm.).

Conclusions

Our results demonstrate that the dominant processes of autotrophy can exhibit extreme seasonality in temperate meromiciic lakes and substantiate the generalization that OP is a more important source of organic matter than AP (e.g Biebl and Pfennig 1979; Parkin and Brock 1980; Steenbergen and Korthals 1982), although the mean annual biomass of phototrophic bacteria exceeds that of phytoplankton. Furthermore, rapid rates of dark CO, assimilation near the oxycline appear to be a consequence of chemoautotrophy (e.g. by nitrifying bacteria), and this process represents a significant source of organic matter to Big Soda Lake. During the winter phytoplankton bloom there may be a large flux of algal-derived particulate organic matter from the epilimnion to the chemocline and sediments, and this large seasonal input of organic matter may cause seasonal variations in processes of anaerobic decomposition. Hence, the winter peak in OP may be followed by enhanced rates of sulfate reduction (e.g. Indrebø et al. 1979*a*), denitrification, and meihanogenesis. Measurements are now under way to test this hypothesis and to determine the role of heterotrophy in Rig Soda Lake.

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