Limnol. Oceanogr., 47(2), 2002, 440–452

Distribution, production, and ecophysiology of *Picocystis* strain ML in Mono Lake, California

Collin S. Roesler[1]
Department of Marine Sciences, University of Connecticut, Groton, Connecticut 06340

Charles W. Culbertson[2]
Water Resources Division, USGS, Menlo Park, California 94025

Stacey M. Etheridge
Department of Marine Sciences, University of Connecticut, Groton, Connecticut 06340

Ralf Goericke
Scripps Institution of Oceanography, La Jolla, California 92093

Ronald P. Kiene
University of South Alabama, Mobile, Alabama 36688

Laurence G. Miller and Ronald S. Oremland
Water Resources Division, USGS, Menlo Park, California 94025

Abstract

A recently described unicellular chlorophytic alga isolated from meromictic Mono Lake, California, occupies a niche that spans two environments: the upper oxic mixolimnion and the deeper anoxic and highly reducing monimolimnion. This organism, *Picocystis* sp. strain ML, accounts for nearly 25% of the primary production during the winter bloom and more than 50% at other times of the year. In incubations, it is heavily grazed by the brine shrimp, *Artemia monica*. We assessed growth and photosynthetic parameters over broad ranges of irradiance, salinity, and pH and under oxic and anoxic conditions. *Picocystis* appears to be particularly adapted to low irradiance; we observed an order of magnitude increase in the cellular pigment concentrations, as well as marked increases in cell-specific photosynthetic parameters for cells acclimated to low-growth irradiance. Growth rates of 0.3–1.5 d^{-1} were observed over a salinity range of 0–260‰ and a pH range of 4–12, with maximal growth at ~50 μmol photons m^{-2} s^{-1}, 40‰, and pH 6–10. Growth and oxygenic photosynthesis were observed under anoxic conditions at rates comparable to those measured under oxic conditions. The ability of the organism to acclimate and grow under such a broad range of environmental conditions makes it an important component of the Mono Lake ecosystem and likely contributes to its dominance of the monimolimnion/mixolimnion interface.

Phototrophic microorganisms exhibit a remarkable capability for adaptation and acclimation that allows them to inhabit niches representing temporally varying biological extremes of light, salinity, pH, and water potential (Gorbushina and Krumbein 1999). In aquatic environments, phytoplankton undergo large increases in cellular pigment concentrations and in photosystem size, number, or both in response to diminished irradiance (e.g., Prézelin 1976; Falkowski and Owens 1980; Perry et al. 1981; Sukenik et al. 1987; Sosik et al. 1989). Photoautotrophs can respond to salinity increases by adjusting their internal solute concentration via synthesis of organic osmolytes (e.g., Kauss 1978; Dickson and Kirst 1986; Keller et al. 1999). Enhanced H^+ and OH^- ion concentrations, which occur at pH extremes, can directly affect cytoplasmic pH and cellular processes (Krulwich 1995 and reference therein), impede trace metal uptake (Peterson and Healy 1985; Gensemer 1991), and change the metal ion chemistry of the aquatic environment, inducing toxic concentrations of metals. Cyanobacteria can shift between oxygenic and anoxygenic photosynthesis as a response to ambient concentrations of sulfide that typically occur over diel cycles in benthic mat communities (Cohen 1989). Given the spectrum of responses to individual environmental parameters, Mono Lake represents a particularly challenging envi-

[1] To whom correspondence should be addressed. Present address: Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine 04575 (croesler@bigelow.org).
[2] Present address: District Office, USGS, Augusta, Maine 04330.

Acknowledgments

The authors thank Jennifer Simeon for culturing assistance, Victor Paganelli (USGS Menlo Park) for processing the total dissolved inorganic carbon samples, and members of the Sierra Nevada Aquatic Research Laboratory for collecting water samples from Mono Lake. The University of Connecticut supported C.S.R. and S.M.E.; the USGS National Research Program supported C.W.C., L.G.M., and R.S.O.; and NSF supported R.P.K. (OCE-9907471).

ronment for autotrophic organisms because of the range in multiple environmental conditions.

Mono Lake is a large (160 km^2), highly stratified, hypersaline (70 to 100‰), alkaline (pH 9.8), carbonate-rich (400 mM) lake located east of the Sierra Nevada Range in California. It has been a closed basin for >500,000 yr; thus, the chemical characteristics and water level are controlled by the balance between the input of freshwater and evaporation. The pH, currently 9.8, is a direct result of the net accumulation of an array of inorganic material (i.e., carbonate and minerals) in the lake and has likely been unchanged for much of this time. Dramatic fluctuations in Mono Lake water level have resulted in large salinity variations over geologic as well as historic time scales, with subsequent influences on lake stratification and chemistry. Forty years of water diversions coupled with a drier climate have resulted in a twofold salinity increase (from ~40 to 80‰) since the early 1940s. During much of this time, the lake was characterized by a monomictic regime, in which annual whole-lake overturn was associated with thermal destratification in late fall. A reduction in diversions, along with increased freshwater runoff associated with the 1982–1983 El Niño, resulted in salinity stratification and ensuing meromixis that persisted for a period of 5 yr (Jellison and Melack 1993*b*). This stratification resulted in complete anoxia in the monimolimnion and accumulation of sulfide (1,800 μM), ammonia (600 μM), and methane (55 μM) because of fluxes from the underlying sediments (Miller et al. 1993). Evaporation over the 1983–1988 meromictic period increased the salinity of the mixolimnion, slowly degrading the stratification. Autumnal surface cooling caused whole-lake overturn in November 1988, thereby ending the meromictic period (Jellison and Melack 1993*b*). During overturn, nearly the entire lake water column became temporarily anoxic as a result of the oxidation of the high concentrations of sulfide, ammonia, and methane that had built up in the monimolimnion (Miller et al. 1993).

Given the climatological and geochemical history of Mono Lake, it is not surprising that organisms with capabilities to adapt to varying chemical and hydrographic conditions would develop. The current ecosystem is comprised of a limited phototrophic community in the mixolimnion dominated by diatoms, mainly *Nitzschia* sp., and occasional cyanobacteria (Lovejoy and Dana 1977; Jellison and Melack 1988, 1993*a*). These phototrophs are grazed upon by a single species of brine shrimp, *Artemia monica* (Lenz 1980; Dana and Lenz 1986), which in turn is an important source of food for migratory birds.

Prokaryotes are usually assumed to be better suited to living in extreme environments than are eukaryotes, but this is not always the case. In 1984 during the meromictic period, we observed a well-defined subsurface chlorophyll *a* (Chl *a*) maximum at the base of the oxycline/chemocline (Oremland et al. 1993), a niche that is generally occupied by anoxygenic photosynthetic bacteria (Walker 1975; Craig 1987; Oremland et al. 1988; Overmann and Tilzer 1989; Hall and Northcote 1990) such as the sulfide-oxidizing photosynthetic bacterium *Ectothiorhodospira* sp. in Big Soda Lake (Cloern et al. 1983; Oremland et al. 1988). However, the organism responsible for the observed maximum in Mono Lake was isolated and determined to be a eukaryotic phototroph. The organism was identified as a relative of the unique, recently described *Picocystis salinarum,* which was isolated from a saline pond at the San Francisco Salt Works, California, in 1991 (Lewin et al. 2000). Analysis of the 18S rRNA sequences indicates that the isolate from Mono Lake is a different strain from that isolated in the saline pond and might be different at the species level as well (J. T. Hollibaugh pers. comm.). We will refer to the organism as *Picocystis* sp. strain ML or, more generally, *Picocystis.* We undertook a study to follow the seasonal distribution and productivity, as well as the physiology of this organism, in an attempt to understand why this unusual eukaryote is able to flourish in the harsh environment of Mono Lake.

Methods

Field observations—A station in the western basin of Mono Lake was sampled quarterly from May 1986 through August 1989. Profiles of temperature and dissolved oxygen were collected with a calibrated Orbisphere thermistor in conjunction with an in situ hydrogen sulfide–insensitive, electrode. The probe was calibrated daily in fresh water at prevailing total pressure yielding an accuracy of ±5% of the reading with zero established and a precision of ±5% at all times. Values were also verified against those determined with the Miller method (Walker et al. 1970). Water samples were collected with a Niskin bottle or with a peristaltic pump. Methods for hydrogen sulfide analysis can be found in Miller et al. (1993). Chl *a* was determined spectrophotometrically (Lorenzen and Jeffrey 1980). Cells were counted by epifluorescence microscopy on samples fixed with 4% formalin within 8 h of collection. Fluorescing microspheres (0.8 μm) were included in cell preparations as a size reference.

Primary production rates were determined from in situ incubations. Water samples were collected before dawn with a peristaltic pump and screened through a 200-μm mesh to remove macrozooplankton. Replicate light bottles and one dark bottle (1-L, filled to overflowing and capped) were incubated at each depth with 100 μCi $NaH^{14}CO_3$ for one photic period (9–11 h). Following the incubation, samples were serially filtered onto 3-μm Nuclepore and Gelman A/E glass fiber filters, which were acidified prior to counting. Activity was corrected for both abiotic uptake (T_0 bottles) and dark uptake at each depth.

Grazing rates by *Artemia monica* on *Picocystis* were measured in May 1987. Replicate bottles containing 800 ml of 3-μm filtered Mono Lake water collected at ~10 m depth were incubated at in situ temperature (~8°C) in the dark. Controls contained only the *Picocystis* cells at in situ concentrations; experimental bottles contained the cells plus 20 viable individuals of the brine shrimp *Artemia monica.* Subsamples were collected at four time points over the 15-h incubation for microscopic cell counts. Grazing rates were calculated from the time course of cell numbers in the experimental treatments.

Laboratory experiments—Cells were isolated from Mono Lake water samples collected from the subsurface chloro-

Table 1. The pigment composition of the Mono Lake *Picocystis* sp. t_r, pigment elution time; UV/Vis_{online}, absorption spectrum on the C18 rp-HPLC system; MS, molecular ion (MS as MH+); MS^2, fragmentation pattern; pigment : Chl *a*, average molar pigment to Chl *a* ratio (± SD for all treatments).

Pigment*	t_r (min)	UV/Vis_{online} (nm)	MS and MS^2 (m/z)	Pigment : Chl *a* (mol : mol)
Neoxanthin(1)	11.24	413, 437, 465	601 → 583, 565, 547, 509, 491	0.12±0.04
Violaxanthin(1)	13.94	417, 440, 470	601 → 583, 565, 547, 509, 491, 221	0.03±0.01
Diadinoxanthin(2)	16.31	(422), 448, 477	583 → 565, 547, 529, 509, 491, 473	0.02±0.01
Violaxanthin(1)	17.15	417, 440, 470	601 → 583, 565, 547, 527, 509, 491	na
Alloxanthin(3)	18.61	(428), 453, 482	565 → 547, 529, 509, 491, 473	0.04±0.01
Monadoxanthin(3)	19.41	(424), 448, 475	567 → 549	0.41±0.11†
Diatoxanthin(2)	19.65	(428), 450, 478‡	567 → 549, 531, 493, 457, 449, 427	na
Lutein(1)	20.48	(424), 446, 474	569 → 551, 533, 477, 476, 459, 429	0.16±0.06
Zeaxanthin(4)	20.75	(428), 454, 479	569 → n.d.	0.01±0.01
Chlorophyll *b*(1)	28.65	460, 599, 648	970 → 885, 847, 629, 597, 569, 541	0.34±0.04
Chlorophyll *a*(1)	31.65	431, 617, 666	893 → 861, 833, 615, 583, 555	1
β,ε-Carotene(3)	38.48	(422), 448, 477	537 → 535, 481, 457, 445, 444, 413	0.21±0.04†
β,β-Carotene(1)	38.74	(428), 454, 479	537 → 435, 481, 457, 445, 444, 413	na

* The following species provided reference pigments: (1) higher plant (grass); (2) diatom (*Thalassiosira pseudonana*); (3) cryptophyte (*Rhodomonas* sp.); (4) cyanobacterium (*Synechococcus* sp., WH7803).

† Quantified concentrations of cell-specific pigment concentrations were not performed on the C30 system; monadoxanthin and diatoxanthin were not separable nor were *β,β*- and *β,ε*-carotene. This number represents the sum of the inseparable pigments.

‡ These on-line spectra represent the beginning and end of the monadoxanthin/diatoxanthin peak. Off-line spectra for these two pigments are monadoxanthin 423, 446, 479 nm (in diethyl ether) and diatoxanthin (432), 454, 482 nm (in acetone).

phyll maximum coincident with the oxycline/chemocline (~15 m) in August 1997. Three-micron filtrate was enriched with 50 μM NH_4 and incubated at 20°C under grow lights at a constant intensity of 27 μmol photons m^{-2} s^{-1} (24-h irradiance). Greening of the culture was observed after a week. This culture was filtered through a 3-μm Gelman Supor® filter a second time, and the filtrate was inoculated into 0.2-μm sterile filtered Mono Lake water enriched with IMR nutrients, vitamins, and trace metals and amended with 50 μM NH_4Cl, 150 μM KH_2PO_4, and 120 μM K_2HPO_4. The size range and unique morphology of *Picocystis* allowed for easy and rapid identification, characterization, and enumeration. Culture purity was assessed using phase contrast and epifluorescence microscopy. Small numbers of bacteria were present but in negligible concentrations (<1% of cell concentrations by number). Semibatch cultures were maintained in logarithmic growth phase for the duration of the physiological experiments. All cultures were allowed to acclimate to their growth conditions for 5–10 d prior to analyses. Cell concentrations in experiments were determined with a Nikon Eclipse E800 epifluorescence microscope using Chl *a* fluorescence.

Pigments: Algal cells from purified cultures were collected on glass fiber filters, disrupted by sonication, and extracted with 100% acetone. Pigments were analyzed on an Alltech C18-HS 3-μm, 4.6-mm, 15-cm Adsorbosphere column [C18rp = HPLC system using as solvents (A) methanol : acetonitrile : 0.5 N aqueous ammonium acetate : water (30 : 30 : 30 : 10 by volume) and (B) methanol : acetonitrile : ethylacetate (10 : 35 : 55 by volume); gradient (time, %A: 0 min, 100%; 3 min, 80%; 21 min, 35%; 30 min, 25%; 33 min, 0%; 35 min, 0%; 35 min, 100%); flow rate 1.5 ml min^{-1}]. The identities of the pigments were established by comparison of their elution times and on-line spectra with those of well-characterized pigments of other algae and higher plants and by coelution of isolated pigments with standards on a C30 column rp–high-performance liquid chromatography (HPLC) system capable of separating chemically similar pigments such as diatoxanthin and monadoxanthin or lutein and zeaxanthin. The identification of all purified pigments (Table 1) was confirmed with mass spectrometry.

Osmolytes: Cellular glycine betaine (GB) and dimethylsulfoniopropionate (DMSP) were measured by collecting 100 ml of culture on a glass fiber filter (Whatman GF/F). Filters were extracted in methanol : chloroform : water (12 : 5 : 1 by volume) for >24 h. The extract was evaporated under nitrogen, reconstituted in 1 ml of MilliQ water, and passed through a 0.2-μm filter before HPLC to separate GB on a cation exchange column as described in Gorham (1984). DMSP in extract was measured by the cold alkaline hydrolysis method (White 1982). Concentrations of the osmolytes were normalized to the number of cells filtered (pg osmolyte $cell^{-1}$).

Experimental treatments: Growth rates, photosynthetic parameters, cell counts, and pigments were measured on cultures grown under a range of light (0.5–100 μmol photons m^{-2} s^{-1}), salinity (0–260‰), and pH (2–12) conditions. Additionally, osmolyte concentrations (GB and DMSP) were measured on cultures grown from 19 to 123‰. Salinity of Mono Lake water was adjusted either by dilution with MilliQ water or by evaporation in a drying oven (80°C) prior to nutrient addition and sterile filtration. Final salinity was measured with a refractometer. The minor pH changes (<0.4) induced by evaporation or dilution were adjusted with small volumes of HCl or NaOH; final pH values ranged between 9.77 and 9.83. For pH tolerance experiments, the alkalinity of Mono Lake waters was adjusted by titration

with either 6 N HCl or NaOH prior to nutrient addition and sterile filtration. These experiments were performed with a salinity of approximately 40‰ (36–41‰), the salinity at which the highest growth rate was observed. Salinity and pH experiments were conducted at 20°C in 24-h light at approximately 25 and 40 μmol photons m^{-2} s^{-1}, respectively. For comparisons of growth under oxic versus anoxic conditions, duplicate cultures were incubated at 22°C under two levels of constant irradiance (13 and 44 μmol photons m^{-2} s^{-1}). Anoxic experiments were performed in an anaerobic glove box and/or stoppered culture tubes that were flushed and bubbled twice daily with O_2-free N_2. Oxygen in the culture tubes (headspace sampled by syringe) was monitored by electron-capture gas chromatography (Perkin-Elmer 3920; Porapak Q column, UHP-N_2 carrier, 250°C detector temperature; 100-μl injections). The media in these incubations was additionally supplemented with NH_4Cl and Na_2S (both to 100 μM final concentrations) to maintain reducing conditions.

Physiological parameters: Growth rates were calculated from the logarithmic change of in vivo fluorescence measured twice per day with a Turner Designs fluorometer. Photosynthetic parameters were determined from small volume, short-term photosynthesis versus irradiance incubations (P-E, 5 ml and 1–2 h, respectively). Samples were inoculated with 5 μCi (SA 56 mCi $mmol^{-1}$ $NaH^{14}CO_3$) and incubated at 10 light levels (from <1 to >500 μmol photons m^{-2} s^{-1}) at 20 $\pm$ 0.3°C. Total ^{14}C activity in each treatment was determined by sacrificing T_0 vials immediately upon inoculation. Dark bottles were used to correct for abiotic and nonphotosynthetic uptake. Incubations were terminated by the addition of 6 or 12 N HCl, depending on the treatment medium. Samples were shaken and vented for 12 h prior to the addition of scintillation cocktail (Ecolume). Corrections for differential quenching by each treatment medium and total dissolved inorganic carbon (DIC, measured by gas chromatography) were applied to each experiment. Equations of Jassby and Platt (1976), without parameterization of photoinhibition, were used to determine the initial slope of the response curve (α) and maximal photosynthetic rate (Pmax). These photosynthetic parameters were scaled to both sample cell and Chl *a* concentrations. P-E incubations were only performed on cultures under oxic conditions over a range of salinity, pH, and irradiance.

Carbon uptake rates were measured on cultures grown under initially anoxic conditions in sterile filtered Mono Lake water, supplemented with nutrients as above, and at 44 μmol photons m^{-2} s^{-1}. Duplicate 10-ml samples were inoculated with 2 μCi and incubated for 1 h at the growth irradiance. Samples were collected onto 0.45-μm Millipore filters, washed at pH 2.95, and fumed for 2 h with concentrated HCl prior to addition of scintillation cocktail. Treatment vials included media blanks and boiled controls, as well as combinations of dark and light incubations, oxic and anoxic conditions, and media supplements of Na_2S and NH_4Cl to final concentrations of 100 μM. Uptake rates were scaled to sample cell concentration.

Results

Field observations—Mono Lake was meromictic during the period of quarterly sampling that began in 1986. Seasonal thermal stratification was clearly evident, with surface water temperature ranging from less than 2°C to greater than 20°C while the monimolimnion exhibited a nearly constant temperature of approximately 4.5°C (Fig. 1A). Mixolimnion DO concentrations ranged from 100 to 200 μM, with wintertime increases associated with the winter phytoplankton bloom and cooler water temperatures (Fig. 1B). The monimolimnion remained anoxic during meromixis and hydrogen sulfide concentrations exceeded 1,800 μM by late 1987 (Fig. 1C).

The November 1988 sampling coincided with a strong storm, which caused whole-lake overturn, ending the 5-yr meromictic period. Following overturn, the water column was isothermal at approximately 8.7°C. All of the DO in the water column was consumed by oxidation of the high concentrations of reduced chemicals that were mixed into the upper water column. Subsequent wintertime mixing resulted in bottom temperatures below 2°C and measurable DO at depth in February 1989. By August 1989, stratification was increasing and anoxic conditions were returning to the deep layer, as evidenced by near zero oxygen values (Fig. 1B) and a build up of sulfide below 15 m.

A narrow Chl *a* layer was observed in May 1986 at a depth of 15 m at the 1% light level (Fig. 1D). This layer was located below the chemocline in the anoxic monimolimnion. Chl *a* concentrations exceeding 50 μg L^{-1} were found in this maximum, whereas the surface waters were nearly devoid of chlorophyll. Microscopic examination indicated dominance by a small (1–3 μm diameter) trilobate phototroph later identified as *Picocystis* (*see* fig. 1 in Lewin et al. 2000). A chloroplast was located in each of the two smaller lobes and the nucleus in the larger third lobe, reminiscent of Mickey Mouse. The unique morphology of the organism allowed for easy identification and enumeration, even within the mixed phytoplankton community encountered in natural samples. *Picocystis* was found in high concentrations associated with the seasonal chlorophyll maxima. Cell maxima were generally found in the oxic mixolimnion in the winter and spring and below the chemocline in the anoxic monimolimnion during summer and autumn (Fig. 1E). During the November 1988 overturn, chlorophyll and *Picocystis* cells were uniformly distributed throughout the water column ($\sim$40 μg L^{-1} and 4.5×10^8 cells L^{-1}, respectively). By the following August, both chlorophyll and *Picocystis* were concentrated below the developing chemocline.

Chlorophyll and in situ primary production were size fractionated to quantify the contribution of *Picocystis* to the biomass and production of the whole-lake algal community. Epifluorescent microscopy confirmed that the <3-μm fraction was dominated by *Picocystis* cells; no other eukaryotic or cyanobacterial phototrophs were observed. We therefore attribute the <3-μm fraction of chlorophyll and primary production rate to *Picocystis.* In terms of total chlorophyll biomass, *Picocystis* represented greater than half of the water column integrated biomass (Fig. 2A,B). The contribution by

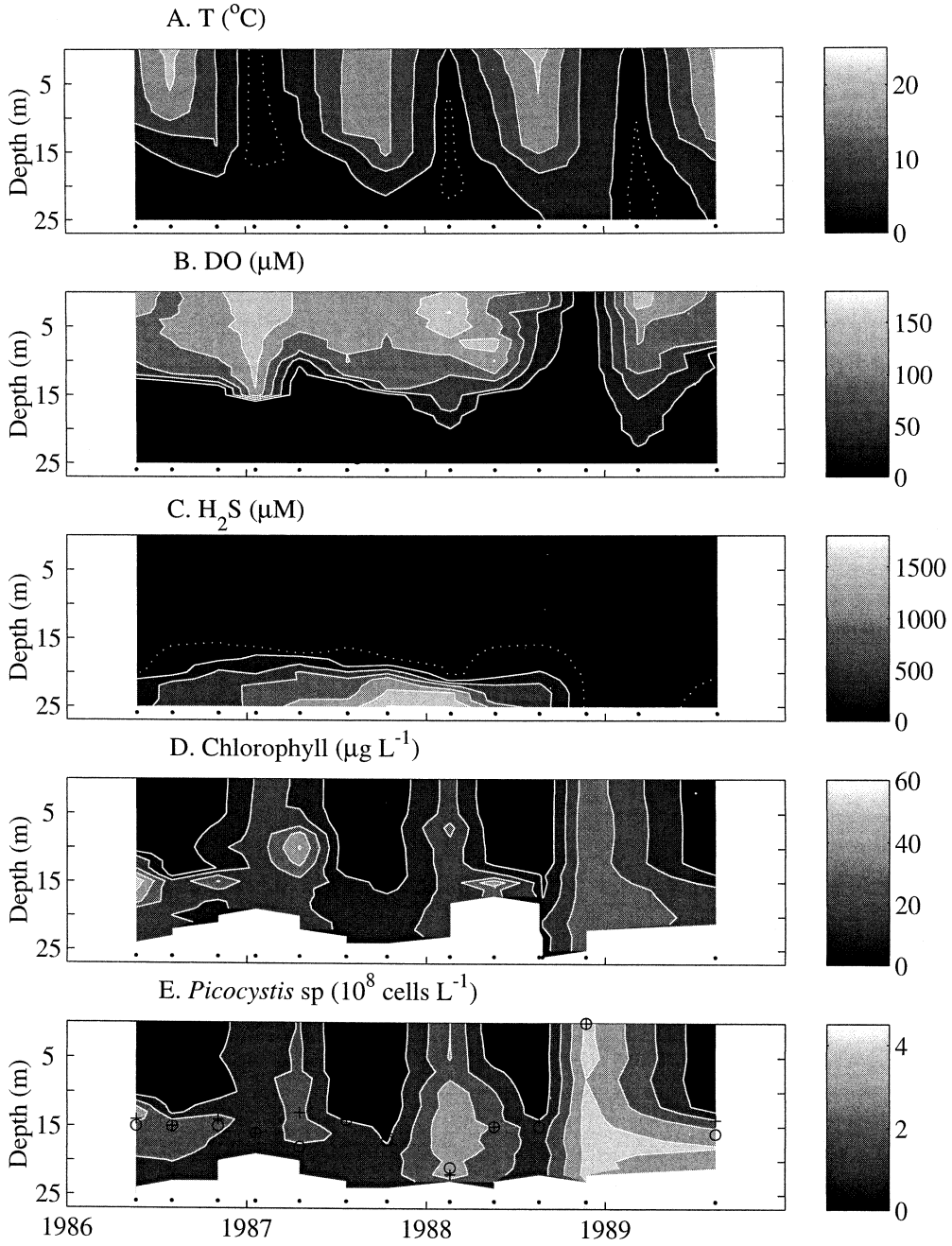

Fig. 1. Time series of (A) temperature (°C), (B) dissolved oxygen (μM), (C) hydrogen sulfide (μM), (D) Chl *a* (μg Chl L^{-1}) and (E) *Picocystis* cell concentrations (10^8 cells L^{-1}) in the western basin of Mono Lake from 1986 through 1989. Contour intervals are 5°C, 30 μM DO, 300 μM H_2S, 10 μg Chl L^{-1} and 1.0 $\times$ 10^8 cells L^{-1}, respectively. Dotted contour lines in (A) and (C) are 2.5°C and 50μM H_2S, respectively. Overturn occurred during the November 1988 sampling. Sampling times are indicated by dots on the time axis; temperature and dissolved oxygen were measured in situ at 1-m intervals; hydrogen sulfide water samples were collected at 2-m intervals throughout the water column, except in the vicinity of the chemocline, at which samples were collected every 0.5 to 1 m. Chl *a* and cell concentrations were sampled at 12 depths in the upper 24 m with higher sampling resolution near the chemocline. Black circles and plus symbols in part (E) indicate the depths of >5 μM H_2S and $<1\mu$M DO, respectively.

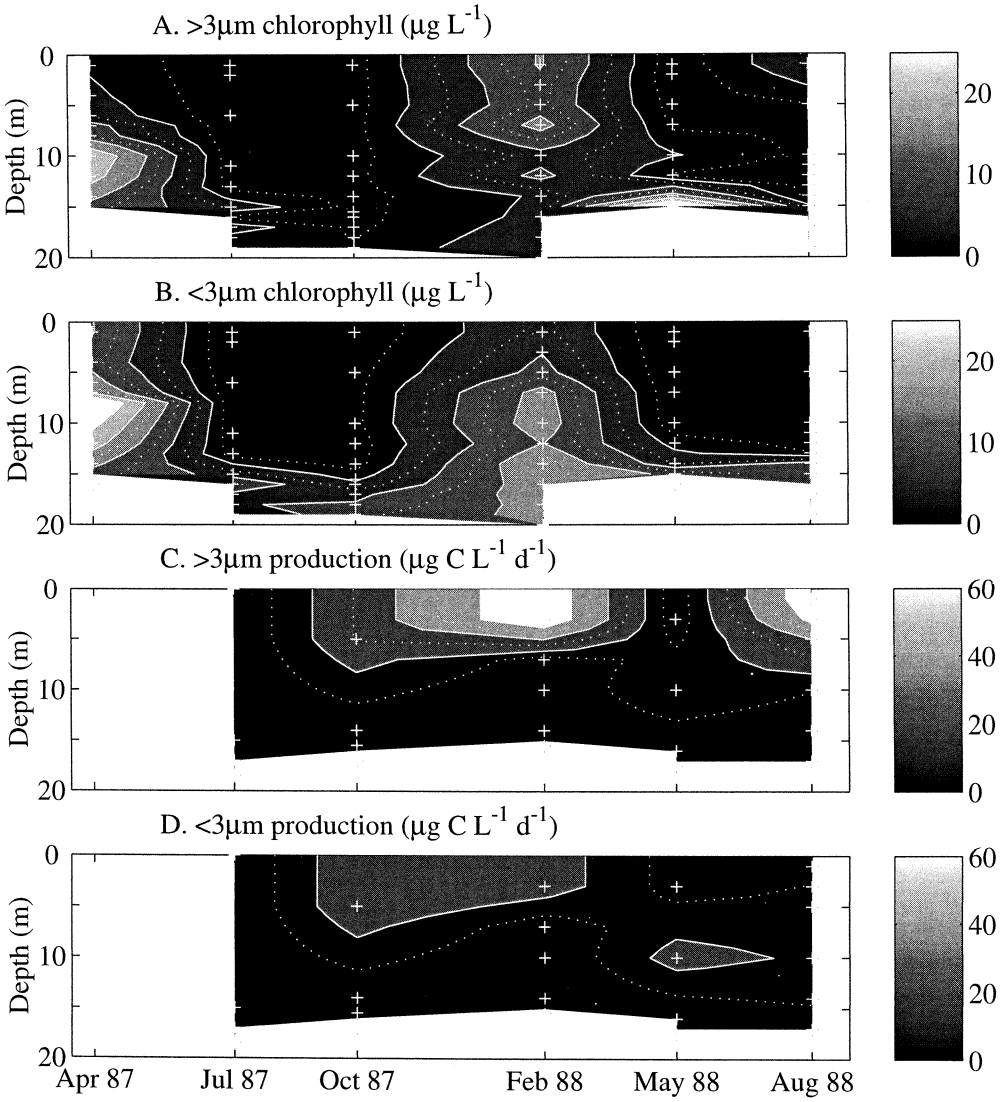

Fig. 2. Time series of size-fractionated Chl *a* (A, B) and primary production (C, D). Solid contour intervals are 5 μg Chl L^{-1} and 20 μg C L^{-1} d^{-1}, respectively, and dotted contour intervals are 2.5 μg Chl L^{-1} and 10 μg C L^{-1} d^{-1}, respectively. Plus symbols indicate sampling dates and depths; note different time interval from Fig. 1.

Picocystis to primary production was smaller on an integrated basis because it tends to inhabit the low-light regions (Fig 2C,D). However, during the winter bloom sampled in February 1987, it was responsible for approximately 25% of the total production, the remainder likely due to large diatoms (Jellison and Melack 1988, 1993*a*). During seasons when the large algal cells were absent or their production was low, the small phototroph was responsible for >50% of the integrated production.

Grazing rates by *Artemia monica* on *Picocystis* averaged $5.47 \pm 0.08 \times 10^5$ cells $shrimp^{-1}$ h^{-1} over the 15-h incubation, with maximal rates of $12.80 \pm 3.47 \times 10^5$ cells $shrimp^{-1}$ h^{-1} in the first 3 h of the incubation (Fig. 3). The initial *Picocystis* cell concentrations were $2.37 \pm 0.07 \times 10^8$ cells L^{-1}, the in situ concentration at the time of sampling. Cells in the control bottles exhibited no change in concentration over the 15-h dark incubation. The brine shrimp removed nearly all the cells from the treatment bottles in the 15-h period.

Laboratory experiments—Pigment composition: The chloropigments of the isolate (Table 1) were found to be identical to higher plant Chl *a* and *b*. The carotenoid complement of this alga consists of the typical green algal carotenoids neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, β,ε-carotene and β,β-carotene. In addition, we found traces of diadinoxanthin and alloxanthin and high concentrations of diatoxanthin and monadoxanthin. The latter two pigments, which elute closely on our C18 rp-HPLC system, were separated on the C30 rp-HPLC system and characterized offline by mass spectrometry.

Pigment variability: Cellular Chl *a* concentrations ranged from 0.04 to 0.45 pg $cell^{-1}$ in response to decreasing growth

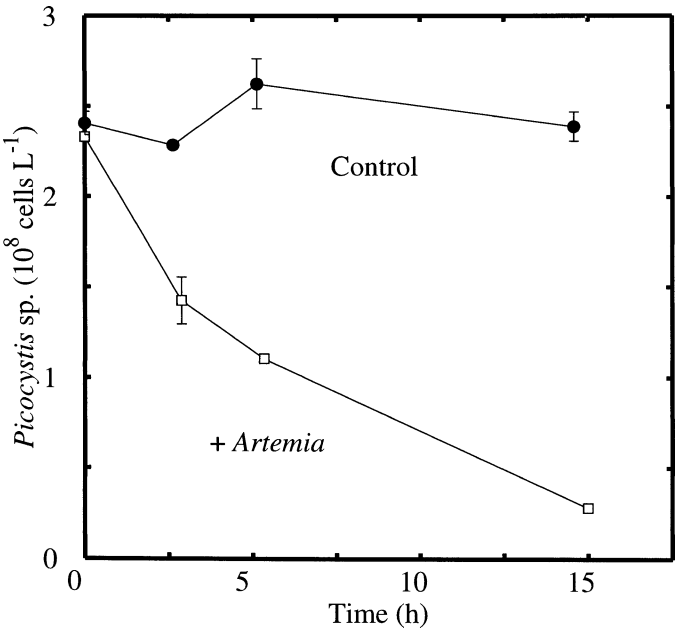

Fig. 3. Time course of in situ concentrations of *Picocystis* incubated in the presence (open squares) and absence (closed circles) of the brine shrimp *Artemia monica.* Error bars indicate the range of duplicate incubations.

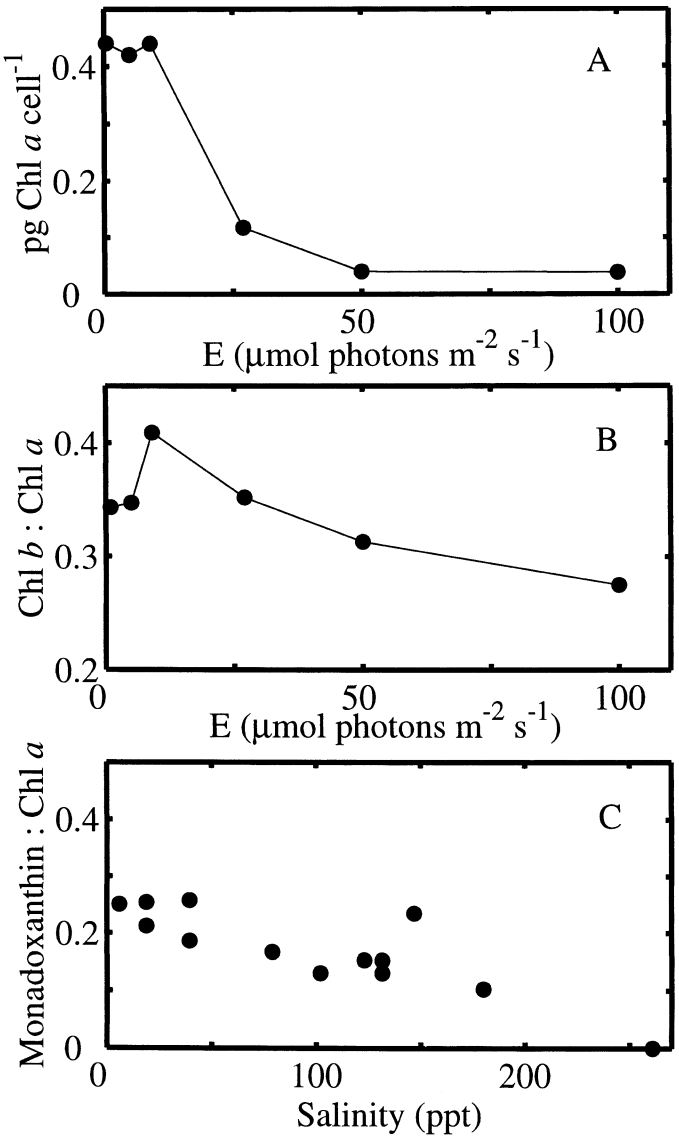

Fig. 4. (A) Cellular Chl *a* concentrations and (B) Chl *b*:Chl *a* ratio as a function of growth irradiance. (C) Monadoxanthin:Chl *a* ratio as a function of growth salinity.

irradiance (Fig. 4A) but exhibited no change with media salinity (0.117 ± 0.023 pg Chl *a* cell^{-1}; $r = 0$ .39; $P = 0.16$) or pH (0.068 ± 0.009 pg Chl *a* cell^{-1}; $r = 0.51$; $P = 0.42$) at respective growth irradiances of 25 and 40 µmol photons m^{-2} s^{-1}. The Chl *b*/*a* ratio decreased from 0.42 to 0.27 with increasing irradiance (Fig. 4B). The ratio of monadoxanthin to Chl *a* decreased linearly with salinity from 0.25 to <0.01 over the 2–260‰ range ($r = 0.86$; $P < 0.001$; Fig. 4C). None of the other accessory carotenoid pigments exhibited variation with respect to Chl *a* in response to growth conditions (Table 1).

Growth rates: Maximal specific growth rates of 1.6 d^{-1} were attained at a growth irradiance of 50 µmol photons m^{-2} s^{-1}, a salinity of 40‰ and a pH of 9.8 (Fig. 5). Growth rates of 0.2 d^{-1} were observed at the lowest growth irradiance, 0.6 µmol photons m^{-2} s^{-1}. Growth rates 0.3–1.6 d^{-1} were observed over the salinity range 0–260‰. Mineral precipitation prohibited concentrating Mono Lake water much past 260‰, preventing the determination of the upper limit for salinity tolerance. Growth rates >1.0 d^{-1} were observed for pH range 4–10. The only experimental condition for which the organism failed to grow was pH 2, but credible growth was observed at pH 12 (0.5 d^{-1}).

Growing *Picocystis* under strictly anoxic conditions was problematic. Oxygen levels, monitored by gas chromatography, indicated that the cells were undergoing oxygenic photosynthesis regardless of the initial oxygen or reducing conditions. Suboxic and reducing conditions were maintained with N_2 flushing and media supplements. Growth rates were not significantly different under anoxic versus oxic conditions at either the higher or lower irradiance levels, and the rates were comparable to those obtained in the irradiance experiment (Fig. 5D).

Osmolytes: Cells grown in saline media (19–123‰) were found to contain substantial concentrations of the osmolytes GB and DMSP (Table 2). There was no relationship between cellular DMSP content and growth salinity over the range tested. The cellular GB content, on the other hand, increased markedly with salinity. At and below 40‰, DMSP was more abundant than GB, but above 40‰, GB exceeded DMSP several-fold. We looked for but did not detect homarine, a nitrogen-containing betaine-like osmolyte that is sometimes found in marine algae (Dickson and Kirst 1986; Keller et al. 1999).

Microscopic observations of cells grown under a range of salinity treatments demonstrated that cells growing at or ex-

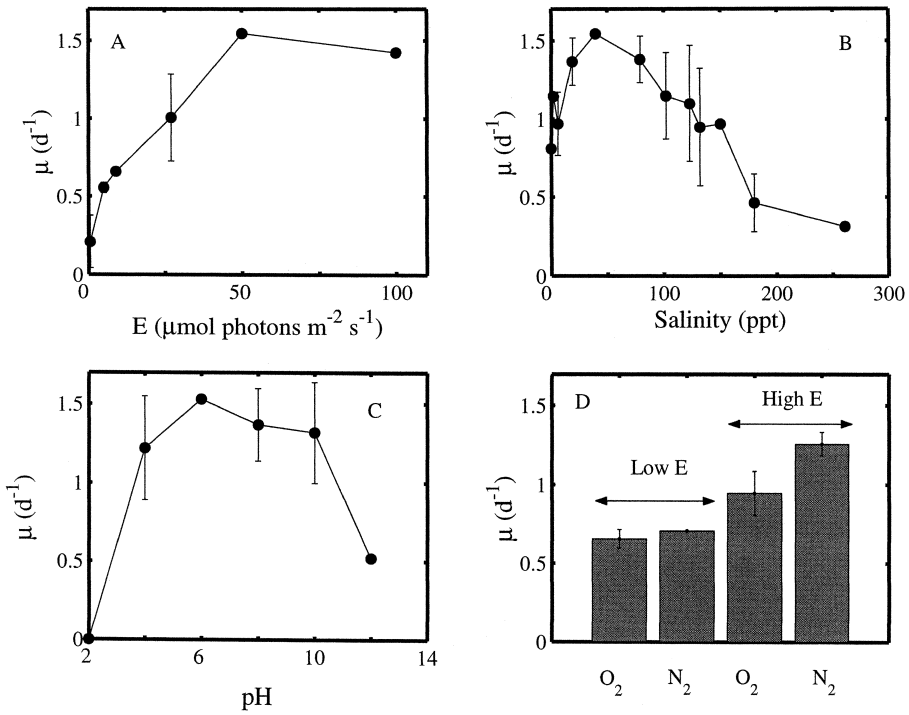

Fig. 5. Specific growth rate determined from time course in vivo fluorescence measurements as a function of (A) growth irradiance, (B) salinity, (C) pH, and (D) aerobic (O_2) versus anaerobic (N_2) conditions at low and high growth irradiance (E, 13 and 44 μmol photons m^{-2} s^{-1}, respectively). Error bars represent range in the case of duplicates (A, C, D) and standard deviations in the case of triplicates (B).

ceeding in situ Mono Lake salinity exhibited the trilobate morphology. As the growth salinity decreased, spherical morphology became more prevalent. Although a quantitative measurement of cytoplasm volume was not performed for each treatment, microscopic observations indicated that there was no apparent change in cell diameter (as measured from lobe edge to lobe edge); rather, at high growth salinity, the cell wall and membrane shrunk to encapsulate the chloroplasts, with a concomitant reduction in cytoplasm (*see* fig. 9 in Lewin et al. 2000). Unlike Lewin et al. (2000), we found no change in morphology as a function of culture age or nutrient stress, only media salinity. Calculations for cell volume were made for the two morphologies (spherical and trilobate) and the range in internal solute concentrations were estimated (Table 2). The combined internal solute concentrations of GB and DMSP nearly balance the external solute concentrations of each growth medium, suggesting that GB is likely the dominant osmolyte for *Picocystis.*

Table 2. Measured cellular osmolyte concentrations for *Picocystis* sp. cultures grown in a range of media salinity and calculated internal solute concentrations assuming the observed minimum and maximum observed cell sizes (see text for details). Computed cell volumes ranged from ~5 to 14 μm^3 for trilobate and spherical morphologies, respectively. DMSP, dimethylsulphoniopropionate; GB, glycine betaine.

	Osmolyte concentration		Solute concentration	
Salinity (ppt)	DMSP (pg $cell^{-1}$)	GB (pg $cell^{-1}$)	DMSP (ppt)	GB (ppt)
19	0.079±0.016	0.000	5.6–15.0	0.0
40	0.141±0.039	0.037	10.0–26.6	2.6–6.9
79	0.106±0.002	0.352	7.5–20.1	24.9–66.3
102	0.082±0.025	0.330	5.8–15.5	23.3–62.2
123	0.143±0.018	0.796	10.1–26.9	56.3–150.2

Photosynthetic rates: All P-E incubations exhibited the characteristic hyperbolic response of photosynthesis to irradiance and resulted in statistically reliable estimates of the maximal rate of photosynthesis, Pmax, and the initial slope of the relationship at limiting irradiance, α. Saturated photosynthetic rates occurred at <200 μmol photons m^{-2} s^{-1} for the cultures acclimated to 100 μmol photons m^{-2} s^{-1} and at ~60 μmol photons m^{-2} s^{-1} for the cultures acclimated to 0.6 μmol photons m^{-2} s^{-1}. The cell-specific maximal photosynthetic rate, $Pmax_{cell}$ approached 1 pg C $cell^{-1}$ h^{-1} for cells grown at 5 μmol photons m^{-2} s^{-1} and under in situ Mono Lake conditions of 70‰ and pH 9.8 (Fig. 6A–C). Both $Pmax_{cell}$ and the cell-specific initial slope, α_{cell}, decreased with increasing irradiance (Fig. 6A,D, filled symbols), indicating high photosynthetic potential and efficiency during growth at low irradiance. Chlorophyll-specific photosynthetic parameters decreased with decreasing growth irradiance in response to increasing cellular Chl *a* concentrations (Fig. 6A,D).

The cell-specific photosynthetic parameters, although reduced in magnitude, exhibited no trend with salinity above

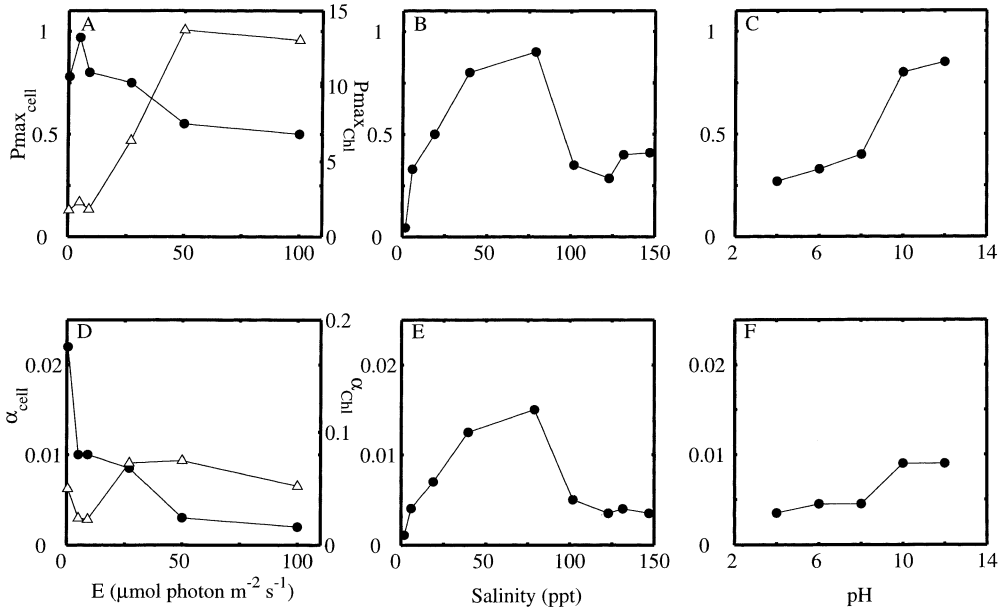

Fig. 6. Cell-specific maximal photosynthetic rates, $Pmax_{cell}$ (pg C cell^{-1} h^{-1}; closed circles) as a function of (A) growth irradiance, (B) salinity, and (C) pH. Cell-specific photosynthetic efficiency factors, α_{cell} (pg C cell^{-1} h^{-1} [μmol photons m^{-2} s^{-1}]$^{-1}$; closed circles) as a function of (D) growth irradiance, (E) salinity, and (F) pH. Chlorophyll-specific photosynthetic parameters as a function of growth irradiance are (A) $Pmax_{Chl}$ (g C [g Chl]$^{-1}$ h^{-1}) and (D) α_{Chl} (g C [g Chl]$^{-1}$ h^{-1} [μmol photons m^{-2} s^{-1}]$^{-1}$) and are indicated by open triangles.

100‰, whereas they decreased with salinity below 80‰ (Fig. 6B,E). The cell-specific photosynthetic parameters exhibited a step-function response to pH, with the critical value between pH 8 and 10 (Fig. 6C,F). Chlorophyll-specific parameters exhibited the same trends as cell-specific parameters, as the cellular Chl *a* concentrations were invariant with growth salinity and pH.

Dark anoxic cell-specific uptake rates were not significantly different from the blank or boiled controls (Wilcoxon rank sum, $\alpha < 0.05$; Fig. 7) and not different from zero within the resolution of the measurement. The uptake rates for anoxic media with different supplements and for oxic media with no supplements were approximately 0.4 pg C cell^{-1} h^{-1}, significantly greater than the controls (Student's *t*-test, $P < 0.0001$). The highest uptake rates, nearly 0.6 pg C cell^{-1} h^{-1}, approaching the observed value of $Pmax_{cell}$ for that growth irradiance, were observed in the anoxic incubation with supplemental NH_4 (100 μM). These were significantly greater than the other light incubations, regardless of oxygen condition ($P < 0.001$).

Growth efficiency: Specific growth rates, μ, were scaled to cell-specific maximal photosynthetic rates, $Pmax_{cell}$, to examine the efficiency of photosynthetic energy partitioning as a function of environmental conditions. Large ratio values indicate efficient transfer of photosynthetically derived energy toward cell division, whereas small values indicate added costs for population growth. The ratio increased hyperbolically with increasing irradiance (Fig. 8A). There was no apparent trend with salinity ($r = 0.30$; $P = 0.48$), indicating that the salinity acclimation does not siphon away photosynthetic energy (Fig. 8B). The response of growth efficiency to pH (Fig. 8C) was more complex. Although cells exhibit the largest values of $Pmax_{cell}$ at pH $\geq$ 10 (Fig. 6C), proportionally less of the photosynthetic energy results in population growth compared to cells grown at pH $\leq$ 8.

Discussion

Pigment composition—Pigments are often used as taxonomic markers because they exhibit relatively distinct distribution patterns. The chlorophyll complement of *Picocystis*, Chl *a* and Chl *b*, is typical for green algae, although the carotenoid complement is extremely complex, comprised of pigments typical of both chlorophytes and cryptophytes. The carotenoid complement of *Picocystis* is clearly unusual, first because it is so diverse, and second because its major carotenoids are monadoxanthin and diatoxanthin, which have only been found in cryptophytes and euglenophytes, respectively. *Picocystis* is a striking exception to the patterns of carotenoid distribution that are used as tools for assigning taxonomic position.

Growth irradiance—Picocystis is adapted to low-light environments and capable of photosynthesis and growth at irradiances <0.1% of the incident summertime solar irradiance in Mono Lake. The ability to grow at such low light levels is due, in part, to the large potential for photoacclimation and shade-adapted photosynthesis. Although the cellular Chl *a* content in *Picocystis* is consistent with that found in similar-sized phototrophs (Waterbury et al. 1986; Chisholm et al. 1988; Sosik et al. 1989), the range in cell-specific Chl *a* concentrations varies by a factor of 10, more than twice the variation observed for other chlorophytes (Sukenik

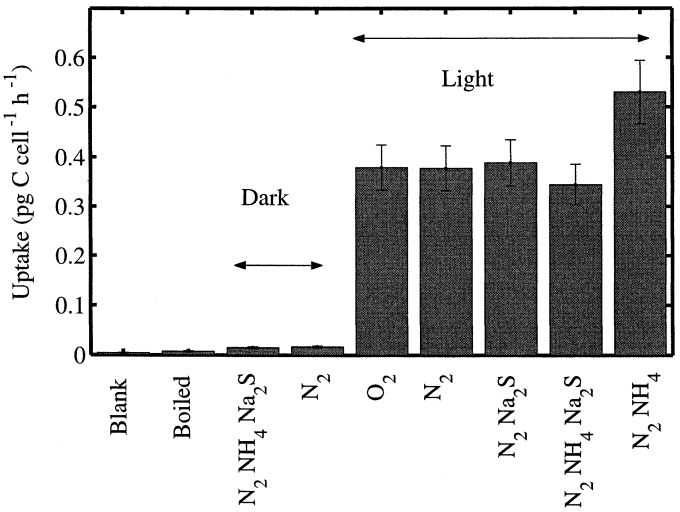

Fig. 7. Cell-specific carbon uptake rates (pg C cell^{-1} h^{-1}) as a function of oxic (O_2) or anoxic (N_2) growth conditions. Additional treatments under anoxic conditions included media supplements of Na_2S, NH_4Cl, or both to final concentrations of 100 μM. Samples were incubated for 1 h; light treatments were conducted at 44 μmol photons m^{-2} s^{-1}.

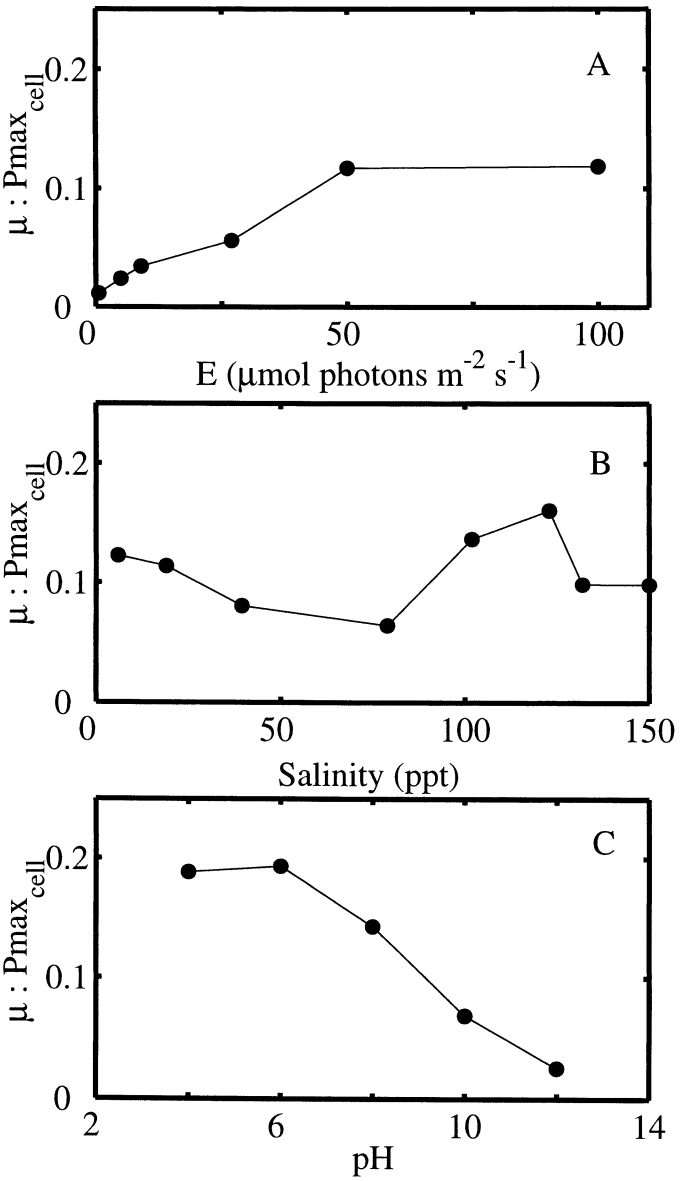

Fig. 8. Specific growth rates, μ, scaled to maximal cell-specific production rates, $Pmax_{cell}$, as a function of (A) growth irradiance, (B) salinity, and (C) pH. Units are (cells [pg C_{fixed}]$^{-1}$).

et al. 1990) or cryptophytes (Sosik et al. 1989). *Picocystis* has a Chl *b* to Chl *a* ratio typical for green algae (Wood 1979) and photoacclimates by preferential synthesis of Chl *b* at low irradiance, similar to the range and strategy observed in *Prochlorococcus* sp. (a small prokaryotic phototroph with Chl *b* and divinyl Chl *a* and *b*; Moore et al. 1995).

The values of the chlorophyll-specific photosynthetic parameters are consistent with published values for nutrient-replete phytoplankton growth in cultures (e.g., Cullen et al. 1992 and references therein) and in the natural environment (e.g., Platt and Jassby 1976). The chlorophyll-specific Pmax increased an order of magnitude with increasing growth irradiance because of the order of magnitude decrease in cellular Chl *a*, to a value of nearly 15 g C (g Chl)$^{-1}$ h^{-1}, which is high but does not exceed the maximal theoretical value (Falkowski 1981). The cell-specific photosynthetic parameters indicated that *Picocystis* sp. is a shade-adapted organism: both the cell-specific photosynthetic efficiency and the cell-specific maximal rate of photosynthesis are higher for low-light–acclimated cells compared to high-light–acclimated cells. Metabolic costs associated with life at low irradiance, such as increased size or number of photosynthetic units, or both, require additional photosynthetic energy and are exhibited in the reduced growth efficiencies that we observed at low irradiances.

Salinity—Picocystis was observed to photosynthesize and grow over the salinity range 0–260‰, with a peak at 40‰. We hypothesize that the cells produced osmolytes to aid them in adapting to ionic stress (Reed 1983; Le Rudulier et al. 1984) as bacteria in Mono Lake have been found to contain a suite of osmolytes (i.e., ectoine, hydroxyectoine, GB, arsenobetaine, and DMSP; Ciulla et al. 1997). *Picocystis* contained DMSP and GB, with internal solute concentrations sufficient to balance external solute concentrations. The observed change in cell size and the associated cytoplasmic volume change could account for a change in solute concentration of nearly a factor of three, which might contribute substantially to balancing osmotic stress in addition to the osmolyte synthesis (Vairavamurthy et al. 1985). Whereas the GB levels were directly related to growth salinity, the DMSP levels were not. This, and the higher per-cell levels of GB, suggests that in *Picocystis* GB is a more important osmolyte at higher salinity than is DMSP (Colmer et al. 1996). Growth efficiencies were high over the 2–150‰ salinity range. This suggests that once acclimated, whether through synthesis of osmolytes or regulation of cytoplasmic volume, there is efficient transfer of photosynthetically derived energy toward growth regardless of external salinity.

pH tolerance—Only the pH 12 treatment yielded reduced growth rates (pH 4–12). The patterns in growth and photosynthesis did not covary with pH but resulted in maximal growth efficiencies at low pH. These experiments were not designed to separate effects of DIC concentration from pH effects. However, although the concentration of DIC changed from 1.3 to 185 mM over the range of pH from 4 to 12 (at 40‰), the dominant species changed from CO_2 to carbonate, respectively. A larger range in DIC, from 10 to 1,240 mM, occurred in the salinity treatments with no change in growth efficiency. Thus the observed changes are more likely due to the pH effects on membrane potential (Krulwich 1995), the details of which were not explored here. Regardless, growth of the organism over a pH range of 4–12 demonstrates its broad adaptive possibilities in aquatic environments.

Anaerobic tolerance—Picocystis was never observed to photosynthesize anoxygenically. However, it does undergo oxygenic photosynthesis and growth under initially anoxic conditions, with rates comparable to those measured under oxic conditions. This suggests that the environment immediately surrounding the cells are likely suboxic, which would affect the reducing environment adjacent to the cell surface, perhaps easing some of the redox stress.

Ecology of Picocystis *in Mono Lake*—Given the experimental results for *Picocystis,* how can we better interpret the observations of its distribution and production in Mono Lake? *Picocystis* cell maxima are found throughout the oxic region of the water column associated with larger algal species and contributing nearly 25% to the winter bloom biomass and primary production. For the remainder of the year, *Picocystis* is a larger contributor to biomass and whole-lake primary production (on the order of 50%). Production in the mixolimnion has been shown to be nutrient limited, and the limitation increases from year to year during meromixis as stratification reduces the nutrient concentrations in the upper layer (Jellison and Melack 1993*a*). Wind-induced and internal wave-induced boundary layer mixing provide upward fluxes of ammonia (on the order of mM m^{-2} d^{-1}) at the chemocline (MacIntyre et al. 1999), where subsurface maxima in *Picocystis* cell concentrations occur throughout the year. Because *Picocystis* can grow and photosynthesize at or below the chemocline in low-light–reducing environments where nutrients are replete, there is always a viable population of cells available for *A. monica,* even during overturn when the lake becomes suboxic. The dynamics of the winter blooms suggests there is some top-down control of phytoplankton biomass (including *Picocystis*). Although the density of *Artemia* declines with depth in the mixolimnion, animals have been recovered routinely from the monimolimnion (Lenz 1980), suggesting that there may be some grazing pressure below the chemocline as well.

Mono Lake differs from other soda lakes in the western U.S., such as Big Soda Lake, Nevada, and Soap Lake, Washington, in that the physical dynamics have varied from periods of monomixis to periods of meromixis on the order of tens of years. Given historic fluctuations in lake level, there have been considerable variations in lake salinity and not just over the last 60 years, but over the last tens of thousands of years associated with climatic variations. Thus, the current regime of short meromictic and monomictic periods has likely persisted for thousands of years. In contrast, the other lakes have been meromictic for decades (Cloern et al. 1983, 1987; Miller and Oremland 1988; Oremland et al. 1988, 1993).

So far, *Picocystis* has not been found in other soda lakes in the western U.S. Tolerance to extreme chemical fluctuations has been observed rarely in other eukaryotic phototrophs (cf. *Cryptomonas phaseolus* in Lake Cisó, Spain; Pedrós-Alió et al. 1987), leading to the dominance by cyanobacteria and small chlorophytes in the aerobic zone and green and purple sulfur bacteria in the anoxic zone (Walker 1975; Cloern et al. 1983; Craig 1987; Oremland et al. 1988; Overmann and Tilzer 1989; Hall and Northcote 1990). Members of all of these types of organisms are absent or are relatively minor components of the phototrophic community in Mono Lake. What controls the photosynthetic community development and distribution in these analogous ecosystems is perplexing. Plates of purple sulfur bacteria in Big Soda Lake, for example, are always observed in the mixolimnion, where salinity is approximately 27‰, and never at the deeper chemocline/pycnocline, where salinity approaches 90‰ (Oremland et al. 1988). The ability of *Picocystis* to photosynthesize and grow at low light *and* high salinity, leads to accumulated biomass at the oxic/anoxic boundary, thereby blocking out available light that would, in the absence of *Picocystis,* have allowed sulfur bacteria plates to form slightly deeper with sufficient light and abundant sulfide.

The ability of *Picocystis* to photosynthesize and grow under such a range of dynamic environmental conditions likely leads to its dominance of the lake phototrophic community, in both the short term (i.e., meromictic period) and the long term (geologic time scales). The resilience of this unique phototroph allows it to persist under a multitude of circumstances, providing a source of primary production and thereby assuring the continued sustenance of the Mono Lake ecosystem during periods of meromixis and the unstable interval following overturn.

References

CHISHOLM, S. W., R. J. OLSON, E. R. ZETTLER, R. GOERICKE, J. B. WATERBURY, AND N. A. WELSCHMEYER. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature **334:** 340–343.

CIULLA, R. A., M. R. DIAZ, B. F. TAYLOR, AND M. F. ROBERTS. 1997. Organic osmolytes in aerobic bacteria from Mono Lake, an alkaline, moderately hypersaline environment. Appl. Env. Microbiol. **63:** 220–226.

CLOERN, J. E., B. E. COLE, AND R. S. OREMLAND. 1983. Autotrophic processes in meromictic Big Soda Lake, Nevada. Limnol. Oceanogr. **28:** 1049–1061.

———, ———, AND S. M. WIENKE. 1987. Big Soda Lake (Nevada). 4. Vertical fluxes of particulate matter: Seasonality and variations across the chemocline. Limnol. Oceanogr. **32:** 815–824.

COHEN, Y. 1989. Photosynthesis in cyanobacterial mats and its relation to the sulfur cycle: A model for microbial sulfur inter-

actions, p. 22–36 *In* Y. Cohen and E. Rosenberg [eds.], Microbial mats: Physiological ecology of benthic microbial communities. American Society of Microbiologists.

COLMER, T. D., T. W. H. FAN, A. LAUCHLI, AND R. M. HIGASHI. 1996. Interactive effects of salinity, nitrogen, and sulphur on the organic solutes in *Spartina alterniflora* leaf blades. J. Exp. Bot. **47:** 369–375.

CRAIG, S. 1987. The distribution and contribution of picoplankton to deep photosynthetic layers in some meromictic lakes. Acta Acad. Aboensis **47:** 55–81.

CULLEN, J. J., X. YANG, AND H. L. MACINTYRE. 1992. Nutrient limitation in marine photosynthesis, p. 69–88. *In* P. G. Falkowski and A. D. Woodhead [eds.], Primary productivity and biogeochemical cycles in the sea. Plenum.

DANA, G. L., AND P. H. LENZ. 1986. Effects of increasing salinity on an *Artemia* population from Mono Lake, California. Oecologia **68:** 428–436.

DICKSON, D. M. J., AND G. O. KIRST. 1986. The role of *β*-dimethylsulphoniopropionate, glycine betaine and homarine in the osmoacclimation of *Platymonas subcordiformis.* Planta **167:** 536–543.

FALKOWSKI, P. G. 1981. Light–shade adaptation and assimilation numbers. J. Plankton Res. **3:** 203–216.

———, AND T. G. OWENS. 1980. Light–shade adaptation: Two strategies in marine phytoplankton. Plant. Physiol. **66:** 632–635.

GENSEMER, R. W. 1991. The effects of pH and aluminum on the growth of the acidophilic diatom *Asterionella ralfsii* var. *americana.* Limnol. Oceanogr. **36:** 123–131.

GORBUSHINA, A. A., AND W. E. KRUMBEIN. 1999. Poikilotrophic response of microorganisms to shifting alkalinity, salinity, temperature, and water potential, p. 75–88. *In* A. Oren [ed.], Microbiology and biogeochemistry of hypersaline environments. CRC Press.

GORHAM, J. 1984. Separation of plant betaines and their sulfur analogues by cation-exchange high-performance liquid chromatography. J. Chromatogr. **287:** 345–351.

HALL, K. J., AND T. G. NORTHCOTE. 1990. Production and decomposition processes in a saline meromictic lake. Hydrobiologia **197:** 115–128.

JASSBY, A. D., AND T. PLATT. 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol. Oceanogr. **21:** 540–547.

JELLISON, R., AND J. M. MELACK. 1988. Photosynthetic activity of phytoplankton and its relation to environmental factors in hypersaline Mono Lake, California. Hydrobiologia **158:** 69–88.

———, AND ———. 1993*a.* Algal photosynthetic activity and its response to meromixis in hypersaline Mono Lake, California. Limnol. Oceanogr. **38:** 818–837.

———, AND ———. 1993*b.* Meromixis in hypersaline Mono Lake, California. 1. Stratification and vertical mixing during the onset, persistence, and breakdown of meromixis. Limnol. Oceanogr. **38:** 1008–1019.

KAUSS, H. 1978. Osmoregulation in algae. Prog. Phytochem. **5:** 1–27.

KELLER, M. D., P. A. MATRAI, AND R. P. KIENE. 1999. Production of glycine betaine and dimethylsulfoniopropionate in marine phytoplankton: I. Batch cultures. Mar. Biol. **135:** 237–248.

KRULWICH, T. A. 1995. Alkaliphiles: 'Basic' molecular problems of pH tolerance and bioenergetics. Mol. Microbiol. **15:** 403–410.

LE RUDULIER, D., A. R. STROM, A. M. DANDEKAR, L. T. SMITH, AND R. C. VALENTINE. 1984. Molecular biology of osmoregulation. Science **244:** 1064–1068.

LENZ, P. H. 1980. Ecology of an alkali-adapted variety of *Artemia* from Mono Lake, California, USA, p. 79–96. *In* G. Persoone, P. Sorgeloos, O. Roesl, and E. Jaspers [eds.], The brine shrimp *Artemia:* Ecology, culturing, use in aquaculture. Universal Press.

LEWIN, R. A., L. KRIENITZ, R. GOERICKE, H. TAKEDA, AND D. HEPPERLE. 2000. *Picocystis salinarum* gen. et sp. nov. (Chlorophyta)—a new picoplanktonic green alga. Phycologia **39:** 560–565.

LORENZEN, C., AND S. W. JEFFREY. 1980. Determinations of chlorophyll in seawater. UNESCO Tech. Pap. Mar. Sci. **35.**

LOVEJOY, C., AND G. DANA. 1977. Primary producer level, p. 42–57. *In* D. W. Winkler [ed.], An ecological study of Mono Lake, California. Univ. of California, Davis, Institute of Ecology **12.**

MACINTYRE, S., K. M. FLYNN, R. JELLISON, AND J. R. ROMERO. 1999. Boundary mixing and nutrient fluxes in Mono Lake, California. Limnol. Oceanogr. **44:** 512–529.

MILLER, L. G., AND R. S. OREMLAND. 1988. Methane eflux from the pelagic regions of four lakes. Global Biogeochem. Cycles **2:** 269–277.

———, R. JELLISON, R. S. OREMLAND, AND C. W. CULBERTSON. 1993. Meromixis in hypersaline Mono Lake, California. 3. Biogeochemical response to stratification and overturn. Limnol. Oceanogr. **38:** 1040–1051.

MOORE, L. R., R. GOERICKE, AND S. W. CHISHOLM. 1995. Comparative physiology of *Synechococcus* and *Prochlorococcus:* influence of light and temperature on growth, pigments, fluorescence and absorptive properties. Mar. Ecol. Prog. Ser. **116:** 259–275.

OREMLAND, R. S., AND OTHERS. 1988. Microbial and biogeochemical processes in Big Soda Lake, Nevada, p. 59–75. *In* A. J. Fleet, K. Kelts, and M. R. Talbot [eds.], Lacustrine petroleum source rocks, Geological Society.

———, AND OTHERS. 1993. Aspects of the biogeochemistry of methane in Mono Lake and the Mono Basin of California, p. 704–744. *In* R. S. Oremland [ed.], Biogeochemistry of global change, Chapman and Hall.

OVERMANN, J., AND M. M. TILZER. 1989. Control of primary productivity and the significance of photosynthetic bacteria in a meromictic kettle lake, Mittlerer Buchensee, West-Germany. Aquat. Sci. **51:** 261–278.

PEDRÓS-ALIÓ, C., J. M. GASOL, AND R. GUERRERO. 1987. On the ecology of a *Cryptomonas phaseolus* population forming a metalimnetic bloom in Lake Cisó, Spain: Annual distribution and loss factors. Limnol. Oceanogr. **32:** 285–298.

PERRY, M. J., M. C. TALBOT, AND R. S. ALBERTE. 1981. Photoadaptation in marine phytoplankton: Response of the photosynthetic unit. Mar. Biol. **62:** 91–101.

PETERSON, H. G., AND F. P. HEALY. 1985. Comparative pH dependent metal inhibition of nutrient uptake by *Scenedesmus quadricauda* (Chlorophyceae). J. Phycol. **21:** 217–222.

PLATT, T., AND A. D. JASSBY. 1976. The relationship between photosynthesis and light for natural assemblages of coastal marine phytoplankton. J. Phycol. **12:** 421–430.

PRÉZELIN, B. B. 1976. The role of peridinen–chlorophyll *a* proteins in the photosynthetic light adaptation of the marine dinoflagellate, *Glenodinium* sp. Planta **130:** 225–233.

REED, R. H. 1983. Measurement and osmotic significance of *β*-dimethylsulfoniopropionate in marine algae. Mar. Biol. Lett. **4:** 173–181.

SOSIK, H. M., S. W. CHISHOLM, AND R. J. OLSON. 1989. Chlorophyll fluorescence from single cells: Interpretation of flow cytometric signals. Limnol. Oceanogr. **34:** 1749–1761.

SUKENIK, A., J. BENNETT, AND P. G. FALKOWSKI. 1987. Light-saturated photosynthesis—limitation by electron transport or carbon fixation? Biochim. Biophys. Acta **891:** 205–215.

———, ———, A. MORTAIN-BERTRAND, AND P. G. FALKOWSKI. 1990. Adaptation of the photosynthetic apparatus to irradiance

in Dunaliella tertiolecta: A kinetic study. Plant Physiol. **92:** 891–898.

VAIRAVAMURTHY, A., M. O. ANDREAE, AND R. L. IVERSON. 1985. Biosynthesis of dimethylsulfide and dimethylpropiothetin by *Hymenomonas carterae* in relation to sulfur source and salinity variations. Limnol. Oceanogr. **30:** 59–70.

WALKER, K. F. 1975. The seasonal phytoplankton cycles of two saline lakes in central Washington. Limnol. Oceanogr. **20:** 40–53.

———, W. D. WILLIAMS, AND U. T. HAMMER. 1970. The Miller method for oxygen determination applied to saline lakes. Limnol. Oceanogr. **15:** 814–815.

WATERBURY, J. B., S. W. WATSON, F. W. VALOIS, AND D. G. GRANKS. 1986. Biological and ecological characterization of the marine unicellular cyanobacterium, *Synechococcus,* p. 71–120. *In* Photosynthetic picoplankton. Can. Bull. Fish. Aquat. Sci. **214.**

WHITE, R. H. 1982. Analysis of dimethylsulfonium compounds in marine algae. J. Mar. Res. **40:** 529–535.

WOOD, A. M. 1979. Chlorophyll *a*:*b* ratios in marine phytoplanktonic algae. J. Phycol. **15:** 330–332.

Received: 7 September 2000
Accepted 19 November 2001
Amended: 22 November 2001