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Lake Kauhakō, Moloka'i, Hawai'i: biological and chemical aspects of a morpho-ectogenic meromictic lake

Stuart P. Donachie[1,*], Robert A. Kinzie III[2], Robert R. Bidigare[3], Daniel W. Sadler[1], David M. Karl[1,3]

[1]Laboratory for Microbiological Oceanography, [2]Department of Zoology and [3]Department of Oceanography, SOEST, University of Hawai'i, Honolulu, Hawai'i 96822, USA

ABSTRACT: We undertook the first combined microbiological and hydrochemical study of the 248 m deep meromictic Lake Kauhakō. Situated at sea level 1.6 km from the sea in the crater of an extinct volcano on the island of Moloka'i, Hawai'i, USA, Lake Kauhakō has the highest relative depth (ratio of depth to surface area, z_r = 374 %) of any lake in the world. The upper 4.5 m were stratified (T = 23 to 26°C; salinity = 6 to 24.5), but below a pycnocline at ~4.5 m temperature and salinity were uniform (T = ~26.25°C; salinity = 32). Seawater likely intrudes by horizontal hydraulic conductivity through rock separating the lake and the Pacific Ocean. Anoxia commenced below 2 m. Hydrogen sulfide was undetectable at 4 m, but averaged ~130 µM between 5 and 28 m. Dissolved inorganic carbon concentrations ranged from ~1.50 mM at the surface to ~3.3 mM below 5 m. Total organic carbon peaked at 0.94 mM above the pycnocline but remained about 0.30 mM below 5 m. Soluble reactive phosphorus and ammonium concentrations, nanomolar above the pycnocline, increased to ~28 and 175 µM, respectively, at greater depth. Nitrate attained 3.7 µM in shallow water, but was ~0.2 µM from the pycnocline to 100 m. Leucine aminopeptidase (LAPase) activity at the surface exceeded 1100 nmol of substrate hydrolyzed l^{-1} h^{-1}. Activities of α- and β-glucosidase were lower, but showed depth distributions similar to that of LAPase. Surface waters hosted large and diverse picoplankton populations; chlorophyll *a* (chl *a*) exceeded 150 µg l^{-1}, and heterotrophic bacteria and autofluorescent bacteria attained 2×10^9 and 9×10^9 l^{-1}, respectively. Filamentous cyanobacteria and '*Prochlorococcus*'-like autotrophs occurred only in the upper 2 m. Chl *a* was the dominant pigment above 2 m, but pigment diversity increased markedly in anoxic waters between 3 and 5 m. Lake Kauhakō is a unique habitat for further studies, particularly of interactions among flora and fauna restricted to a shallow water column within a single basin.

KEY WORDS: Lake Kauhakō · Meromixis · Moloka'i · Carbon · Nutrients · Pigments · Ectoenzyme · Bacteria

'...the great intellectual fascination of limnology lies in the comparative study of a great number of systems, each having some resemblance to the others and also many differences.'

Hutchinson (1964)

INTRODUCTION

Lake Kauhakō is situated in the crater of an extinct, late Pleistocene volcano on the Kalaupapa Peninsula, on the north shore of Moloka'i, Hawai'i, USA (Stearns & Macdonald 1946) (Fig. 1). At 248 m deep, it is believed to be the 4th deepest lake in the United States. The lake is meromictic, a condition here considered to be morphogenically derived (Maciolek 1982), i.e. the small surface area (3500 m^2) and relatively great depth limit vertical mixing to the upper 2% of the water column. The lake is bounded by high (~130 m), steep, and heavily wooded slopes, a topography that restricts wind-driven mixing to a shallow surface layer and promotes the persistence of meromixis. As the lake is in

*Present address: Environmental Microbiology Laboratory, Department of Microbiology, Snyder Hall, University of Hawai'i, The Mall, Honolulu, Hawai'i 96822, USA.
E-mail: donachie@soest.hawaii.edu

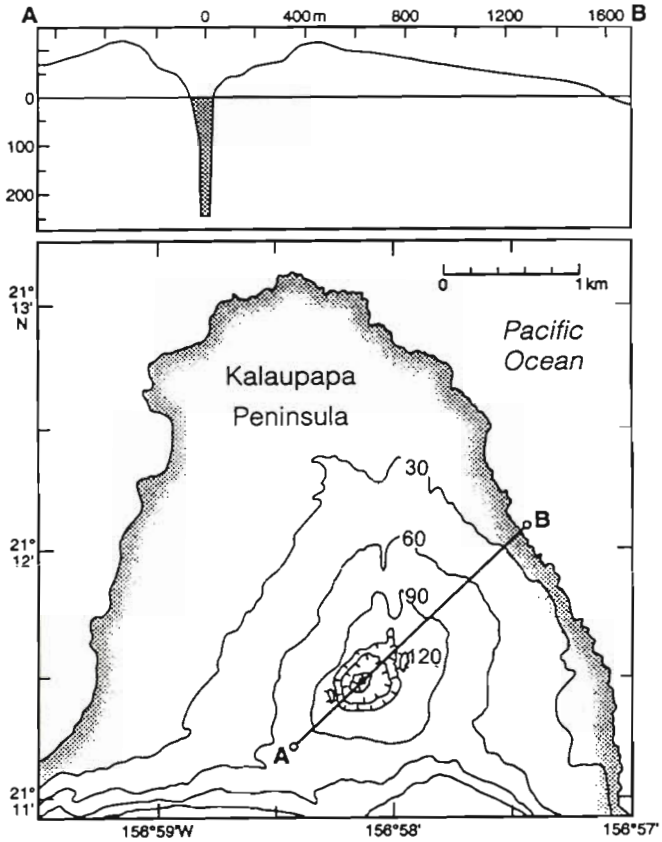

Fig. 1. Lake Kauhakō is situated on the Kalaupapa Peninsula on the north shore of Moloka'i, Hawai'i. The upper panel shows a vertical profile along AB on the map. Scale interval in hundreds of meters. Contour interval in meters. Redrawn from Maciolek (1982)

close proximity to the Pacific Ocean, meromixis may also be ectogenic (derived and sustained through inflow of both fresh- and seawater). Freshwater input amounts to ~1 m yr^{-1} of rainfall (Giambelluca et al. 1986) over the lake surface and catchment area, the latter comprising only the 0.26 km^2 crater opening, and groundwater intrusion from the freshwater table. The lake surface is at sea level; in view of the local geology, the lake's proximity to the Pacific Ocean (1.6 km) and the fact that the lake basin is fully cryptodepressed (below sea level), some exchange of water with the sea is likely.

Meromixis in a water body is characterized by the formation of a layer of oxygenated surface water (the mixolimnion) separated by a chemocline or pycnocline from anoxic and denser water known as the monimolimnion. In response to steep light intensity and/or chemical gradients across depth intervals that may cover only centimeters (Fry 1986), diverse and highly productive microbial populations develop above and in the vicinity of the chemocline (Pfennig 1967, Takahashi & Ichimura 1970).

In light of the conditions which prevail in such lakes and the fact that interest has heightened recently in environments considered physically and/or chemically extreme, a more detailed study of Lake Kauhakō appeared warranted. The lake is not easily accessible, and few data exist concerning its hydrology, particularly below a few meters. We applied a range of modern methods and measurement techniques to define aspects of the lake's chemistry and biology that have to date been undescribed or poorly documented.

MATERIALS AND METHODS

All water samples were collected from the center of Lake Kauhakō between 23 and 25 March 1997. Water at various depths in the upper 100 m was collected in a 5 l Go-Flo or 1 l plastic bathymeter lowered by hand on a Kevlar line from a small dinghy. Vertical sampling resolution of the bathymeter was 12 cm. Samples were immediately returned to the shore and subsamples taken for determination of conductivity, dissolved oxygen, sulfide, nitrous oxide, dissolved inorganic carbon (DIC) and alkalinity, dissolved organic carbon (DOC), nutrients (soluble reactive and total dissolved phosphorus [SRP, TDP], NO_3^-, NH_4^+, total dissolved nitrogen [TDN] and soluble reactive silica [SRSi]), eukaryotic and prokaryotic pigments, microbial ectoenzyme activities (leucine aminopeptidase [LAPase], and α- and β-glucosidase), and cell numbers (heterotrophic and autotrophic *Bacteria*, other autotrophs, picophytoplankton). Data for a temperature profile (0 to 100 m) were collected by a datalogger, which recorded temperature as a function of pressure.

Salinity. A hand-held conductivity meter (Oakton WD-35607-10) with an integral temperature probe was used to determine conductivity in samples to 100 m. Salinities were derived from the conductivity and temperature measured at 1 atmosphere, according to standard procedures (Perkin & Lewis 1980).

Dissolved oxygen. Subsamples for dissolved oxygen were collected to 4 m and were the first taken from the water sampler. They were collected in calibrated iodine flasks and the dissolved oxygen chemically bound in the field by addition of 1 ml each of $MnSO_4 \cdot H_2O$ and alkaline iodide. The floc was dispersed through vigorous agitation (20 s) and the sample sealed and stored in darkness until analysis within 5 h using the Winkler titration method (Carpenter 1965).

Sulfide. The presence of sulfide at 3 m was obvious from the characteristic odor. At both 3 and 4 m, sulfide subsamples were taken from the sampler after those for dissolved oxygen. From 4.5 to 20 m inclusive, sulfide subsamples were drawn first.

Subsamples for sulfide were collected in 125 ml reagent bottles with ground glass stoppers. Sulfide was immediately precipitated by addition of 1 ml of a zinc acetate solution in oxygen-free deionized water containing 2 g l^{-1} gelatin (Fonselius 1983). The tops were wrapped tightly with Labfilm®, and the bottles stored in darkness until analyzed. Sulfide was determined by titration with an acidic standard iodine solution, followed by back titration with standard sodium thiosulfate (Skoog et al. 1988).

Nitrous oxide. Subsamples (0.1 to 5 m) for nitrous oxide (N_2O) were collected in 60 ml glass bottles, fixed with 100 µl saturated mercuric chloride, and sealed with rubber stoppers and aluminium crimp-seal caps. Prior to analysis the sample was equilibrated with an equal volume of helium. Nitrous oxide was separated by gas chromatography and detected using an electron capture detector (Elkins 1980).

Dissolved inorganic carbon and alkalinity. DIC samples (0 to 100 m) were collected in combusted 180 ml glass bottles. After allowing the bottle to overflow twice, a small volume of sample was removed using a plastic pipette and 100 µl of saturated $HgCl_2$ added. The bottles were sealed with rubber stoppers and aluminium crimp-seal caps. DIC was measured by coulometric determination of extracted CO_2 after Johnson et al. (1987). Total alkalinity in each sample was determined by potentiometric titration with hydrochloric acid (0.1 M HCl in 0.6 M NaCl) after Winn et al. (1994) and calculated according to standard procedures described in DOE (1994).

Total organic carbon (TOC). Subsamples for TOC (0 to 100 m) were collected in sterile 50 ml polypropylene centrifuge tubes. The sampler spout was rinsed by allowing water to flow freely (~10 s), after which the tubes were filled without rinsing. These samples were immediately placed in a cooler, and frozen upright upon return to the laboratory. They were stored frozen (Tupas et al. 1994) until analyzed in an MQ1001 TOC analyzer (MQ Scientific Inc., Pullman, WA) after Qian & Mopper (1996).

Dissolved nutrients. Subsamples for dissolved inorganic nutrient (NO_2^-, NO_3^-, NH_4^+, SRP and SRSi; 0 to 100 m) determinations were passed from a sterile syringe through combusted Whatman GF/F filters in a Millipore disk filter holder. Filtrates were collected in acid-washed (3× with 1 M HCl) and rinsed (3× with double distilled H_2O) 125 ml high density polyethylene (HDPE) bottles, placed in a cooler, and frozen upon return to the laboratory (–20°C) (Dore et al. 1996). They were analyzed according to Technicon Industrial systems (1973, 1977, 1979) after Armstrong et al. (1967) for N, Murphy & Riley (1962) for SRP, and Strickland & Parsons (1972) for SRSi, with slight modifications to surfactant concentrations, sampling rate, and pump tube size (T. Walsh pers. comm.). SRSi was determined in thawed samples maintained at room temperature for several weeks. TDP and TDN were measured after ultraviolet light photo-oxidation (Walsh 1989, Karl et al. 1993). Dissolved organic nitrogen (DON) was considered equal to TDN – (NO_3^- + NH_4^+), and soluble nonreactive phosphorus (SNP) considered equal to TDP – SRP.

Pigments. Total chlorophyll *a* (chl *a*) (i.e. monovinyl plus divinyl chl *a*) and accessory pigment concentrations were determined in water samples collected from 0.5 to 100 m. Samples were filtered through 25 mm Whatman GF/F filters and stored at –20°C until analyzed. Filters were placed in 3 or 6 ml acetone, depending on filter loading, and extracted for 24 h (0°C, dark). Prior to analysis, pigment extracts were vortexed and centrifuged to remove cellular and filter debris. Samples (200 µl) of a mixture of 0.3 ml H_2O and 1 ml extract were injected into a Varian 9012 HPLC system equipped with a Varian 9300 autosampler, a Timberline column heater (26°C), and a Spherisorb 5 mm ODS2 analytical (4.6 × 250 mm) column and corresponding guard column. Pigments were detected with a ThermoSeparation UV2000 detector (λ = 436 and 450 nm) and analyzed after Wright et al. (1991) and Latasa et al. (1997). The HPLC method used cannot separate monovinyl chl *a* from divinyl chl *a*. Concentrations of these pigments were determined by monitoring the monovinyl chl *a* plus divinyl chl *a* peak at 2 wavelengths (436 and 450 nm) and deriving their respective concentrations via dichromatic equations given by Latasa et al. (1996). Pigment peaks were identified by comparing retention times with those of extracts prepared from algal cultures of known pigment composition (Latasa et al. 1996). Pigment concentrations were calculated using pure external standards.

Cell numbers. Autofluorescent *Bacteria*, filamentous cyanobacteria and '*Synechococcus*'-like autotrophic picoplankton collected between 0.1 and 100 m were counted in 0.5 ml samples fixed with paraformaldehyde (final concentration 0.2%) by epifluorescence microscopy using DAPI (4'6-diamidino-2-phenylindole) (Porter & Feig 1980). Samples for cell counts were stored frozen in sterile 2 ml polypropylene cryovials (–20°C) until analyzed. Picoeukaryotes (e.g. cryptophytes) and other autofluorescent cells not considered *Bacteria*, filamentous cyanobacteria, or '*Synechococcus*'-like were counted as 'other autofluorescent cells'. Numbers of unpigmented *Bacteria* plus *Archaea* (hereafter referred to as heterotrophic bacteria) to 100 m, and of cells we tentatively consider as '*Prochlorococcus*'-like to 2 m, were determined by flow cytometry (Monger & Landry 1993) in 1 ml samples fixed in sterile 2 ml polypropylene cryovials with paraformaldehyde (0.2%). *Prochlorococcus* is undetectable by epifluorescence microscopy due to its weak fluorescence, but may be enumerated by flow cytometry through a

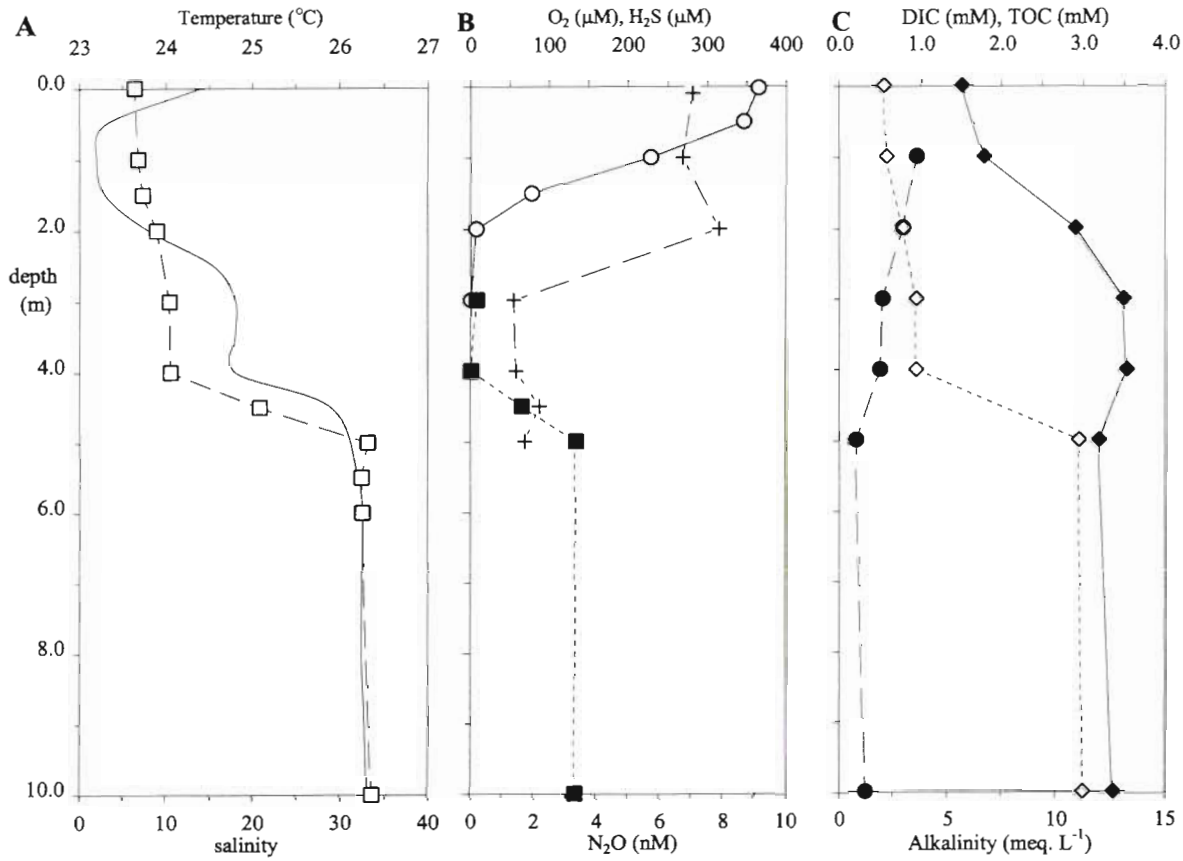

Fig. 2. (A) Salinity (□) and temperature (solid line) profiles of Lake Kauhakō. (B) Concentrations of dissolved gases (○: oxygen, ■: hydrogen sulfide, +: nitrous oxide) in Lake Kauhakō . (C) DIC (◆), TOC (●) and alkalinity (◇) in Lake Kauhakō . (Data below 10 m not shown)

characteristic signature based on forward light scatter and chlorophyll fluorescence (Chisholm et al. 1988). The flow cytometric assignment of *Prochlorococcus* in Lake Kauhakō samples is based on previous experience with *Prochlorococcus* in oceanic samples and remains tentative, but the detection of marker pigments (divinyl chlorophylls) by HPLC analysis can indicate the presence of these cells.

Ectoenzyme activities. The potential activities (0.1 to 5 m) at ~25°C of LAPase (EC 3.4.1.1) and α- and β-glucosidase (EC 3.2.1.20 and EC 3.2.1.21 respectively) were determined using fluorogenic substrate analogs (Hoppe 1983, Somville & Billen 1983, Christian & Karl 1995). Fluorescence was measured in a Perkin-Elmer LS-5 spectrofluorometer. Activities are potential since substrate analogs were applied at above trace concentrations, 1 mM for LAPase, and 1.6 μM for the glucosidases.

LAPase activity in aquatic habitats is not solely of heterotrophic bacterial origin and may be derived from photosynthetic *Bacteria* such as *Synechococcus* (Martinez & Azam 1993) and *Eucarya* such as phagotrophic nanoflagellates (Karner et al. 1994). Cell-specific activities described in this paper were calculated on the basis of the combined numbers of heterotrophic and autofluorescent *Bacteria*, together with those of the filamentous cyanobacteria, '*Prochlorococcus*'- and '*Synechococcus*'-like picoplankton; marine heterotrophic bacteria and *Synechococcus* spp. may express similar levels of this enzyme (Martinez & Azam 1993) and there is uncertainty about the contribution, if any, to this activity from *Prochlorococcus* because this organism has not been available in pure culture until recently. Activities per cell of α- and β-glucosidase are based only on the numbers of heterotrophic bacteria.

RESULTS

Hydrography

The upper 4 m of Lake Kauhakō were stratified with respect to temperature and salinity (Fig. 2A); euhaline

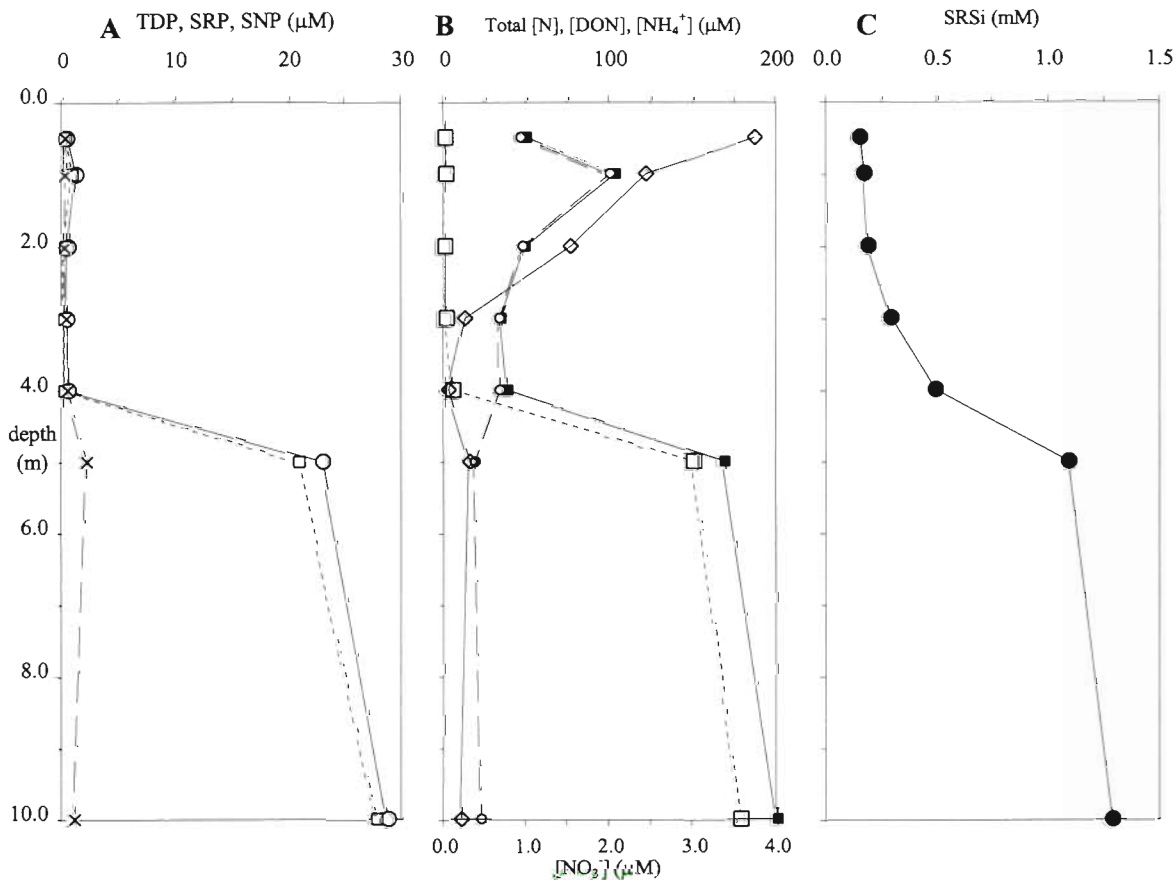

Fig. 3. Vertical distribution of (A) dissolved phosphorus (○: TDP, □: SRP, ×: SNP), (B) nitrogen species (■: TDN, ○: DON, □: NH_4^+, ◇: NO_3^-) and (C) soluble reactive silica in Lake Kauhakō

and isothermic water then extended to 100 m. Dichothermy was pronounced, with a temperature minimum at 1 m. Salinity ranged from 6.4 at the surface to 34.4 between 5.5 and 80 m. A pronounced temperature and salinity gradient around 4.5 m signaled the presence of a pycnocline.

Dissolved oxygen, sulfide and nitrous oxide

Dissolved oxygen peaked at 365 μM (50% above air saturation) at the surface (Fig. 2B). Water below 3 m was anoxic. Sulfide concentrations between 5 and 28 m were 126 to 133 μM (Fig. 2B). Surface water, with 7 nM N_2O, was ~2% supersaturated with respect to atmospheric N_2O (Fig. 2B).

Carbonate system parameters

DIC concentrations increased with depth in the mixolimnion but were stable at 3.1 to 3.5 mM between 5 and 20 m (Fig. 2C; data below 10 m not shown). Alkalinity increased from 2.0 meq. l^{-1} at the surface to 3.5 meq. l^{-1} at 4 m. Below 5 m inclusive, alkalinity was stable at ~11.1 meq. l^{-1} (Fig. 2C). TOC peaked at 0.94 mM (11.2 mg C l^{-1}) at 1 m, the shallowest sample taken (Fig. 2C), falling sharply thereafter to 0.20–0.30 mM between 10 and 40 m (data below 10 m not shown).

Nutrients

SRP comprised the bulk of TDP at most depths (Fig. 3A); it was depleted in the upper 4 m (<0.08 μM) but attained >20 μM by 5 m and was stable at ~28 μM between 10 and 100 m (data below 10 m not shown).

TDN measured 35 to 100 μM above 4 m, and ~160 to 200 μM below 5 m (Fig. 3B); DON comprised up to 97% of TDN to 4 m, but only ~7 to 14% below the pycnocline (Fig. 3B). NH_4^+ concentrations were <1 μM above 3 m, ~150 μM by 5 m (Fig. 3B), and

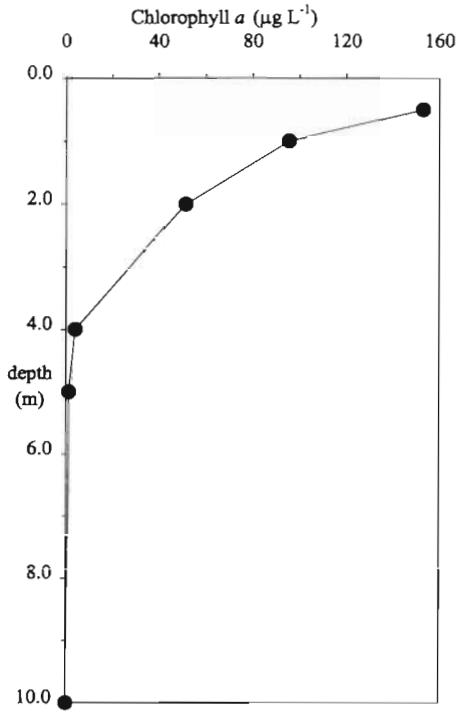

Fig. 4. Vertical distribution of chlorophyll *a* in Lake Kauhakō

~175 µM between 10 and 100 m (data below 10 m not shown). NO_3^- concentrations fell sharply with depth, from ~3.75 µM at 0.5 m to <1 µM at 3 to 100 m (Fig. 3B).

Lowest SRSi concentrations were recorded in surface waters, with ~0.15 to ~0.5 mM between 0.5 and 4 m (Fig. 3C). Below 5 m, SRSi varied between 1.1 and 1.3 mM.

Pigments

Total chl *a* concentration peaked at over 150 µg l^{-1} at 0.5 m, and fell to ~2 µg l^{-1} at 4 m (Fig. 4). At 10 and 40 m, chl *a* concentrations were ~0.1 µg l^{-1}. A number of oxygenic photoautotroph accessory pigments were present in the upper 2 m, including zeaxanthin (cyanobacteria marker), alloxanthin (cryptophyte marker), chl *c* and diadinoxanthin (chromophyte markers), fucoxanthin (diatom marker), and β-carotene (Fig. 5A). A large number of pigment peaks were detected in samples collected from 3 to 5 m (Fig. 5B–D), but only a subset was found below 10 m (data not shown). Divinyl chl *a*, a pigment so far found only in *Prochlorococcus* in aquatic ecosystems, was detected at 0.5 and 1 m, and comprised 2.1 and 5.6% of the chl *a* at these depths, respectively. This percentage dropped to ~1.5% by 2 m and decreased to a trace level at 4 m.

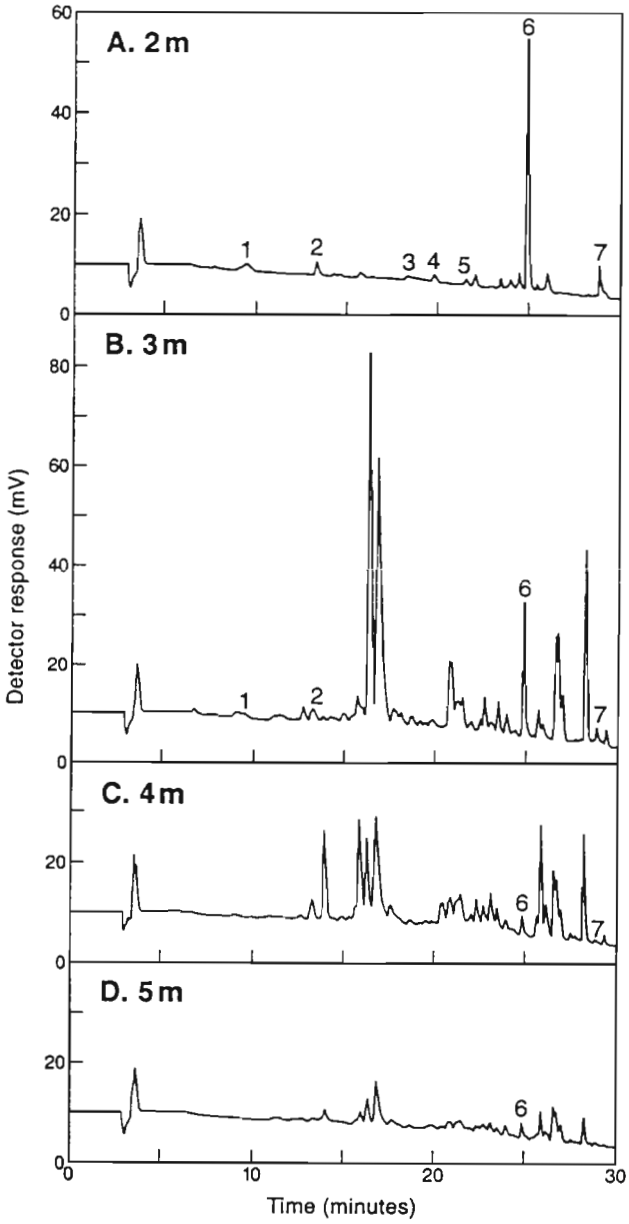

Fig. 5. Reverse-phase HPLC chromatograms (436 nm) obtained for pigment extracts prepared from samples collected at (A) 2 m, (B) 3 m, (C) 4 m and (D) 5 m depth from Lake Kauhakō. Peak identities: (1) chlorophyll *c* (chromophyte marker), (2) fucoxanthin (diatom marker), (3) diadinoxanthin (chromophyte marker), (4) alloxanthin (cryptophyte marker), (5) zeaxanthin (cyanobacteria marker), (6) monovinyl plus divinyl chlorophyll *a*, and (7) β-carotene

Sinking cells or cell debris may account for the divinyl chl *a* at 3 and 4 m; this pigment was undetectable below 5 m. Most pigments detected between 3 and 5 m do not correspond to those found in oxygenic photoautotrophs; they likely comprise bacteriochlorophyll and bacteriocarotenoid pigments. A lack of authentic bac-

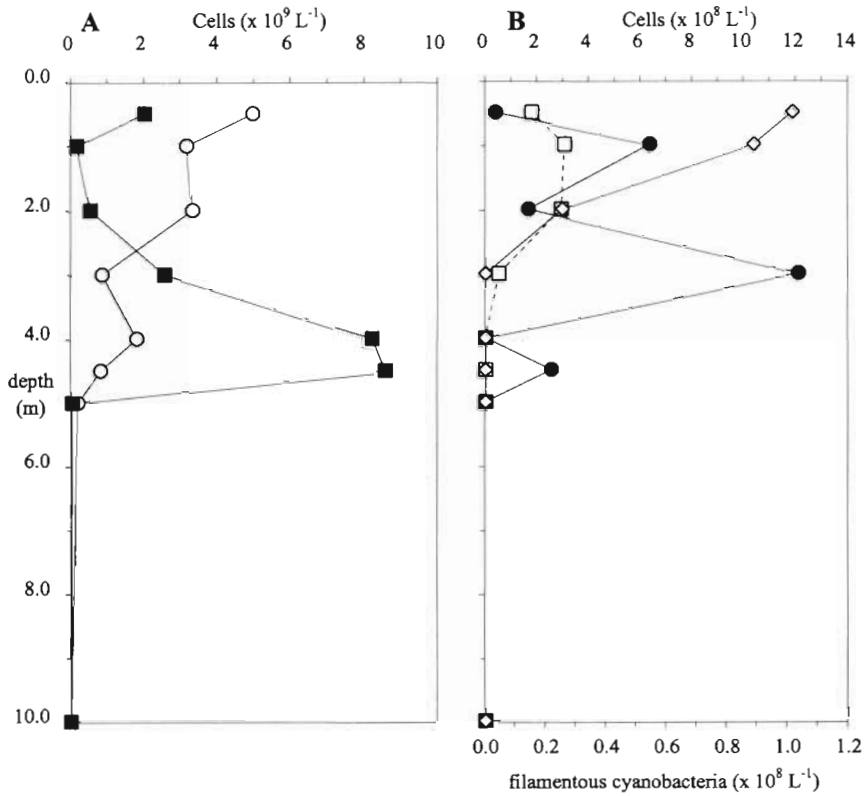

Fig. 6. Vertical distribution of (A) heterotrophic (○) and autofluorescent (■) *Bacteria*, and (B) '*Synechococcus*'-like cells (□), filamentous cyanobacteria (◇) and 'other autofluorescent cells' (●) in Lake Kauhakō

teriopigment standards in our laboratory precluded identification of these compounds, which were distributed heterogeneously with respect to depth, indicating that the photosynthetic *Bacteria* taxa were segregated into distinct layers.

Cell numbers

The distribution of heterotrophic and autofluorescent *Bacteria* contrasted with depth (Fig. 6A). Numbers of heterotrophic bacteria fell from ~5×10^9 l^{-1} at 0.5 m to ~1.9×10^8 l^{-1} between 5 and 100 m. Autofluorescent *Bacteria* numbers peaked at >8.0×10^9 l^{-1} in the vicinity of the pycnocline but were only ~10^7 ml^{-1} between 5 and 100 m.

Filamentous cyanobacteria attained ~1.0×10^8 l^{-1} at 0.5 m (Fig. 6B). Filaments comprised 4 to 12 cells of ~10 to 15 × 5 µm each, each filament terminating in a non-autofluorescent heterocyst. These filaments were not seen below 2 m. Cells of ~1 × 0.75 µm, morphologically similar to *Synechococcus* spp. (uniform autofluorescence, circular to ovoid), numbered 2.0 to 3.0 × 10^8 l^{-1} between 0.5 and 2 m (Fig. 6B). They were not detected below 3 m. Cells considered 'other autofluorescent cells' were not uniformly distributed with depth; lowest numbers occurred at the oxycline (1.65×10^8 l^{-1}) but they peaked at 3 m (Fig. 6B). No 'other autofluorescent cells' were observed below 5 m inclusive.

Cells considered flow cytometrically as '*Prochlorococcus*'-like numbered 1.3×10^8 l^{-1} at 0.5 m and 1.1×10^7 l^{-1} at 2 m (data not shown); their order of magnitude reduction in abundance with depth accompanied a decrease in the divinyl chlorophyll concentration. At ~1.0 µm diameter, these cells were larger than *Prochlorococcus* in oceanic samples (0.6 to 0.8 µm in diameter and 1.2 to 1.6 µm in length; Chisholm et al. 1992).

Ectoenzyme activities

The highest total activity of each ectoenzyme was recorded at 0.1 m and fell with increasing depth (Fig. 7). Total LAPase decreased by nearly 200-fold from ~1.05 µmol substrate hydrolyzed l^{-1} h^{-1} at 0.5 m to ~7.49 nmol l^{-1} h^{-1} at 5.0 m; activity per prokaryote ranged from ~122 to 4.70 amol $cell^{-1}$ h^{-1} at these depths, respectively (Fig. 7A). Total α-glucosidase activity attained 10.79 nmol substrate hydrolyzed l^{-1} h^{-1} at 0.5 m, and ranged from 1.90 to 0.17 amol h^{-1} per heterotrophic bacterium (Fig. 7B). Total β-glucosidase activity attained 13.96 nmol substrate hydrolyzed l^{-1} h^{-1} and ranged from 2.29 to 0.17 amol $cell^{-1}$ h^{-1} (Fig. 7C).

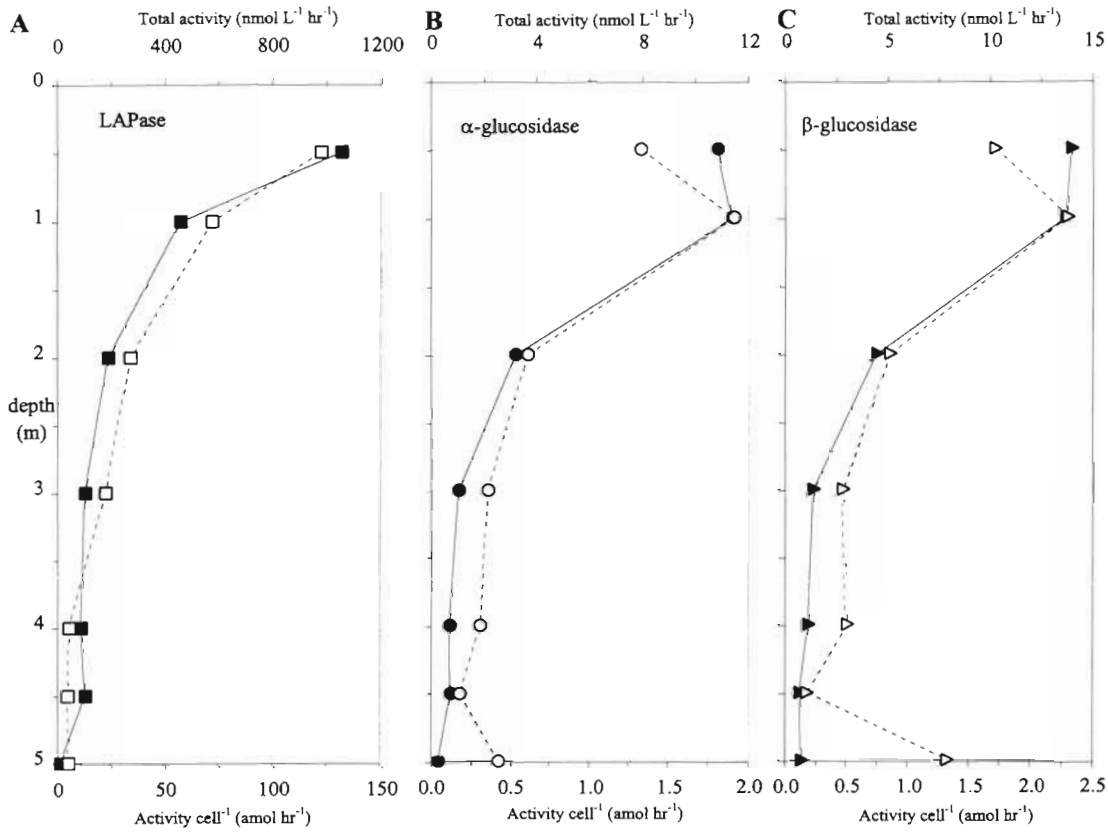

Fig. 7. Vertical distribution of ectoenzyme activities in Lake Kauhakō. Solid symbols indicate total activity in water; open symbols indicate activity per cell

DISCUSSION

We have described a range of hydrochemical and microbiological aspects of Lake Kauhakō, a lake only briefly reported in the limnological literature. The lake is characterized by oxygenated, brackish water overlying saline anoxic water. Our data for water column temperature and chemistry profiles are similar to those of R. A. Kinzie III et al. (unpubl. data), who sampled Lake Kauhakō in 1995 and 1996. These combined observations suggest that the stratification of Lake Kauhakō is persistent.

Salinity through much of the lake was similar to that of seawater. The lake surface is at sea level but does not respond synchronously with nearby ocean tides despite its proximity to the sea (<2 km). This observation suggests the absence of an 'open' connection to the sea. Anoxia in the monimolimnion also implies that any connection to the ocean has little effect in terms of introducing large volumes of oxygenated water to the lake. Exchange by horizontal hydraulic conductivity through the rock or fractures between the lake and the sea is possible; seawater passing through ~150 m d^{-1} of the rock comprising the Kalaupapa Peninsula would have an ~11 d transit time to the lake, and derive a surface fluctuation in a standing body of water 1.6 km from the sea of 5 to 10% of the adjacent ocean tide (Oki 1997). In Lake Kauhakō this would equate to the 'few centimeters' reported by Maciolek (1982). The basin's extreme declivity and the protection from winds offered by the high crater walls ensure the persistence of meromixis. The freshwater table on the peninsula is located at 1.5 to 2.0 m (Oki 1997), the depth of the oxic layer during our study. Meromixis in Lake Kauhakō can therefore be considered morphogenic (Maciolek 1982) and ectogenic.

Limited aeolian influence will preclude mixing and ensure that the oxic layer remains shallow. Oxic layer depth and the minimum depth at which sulfide was detected during our visit were each less than reported by Maciolek (1982) based on a 1974 survey. Our salinity, temperature and nutrient data indicate that mixing occurs at least between 4.5 and 100 m. Nitrous oxide is a greenhouse gas and is involved in ozone depletion (Dore & Karl 1996); on the basis of N_2O solubility in water (Weiss & Price 1980) surface water of Lake Kauhakō was 2% supersaturated with respect to the atmosphere. The error associated with the analysis is

2 to 4 % so we cannot say if the lake constitutes either an N_2O source or a sink, although both would be insignificant considering the lake's small surface area.

The DIC concentration at the surface of Lake Kauhakō was about 3/4 that in Hawaiian coastal waters (Winn et al. 1994), reflecting high primary productivity and rapid uptake of inorganic carbon. That CO_2 produced during nitrate or sulfate respiration by heterotrophic bacteria in anoxic waters may account for the high DIC concentrations below the oxycline. Since CO_2 must have a finite diffusive lifetime, the high DIC concentration at the oxycline implies that it is produced very rapidly indeed. As we did not collect dissolved nutrient samples at 4.5 m, we cannot say if there were similar distributions of NO_3^-, NH_4^+ and SRP. The rapid increase in alkalinity across the pycnocline of Lake Kauhakō is consistent with the production of alkalinity in anoxic waters by organic matter oxidation through sulfate reduction, as reported for the Black Sea (Goyet et al. 1991).

Although TOC peaked in the shallowest sample taken, we had no parallel sample from the pycnocline. As the combined heterotrophic and autofluorescent *Bacteria* cell numbers increased 2.75-fold from 3 to 4.5 m it is likely that the TOC concentration increased at the pycnocline, especially since bacterial cells in anoxic lake waters may be larger than in oxic waters (Cole et al. 1993). Heterotrophic and photosynthetic activities in surface waters of Lake Kauhakō probably keep NH_4^+ and SRP low and maintain rapid turnover rates. Assuming a maximum photosynthetic uptake of 20 g C g^{-1} chl h^{-1} (Falkowski 1981) and a Redfield C:N:P molar ratio of 106:16:1, N and P demand would be 38 and 2.4 µmol h^{-1}, respectively. Ammonium oxidation by nitrifiers in the lake's surface waters may deplete NH_4^+ to the low levels we detected, and denitrification (Codispoti et al. 1991) may also deplete NO_3^- in anoxic waters above the pycnocline.

Order of magnitude differences in SRP concentrations between 0.5 and 4.0 m reflect the activities of different microbial communities and the absence of vertical advective processes. The latter may occasionally affect productivity in surface waters of Lake Kauhakō through advection of bacterial cells from P-rich water. Some lake sediments are an important P source when the entire water column mixes (Brooks & Edgington 1994) but P from Lake Kauhakō benthic sediments is unlikely to reach surface waters. Phosphorus (as P_2O_5) comprises ~0.3% by weight of the Kalaupapa basalt (Clague et al. 1982) and could constitute a relatively large supply to the water column if leached from surrounding rocks. Additional utilizable P is likely found in vegetation debris entering the lake (Vogt et al. 1986).

SRSi concentrations in the upper meter of Lake Kauhakō exceeded those usually found in rivers and lakes (2 to 5 µM), while SRSi concentrations below the pycnocline compared to those in anoxic waters of meromictic Lake Nordbytjernet (to 1.4 mM; Hongve 1997). In the subtropical gyre north of Hawai'i, SRSi concentrations of 0.15 to 0.16 mM between 2000 and 4000 m have been reported, while concentrations did not exceed 0.02 mM above 500 m (Roemmich et al. 1991). SRSi concentrations in seawater immediately off the Kalaupapa Peninsula are unlikely to differ significantly from open ocean surface values, so SRSi accumulation in the Lake Kauhakō monimolimnion may arise through removal from the surrounding rejuvenated-stage alkalic basalt and basanite rocks (Langenheim & Clague 1987) and dissolution of sinking diatom frustules. We observed diatoms by epifluorescence microscopy in Lake Kauhakō samples. Although our samples for SRSi were frozen, a practice which may lead to underestimation by 10 to 100% through polymerization in saline waters with high SRSi concentrations (MacDonald et al. 1986), extended storage at room temperature results in partial solubilization (Walsh 1989).

Pigment diversity in the upper 2 m of Lake Kauhakō was low and dominated by chl *a*. By 3 m chl *a* had largely been replaced by a diverse suite of bacterial pigments. The detection of *Prochlorococcus* spp. in Lake Kauhako by flow cytometry was supported by the presumptive detection of divinyl chl a (Goericke & Repeta 1992), with sinking cells likely accounting for the trace levels of these pigments at 3 and 4 m. *Prochlorococcus* has not been previously reported in brackish waters such as those at the surface of Lake Kauhakō.

In the upper 4.5 m of Lake Kauhakō, heterotrophic *Bacteria* numbers were similar to those in non-oligotrophic lakes (Zehr et al. 1987, Münster 1991). The fact that heterotrophic bacterial numbers were high in anoxic water above the pycnocline suggests a switch to nitrate respiration (Overbeck 1993). Chroococcoid cyanobacteria similar to those described here as '*Synechococcus*'-like are found in lakes (Cole 1983) and oligotrophic seawaters (Campbell & Vaulot 1993). In Lake Kauhakō, large '*Synechococcus*'-like unicells, together with an unidentified filamentous cyanobacterium, dominated the picophytoplankton in the upper 2 m. Water samples from 4.5 to 4.75 m appeared brown, a coloration that may have arisen from brown pigmented *Chlorobium* spp. (Guerrero et al. 1985, García-Gil et al. 1993). These bacteria usually occur in meromictic lakes in well-defined, colored 'plates' (Takahashi & Ichimura 1970, van Gemerden et al. 1985). This work is the first report of autofluorescent *Bacteria* in Lake Kauhakō. These cells peaked numerically at the pycnocline (4.5 m). Since most of the *Bacteria* from 4 m observed by epifluorescence microscopy

were associated with particles, this part of the lake may be a major site of organic matter recycling.

Proteolytic enzyme activities in Lake Kauhakō exceeded those in meromictic Mahoney Lake (Overmann et al. 1996) and Mekkojärvi (Münster 1991). Although we applied substrate analogs at concentrations considered saturating in oceanic samples, they may not have been saturating in any or all our Lake Kauhakō samples; enzyme activities may therefore have been underestimated. Total glucosidase activities were high compared to those found in other aquatic habitats (Münster 1991, Karner & Rassoulzadegan 1995) although per cell activities of each enzyme were within the range reported for pelagic marine bacteria (Donachie et al. unpubl.).

Kauhakō Crater '...contains one of the finest examples of... windward dryland forest [in] the state of Hawaii' (Medeiros et al. 1996) and was designated a Special Ecological Area in 1994. Anthropogenic impact on the lake may be minimal; it represents a unique site in which to study interactions among relatively isolated flora and fauna, much of which appears restricted to a shallow water column within a single basin.

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