IN SITU NITROGEN(C_2H_2)-FIXATION IN LAKES OF SOUTHERN VICTORIALAND, ANTARCTICA

by

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INTRODUCTION

Primary production in antarctic habitats may be limited by nitrogen (Goldman, 1970; Samsel and Parker, 1971, 1972), phosphorus (Hoehn et al., 1977), and for some organisms perhaps silicon (Parker et al., 1972; Seaburg et al., 1979). In addition the biota also may be subjected to low temperature, light and moisture (Parker, 1978). If there is a nitrogen deficiency in these habitats, it should play an important role in community development, which may indicate the presence of nitrogen fixing organisms. Studies in subantarctic island lakes and terrestrial habitats have shown nitrogen fixation in the algal mat community (Croome, 1973; Fogg and Stewart, 1968; Horne, 1972), while in situ nitrogen fixation on the antarctic continent has not been reported previously.

The objectives of this study included the determination of the extent of nitrogen fixation in select habitats in Southern Victorialand and the identification of the key organisms involved. The habitats studied were littoral or temporary ice-free moat areas, soils and plankton of a number of lakes during the 1977-78 austral summer. Also, the investigation included a more intensive evaluation of lakes Chad, Hoare, and Fryxell during the 1978-79 austral summer which added the benthic attached algal mat as a study site. Another aim of this study was to identify any spatial patterns of nitrogenase activity within the lakes.

LITERATURE REVIEW

Nitrogen Fixation - General

Biological nitrogen fixation is the reduction of nitrogen gas (N_2) to ammonia (NH_3) catalyzed by the nitrogenase enzyme complex. Nitrogenase receives electrons from a donor molecule (e.g., pyruvate) via a carrier molecule (e.g., ferredoxin) to reduce the triple bonded N_2 by adding six hydrogen atoms (H) to synthesize ammonia. Energy as 12 to 24 molecules of adenosine triphosphate (ATP) is expended per molecule of N_2 reduced. Recycling of reducing power from H_2 may allow more efficient utilization of this energy. Numerous authors have reviewed the biochemistry of N_2 fixation which will not be further discussed here (Mortenson and Thorneley, 1979; Orme-Johnson and Davis, 1977; Winter and Burris, 1976; Zumft and Mortenson, 1975).

Certain bluegreen algae and bacteria fix N_2 as free-living organisms or in symbiotic associations. Symbioses involving bacteria or bluegreen algae with higher plants and fungi are considered responsible for at least 50% of the total worldwide N_2 fixation, as reviewed by Postgate (1972).

The correlation of aerobic N_2 fixation in bluegreen algae with the presence of heterocysts is well-known (Fogg, 1944, 1949, 1951). In fact, heterocysts probably are the site of N_2 fixation in most bluegreen algae (Fay, 1973; Wolk, 1974). Anaerobic or microaerophilic N_2 fixation has been observed in non-heterocystous bluegreen algae, as well as, the filaments of heterocystous algae (Fogg, 1974). Oxygen apparently inactivates nitrogenase activity, which is countered by the differentiated

heterocysts.

Dark N_2 fixation is common among heterotrophic bacteria, but only recently has evidence been provided for dark N_2 fixation by bluegreen algae (Khoja and Whitton, 1971). Fogg (1974) has reviewed the evidence for a loose relationship between photosynthesis and N_2 fixation. Photosynthetically produced reductant is not necessary but a reductant (e.g., pyruvate) and carbon chains, which may be synthesized indirectly from photosynthate, are required for N_2 fixation in both bacteria and bluegreen algae. Although bluegreen algae can fix N_2 in dark, the rates are lower than light N_2 fixation rates and often depend on previous exposure to light (Fay, 1965; Khoja and Whitton, 1971; Granhall and Lundgen, 1971).

Nitrogenase activity has been observed in hot thermal springs (Stewart, 1970) and in temperatures nearing 0 C (Fogg and Stewart, 1968), however, the optimum nitrogen fixation occurs between 37-25 C. A biphasic curve showing temperature versus nitrogenase activity follows a normal curve with higher activities between 25 and 5 C decreasing at either extreme (Knowles et al., 1973). The azoferredoxin or Fe-protein has been shown to be cold labile but it is unknown if the entire system is cold labile for photosynthetic organisms (Stewart, 1973). Reviews of bluegreen algal nitrogen fixation are numerous and informative (Fogg and Stewart, 1965; Stewart, 1970, 1973).

Beijerinck (1901) isolated two N_2 fixing aerobic bacteria (Azotobacter spp.), and Winogradsky (1893) isolated the anaerobic N_2 fixing bacterium Clostridum pasteurianum. After these discoveries, many other

bacteria were found to fix nitrogen: Rhodospirillum rubrum, a photosynthetic bacterium (Kamen and Gest, 1949); the enteric bacterium Klebsiella pneumoniae (previously Aerobacter aerogenes, Pengra and Wilson, 1958); certain strains of sulfate reducing bacteria (LeGall et al., 1959; Sisler and ZoBell, 1951). Because many other bacteria fix N_2, nitrogenase obviously occurs widely. As noted, pyruvate or some other reductant acquired through photosynthesis, respiration, or fermentation is required. Photosynthesis is the ultimate source of energy, reductant and acceptor molecules for heterotrophic nitrogen fixation but the link is indirect.

Antarctic Nitrogen Fixation

N_2 fixation has been demonstrated in in situ studies on subantarctic islands (Fogg and Stewart, 1968; Croome, 1973; and Horne, 1972). Collections and culturing from Southern Victorialand, continental Antarctica, showed that Nostoc commune and other heterocystous bluegreen algae were present in the terrestrial and littoral habitats of the Taylor and Wright Valleys, and N_2 fixation was demonstrated for one unialgal culture (Holm-Hansen, 1963). Azotobacter chroococcum, A. indicus, Achromobacter sp., and Bacillus sp. have been isolated from antarctic soils (Boyd and Boyd, 1962), while attempts to isolate N_2 fixing bacteria from the McMurdo Sound area were unsuccessful (Sieburth, 1965). In the terrestrial habitats on Signey Island, in situ assays for N_2 fixation proved nitrogenase activity correlated with the bluegreen alga Nostoc commune, both free-living and as the algal associate in the

lichen Collema pulposum (Fogg and Stewart, 1968; Horne, 1972). Blue-green algae were considered the most important N_2 fixing organisms in this region. In situ N_2 fixation in Marion Island mires occurred with the presence of the bluegreen alga Stigonema ocellatum, while Nostoc commune, Tolypothrix and Calothrix were also found (Croome, 1973). Temperature and light were considered the major influencing variables of N_2 fixation in these habitats (Fogg and Stewart, 1968; Croome, 1973). As had been shown previously for algal mats on Ross Island (Goldman, 1972), those on Signey Island reached elevated temperatures in the microhabitats where N_2 fixation occurred (Fogg and Stewart, 1968).

Assay Techniques for Nitrogen Fixation

N_2 fixation rates have been assayed by the increase in nitrogen, using Kjeldahl nitrogen determinations (Fogg, 1942), demonstration of growth in nitrogen-free culture media, manometric determination of the consumption of nitrogen gas (Cox, 1966), uptake of isotopic nitrogen gas ($^{15}N_2$) (Burris et al., 1943), and C_2H_2 reduction to ethylene (Stewart et al., 1967). Determination of nitrogen fixation using increase in nitrogen using Kjeldahl analyses or growth in nitrogen-free media was subject to error due to absorption of ammonia from the air during incubation. The advent of the stable isotope method for N_2 fixation provided a direct, more sensitive and less troubleprone measurement. Incubations for $^{15}N_2$ uptake required shorter periods, eliminated the $^{14}NH_3$ adsorption problem, and allowed convenient in situ N_2 fixation studies. However, laborious hydrolytic digestion and expensive mass

spectrometry were necessary. Acetylene reduction to ethylene (i.e., $C_2H_2 \rightarrow C_2H_4$) by nitrogenase was economical and more sensitive than $^{15}N_2$ uptake. However, it was an indirect measurement of N_2 fixation, requiring that the theoretical unconfirmed equivalent of 3 moles of C_2H_2 reduced per mole of N_2 reduced be applied. This assumption later proved to be an overestimate of the N_2 actually reduced; it is now recognized that the arbitary use of this 3:1 ratio is inaccurate without parallel $^{15}N_2$ fixation data since the ratio of C_2H_2 reduced to N_2 fixed varies from 4:1 to 25:1 (Bergersen, 1970; Petersen and Burris, 1976).

Widespread application of the C_2H_2 reduction assay to numerous habitats using a diversity of methods requires caution in comparing results among investigators. Assay problems have been discussed by numerous investigators, the main problem being that prolonged incubation in C_2H_2 stimulates nitrogenase activity (David and Fay, 1977). Thus, the estimation of naturally occurring nitrogenase activity may be overestimated. The many modifications of the assay lead to data of an inaccurate and non-comparable nature with often an overestimation of the N_2 fixation rate (Flett et al., 1976). For example, incubation under C_2H_2 at 25 C for over 6 h stimulated nitrogenase activity (David and Fay, 1977). Other temperatures were not examined in this study. Thus, comparison of C_2H_2 results not accompanied by $^{15}N_2$ results must recognize the potential error. Furthermore, the aqueous assays are most susceptable to error due to C_2H_2 solubility in water and equilibration problems. These problems can be corrected partly by calculations or by keeping the aqueous phase well below 30% of the total reaction chamber volume and

allowing the material to equilibrate before sampling (Flett et al., 1976). Hardy (1973) has proposed a specific terminology to differentiate nitrogen fixation measurements extrapolated from C_2H_2 reduction (i. e., Nitrogen(C_2H_2)-fixation) that will be used in this paper.

MATERIALS AND METHODS

Physical Measurements

An instrument package, allowing measurement of light, salinity, conductivity and temperature beneath the permanent ice of lakes was designed by D.P. Brown for the VPI & SU research program. Photosynthetically available radiation was measured by an LI-185A Quantum/Radiometer/Photometer (LiCor Corporation) equipped with surface and subsurface sensors to allow concurrent readings for determination of the percentage of incident radiation reaching the submerged probe. Salinity, conductivity, and temperature were measured on a Yellow Springs Instrument Company 3a conductivity meter. Long stemmed thermometers and thermisters were used initially, but instrument failures with both these methods caused heavy reliance on the temperatures determined with the S-C-T meter mentioned previously.

Water Chemical Analyses

Kemmerer, Niskin, or Van Dorn water bottles were used for water sampling, as available. Water from shallow areas was collected directly in an acid washed 1 l plastic bottle. Some water collections under ice also were made by SCUBA diving during the 1978-79 austral summer. A diver took 1 l acid washed plastic bottles down to the sampling site where they were opened and filled. All water samples were returned to McMurdo Station where they were refrigerated at ca. 20 C until analyzed. Analyses postponed more than 48 h were conducted on water frozen and thawed before use.

Dissolved oxygen (O_2) was determined in a number of ways during the 1977-78 and 1978-79 field seasons. During the 1977-78 season a Yellow Springs Instrument Company O_2 meter 51A was used initially but abandoned since a majority of the waters surveyed contained supersaturated O_2 concentrations which read offscale on this instrument. During both field seasons the modified Winkler O_2 determination was used (Strictland and Parsons, 1968). O_2 in the mat was estimated using the modified Winkler method, specially adapted by Simmons (Appendix 8).

Analyses for nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) ions were performed in acid washed 10 dram vials. Initially samples were analysed both as filtered and unfiltered samples. Many samples when filtering with Millipore 0.45 μm filters (both acid washed and unwashed) showed trace additions of NO_3^- and NH_4^+, while equal concentrations were obtained on ashed (Whatman 984H) glass fiber filters. Subsequent analyses were done on unfiltered lake water. Determination of NO_3^- present involved the cadmium reduction technique (Strictland and Parsons, 1968) but using smaller columns and 15 ml samples. NO_2^- was determined by azo dye formation (Strictland and Parsons, 1968) using 15 ml samples. NH_4^+ was measured, using the phenol hypochlorite method (Weatherburn, 1967) on 15 ml samples. In all cases a standard curve was used and checked periodically with internal standards.

Dissolved and total organic carbons (DOC and TOC) were determined as follows: For TOC, 10 ml of unfiltered water was put into an ashed 30 ml serum bottle, then preserved with 1.0 ml of 1.0 N HCl and 1.0 ml of 50 μg/ml $HgCl_2$. DOC involved filtration through 42.5 mm Whatman 984H

ashed glass fiber filters at less than 10 cm Hg vacuum. Ten ml of this filtrate was preserved as with TOC. Sealed vials were returned to VPI & SU and analysed on a Oceanography International Corporation 525 Total Carbon Analyzer after a chemical digestion with phosphoric acid and potassium persulfite described by Strictland and Parsons (1968). This method differs from that described by Strictland and Parsons (1968) in that the sample was injected into the chamber to be sparged free of CO_2 during analysis (D.P. Brown, personal communication).

Field Collections and Culturing

Samples of mat and sediment were collected in sterile jars. Jars were returned to McMurdo Station and stored at 2 C or -20 C in the dark. Samples were shipped frozen to VPI & SU for culturing and identification. Mat pieces from the 1977-78 season were also placed in a primary enrichment medium, namely Guillard's (Nichols, 1973) without the nitrate salt (Seaburg et al., 1979). After growth at 2 or 15 C the algae were transferred to the same medium and manipulated until unialgal cultures were obtained. Unialgal cultures were propagated on Guillard's medium with and without nitrate salts. Mat pieces also were inoculated on Döbereiner's semisolid medium with glucose and succinate as the carbon sources (Day and Döbereiner, 1976) and Burk's nitrogen free medium with sucrose as the carbon source (Page and Sadoff, 1976). These enrichment media for nitrogen fixing bacteria were incubated over two weeks at 15 C in cool white fluorescent light. Isolates were obtained from these enrichments by transfer to the same media on which they grew. Many iso-

lates failed to grow on the transfer plates, suggesting that nitrogen was obtained from the mat added to the enrichment. Cultures were tested for nitrogenase activity.

Acetylene Reduction

Three methods for *in situ* C_2H_2 reduction were used. Method I utilized serum bottle reaction chambers for algal mat pieces and the planktonic communities. This method also was employed for assaying bacterial and bluegreen algal cultures. Determinations of C_2H_2 reduction in soils utilized a SARAN chamber (Method II). Method III required a coring apparatus to act as the collection and reaction chamber and was used for the mat and sediment assays. Since the basic incubation and analysis techniques were the same for all three *in situ* methods, only Method I will be fully described, while mechanical differences will be pointed out for the remaining methods.

Method I - Serum Bottles

Borosilicate glass serum bottles of various volumes (10, 30 and 150 ml) were used for *in situ* incubation chambers. Algal mat pieces were collected by scraping a sterile jar across the mat or pushing the mat into the jar with hand or wet suit glove. Mat pieces were then placed in a sequential fashion (bottom, then upper layers), into the presterilized serum bottles. After a sufficient amount of mat was distributed to each serum bottle, water from the collection site was added to bring the vapor phase to a specific volume. The bottles were sealed with a

butyl rubber stopper and aluminum cap using crimpers. In some cases a preincubation was done in situ to deplete the photosynethetic reserves of the dark bottles. Air was withdrawn with a syringe, and the same volume C_2H_2 plus other additions replaced. Samples were treated variously. Some were brought to either 40-90 mM NH_4Cl, others received 10% 5N H_2SO_4, 4% organic solution (2.5% glucose and 2.5% succinate), or none of these treatments. C_2H_2 was generated by calcium carbide and water in a 300 ml bottle reacting to form fairly pure C_2H_2. The freshly prepared C_2H_2 was then injected to 0.15 atm into all reaction chambers except a control, no C_2H_2, chamber. Chambers were returned to the collection site for in situ incubation. Incubation times ranged from 4-24 hr in 1977-78 and 12-35 hr in 1978-79 seasons. After the incubation was complete, the bottles were either inactivated with 5 N H_2SO_4 or the sample was agitated and the vapor phase subsampled into 10 ml red stoppered Vacutainers (Becton-Dickenson Corp.). Caps were sealed with a silicone sealant with an acetic acid base (Note: This is not the ideal sealant to use since traces of acetic acid leak into the vacutainer giving an interfering peak during gas chromatographic analysis). After in situ incubation and fixation the serum bottles were returned to the Eklund Biological Laboratory. Pieces of algal mat were analyzed for dry and ash free weights to determine biomass and organic carbon content (Rand et al., 1976). Vapor phase samples were analyzed at the Eklund Laboratory during the 1977-78 season, using a Kontes model 2000 gas chromatograph (Kontes Glass, Vineland, NJ) equipped with an argon ionization detection system. The column was glass and 6 ft long packed with

Porapak R using argon carrier gas. Data obtained from this instrument was qualitative due to poor stability of the instrument. Remaining samples from 1977-78 and all of the samples for the 1978-79 season were returned to VPI & SU for analysis with a Beckman GC-4 gas chromatograph (Beckman Instruments, Inc., CA) equipped with a hydrogen flame ionization detector and a 6 ft glass column. Porapak T was the packing used, helium the carrier gas, column oven was at 55-60 C and the detector was at 120 C. C_2H_4 standards were run by making serial dilutions of 99.9% pure C_2H_4 (Matheson Gas Products, NJ). One ml of standard or sample was run through the gas chromatograph and the peak height used to compare the standards to the sample. No correction factor for C_2H_4 solubility in the aqueous phase was applied, because the error is considered less than 10% (Flett et al., 1975). That this assumption is correct is borne out by the aqueous phase being less than 30% of the total reaction chamber volume and the routine equilibration of samples. This method was modeled after that described by Burris (1972). A modification of Method I was used to test bacterial and algal cultures for C_2H_2 reduction. The reaction chamber was made out of a 100 by 15 mm test tube into which the sample was added or in which it was grown. The sample chamber was sealed with a serum stopper and treated as described for Method I.

Method II - SARAN Bag Chambers

A core of soil was taken, using a 5 cm diam steel core fitted with a plunger for core extrusion. The core was placed in a double thickness SARAN bag (Burris, 1974) (Cryovac Division of W.R. Grace and Co., IA)

and fitted with a septum as shown in Figure 1. Black elastic tape was often used in conjunction with plasticine clay as a sealant. A hand vacuum pump was used to achieve a partial vacuum of not more than 10 cm Hg. These chambers were treated as described in Method I, but air also was added to give a final vapor phase of 200 ml. Incubations were carried out *in situ* and under elevated temperatures *in vitro*.

Method III - Sediment Coring Apparatus

A coring apparatus was used for mat and sediment C_2H_2 reduction assays (Stavros, 1976). A one way valve allowed sampling from the dive hole and moat areas. The vapor phase was adjusted for all cores of one site by allowing sediment to escape from the bottom. Water was aspirated off to give the exact vapor phase required and leave between 0.5 and 1.5 cm of water above the mat surface. Cores were treated as described for Method I and incubated in a rack that kept cores in a vertical position to prevent shading by adjacent cores. This method gave some qualitative data, but Method I was used primarily for quantitative determination of C_2H_2 reduction in the mats.

$^{15}N_2$-Uptake Assay

Measurements of stable isotopic uptake by algal mats were performed in serum bottles as described by Burris (1972). Incubation was run parallel with C_2H_2-reduction assays.

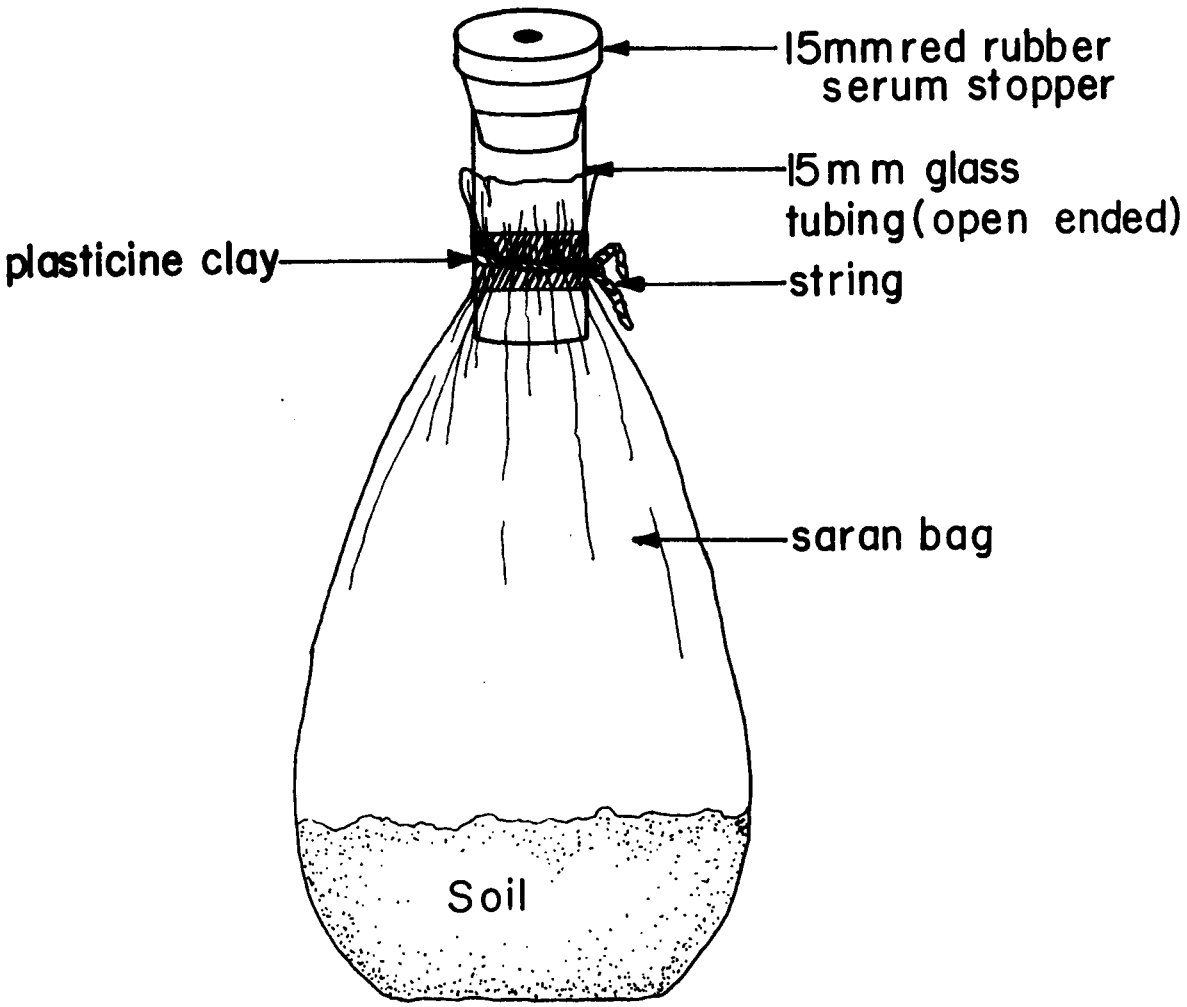


FIGURE 1. SARAN bag assay chamber for acetylene reduction on soil samples (Burris, 1974).

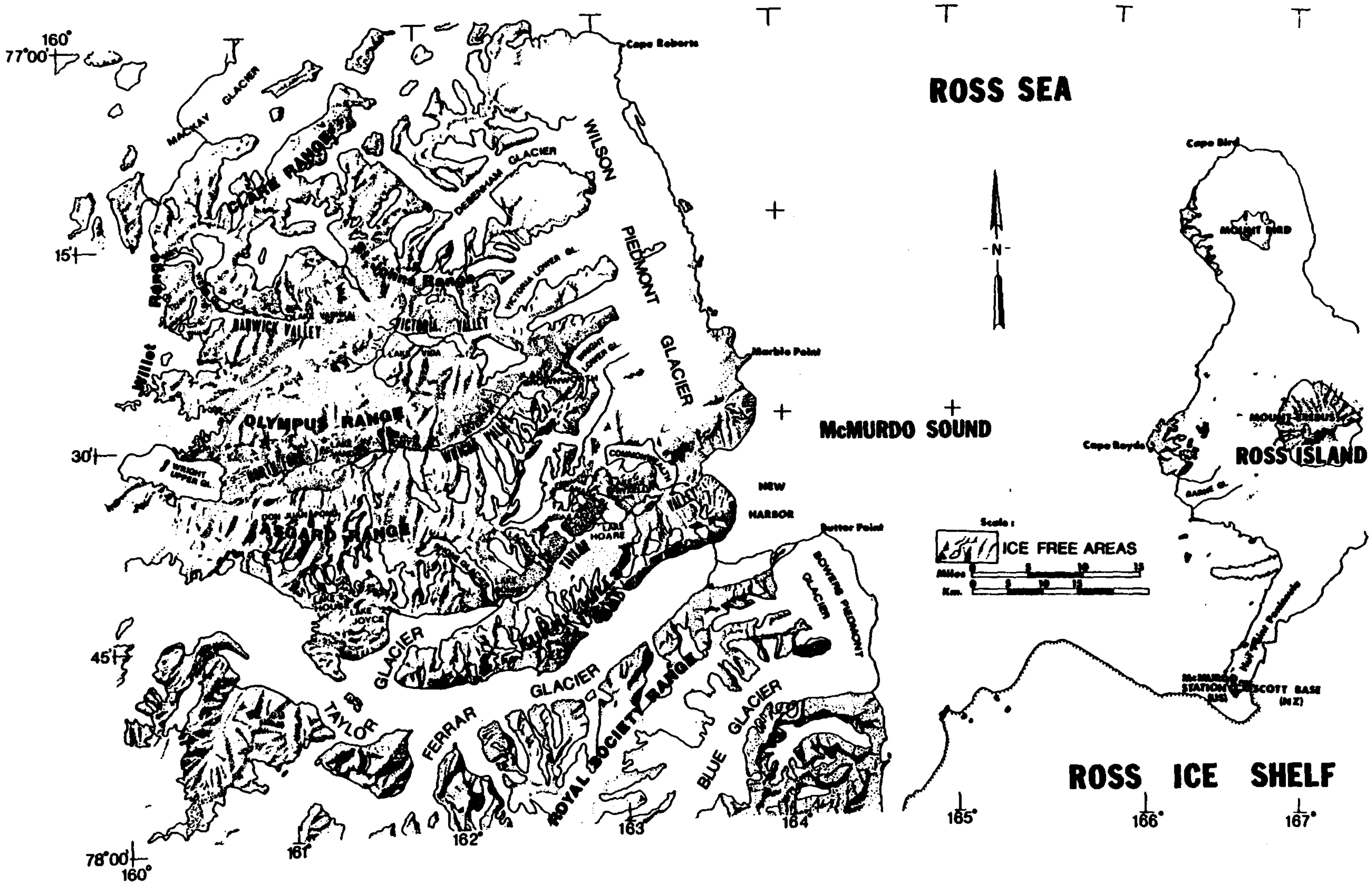
Statistical Analyses

These data were compared using the least squared means function of the Statistical Analysis System (LSMEANS). A series of T-tests were run on samples by this procedure and the differences were printed out as the confidence of rejection of the H^{o} that the mean is equal to the compared mean (Barr et al., 1976).

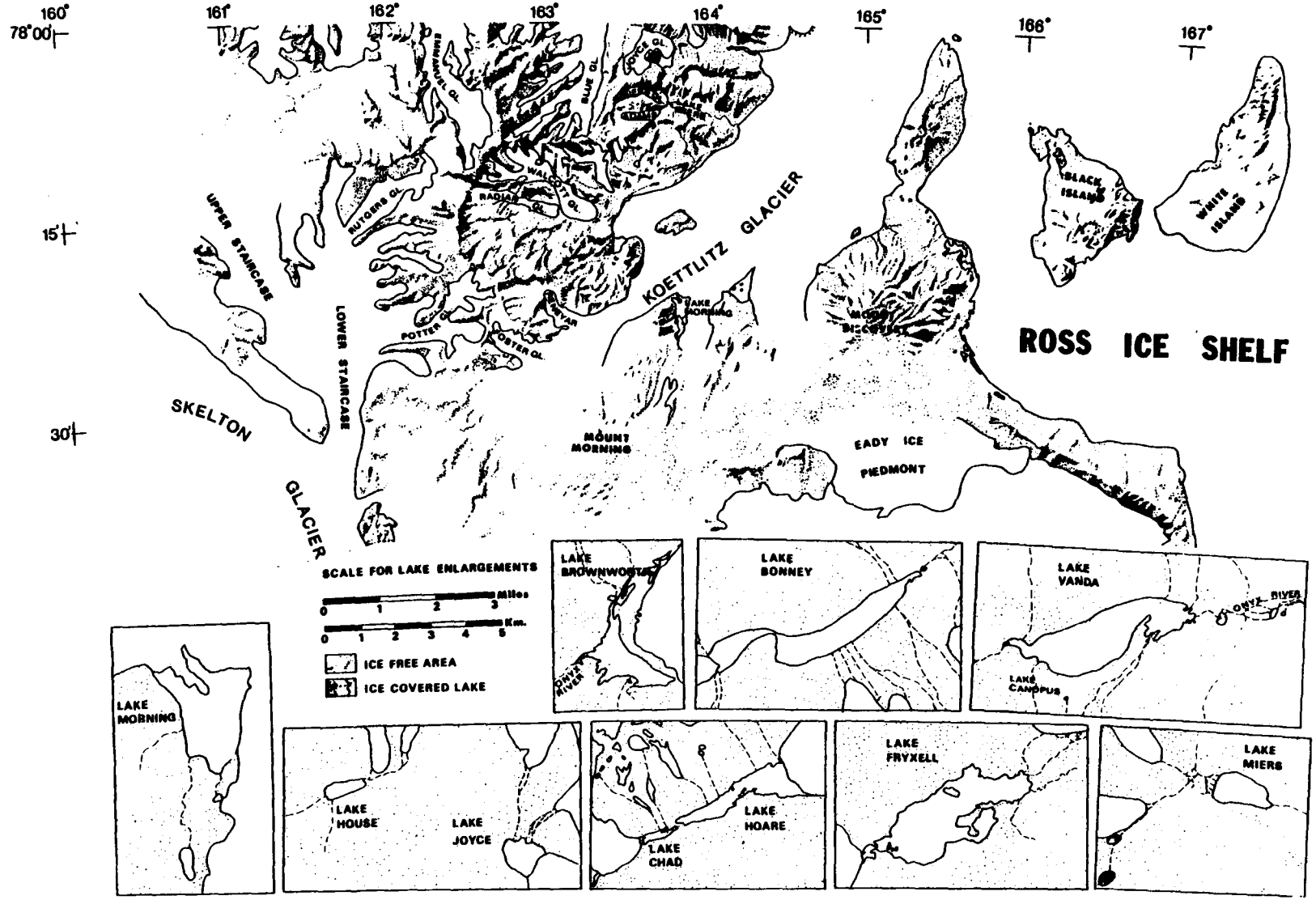
SITE DESCRIPTION - LITERATURE REVIEW

Southern Victorialand (Figure 2), one of several antarctic dry valley oases (Clark, 1965; Heywood, 1977), is an ice-free region of extremely harsh environments in terms of temperature, light, moisture, and salinity (Parker, 1978). These unusual oases of Antarctica are ice-free apparently from blockage of the polar ice sheet by moranic material and/or differential recession of the glaciers due to the low albedo and consequent higher temperatures in them (Wilson, 1970). Southern Victorialand, the Bunger Hills, the Vestfold Hills and the Schirmacheroasen are the four chief ice-free areas and comprise <4% of the antarctic land mass. Southern Victorialand is the largest of the four and within 100 km of McMurdo Station. It is the most intensively studied oasis. The mean annual temperature is -18 C, the relative humidity below 45% and an annual precipitation of ca. 15 cm of water. Desiccation is one biological stress since sublimnation losses sometimes exceed the moisture gains through precipitation. This is hastened furthermore by a dry prevailing wind off the Polar Plateau (Heywood, 1977). Within these dry valley oases a diverse group of lakes are found ranging from fresh to saline, ice-free to permanently ice covered, small and shallow pools to large relicit seawater lakes. Lakes Bonney, Fryxell, and Vanda are considered ectogenic, meromictic lakes thought to be formed from fresh glacial meltwater flowing on top of relicit evaporated and concentrated seawater or brine layers (Heywood, 1977). Other lakes may have formed by meltwater influx into a relatively nonsaline basin as there is little chemical stratification or brine (i.e., Lakes Chad and Hoare). Canopus

FIGURE 2. Southern Victorialand, Antarctica with inset maps of lakes studied during the 1977-78 and 1978-79 austral summer.



ROSS SEA
Cape Roberts
MACKAY GLACIER
CLARE RANGE
DEBENHAM GLACIER
WILSON PIEDMONT GLACIER
VICTORIA LOWER GL.
VICTORIA VALLEY
BARWICK VALLEY
LAKE VIDA
Marble Point
OLYMPUS RANGE
WRIGHT UPPER GL.
ASGARD RANGE
LAKE HOARE
LAKE JOYCE
McMURDO SOUND
NEW HARBOR
Butter Point
TAYLOR GLACIER
FERRAR GLACIER
ROYAL SOCIETY RANGE
BLUE GLACIER
BOWERS PIEDMONT GLACIER
Cape Bird
MOUNT BIRD
MOUNT EREBUS
Cape Royds
ROSS ISLAND
BARNE GL.
McMURDO STATION (US)
SCOTT BASE (NZ)
ROSS ICE SHELF
N
Scale:
ICE FREE AREAS
Miles
Km.
160°
77°00'
15'
30'
45'
78°00'
161°
162°
163°
164°
165°
166°
167°


160°
78°00'
161°
162°
163°
164°
165°
166°
167°
15'
30'
UPPER STAIRCASE
LOWER STAIRCASE
SKELTON GLACIER
KOETTLITZ GLACIER
RUTORS GL.
SAMUEL GL.
BLUE GL.
RADIAN GL.
POTTER GL.
FOSTER GL.
LAKE MORNING
MOUNT MORNING
EADY ICE PIEDMONT
ROSS ICE SHELF
BLACK ISLAND
WHITE ISLAND
SCALE FOR LAKE ENLARGEMENTS
Miles
0 1 2 3
Km.
0 1 2 3 4 5
ICE FREE AREA
ICE COVERED LAKE
LAKE MORNING
LAKE BROWNWORTH
ONYX RIVER
LAKE BONNEY
LAKE VANDA
ONYX RIVER
LAKE CANOPUS
LAKE HOUSE
LAKE JOYCE
LAKE CHAD
LAKE HOARE
LAKE FRYXELL
LAKE MIERS

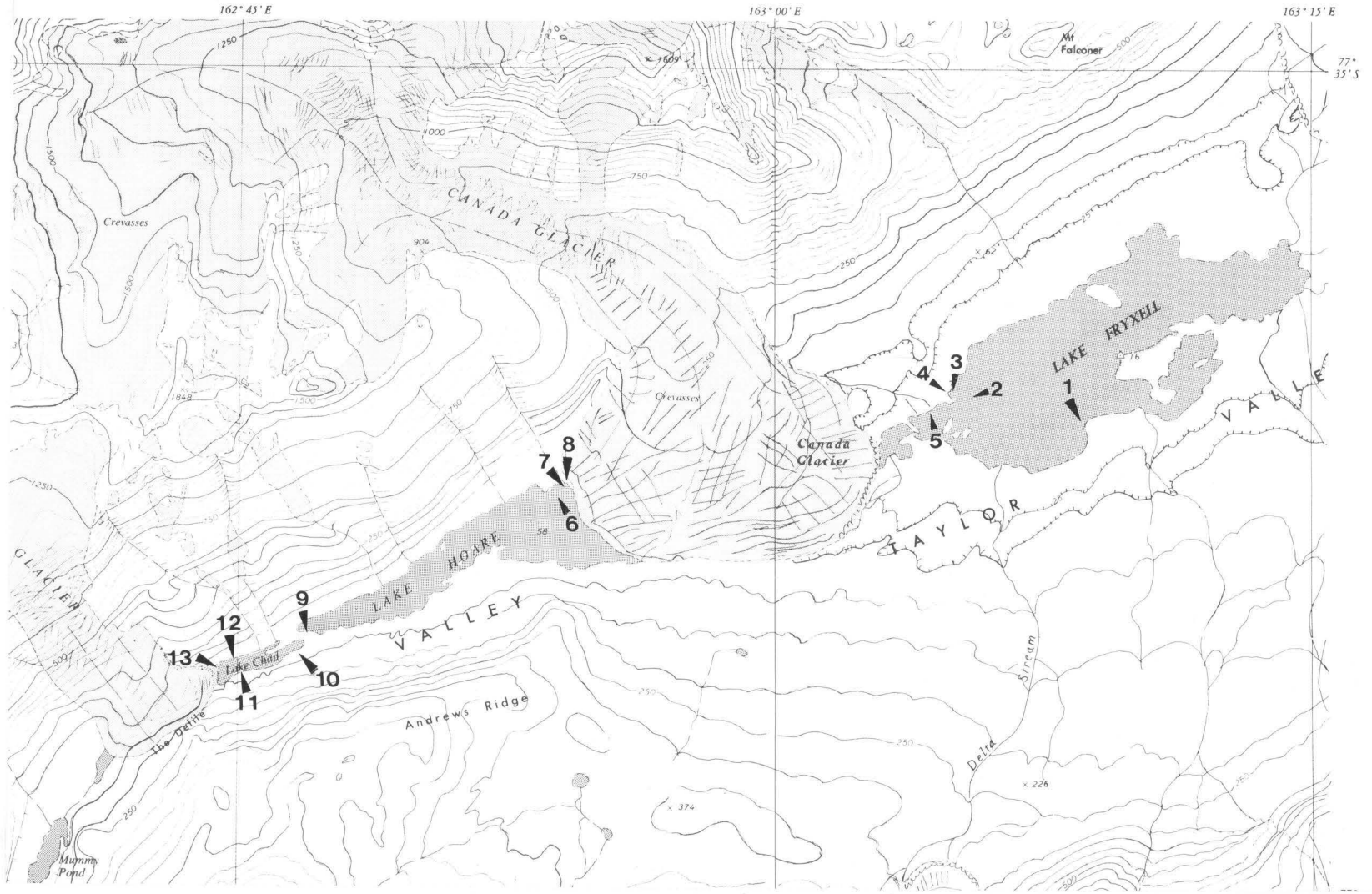
Lake and other small pools are found to melt completely in the austral summer and freeze solidly in the winter. The larger lakes have a permanent ice cover of about 4 m (Armitage and House, 1962; VPI & SU field data, 1978). The ice cover of these lakes acts as an insulator to trap solar heat, maintaining a more constant temperature (0-7 C) than ice-free or seasonally thawed areas (Heywood, 1977). Photosynthesis is reportedly favored for all but 2-4 months of the year (Heywood, 1977), but evidence of extremely low light levels in the water column and benthic regions during the austral summer peak might cause questioning of this assumption (VPI & SU field data, 1978). The previous data for these lakes are summarized in Table 1. Lakes Bonney, Fryxell, and Vanda have pronounced chemical and physical stratification which is reflected in the temperature and conductivity profiles. The smaller, entirely freshwater lakes, Lakes Brownworth, Morning, Vida, and possibly Chad, appear to freeze to the bottom during the winter. Lakes Miers and Hoare, larger freshwater lakes, show less stratification but nevertheless have permanent liquid water beneath 5 m of ice.

TABLE 1. SUMMARY OF PHYSICAL DATA FOR SEVEN SOUTHERN VICTORIALAND LAKES.[1]

Lake Name	Latitude South	Longitude East	Valley	Lake Area (km^2)	Max. Depth (m)	Temp. Range (C)	Conductivity Range ($\mu mhos \cdot cm^{-2}$)
Fryxell	77°37'	163°07'	Taylor	5.366	16.0	0.2-- 3.24	1520-22727
Vanda	77°32'	161°33'	Wright	5.038	70.0	0 --25.0	112-250000
Bonney	77°43'	162°23'	Taylor	3.845	33.4	-4.6-- 8.0	1-212765
Brownworth	77°26'	162°45'	Wright	2.027	4-5?	-	
Miers	78°07'	163°54'	Miers	1.066	19.5	0 -- 5.8	90-300
Hoare	77°38'	162°53'	Taylor	1.770	31.0	0.8-- 1.0	240-1200
Chad	77°38'	162°45'	Taylor	0.173	6.0	-	-

[1]Data sources: VPI & SU field data; Angino et al., 1962, 1965; Armitage and House, 1962; Bell, 1967; Torii et al., 1975.

FIGURE 3. Diagram showing the study sites on Lakes Chad, Fryxell and Hoare during the 1978-79 austral summer. This map removed from a United States Geological Survey map.


162° 45' E
163° 00' E
163° 15' E
77° 35' S
Mt Falconer
CANADA GLACIER
Crevasses
LAKE FRYXELL
Canada Glacier
TAYLOR VALLEY
LAKE HOARE
VALLEY
Andrews Ridge
Lake Chad
Delta Stream
Mummy Pond
1
2
3
4
5
6
7
8
9
10
11
12
13

RESULTS AND DISCUSSION

During the 1977-78 austral summer eight lakes in Southern Victoria-land were examined to assess the extent of nitrogen(C_2H_2)-fixation in these unique ecosystems. The communities examined were the littoral or moat algal mats in the shallow lake shoreline areas, the plankton, the meltpools and surrounding moist soils. The 1977-78 field season data showed that acetylene (C_2H_2) reduction occurred only in the littoral algal mat communities. During the 1978-79 season, major emphasis was placed on the littoral moat and the deep benthic, previously unstudied, mat communities of Lakes Chad, Fryxell and Hoare. Less attention was paid to other communities in these lakes.

Incubation periods during the first field season varied from 2-24 h, while those from the 1977-78 field season were as close to 24 h as possible. Incubation periods less than 6 h had unmeasureable C_2H_4 production and longer incubations had given more accurate quantitative results. While long incubation periods have been reported to overestimate the N_2 fixation using C_2H_2 reduction (David and Fay, 1977), the longer incubation periods were necessary to get any measureable ethylene. Also, it is likely that the 24 h incubation at 0-5 C would not show as great an artifact over this period as incubations at warm temperatures. This is supported by the following: C_2H_2 reduction in a unialgal culture of Anabaena aequalis previously isolated from Lake Miers proved significant at and above 5 C. A lag period occurred initially after which a steady rate with little stimulation over 24 h incubation was seen at the lower temperatures (i.e., 5 and 8 C). The rates achieved by these

incubations show a decrease with decreasing temperature in C_2H_2 reduction (Figure 4). There appears to be a very significant temperature decrease as expected but no great stimulation in C_2H_2 reduction over time (Table 2). These results, while not conclusive, show a potential for fixation at low temperatures by antarctic bluegreen algae, as well as, suggesting further work is necessary regarding stimulation under C_2H_2 at different temperatures closer to those found in antarctic habitats.

C_2H_2 Reduction Data

Soils

Nitrogen(C_2H_2)-fixation was not detected in the soils surrounding these lakes (Table 3). The low moisture content and temperature of some of these soils reflect an environment not conducive to nitrogen fixation or other biological activity. The inability to detect C_2H_2 reduction also may be due to slow metabolic rates or the absence of actively metabolizing microorganisms. Heterocystous bluegreen algae have been found in these and other soils in Antarctica (Cameron, 1971; Holm-Hansen, 1963; Seaburg *et al.*, 1979). While no nitrogen fixing bacteria have been isolated from these soils (Sieburth, 1965), they have been isolated from other antarctic soils (Boyd and Boyd, 1962). The presence of nitrogen fixing bluegreen algae and possibly bacteria in these soils indicates a potential for nitrogen fixation, but these organisms may be inactive forms introduced by wind or animals (Benninghoff, 1978). More extensive incubation periods or a more sensitive assay may show small

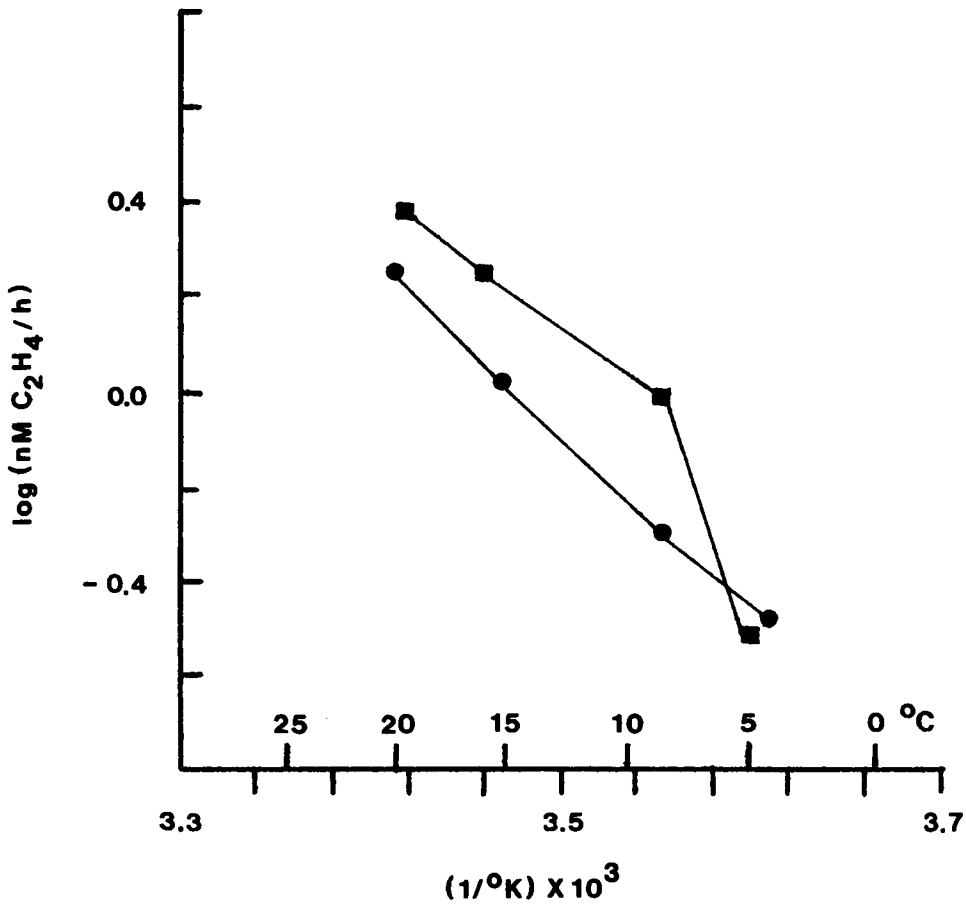


FIGURE 4. Acetylene reduction versus temperature in a unialgal culture of Anabaena aequalis isolated from Lake Miers, Antarctica. Squares = nM $C_2H_4/h/10^3$ heterocysts; Circles = nM $C_2H_4/h/10^6$ cells.

TABLE 2

ACETYLENE REDUCTION RATES AT FOUR TEMPERATURES AND DIFFERENT INCUBATION TIMES (h) USING A UNIALGAL CULTURE OF ANABAENA AEQUALIS ISOLATED FROM LAKE MIERS, ANTARCTICA.

Temp (C)	Time Period[1]	Vegetative Cells[2]	Heterocysts[3]
18	0 - 1	0.34	0.51
	1 - 2	0.90	1.35
	2 - 4	1.46	2.17
	4 - 6	1.64	2.44
15	0 - 1	0.53	0.88
	1 - 2	0.94	1.57
	2 - 4	1.03	1.73
	4 - 6	0.87	1.46
	6 - 12	1.10	1.84
	12 - 24	1.21	2.02
8	0 - 1	0.21	0.31
	1 - 2	0.46	0.68
	2 - 4	-	-
	4 - 6	0.57	0.85
	6 - 12	0.42	0.62
5	0 - 2	0.18	0.22
	2 - 6	0.20	0.25
	6 - 12	0.24	0.29
	12 - 24	0.31	0.25

[1]Time period expressed in hours and is the period used for calculation of the rate.

[2]Rate as nmoles $C_2H_4 \cdot h^{-1} \cdot 10^5$ cells^{-1}

[3]Rate as nmoles $C_2H_4 \cdot h^{-1} \cdot 10^3$ heterocysts^{-1}

TABLE 3

A QUALITATIVE SURVEY OF C_2H_2 REDUCTION IN A NUMBER OF HABITATS IN SOUTHERN VICTORIALAND LAKES, 1977-78 AUSTRAL SUMMER.[1]

Lake[2]	Littoral/Moat Mats	Plankton	Soil
Miers (14)	+	-	
Brownworth (15)	±		-
Chad (11)	+		-
Hoare (16)		-	-
Bonney (17)	-		
Fryxell (1)	+		-
Vanda (18)	-		

[1] + = C_2H_2 reduction found; ± = slightly positive results could indicate C_2H_2 reduction present; - = no measureable C_2H_2 reduction.

[2] Numbers in parenthesis refer to site description in Appendix 7.

amounts of acetylene reduction, but the amount of dinitrogen fixed would only be important to the microhabitats and intimately associated organisms within the soils.

Plankton

Nitrogenase activity was not detected in the plankton communities of Lakes Hoare and Miers (Table 3). The temperatures in Lake Hoare and Miers water columns were low (never exceeding 4 C) and do not have a capacity for microhabitat warming found in the soils and mats. Since the water column is covered by 5-6 m of permanent ice, the photosynthetically available radiation also is reduced (less than 1% incident light) in these lakes which may be a major limiting factor to nitrogen fixation via photosynthesis (Fogg and Stewart, 1968; Holm-Hansen, 1977). Few heterocystous bluegreen algae have been observed in the plankton of either lake with only one species of Anabaena being found in Lake Hoare plankton (Seaburg et al., 1979). A more sensitive method might have detected some potential for nitrogen fixation but the low cell numbers chiefly of eukaryote algae, the low light levels, the low nutrient levels, and the supersaturated oxygen apparently mediate against any significant nitrogenase activity in the plankton.

Littoral Moat Algal Mats

Nitrogenase activity in the littoral moat mats probably is the most significant in these antarctic lakes. During the 1977-78 season the algal mats of Bonney, Brownworth, Chad, Fryxell, Miers, and Vanda were

assayed for nitrogenase activity. Lakes Brownworth, Chad, Fryxell, and Miers had C_2H_2 reduction in their littoral mats (Table 3), while mats from Bonney and Vanda showed no C_2H_2 reduction. A more detailed study of the littoral algal mats of Lakes Chad, Fryxell, and Hoare was undertaken during the 1978-79 austral summer to augment the data collected the previous season. The well developed littoral mats of Chad and Fryxell showed significant nitrogen(C_2H_2)-fixation, and those in Fryxell were greater than those in Chad (Table 4). However, Lake Chad had the most extensive mats, perhaps because of its relatively shallow nature which would allow more light and/or due to the flow of fresh glacial meltwater through this lake during the austral summer. Interestingly more difference was seen in simultaneous runs on the littoral moat mats in Lake Fryxell than between the earliest and latest runs on the same habitat. Seasonal differences were not apparent when compared to the spacial heterogeneity of the mat communities. A study encompassing dates very early in the austral summer (i.e., September) and very late (i.e., late February) would very possibly show a seasonal difference not seen here. Unfortunately no assays were run on the littoral algal mats of Lake Hoare since they were not discovered until very late in the season.

A parallel dark incubation showed dark nitrogen(C_2H_2)-fixation was significant yet considerably less than the light nitrogen(C_2H_2)-fixation (Table 5). The organically enriched dark incubated samples were significantly higher in activity than unenriched samples which indicated bacterial or bluegreen algal heterotrophic nitrogen fixation. The

TABLE 4. NITROGEN(C_2H_2)-FIXATION RATES FROM IN SITU INCUBATIONS OF BLUEGREEN ALGAL MATS OF LAKES CHAD, FRYXELL AND HOARE, 1978-79 AUSTRAL SUMMER.[1]

Lake	Habitat[2]	Date	Control	nmoles $C_2H_4 \cdot day^{-1} \cdot mgC^{-1}$		nmoles $C_2H_4 \cdot day^{-1} \cdot gdw^{-1}$	
				$-NH_4^+$	$+NH_4^+$	$-NH_4^+$	$+NH_4^+$
Hoare	Benthic (6)	1/14/79	0	0.004	0.005	0.309	0.615
	Peripheral (8)	1/14/79	0	3.359*	0.020	144.66*	2.06
Chad	Littoral moat (11)	1/23/79	-	0.300*	0.070	-	-
	Outflow (9)	1/26/79	-	0.078*	0.021	6.98*	2.45
	Peripheral (10)	1/26/79	-	1.279*	0.144	170.62*	8.21
	Littoral moat (11)	1/26/79	0	0.043	0.012	20.69	6.63
	Littoral moat (12)	1/26/79	-	0.195	0.131	81.02*	46.68
Fryxell	Littoral moat (1)	1/14/78	-	0.431*	0.012	-	-
	Benthic (2)	12/28/78	0.14	0.276	0.306	3.99	3.07
	Benthic (2)	1/7/79	0	0.069	0.017	14.8	2.1
	Littoral moat (3)	1/7/79	0	1.062*	0.046	151.6*	5.1
	Littoral moat (3)	1/22/79	0.9	3.199*	0.802	224.1*	28.9
	Littoral moat (3)	1/22/79	0	1.472*	0.736	117.6*	47.4

TABLE 4. CONTINUED.

Lake	Habitat[2]	Date	Control	nmoles $C_2H_4 \cdot day^{-1} \cdot mgC^{-1}$ $-NH_4^+$	nmoles $C_2H_4 \cdot day^{-1} \cdot mgC^{-1}$ $+NH_4^+$	nmoles $C_2H_4 \cdot day^{-1} \cdot gdw^{-1}$ $-NH_4^+$	nmoles $C_2H_4 \cdot day^{-1} \cdot gdw^{-1}$ $+NH_4^+$
Fryxell	Peripheral (4)	12/27/78	0	0.223*	0	13.5*	0

[1]mgC = milligrams lost on ignition at 500 C; gdw = grams dry weight of samples; $-NH_4$ = not enriched by addition of ammonia; $+NH_4$ = enriched by addition of ammonia; all samples which were determined to by significantly different at the 95% confidence level from the corresponding ammonia control are signified with an *.

[2]Number in parentheses refers to site described in Appendix 7.

TABLE 5. LIGHT AND DARK C_2H_2 REDUCTION IN LAKE FRYXELL'S LITTORAL MOAT ALGAL MAT, JANUARY 1979.

Incubation	Control[1,2]	$-NH_4^+$	$+NH_4^+$	+ Organics
Light	0.9**	3.20***	0.80**	1.57***
Dark		0.06*	0.01*	0.18*

[1] No C_2H_2 added; thus, metabolicaaly produced ethylene, expressed as nmoles $C_2H_4 \cdot day^{-1}$. All others expressed as nmoles $C_2H_4 \cdot day^{-1} \cdot mgC^{-1}$; for details on $+NH_4^+$ and + organics, see Materials and Methods.

[2] all samples marked with an asterik are significantly different; ** are not significantly different from each other but are from the other samples; *** are not significantly different from each other but are from the other samples.

extremely low levels of activity in the dark, however, indicate that photoautotrophic organisms are responsible for the major C_2H_2 reduction occurring in the littoral algal mats.

Peripheral Habitats

A number of surrounding meltpool and overflow areas had well developed algal mat communities which showed nitrogen(C_2H_2)-fixation capabilities (Table 4). At Lake Fryxell the littoral moat mats had greater C_2H_2 reduction than the nearby peripheral water mats, while the highest activity measured was in the peripheral water mats of Lake Hoare. In Lake Chad meltpools and the Lake Chad-to-Hoare overflow, significant nitrogen(C_2H_2)-fixation was found. The widespread positive C_2H_2 reduction results indicate that nitrogen fixation is common in habitats such as the meltpools and shallow streams assayed here.

Benthic Algal Mats

No significant nitrogen(C_2H_2)-fixation was detected in the benthic algal mats of Lakes Hoare and Fryxell (Table 4). In the assay at Lake Hoare no differences were seen between any of the treatments except that the organically enriched samples showed greater C_2H_2 reduction than the control. There was no difference between treatments in Lake Fryxell benthic mats. The possibility that anaerobic or microaerophilic N_2 fixation occurs cannot be ruled out since the Lake Fryxell benthic mats were anaerobic below a few centimeters and microhabitats within the mat at Lake Hoare may exist, allowing anaerobic N_2 fixation. The C_2H_2

reduction method used (Method I) would destroy anaerobic fixers during processing because mats would be disrupted on collection.

Habitat Descriptions

Littoral Moat Communities

The littoral moat regions are ice-free during a part of the short austral summer and are frozen to the sediment during the remainder of the year. Usually these areas are very shallow and gently sloping, as shown schematically in Figure 5. Lakes Fryxell (Sites 1, 3 and 5) and Chad (Sites 11, 12 and 13) have the typical littoral moat described above. Lake Hoare has a moat region (Site 7) that slopes sharply down from the shoreline reaching a depth of 1.5 m, 1 m from shore. The steeper grade and seasonal influx of nitrate-rich meltwater and subsequent scouring by ice may relate to the poor algal mat development in the Lake Hoare moat. In all moat areas studied except Lake Hoare, well developed algal mat communities developed unless influenced by extensive shading (e.g., Lake Bonney mats). Little fixed nitrogen was detected in these lake moat waters (Table 6), except after influx of meltwater to the Lake Hoare moat which resulted in high nitrate values. No significant differences could be seen in the dissolved and particulate organic matter between these lakes. The shallowness of these moat areas, with the exception of Lake Hoare, allowed nearly all of the surface photosynthetically available radiation to reach the algal mat community (e.g., 93% at Lake Brownworth moat). These areas receive maximum radiation, have very little combined nitrogen and have the capacity for higher

FIGURE 5. Schematic representation of the littoral and benthic regions of Lakes Fryxell and Hoare.

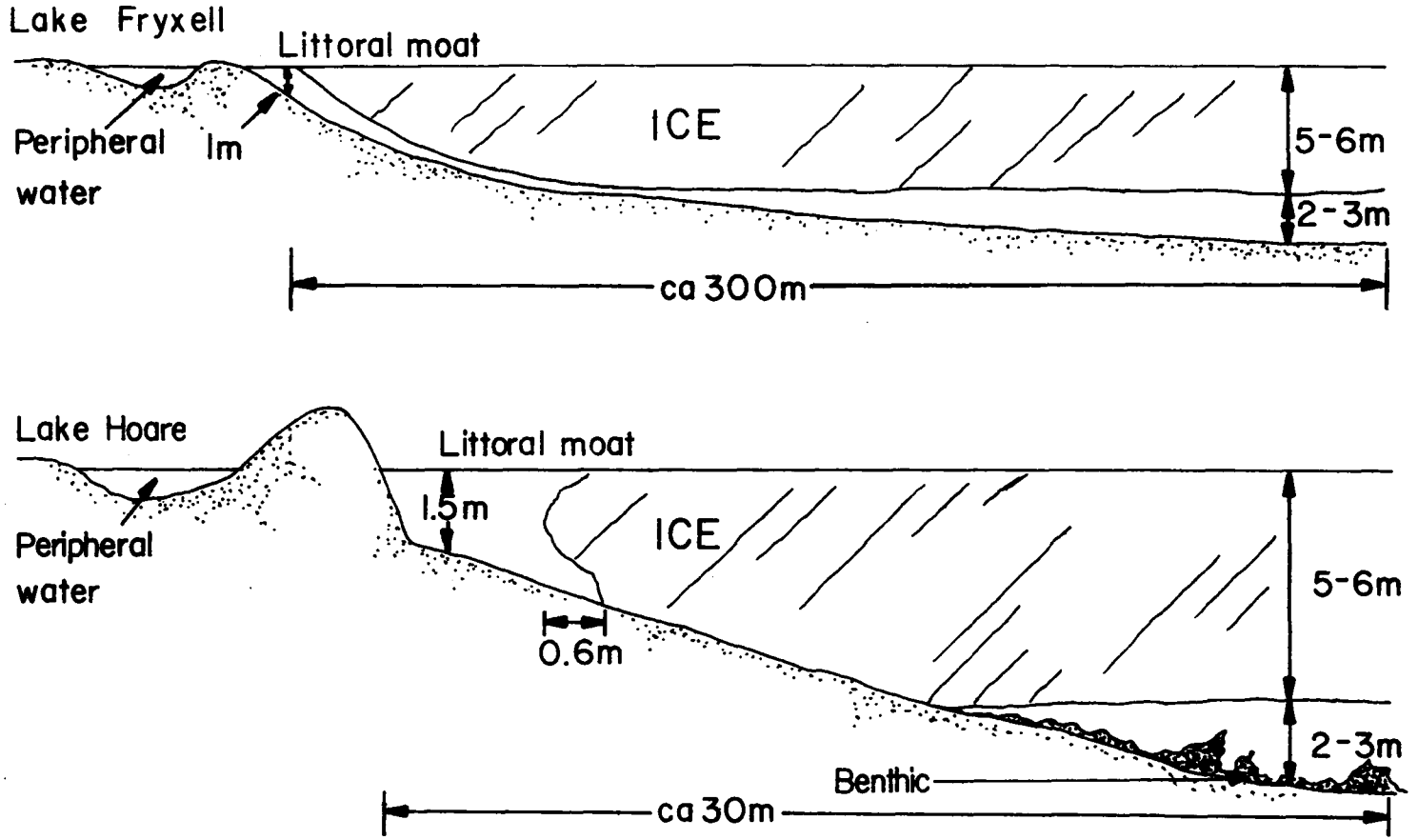
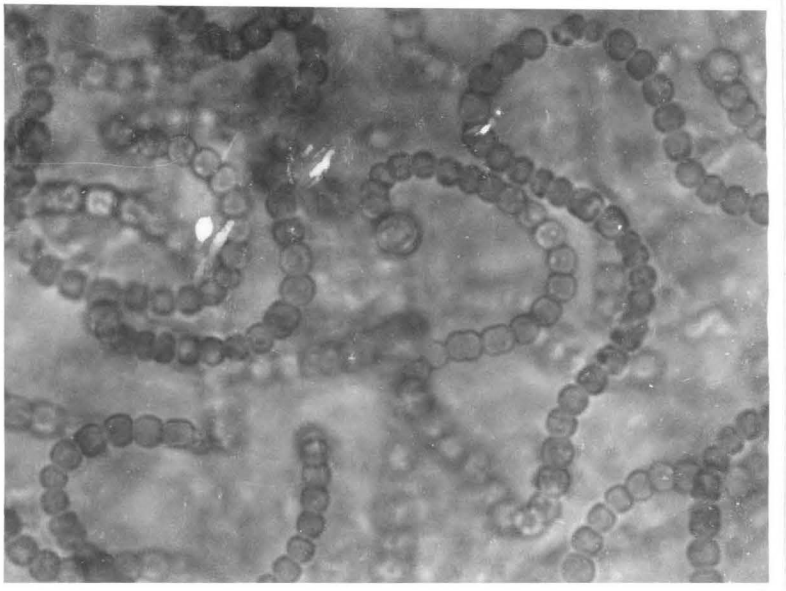


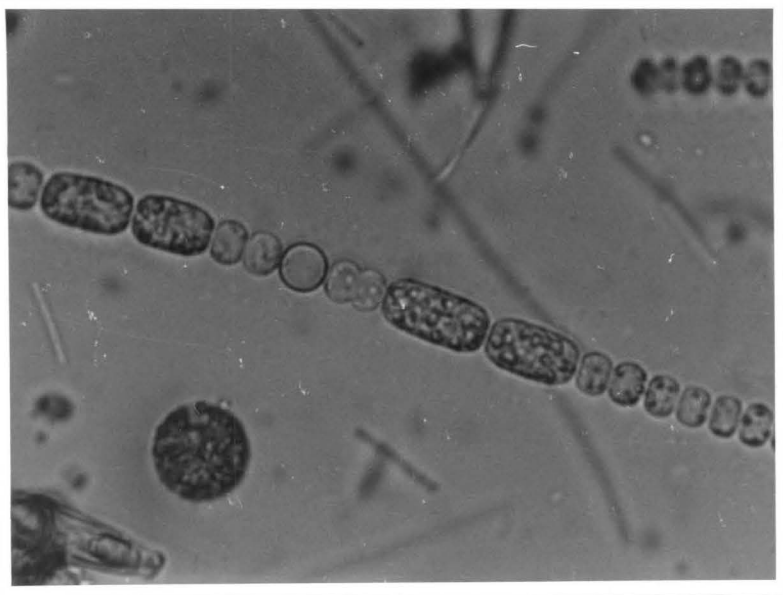
TABLE 6. DISSOLVED AND TOTAL ORGANIC CARBON, AMMONIA AND NITRATES FOUND IN THE WATERS OF SITES STUDIED DURING THE 1978-79 AUSTRAL SUMMER.[1]

Lake	Site	Date	DOC	TOC	NH_4-N	NO_3-N
Fryxell	Benthic area (Site 2)	1/6/79	82	68	0.23	0
		1/20/79	-	-	-	0.32
Fryxell	Littoral moat (Site 3)	12/28/78	38	48	0.405	0.33
		1/6/79	70	78	0.51	0
		1/20/79	48	31	-	-
Chad	Littoral moat (Site 11)	1/30/79	-	-	0.49	0.35
Hoare	Benthic area (Site 6)	12/30/78	45	43	0.47	3.53
		1/3/79	42	-	0.02	-
		1/14/79	-	23	0.01	-
Hoare	Littoral moat (Site 7)	12/30/78	39	43	1.06	0.33
		1/3/79	-	70	0.31	2.52
		1/14/79	-	30	0	2.28
Hoare	Meltstream	1/14/79	31	22	0.38	4.37

[1] DOC = dissolved organic carbon expressed in mg/l; TOC = total organic carbon expressed in mg/l; NH_4-N = ammonia nitrogen expressed in µM nitrogen; NO_3-N = nitrate and nitrite nitrogen expressed as µM nitrogen.

FIGURE 6. Heterocystous bluegreen algae found in algal mats of Lakes Hoare, Chad and Fryxell: *Nostoc commune* (top), *Anabaena* sp. (bottom).





temperatures due to light absorption and microclimates within the mat. All these factors would favor the presence of nitrogenase activity.

The littoral moats have a well developed bluegreen algal mat community with abundant and often dominant heterocystous (i.e., nitrogen fixing) forms. Anabaena sp. and Nostoc commune (Figure 6) are the major algae found, while other heterocystous algae were also present. The photographs in Figure 6 were taken from live mats and show the presence of heterocysts, an indication that nitrogen may be limiting and that nitrogen fixation may be occurring. In all cases where C_2H_2 reduction occurred, heterocystous bluegreen algae were identified (Table 7). Three types of mat communities were found in these littoral moats: (1) mats flattened and grey with a grainy texture, found in Lakes Bonney and Vanda (Figure 7), (2) mats thick and spongy with reddish to grey-green coloration and found in Lakes Brownworth and Chad (Figure 7), and (3) mats flattened with loosely associated Nostoc colonies bearing a grey-green coloration found in Lakes Fryxell and Miers (Figure 7). The first type of mat may have lacked nitrogen fixing organisms since no C_2H_2 reduction was found; unfortunately no identifications were done. However, NH_4^+ and NO_3^- ion levels in Lake Bonney were reported to be fairly high (Hoehn et al., 1977). The other mats described were found to reduce C_2H_2 with the mats from Lakes Fryxell and Miers being most active.

Communities of Lake Peripheral Regions

All of the regions peripheral to the lakes surveyed were shallow areas of water surrounding the lakes. These are seasonally affected

TABLE 7

DOMINANT BLUEGREEN ALGAE SPECIES, ACETYLENE REDUCTION AND THE PRESENCE OF HETEROCYSTOUS BLUEGREEN ALGAE AT SITES STUDIED DURING THE 1978-79 AUSTRAL SUMMER.

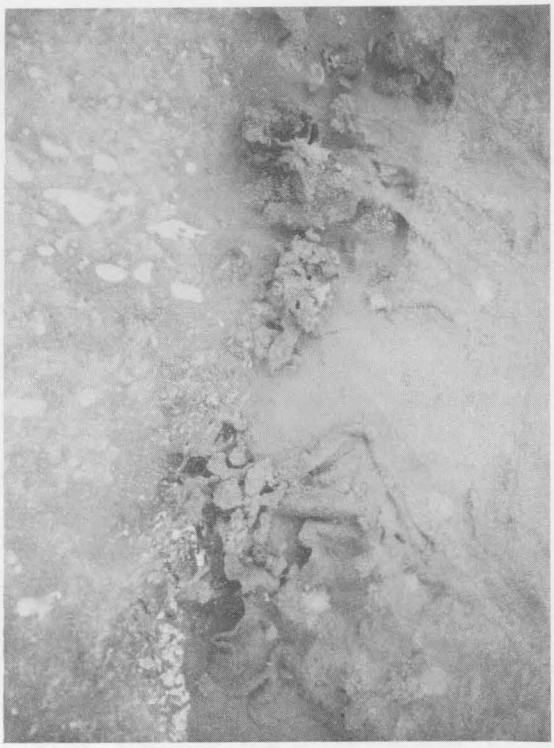
Lake	Habitat[1]	Dominant Algae[2]	Heterocystous Algae	Nitrogen(C_2H_2)-Fixation
Fryxell	moat (1)	Nostoc commune Phormidium frigidum	+	+
	benthic (2)	Phormidium frigidum Lyngbya martensiana	-	-
	moat (3)	Nostoc commune Phormidium frigidum	+	+
	peripheral (4)	Nostoc[3] Phormidium	+	+
	moat (5)	Nostoc commune	+	+
Hoare	benthic (6)	Phormidium frigidum	-	-
	moat (7)	Phormidium frigidum Lyngbya martensiana Nostoc commune Anabaena sp.	 + +	
	peripheral (8)	Anabaena sp. Lyngbya martensiana	+	 +
Chad	outflow (9)	Nostoc commune Calothrix braunii Schizothrix antarctica Phormidium frigidum Microcoleus paludosus Oscillatoria priestleyi	+ +	+
	peripheral (10)	Nostoc commune Microcoleus paludosus Lyngbya martensiana	+	+

TABLE 7. CONTINUED.

Lake	Habitat[1]	Dominant Algae[2]	Heterocystous Algae	Nitrogen(C_2H_2)-Fixation
Chad	moat (11)	Phormidium frigidum Nostoc commune	+	+
	moat (12)	Nostoc commune	+	+
	moat (13)	Nostoc commune Microcoleus paludosus Lyngbya martensiana Oscillatoria limosa	+	+

[1]Numbers refer to a more detailed description of the sites in Appendix 7.
[2]Appendices 1-6 summarize the algal species present in every site.
[3]Tentative identification sample lost.

FIGURE 7. Bluegreen algal mat communities from the littoral moat and peripheral regions. From the upper right hand corner clockwise: Littoral moat mat from Lake Bonney, Lake Fryxell, and Lake Chad; upper left, peripheral water mat from Lake Fryxell.



areas that may depend on meltwater influx for maintenance of existing nutrient levels. They are totally melted during most of the austral summer except during cold periods when a surface crust of ice rapidly forms. The only area of rapidly moving water is the flow from Lake Chad-to-Lake Hoare that developed during the 1978-79 season in late December, while the other areas are small pools of water. Unfortunately no chemical analyses were run on these areas. Radiation reaching these areas for the most part is unrestricted as the maximum water depth in these melted peripheral pools never exceeded 0.5 m. The algal mats developed in a flattened form (Figure 7) except in a site on Lake Chad (Site 10) where a dark raised Nostoc community was located. The main mat components were either filamentous heterocystous or non-heterocystous bluegreen algae (Table 7) and C_2H_2 reduction was found in every site.

Benthic Community

This benthic region is a well insulated area from the antarctic environment due to a 5-6 m permanent ice cover. However, this ice cover may have deleterious effects too. The water beneath has a stable temperature. Lake Hoare has a lower temperature than Lake Fryxell (Table 8) which may be due to the much larger volume of water in Lake Hoare. The amount of light reaching the mats in these benthic regions is well below 1% of surface photosynthetically available radiation (Table 8) which may explain the lack of nitrogenase activity at these sites. The supersaturated O_2 and cooler temperatures also may be involved, however,

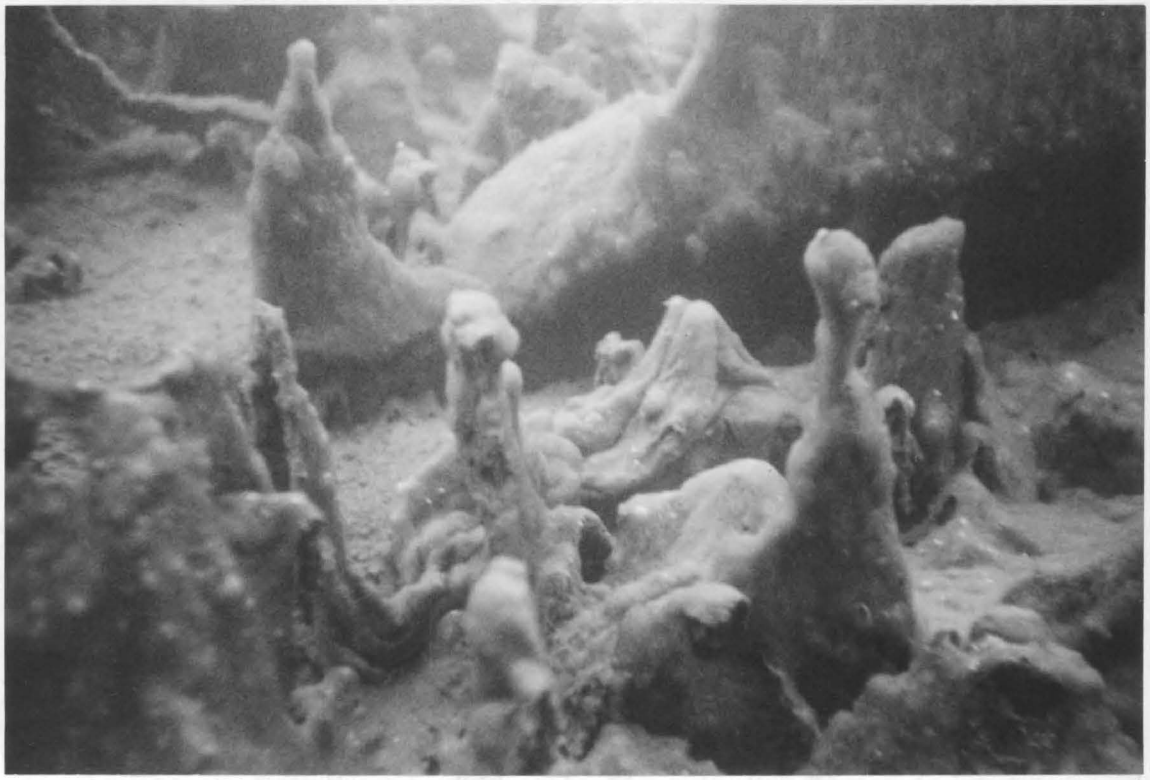
TABLE 8

PHYSICAL MEASUREMENTS FROM THE WATER ABOVE BENTHIC MATS IN LAKE FRYXELL AND LAKE HOARE, 1978-79 AUSTRAL SUMMER.[1]

	Date	Temperature	Conductivity	Salinity	%PAR
Fryxell					
ice/water interface	1/5/79	2.0	390	0.2	0
	1/6/79	1.3	930	0.8	1.4
mat surface	1/5/79	4.0	4700	4.75	0
	1/6/79	3.8	4200	4.5	0.13
Hoare					
ice/water interface	12/30/78	0	340	0	0.18
	1/11/79	-0.25	350	0.5	0.87
mat surface	12/30/78	0	465	0.1	0.13
	1/11/79	-0.25	450	0.5	0.78

[1] %PAR = % of photosynthetically available light reaching the mats; temperature as ^{o}C; conductivity as $\mu mhos/cm^2$; salinity as ppt.

FIGURE 8. Benthic or attached algal community beneath the permanent ice layer of Lake Hoare.



and no data was collected on the N_2 composition of these waters which are effectively sealed by a permanent cap of ice. The amounts of combined nitrogen are fairly low in Lake Fryxell, while nitrates exceeded 3 μM NO_3-N in Lake Hoare (Table 6). A well developed benthic algal mat community was found that was strikingly different in appearance (Figure 8) and composition (Table 7) than those in the littoral and peripheral waters. A lack of heterocystous bluegreen algae and abundance of diatoms in these mats was the most significant observation and related directly to the absence of C_2H_2 reduction. These algal mats have the capacity for anaerobic microenvironments within the mat that could allow nitrogenase activity. At Lake Fryxell an anaerobic layer was found to develop 2-4 cm below the surface of the mats, while none was detected in the mats from Lake Hoare. If anaerobic fixation occurs, it would not have been detected by this assay. The absence of heterocystous bluegreen algae and low light levels indicate aerobic N_2 fixation was not present or undetectable in the benthic communities studied.

Few differences occurred in the water chemistry that would explain the localization of nitrogenase activity, while areas receiving maximal sunlight had nitrogen(C_2H_2)-fixation and heterocystous bluegreen algae. The possibility that mats are able to absorb light and create elevated temperatures in microhabitats has been proposed (Goldman, 1972; Fogg and Stewart, 1968) and may explain why littoral and peripheral regions are the sites of C_2H_2 reduction. Further work is necessary to determine if temperatures in the mat are actually elevated relative to the ambient temperature and see if this relates to the differences seen between the

mats of Chad and Fryxell for instance.

^{15}N Fixation Attempts

The direct assay for nitrogenase activity using $^{15}N_2$ showed no detectable uptake after 12 h incubation *in situ*. The low C_2H_2 reduction rates seen in a parallel run indicate that a longer incubation period would have been necessary for detection by this less sensitive stable isotope method. Future work would be improved by determinations of a ratio between nitrogen fixed and C_2H_2 reduced in order to directly verify N_2 fixation and estimate the contribution of fixed nitrogen by the mat communities to the lakes.

CONCLUSIONS

Nitrogen(C_2H_2)-fixation is not a phenomenon localized in one lake in Southern Victorialand, but is likely to occur in any available site that provides sufficient moisture and light along with low fixed available nitrogen. It is possible that areas with a large drainage area such as Canopus Lake would have concentrations of fixed nitrogen which would repress nitrogenase synthesis. However, few areas were seen where this occurred. In none of the sites studied was repression by natural concentrations of fixed nitrogen conclusively shown. However, the high nitrate concentration in the benthic region of Lake Hoare might partially explain the lack of nitrogenase activity. The littoral moats and peripheral waters had maximal sunlight and moisture during the austral summer and were the sites where nitrogen(C_2H_2)-fixing activity was located. Heterocystous bluegreen algal species were found at sites where C_2H_2 reduction occurred (i.e., Nostoc commune, Anabaena sp. and Calothrix braunii). These algal genera are known nitrogen fixers and are probably responsible for the observed nitrogen fixation. Nitrogen fixation by heterotrophic bacteria probably was insignificant since there was no stimulation by glucose or succinate additions, while the absence of light drastically reduced the observed nitrogenase activity. Nitrogen fixation by photosynthetic bacteria and anaerobes cannot be ruled out since they were not isolated and the C_2H_2 reduction method used would inactivate them. Significant C_2H_2 reduction occurs in the littoral moat mats and mats in the peripheral lake waters, while no nitrogenase activity was detected in the benthic algal mats. Greater rates of

C_2H_2 reduction occur in the peripheral waters of Lake Hoare than in the other peripheral water areas. Thus, this site had the greatest potential for fixation studied. Fryxell littoral moat mats had significantly greater nitrogen(C_2H_2)-fixation than the littoral mats of Lake Chad, while no assays were performed on the moat mats of Lake Hoare. The observed nitrogen(C_2H_2)-fixation may contribute significantly to the nitrogen balance in the mat community. If a conservative estimate of nitrogen fixation was extrapolated from these data (i.e., using 25:1 ratio of C_2H_2 reduced to N_2 fixed), however, the values are extremely low; Only 7 x 10^{-6}ng N_2/m^2/day in Chad littoral moat mats and 1 x 10^{-3} ng N_2/m^2/day in Lake Fryxell littoral moat mats would be expected. Even if the entire littoral moat area of these lakes is considered, the ecological importance of N_2 fixation remains small or only locally important over a short period of time. However, if the small amounts of nitrogen fixed annually were not lost from these lakes, the accumulation over many years might well be significant ecologically.

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APPENDIX 1. EUKARYOTIC ALGAL SPECIES IN THE ALGAL MATS OF LAKE FRYXELL, 1978-79 AUSTRAL SUMMER.[1]

	SITE 2 - BENTHIC SITE					
DATE:	1-5-79	1-7-79	1-7-79	1-7-79	1-7-79	1-7-79
Crysophyta						
Fragilaria virescens var. *capitata*		+	+	+	+	+
Stauroneis anceps	+	+	+	+	+	+
Caloneis ventricosa	+	+	+	+	+	+
Navicula muticopsis	+	+	+	+	+	+
Navicula cryptocephala	+	+	+	+	+	+
Navicula laticeps		+	+	+	+	+
Navicula fragilarioides						
Navicula pelliculosa						
Navicula ilopangoensis	+					
Nitzschia frustulum			+	+	+	+
Hantzschia amphioxys	+	+	+	+	+	+
Pyrrhophyta						
Chlorophyta						
Non-Pigmented Flagellates						
Bodo sp.	+					
Monas sp.	+					
			A*	B*	C*	D*

[1] + = identified from sample; * = A through D are samples from a mat profile, A being mat surface and D deepest layer in the mat.

APPENDIX 1. CONTINUED.

	SITE 3 - LITTORAL MOAT				
DATE:	12-28-78	1-6-79	1-6-79	1-7-79	1-20-79
Crysophyta					
Fragilaria virescens var. *capitata*					
Stauroneis anceps	+		+		+
Caloneis ventricosa		+	+		+
Navicula muticopsis		+	+		+
Navicula cryptocephala			+		
Navicula laticeps		+			
Navicula fragilarioides				+	
Navicula pelliculosa		+	+		
Navicula ilopangoensis					
Nitzschia frustulum					
Hantzschia amphioxys	+	+	+	+	+
Pyrrhophyta					
Chlorophyta					
Non-Pigmented Flagellates					
Bodo sp.					
Monas sp.				+	

APPENDIX 2. EUKARYOTIC ALGAL SPECIES IN THE ALGAL MATS OF LAKE CHAD, 1978-79 AUSTRAL SUMMER.[1]

	SITE 11 - LITTORAL MOAT					
DATE:	1-26-79	1-26-79	1-26-79	1-26-79	1-26-79	1-26-79
Crysophyta						
Stauroneis anceps	+					+
Caloneis ventricosa		+	+	+	+	+
Navicula muticopsis						+
Navicula laticeps						+
Hantzschia amphioxys						+
Gloeochrysis pyreniger						+
Ochromonas sp.				+	+	
Pyrrhophyta						
Chlorophyta						
Chlamydomonas acuta			+	+	+	+
Chlamydomonas intermedia						+
Chlorella vulgaris		+				
Chlorococcum sp.				+	+	
Tetracystis sp.	+					
Chlorosarcinopsis sp.		+	+			
Cylindrocystis brebissonii				+	+	
Non-Pigmented Flagellates						
Bodo sp.						+
Monas sp.						
	A*	B*	C*	D*	E*	

[1] + = identified from sample; * = A through E are samples from a mat profile, A being surface and E being deepest portion of the mat.

APPENDIX 2. CONTINUED.

	SITE 9 overflow	SITE 10 peripheral water	SITE 11 moat	SITE 12 meltstream
DATE:	1-26-79	1-26-79	1-26-79	1-26-79
Crysophyta				
Stauroneis anceps				+
Caloneis ventricosa				+
Navicula muticopsis				+
Navicula laticeps				
Hantzschia amphioxys		+		+
Gloeochrysis pyreniger				
Ochromonas sp.				
Pyrrhophyta				
Chlorophyta				
Chlamydomonas acuta				
Chlamydomonas intermedia				
Chlorella vulgaris				
Chlorococcum sp.				
Tetracystis sp.				
Chlorosarcinopsis sp.				
Cylindrocystis brebissonii	+			
Non-Pigmented Flagellates				
Bodo sp.				
Monas sp.			+	

+ = identified from sample

APPENDIX 3. EUKARYOTIC ALGAL SPECIES IN THE ALGAL MATS OF LAKE HOARE, 1978-79 AUSTRAL SUMMER.[1]

	SITE 6 benthic site				SITE 7 moat	SITE 8 peripheral water
DATE:	1-11-79	1-13-79	1-18-79	1-18-79	1-18-79	1-18-79
Crysophyta						
Fragilaria virescens var. *capitata*		+		+		
Cymbella sp.				+		
Stauroneis anceps		+	+		+	+
Caloneis ventricosa	+	+	+	+	+	+
Navicula muticopsis	+	+	+	+		
Navicula mutica var. *cohnii*				+		
Navicula cryptocephala	+	+	+			
Navicula laticeps		+		+		
Navicula pelliculosa		+	+			
Nitzschia frustulum		+				
Hantzschia amphioxys	+	+	+	+	+	+
Pyrrhophyta						
Gymnodinium sp. (antarctica?)		+				
Chlorophyta						
Chlorococcum sp.			+			
Non-Pigmented Flagellates						
Bodo sp.				+		
Monas sp.	+					

[1] + = identified from sample

APPENDIX 4. BLUEGREEN ALGAL (CYANOBACTERIAL) SPECIES AS A PERCENTAGE OF TOTAL CELL NUMBER IN THE ALGAL MATS OF LAKE FRYXELL, 1978-79 AUSTRAL SUMMER.[1]

	SITE 2 - BENTHIC SITE					
DATE:	1-5-79	1-7-79	1-7-79	1-7-79	1-7-79	1-7-79
Heterocystous						
Nostoc commune						
Nostoc longistaffi						
Anabaena sp.						
Non-Heterocystous						
Schizothrix antarctica			+			
Phormidium angustissimum		2%		+		
Phormidium frigidum	98%	98%	20%	10%	2%	2%
Phormidium fragile						
Phormidium priestleyi			+			
Microcoleus paludosus						
Porphyrosiphon notarisii						
Lyngbya martensiana			10%	+		
Oscillatoria retzii						
Oscillatoria limosa						
Chroococcus turgidus						
Pleurocapsalean BG						2%
			A[2]	B[2]	C[2]	D[2]

[1] % = the contribution of the indicated blue-green alga to the total algal biomass is estimated by the number to the left of the percentage sign. + = the alga indicated was identified from the sample but was not a significant portion of the total biomass.

[2] These samples were taken at decreasing depth in the mat with A being surface and D being sediment.

APPENDIX 4. CONTINUED.

	SITE 3 - LITTORAL MOAT				
DATE:	12-28-79	1-6-79	1-6-79	1-7-79	1-20-79
Heterocystous					
Nostoc commune	90%	50%	90%	90%	75%
Nostoc longistaffi			+		
Anabaena sp.				+	+
Non-Heterocystous					
Schizothrix antarctica					
Phormidium angustissimum		+	+		+
Phormidium frigidum	5%	48%	2%	5%	23%
Phormidium priestleyi		+			
Microcoleus paludosus		+	3%	2%	1%
Porphyrosiphon notarisii					+
Lyngbya martensiana	5%	2%	3%	3%	1%
Oscillatoria retzii					+
Oscillatoria limosa	+	+	2%		
Chroococcus turgidus	+				
Pleurocapsalean BG					

% = the contribution of the blue-green alga indicated to the total algal biomass is estimated by the number to the left of the percentage sign. + = the alga indicated was identified from the sample but was not a significant portion of the total biomass.

APPENDIX 5. BLUEGREEN ALGAL SPECIES AS A PERCENTAGE OF TOTAL CELL NUMBER IN THE ALGAL MATS OF LAKE CHAD, January 26, 1979.[1]

	SITE 11 - LITTORAL MOAT					
	A	B	C	D	E	F
Heterocystous						
Nostoc commune	15%	4%	24%	1%	1%	17%
Nostoc longistaffi		+				+
Anabaena sp.						+
Calothrix braunii	+			+	+	2%
Non-Heterocystous						
Schizothrix antarctica	+					
Phormidium angustissimum	+	5%	3%			1%
Phormidium frigidum	85%	90%	72%	95%	95%	80%
Phormidium fragile						
Microcoleus paludosus			+	+	+	
Porphyrosiphon notarisii						+
Lyngbya martensiana	+			1%	1%	+
Oscillatoria priestleyi						
Oscillatoria limosa	+	+	1%			
Plectonema nostocorum				3%	+	
Microcystis stagnalis				+	+	+
Chroococcus pallidus			+	+	+	+
Chroococcus turgidus			+			+
Eucapsis alpina						
Pleurocapsalean BG						
	A[2]	B[2]	C[2]	D[2]	E[2]	

[1]% = the proportion of the blue-green algal species to the total algal biomass; + = species present but in trivial portion to the total algal biomass. Samples A-F taken at decreasing depth in the mat, A at the surface to F at the bottom.

APPENDIX 5. CONTINUED

	SITE 9 overflow	SITE 10 peripheral	SITE 12 moat	SITE 13 meltstream
DATE:	1-26-79	1-26-79	1-26-79	1-26-79
Heterocystous				
Nostoc commune	10%	65%	98%	10%
Nostoc longistaffi				
Anabaena sp.				1%
Calothrix braunii	10%	+		
Non-Heterocystous				
Schizothrix antarctica	20%			
Phormidium angustissimum	1%	+	+	+
Phormidium frigidum	9%	5%	2%	+
Phormidium fragile		+		
Microcoleus paludosus	10%	20%		25%
Porphyrosiphon notarisii	+	+		
Lyngbya martensiana		10%		25%
Oscillatoria priestleyi	10%			
Oscillatoria limosa				25%
Plectonema nostocorum				
Microcystis stagnalis				
Chroococcus pallidus				
Chroococcus turgidus	5%	+		5%
Eucapsis alpina				+
Pleurocapsalean BG	10%			

APPENDIX 6. BLUEGREEN ALGAL (CYANOBACTERIAL) SPECIES AS A PERCENTAGE OF TOTAL CELL NUMBER IN THE ALGAL MATS OF LAKE HOARE, 1978-79 AUSTRAL SUMMER.[1]

	SITE 6 benthic site				SITE 7 moat	SITE 8 peripheral
DATE:	1-11-79	1-13-79	1-18-79	1-18-79	1-18-79	1-18-79
Heterocystous						
Nostoc commune					4%	
Anabaena sp.					1%	5%
Non-Heterocystous						
Schizothrix antarctica				+		
Phormidium angustissimum				5%		
Phormidium frigidum	30%	10%	99%	95%	80%	+
Microcoleus paludosus					5%	+
Lyngbya martensiana	+	5%		+	10%	90%
Oscillatoria limosa						+
Chroococcus pallidus					+	+
Chroococcus turgidus						+
Eucapsis alpina						+

[1]% = the contribution of the blue-green alga indicated to the total algal biomass is estimated by the number to the left of the percentage sign. + = the alga indicated was identified from the sample but was not a significant portion of the total biomass.

APPENDIX 7

SITE DESCRIPTIONS OF THE AREAS STUDIED DURING THE 1977-78 AND 1978-79 AUSTRAL SUMMERS.

Sites 1 and 3. These two sites on Lake Fryxell's littoral moat vary only in their location on the lake. The sites melt during the short austral summer and freeze solidly the rest of the year. Moat water was fresh and less than 1 m deep. A thin but well developed algal mat covered the entire bottom of this site except for large rocks. This thin mat had two distinct layers - a fibrous underlying component and a loosely associated group of algae consisting mainly of Nostoc colonies. These Nostoc colonies grew up to 3 cm in diameter, indicating a relatively undisturbed system. Samples from Site 3 revealed that the blue-green algae present in the fibrous layer were Nostoc commune and Phormidium frigidum while the floating component was Nostoc commune.

Site 2. At this site in Lake Fryxell, whose mats were below 5 m of ice and in 0.3-3.0 m of additional water, SCUBA was used for the investigations. Only a small amount of light reached these mats, an important factor regulating this community's structure and function. Fresh water does not bathe these deeper mats. While the deep benthic algal mats covered most of the visible lake bottom at this site, mat clumps were lifted off, apparently by the upward force of entrapped gas bubbles within their matrices, leaving bare areas. Many such loose pieces of mat rose to the bottom of the ice. In some areas the mats were soft and over 8 cm thick, while in others a white mineral deposit formed a hard layer limiting the mats to a few centimeters in thickness. Observed temperature of the water ranged from 3.8-4.0 C (1/6/79). The

bluegreen algal species present in these mats were predominately Phormidium frigidum with one case of codominance with Lyngbya martensiana. No heterocystous bluegreen algae were seen at this site but a large number of diatoms were found.

Site 4. This was a very shallow (mean depth 10 cm) body of water separated from the rest of Lake Fryxell by a small barrier of soil. The entire bottom of the pool was covered by a thin red colored algal mat which, when peeled away from the substrate, showed green underneath. The water temperature was 4.5 C during the assay. The algal mat probably was composed of species of Nostoc and Phormidium, but identifications were not done.

Site 5. This site consisted of a wide area of shallow trickling meltstream entering Lake Fryxell from the Canada Glacier. Proceeding from the moraines of the glacier, the meltstream fanned out into a large area of moist soil, probably rich in nutrients, that supported a large expanse of algal and moss growth. Mats of Nostoc had developed in the shallow moat where the meltstream entered Lake Fryxell.

Site 6. The deep mat community of Lake Hoare was studied by SCUBA under a permanent ice layer 5-6 m thick. The benthic mats almost totally covered the bottom up to 3.5 m of free water, and no cessation of mat growth was observable. The water temperature above the mats ranged from 0 to 1.0 C. As with Lake Fryxell, the light penetrating to this depth was very low ranging from 0.11 to 0.78% of the photosynthetically available radiation. The low salinity and conductivity indicate fresher water over the deep mats of Hoare than Fryxell. Mats were observed to

detach and rise off the bottom where the clumps were trapped under the ice. Two macroforms of mat were observed. One was flattened and smooth but would occasionally pull up and be suspended by a piece of mat until it became free of its attachment and rose to the under ice surface. The second appeared to be a piled up smooth mat or a form of mat that became rougher and thicker than the first. The bluegreen algae in all these mats were primarily Phormidium frigidum accompanied by a large variety and number of diatoms, the latter especially in the gelatinous top layer of mat (ca. 1 cm).

Site 7. This site, located in the littoral ice-free moat of Lake Hoare, was seasonally affected. When the meltstreams from the Canada Glacier achieved maximum flow in late December, extensive melting occurred and pieces of ice scoured the area. With cessation of glacier inflow in mid-January the moat froze on the surface. The moat was explored using SCUBA and found to be completely cut off from the deeper waters and had mat growing in a shelf under the ice layer. There was little mat in this moat area except in the shelf mentioned. The paucity of mat could be due to the scouring by ice or the deepness of the moat with resulting loss of light insensity. The mats were found to contain mostly Phormidium frigidum and Lyngbya martensiana, but Nostoc commune and Anabaena sp. were also present.

Site 8. This shallow pool, separated by moraine material from the main body of Lake Hoare, had a maximum water depth of 0.6 m. The pool was frozen to the bottom on the first of December but by late-December only a thin layer of ice remained. The bluegreen algal mat present was

similar to that at Site 4, red on the surface and thin (ca. 5 mm). The mat covered the pool edges and down towards the center about 3 m. Identification of the algal species from freshly collected mat showed an abundance of Anabaena not reflected in subsequent identifications at VPI & SU. The dominant bluegreen was probably Anabaena along with Lyngbya martensiana as a codominant or subdominant.

Site 9. Seasonal overflow occurred from Lake Chad to Lake Hoare reaching a maximum of less than 3 cm depth and trickling at a rapid rate. Flow began in early January after the meltstream input from the Suess Glacier into Chad became heavy and continued to flow as late as 26 January 1979. On the bottom of this interlake overflow a reddish colored algal mat developed which was less than 1 cm thick. A wide variety of bluegreen algae occurred in the community, including two heterocystous forms with codominant non-heterocystous forms.

Site 10. This site consisted of a small pool (less than one square meter) off the edge of Lake Chad. The water was less than 8 cm deep. The algal community was black to dark green in color attached to the north side of a rock with part of the mat exposed above water. The major bluegreen algal component was Nostoc commune with Microcoleus paludosus and Lyngbya martensiana as codominants. Only one eukaryotic alga was identified here, the diatom Hantzschia amphioxys.

Site 11. In this moat area of Lake Chad with about 1 m of free water an extensive spongy mat community with red coloration had developed. The red coloration began at the lake edge and began turning grey-green at 1 m, by 1.5 m there was no red coloration. These mats covered

all of the eastern shoreline at Lake Chad as well as parts of the western shore. Phormidium frigidum was the major bluegreen alga with Nostoc commune as a codominant.

Site 12. This site comprised a moat region on the western shore of Lake Chad. The mat community was well developed as on the eastern shore but had clusters of black colored Nostoc commune colonies clinging to the spongy mat surface. There was little intermingling of these clusters of Nostoc with the rest of the mat. The mat extended from Site 13 to 60 m beyond Site 12 (Figure 3). The clusters of Nostoc associated with the spongy mat extended out into the lake 3 to 5 m where they disappeared, however, the spongy reddish colored mat continued out beyond this depth.

Site 13. At this delta region, very little mat development occurred. One of the seasonal meltstreams from the Suess Glacier entered Chad at this site, and the mat community was quite different, having four bluegreen algal codominants (i.e., Nostoc commune, Microcoleus paludosus, Lyngbya martensiana, and Oscillatoria limosa).

Site 14. Lake Miers, visited 14 December 1977, lacked a well developed littoral moat region. A small meltpool south of the lake was partially melted and possessed extensive algal mat with Nostoc commune dominating.

Site 15. Lake Brownworth, visited 21 December 1977, had a discontinuous ice-free moat region. The algal mat community in these regions, attached below 15-30 cm of water, spread throughout the moat area. These mats were 2.4-5.0 cm thick and had an orange coloration. Water

above these mats was at 3.0 C with 13.2 mg/l dissolved oxygen, 0.25 ppt salinity, and 350 μmhos/cm^2 conductivity. Light does not appear limiting since 98% of the photosynthetically available radiation reached the mat surface. The soil assay site was 2 m from the shore where the soil contained 8.4% moisture and was slightly above freezing during the assay (ca. 0.25 C). A 1:5 slurry of soil had a pH of 7.95, while the soil lost 1.0% on ignition.

Site 16. Lake Hoare, visited 18 January 1978, lacked melted littoral moat regions. No algal mat was found except in the meltstream beds. Soil from one of these meltstreams was above 0 C and contained 13.8% moisture.

Site 17. Lake Bonney, west lobe, studied 16 January 1978, was found to contain poorly developed algal mats. The mat was under ice near the Taylor Glacier and was grey and less than 0.5 cm thick.

Site 18. Lake Vanda, visited 20 January 1978, was found to have poorly developed algal mats similar to those described for Lake Bonney.

APPENDIX 8

METHOD FOR QUALITATIVE DETERMINATION OF OXYGEN IN MATS

Water from the mat was obtained by withdrawing a sample from a coring apparatus (Figure 1) with a plastic syringe fitted with a special needle (16 gauge, 7 inches long). Since only small samples were possible a qualitative modification of the Winkler techniques (G.M. Simmons, personal communication) was used to estimate the oxygen in the inter-mat water.

Water was withdrawn from the corer and put into a 7 ml vial without aeration. After flushing once with sample add the following Winkler reagents: 0.2 ml manganous sulfate reagent and 0.2 ml alkaline iodide solution. Seal with Parafilm and mixed by inversion. The manganous-manganic hydroxide floc was allowed to settle halfway and the sample remixed. After the floc settled for the second time 0.2 ml of conc. sulfuric acid was added and the vial resealed with Parafilm and a cap, then mixed. All samples were returned to Eklund Biological Laboratory and titrated with 0.0025 N sodium thiosulfate using a starch indicator. The milliliter of titrant used was multiplied by a correction factor of 4 to approximate the mg/l dissolved O_2.

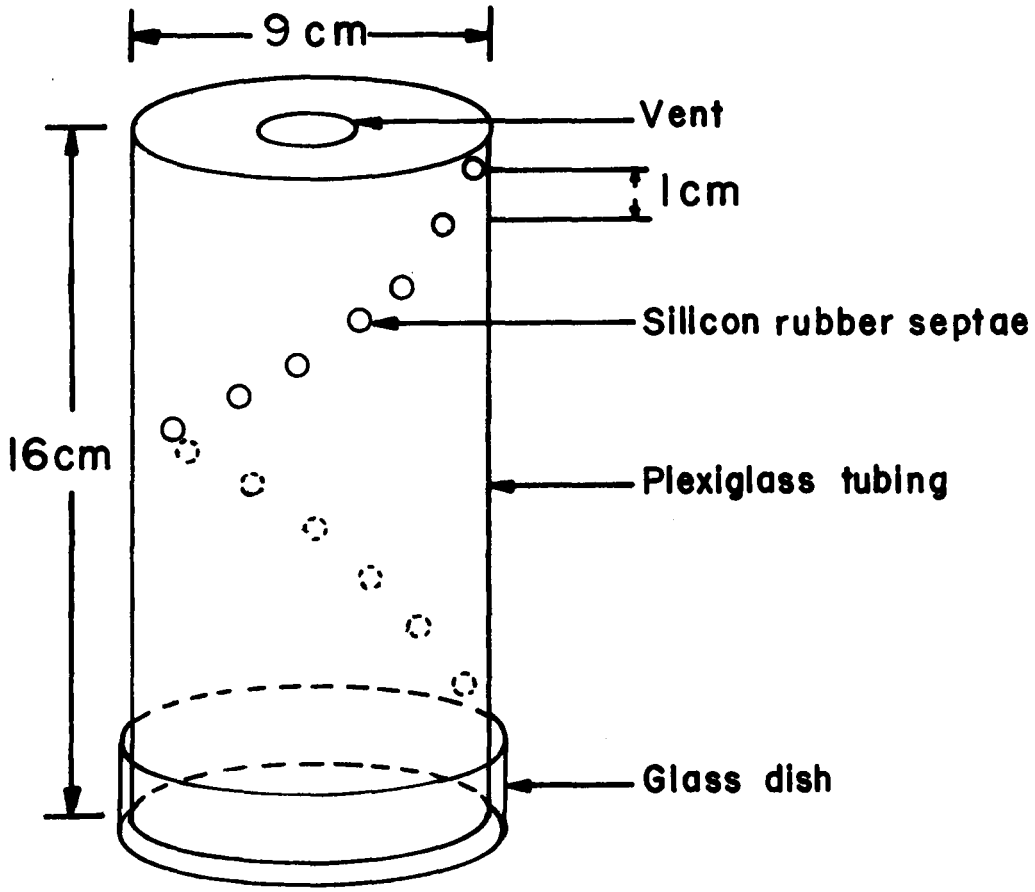

FIGURE 9. Coring apparatus for determination of oxygen content within algal mats.

APPENDIX 9

STANDING CROP OF VARIOUS ALGAL MAT COMMUNITIES OF LAKES CHAD, FRYXELL AND HOARE, 1978-79 AUSTRAL SUMMER.[1]

Lake	Date	Site	mgC/cm^2	s	n
Chad	1/26/79	Littoral moat	152.8	57.2	7
Fryxell	12/9/78	Benthic	66.8	34.0	7
	12/14/78	Benthic	59.6	12.0	8
	1/7/79	Benthic	60.4	12.2	8
	12/28/78	Littoral moat	100.3	19.6	8
	1/22/79	Littoral moat	47.4	14.7	6
Hoare	12/1/78	Benthic	138.6	11.2	3
	12/21/78	Benthic	55.7	11.3	7
	1/13/79	Littoral moat	43.8	28.0	8

[1]Standing crops determined using a 2.54 cm diameter core of mat which was analyzed for ash for dry weight using the method described in Rand (1975); s = standard deviation, n = number of observations.

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IN SITU NITROGEN(C_2H_2)-FIXATION IN LAKES OF SOUTHERN VICTORIALAND, ANTARCTICA

by

F.C. Thomas Allnutt

(ABSTRACT)

Nitrogenase fixation occurred in a number of habitats in and nearby several antarctic lakes. The observed acetylene reduction occurred in bluegreen algal mats in littoral areas that received maximal sunlight. The benthic bluegreen algal communities in reduced light under 5-6 m of permanent ice showed no detectable nitrogenase activity. The observed nitrogen fixation potential correlated with the presence of heterocystous bluegreen algae considered to be the major nitrogen fixing organisms in these habitats. The relatively low acetylene reduction rates suggest that a small but significant contribution of ammonia to these environments deficient in nitrogen may occur through nitrogen fixation.