Temporal and spatial variation of light, nutrients and phytoplankton production in Lake Bonney, Antarctica

by Thomas Robert Sharp

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences
Montana State University

Abstract:
Lake Bonney, Antarctica is characterized by a permanent 4 m thick ice cap, a dichothermic temperature profile and strong vertical conductivity, oxygen and nutrient gradients. The ice cap reduces under-ice irradiance to less than 50 μE m^12 s^-1 and, together with the chemical stratification, create a non-turbulent environment. Hypothetically, phytoplankton communities can be separated along resource gradients, because of the non-turbulent environment. Photosynthesis, growth and nutrient concentrations within these stratified phytoplankton communities were routinely measured over the 1989-1990 and 1990-1991 austral spring and summer and during the winter spring transition of 1991, to determine how these parameters vary during the phytoplankton growing season. Nutrient enrichment bioassays were used to test for nutrient deficiency of phytoplankton photosynthesis. A light-dependent phytoplankton growth model was developed. The results of this study indicate that 1) photosynthesis in the surface phytoplankton populations may intermittently be enhanced by N and P enrichments 2) maximum phytoplankton growth rates occur in December and January and 3) the efficiency of light utilization by the phytoplankton is high in comparison to other marine or freshwater phytoplankton. Net chlorophyll specific growth rates range from -0.071 to 0.151 d^-1. Model results indicate that gross chlorophyll specific growth rates range between 0.002 and 0.017 d^-1. The significance of the research is most applicable to other pelagic polar ecosystems and deep chlorophyll maxima in general.

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Thomas Robert Sharp

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style and consistency, and is ready for submission to the College of Graduate Studies.

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Date 30 JUNE 1993

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TABLE OF CONTENTS

TABLE OF CONTENTS-continued

LIST OF TABLES

LIST OF TABLES-continued

LIST OF TABLES-continued

LIST OF FIGURES

LIST OF FIGURES-continued

LIST OF FIGURES-continued

LIST OF FIGURES-continued

ABSTRACT

Lake Bonney, Antarctica is characterized by a permanent 4 m thick ice cap, a dichothermic temperature profile and strong vertical conductivity, oxygen and nutrient gradients. The ice cap reduces under-ice irradiance to less than 50 $\mu E\ m^{-2}\ s^{-1}$ and, together with the chemical stratification, create a non-turbulent environment. Hypothetically, phytoplankton communities can be separated along resource gradients, because of the non-turbulent environment. Photosynthesis, growth and nutrient concentrations within these stratified phytoplankton communities were routinely measured over the 1989-1990 and 1990-1991 austral spring and summer and during the winter spring transition of 1991, to determine how these parameters vary during the phytoplankton growing season. Nutrient enrichment bioassays were used to test for nutrient deficiency of phytoplankton photosynthesis. A light-dependent phytoplankton growth model was developed. The results of this study indicate that 1) photosynthesis in the surface phytoplankton populations may intermittently be enhanced by N and P enrichments 2) maximum phytoplankton growth rates occur in December and January and 3) the efficiency of light utilization by the phytoplankton is high in comparison to other marine or freshwater phytoplankton. Net chlorophyll specific growth rates range from -0.071 to 0.151 d^{-1}. Model results indicate that gross chlorophyll specific growth rates range between 0.002 and 0.017 d^{-1}. The significance of the research is most applicable to other pelagic polar ecosystems and deep chlorophyll maxima in general.

INTRODUCTION

The permanently ice-covered lakes of the dry valleys region of Antarctica have unusual physical, chemical and biological characteristics, making these lakes ideal systems for studying phytoplankton ecology, particularly the relationship between phytoplankton production and light. Photoautotrophic growth is restricted to the spring, summer and fall, because of the seasonal variation of light that these polar ecosystems receive. Photosynthesis is the primary source of organic carbon in most ecosystems (Odum 1959; Whittaker et al. 1975). This is especially true for antarctic lake ecosystems where allochthomous organic carbon inputs are low (Aiken et al. 1990). Therefore, as the first step to gaining a better understanding of the flux of carbon and energy within these lake ecosystems, it is necessary to determine the temporal and spatial variation of photoautotrophic production and the environmental factors which govern it.

Attempts to predict phytoplankton production from incident light have long been one of the primary objectives of limnologists and oceanographers (reviewed by Cullen 1990). Inductive (Talling 1957) and deductive (Platt et al. 1988) methods have been used to derive mathematical formulations for the relationship between incident light and primary production. Random vertical displacement of

the phytoplankton from turbulence limits the ability of the light-dependent phytoplankton production models to make accurate predictions (Marra 1978, Harris 1986, Vincent 1991).

The underwater light field to which phytoplankton photosynthesis is coupled varies from first and second order effects. First order effects that influence light availability to phytoplankton include latitude, meteorological events, and seasonal and diurnal variation in irradiance (flux). Second order effects influencing light availability to phytoplankton are the vertical displacement of phytoplankton in the water column, which may result from either turbulent mixing or the migration of motile phytoplankton (Harris 1986).

Variations in light field experienced by phytoplankton are partially dependent on the physical structure of the water column. Turbulent mixing will occur, if the stress generated by the wind has sufficient force to overcome the potential energy of the density stratification of the water column. Phytoplankton will undergo random vertical displacement and experience a fluctuating light field when the water column is mixed (Spigel and Imberger 1987).

Phytoplankton communities growing below the mixed layer have been termed deep chlorophyll maxima and are common features of both freshwater and marine systems (Cullen 1982, Priscu and Goldman 1983). Other analogous

situations where algae are not subject to turbulent mixing and experience only first order variations in light are sea ice algae (Palmisano et al 1985, Lizotte and Sullivan 1991), snow algae (Thomas 1972, Mosser et al. 1977) and the phytoplankton communities of the dry valley lakes (Koob and Leister 1972, Vincent 1981, Priscu et al. 1987, 1988, Lizotte and Priscu 1992).

The constancy of the light field, experienced by these spatially stable algal communities (compared to phytoplankton in a turbulent environment), offers the opportunity for the phytoplankton to optimize photosynthesis by acclimating their photosynthetic apparatus to a specific light field. When the underwater light field varies at long time scales (months) a phytoplankton community responds by successional changes in community structure (Harris 1986). At short time scales (hours) phytoplankton respond by adjusting their photosynthetic apparatus (Perry et al 1981, Harding et al 1985, Sukenik et al 1990). Examples of short-term variations in photoacclimation resulting from varying underwater irradiance include changes in photosynthetic capacity (Marra 1978, Neale and Marra 1985) and the kinetics of photoinhibition (reviewed by Neale 1987). These short-term variations may significantly influence <u>in situ</u> phytoplankton production (Neale et al. 1991).

In the non-turbulent antarctic dry valley lakes, phytoplankton communities experience a light field that varies on daily and seasonal time scales only (Vincent 1981, Priscu et al. 1987). This constancy should eliminate the need for short-term variations in the photosynthetic response of phytoplankton. In this study, Lake Bonney (one of the lakes in the dry valleys) was examined over three field spring-summer seasons (September through January) to determine which environmental factors may control phytoplankton production and how phytoplankton production responds to the seasonal variation in irradiance and day length.

In many respects, because of the permanent ice cover, amixis, and simplified food webs, the dry valley lakes resemble experimental mesocosms (Goldman 1964). Vertical water movement in the water column of Lake Bonney is on the scale of molecular diffusion because the permanent ice cap prevents wind induced turbulence and strong density gradients over the water column increase stability (Spigel et al. 1990). Owing to its latitude, approximately 77° S, the phytoplankton experience continuous dark for four months and continuous light for four months separated by two months of twilight (Priscu 1989). Lake Bonney also lacks crustaceous zooplankton and fish, which may graze on the phytoplankton (Parker and Simmons 1985) and confound the determination of phytoplankton production and growth

(Welschmeyer and Lorenzen 1985). Lake Bonney is essentially a closed hydraulic system lacking a surface outlet and receiving intermittent stream inflow for only about two months during the summer. Additionally, the layered phytoplankton communities in Lake Bonney (Koob and Leister 1972) have been compared to a series of deep chlorophyll maxima (Lizotte and Priscu 1992). Finally, these communities are among the most shade adapted yet recorded for phytoplankton. Physiological studies have sought to reveal photosynthetic mechanisms which enable the phytoplankton to survive in this low light environment (Goldman 1964, Goldman et al. 1967, Parker et al 1977, Vincent 1981, Parker et al. 1982, Seaburg et al. 1983, Vincent and Vincent 1983, Priscu et al. 1987 and 1988, Neale and Priscu 1990, Priscu et al. 1990, Lizotte and Priscu 1992).

While most of the recent research on the phytoplankton of the dry valley lakes has examined the factors which influence photosynthesis, very little is known about the seasonal and vertical development of phytoplankton biomass and production (Priddle et al. 1986). Vincent (1981) suggested that the period of maximum growth for phytoplankton in the dry valley lakes occurs during the winter-spring transition after nutrients have accumulated from remineralization over the winter and irradiance rises above the compensation point for net photosynthesis.

However the data to test this hypothesis were not available to Vincent. Also with one exception (Priscu et al. 1987), no estimates of *in situ* specific growth rates have been reported.

Priscu et al. (1987) found that phytoplankton from two dry valley lakes had surprisingly high growth rates (0.002-0.046 d^{-1}), despite the low irradiances. They speculated that the high growth rates resulted from the extreme hydraulic stability of the water column which allows the phytoplankton to acclimate to the environmental conditions, especially light, at a specific depth.

HYPOTHESES AND OBJECTIVES

In this study, the following hypotheses were tested:

1) Phytoplankton growth rates in Lake Bonney are greatest during the winter-spring transition when nutrient concentrations should be highest after remineralization over the winter and irradiance rises above the compensation irradiance for net photosynthesis;

2) The high degree of photoacclimation by Lake Bonney phytoplankton is reflected by a high efficiency of light utilization.

To test these hypotheses the following objectives were undertaken:

1) Describe the temporal and spatial distribution of nutrients, phytoplankton biomass and production in terms of the seasonal variation in light;

2) Use nutrient enrichment bioassays to test for temporal variation of nutrient deficiency of phytoplankton photosynthesis;

3) Develop a light-dependent phytoplankton growth model to predict gross specific growth rates.

DESCRIPTION OF STUDY SITE

Geography and Geology

Lake Bonney is located at the head of the Taylor Valley in the dry valleys region of Southern Victoria Land, Antarctica (77°10′-77°45′ S, 160°20′-160°00′ E). The dry valleys region of Southern Victoria Land is an ice-free area of the antarctic continent covering about 3700 km^2. The Taylor Valley extends from McMurdo Sound to the terminus of the Taylor Glacier. Lakes Fryxell, Hoare, Chad, and Mummy Pond are also located in the Taylor Valley. The annual mean temperature is between -20 and -25°C and annual mean humidity is about 50% (Heywood 1984). The area receives about 10 cm of precipitation per year, making it the most arid region in Antarctica (Heywood 1984). Mummified seal carcasses have been radio-dated at between 300 and 1250 years old, indicating the area has been arid for at least 300 years (Olsen and Broecker 1961).

Bedrock consists of gneiss, marble, schist, and metagreywacke, which have been intruded by granite and covered by shales and sandstones (Clark 1965). Plutonic intrusions of dolerite are common and break up the tan color of the peri-glacial landscape.

Previous Limnological Studies on Lake Bonney

Scientific study of the dry valley lakes began in 1961 when the ice cover of Lakes Bonney and Vanda was first penetrated (Angino et al. 1963). Initial studies on the dry valley lakes were primarily geochemical in nature. Goldman (1964) conducted the first biological investigations in Lake Bonney in the austral summer of 1961-62 and concluded that the phytoplankton were highly shade-adapted. Koob and Leister (1972) made a more detailed study of the phytoplankton populations and production during 1964-65 austral summer. They discovered three distinct phytoplankton communities and primary productivity maxima. An extensive algal mat community was reported in the benthos on the eastern end of Lake Bonney (Parker et al. 1977).

METHODS

Routine Data Collection

Data collected from the center of the east lobe are presented in this thesis (station E30, see Spigel et al in press). Samples were collected over the austral spring and summer (October-January) during 1989-1990 and 1990-1991, and over the winter-spring (September-December) transition during 1991. To reduce confusion with the 1990-1991 season, I will refer to the 1991 winter-spring transition as the 1991-1992 sampling season, although no data were collected in 1992. The only data presented from the 1989-1990 season are nutrient concentrations of lake water and glacial stream samples and phytoplankton biovolume. The dates and routine sampling depths for chlorophyll a (CHL), primary production (PPR), NH_4^+, NO_2^- + NO_3^-, and soluble reactive phosphorus (SRP) are listed in Table 1.

The ice of Lake Bonney was penetrated with a 25 cm or a 10 cm ice auger. When the 10 cm ice auger was used, the hole was enlarged by melting with a copper pipe through which heated glycol was circulated. The ice hole was allowed to cool before sampling. All depths are measured from the piezometric water level in the ice hole (usually about 25 cm below the ice surface). This avoids confusion caused by measuring depths from the top or bottom of the

Table 1. Inventory of depths analyzed for CHL, PPR, and nutrients.

Sampling Dates	CHL	PPR	NH_4^+	$NO_2^- + NO_3^-$	SRP
24 Nov 1989	A	ND	A	A	A
14 Dec	A	ND	A	A	A
16 Jan 1990	A	ND	A	A	A
30 Oct	B	B	B,C	ND	B,C
6 Nov	B	B	B,C	ND	B,C
13 Nov	B,H	B	ND	ND	ND
20 Nov	B	B	B,C	ND	B,C
27 Nov	B,C	B	B,C	ND	B,C
4 Dec	B,C	B	ND	ND	ND
11 Dec	B,C	B	ND	ND	ND
20 Dec	B,C	B	B,C	ND	B,C
3 Jan 1991	B,C	B	ND	ND	ND
11 Jan	B,C	B	ND	ND	ND
22 Jan	B,C	ND	ND	ND	ND
9 Sep	C,D	E	C,D	C,D	C,D
14 Sep	C,D	E	C,D	C,D	C,D
19 Sep	C,D	E	C,D	C,D	C,D
24 Sep	C,D	E	C,D	C,D	C,D
29 Sep	C,D	D	C,D	C,D	C,D
3 Oct	C,D	E	C,D	C,D	C,D
10 Oct	C,D	E	C,D	ND	C,D
17 Oct	C,F	F	C,F	ND	C,F
24 Oct	C,F	F	C,F	C,F	C,F
31 Oct	C,F	F	C,F	ND	C,F
6 Nov	C,G	G	C,G	C,F	C,G
13 Nov	C,G	G	C,G	ND	C,G
19 Nov	C,G	G	C,G	C,F	C,G
25 Nov	C,G	G	C,G	ND	C,G
1 Dec	C,G	G	C,G	C,F	C,G

A=4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 35 m
B = 4.5, 6, 8, 10, 13, 15, 16, 17, 18 and 20 m
C = 23, 26, 29, 32, and 35 m
D = 3.9, 4.5, 6, 8, 10, 13, 15, 16, 17, 18 and 20 m
E = 3.9, 4.5, 6, 8, 10, 13, 15, 16, 17, and 20 m
F = 3.9, 4.5, 6, 8, 10, 12, 13, 16, 17, 18 and 20 m
G = 3.9, 4.5, 6, 8, 10, 12, 13, 15, 16, 17, 18 and 20 m
H = 23 and 26 m
ND = not determined

ice as the ice thickness may change over the summer; however the depths were displaced when substantial run-off occurred in January of 1991.

Physical and Chemical Measurements

Water temperature was measured from under the ice to the bottom with a YSI model 57 temperature probe. Dissolved oxygen concentrations were determined by a modified (for supersaturation) Winkler titration method (APHA 1985).

During 1990-1991, surface irradiance was monitored with a Li-Cor 2π quantum sensor (400-700 nm) and logged onto a Campbell data logger, while under-ice irradiance was monitored at 10 m depth with a Li-Cor 4π quantum sensor and logged with a Li-Cor model Li-1000 data logger. In 1991-1992 surface and underwater irradiance were logged contemporaneously with a Li-Cor model LI-1000 data logger connected to both the 2π and 4π Li-Cor quantum sensors, respectively.

Water column transparency was determined at approximately weekly intervals from irradiance profiles (400-700nm) made with a 4π quantum sensor. Because light is attenuated exponentially in the water column, the data were log transformed and a linear regression model was applied over the depths where the relationship between log transformed data and depth appeared linear. This gave the

diffuse attenuation coefficient for photosynthetically available radiation (the intrinsic rate at which light is attenuated) over the water column (Wetzel 1983). The extinction coefficients were used to extrapolate from irradiance monitored at 10 m to other depths.

A 2.2 L Niskin bottle with a teflon coated spring was used to collect water from each of the routine sampling depths for measurement of nutrient concentrations, dissolved inorganic carbon (DIC), chlorophyll a (CHL) concentrations, phytoplankton numbers, biomass and photosynthesis. One liter high density polyethylene (HDPE) bottles were rinsed 3 times with sample, filled and placed in a cooler. The remainder of the sample in the Niskin bottle was used to measure photosynthesis in situ.

The cooler containing the samples was returned to a shoreside laboratory. One to three hundred mL of sample was vacuum filtered (< 0.3 atm) through 2.5 cm Whatman GF/F (1989-1990) or GF/C (1990-1991 and 1991-1992) filters. The filtered sample was used for chlorophyll a determinations. Filtrate was collected in acid washed 250 mL sidearm flasks. The filtrate was then transferred to acid washed 125 mL HDPE bottles and frozen until analysis for NH_4^+, NO_2^- + NO_3^-, and soluble reactive phosphorus (SRP) at the Eklund Biology Center (EBC), McMurdo Station. Nutrient concentrations for each of the glacial meltwater streams flowing into Lake Bonney were analyzed once in 1989-1990.

The limit of detection (defined as three times the sample standard deviation plus the blank signal, see Miller and Miller 1988) of each method is listed in Table 2.

Ammonium concentrations were determined in 10 mL samples by the blue indophenol reaction between NH_4^+, phenol and hypochlorite at high pH (Solorzano 1969). Water samples below 14 m in 1989-1990 and 1990-1991 and below 16 m in 1991-1992 were diluted 9:1 with deionized water (9 parts deionized water (18 Mohm) to 1 part sample), to prevent salt interference of the color forming reaction. A 1 cm pathlength cell in a Beckman DB-G spectrophotometer was used for all determinations.

Table 2. The average limit of detection for NH_4^+, NO_2^- + NO_3^-, and SRP methods. L.O.D. = limit of detection.

Nutrient	Season	L.O.D. (μM)
NH_4^+	1989-1990	1.541
	1990-1991	0.850
	1991-1992	0.474
NO_2^- + NO_3^-	1989-1990	1.870
	1991-1992	0.805
SRP	1989-1990	0.112
	1990-1991	0.071
	1991-1992	0.040

The effect of freezing the sample on NH_4^+ concentrations was tested on samples representative of the upper freshwater layer (4.5 m), chemocline (17 m) and deep salt layer (35 m). Samples were refrigerated (4°C) or frozen (-80°C) for five days before analysis. There was no

significant difference between frozen and refrigerated samples (Table 3).

Table 3. Comparison of frozen and unfrozen samples analyzed for NH_4^+ (μM). S.D. = standard deviation.

Depth (m)	Refrigerated Average ± S.D.	Frozen Average ± S.D.
5	0.78 ± 0.03	0.72 ± 0.07
17	27.37 ± 0.34	27.77 ± 0.35
35	133.48 ± 5.327	128.44 ± 2.43

In 1989-1990, NO_3^- and NO_2^- concentrations were determined by reducing NO_3^- to NO_2^- by a cadmium-copper reduction column at pH = 8.3, with subsequent measurement of NO_2^- by diazotizing with sulfanilamide and coupling with NAD dihydrochloride. The method was followed as outlined in Standard Methods (APHA 1985), 20 mL of the ammonium chloride EDTA buffer was added to each 10 mL sample before the reduction step. In 1991-1992, NO_2^- + NO_3^- was measured by reducing NO_3^- to NO_2^- with spongy cadmium (Jones, 1984) and measuring the resulting NO_2^- with the NED/sulfanilamide method. Samples from 1990-1991 were not analyzed for NO_2^- + NO_3^- at the time of data analysis. A one cm cell pathlength in a Beckman DB-G was used in all determinations.

The ammonium molybdate/potassium antimonyl tartrate method was used for the determination of soluble reactive phosphorus (SRP). The method was followed as in Downes (1978) with the exception that the reduction step, used to

remove arsenate interference, was omitted. In 1989-1990 and 1990-1991, water samples from depths below 15 m were diluted 1:1 (1 part sample:1 part deionized water) and below 20 m were diluted 9:1 (deionized water to sample). A 1 cm pathlength cell was used in a Perkin-Elmer λ 4 Spectrophotometer for color determination. In 1991-1992 none of the water samples for SRP were diluted and a 10 cm pathlength cell in a Perkin-Elmer λ 4 Spectrophotometer was used.

Average recovery efficiencies for the three nutrient analyses were determined on samples collected from depths representing the upper freshwater layer (4.5 m), chemocline (17 m) and deep saline layer (35 m) (Table 4). Recovery efficiencies were not used to correct the sample concentrations.

Table 4. Average recovery efficiencies + coefficent of variation for NH_4^+, NO_3^-, and SRP from 4.5, 17, and 35 m samples. The number of replicate groups tested for each assay are in parenthesis. ND = not determined.

Assay	Season	4.5 m	17 m	35 m
$NH4^+$	1989-1990/ 1990-1991/ 1991-1992	113 ± 9 (4)	122 ± 28 (4)	103 ± 33 (4)
NO_3^-	1989-1990	110 (2)	116 ± 6 (3)	96 (2)
	1991-1992	133 (1)	100 (1)	105 (1)
SRP	1989-1990/ 1990-1991	110 (2)	96 ± 2 (3)	100 (1)
	1991-1992	99 (1)	100 (1)	ND

Biological Data Collection

Chlorophyll a

Chlorophyll a (CHL) concentrations were determined from 100 to 300 mL of lake water filtered through 2.5 cm Whatman GF/C glass fiber filters. Samples were immediately frozen until analysis at the Eklund Biology Center (<2 months). Samples were not routinely duplicated until after 27 November 1991.

CHL was extracted from the frozen sample filters in 10 mL of 90% acetone in 20 mL glass scintillation vials. Samples were vortexed for 1 minute and then allowed to extract for 12-24 hours at 4° C in the dark. Tests showed that this extraction procedure was as efficient as extraction by homogenization (Lizotte and Priscu, unpublished data). Extracted CHL was measured fluorometrically in a Turner Design model 10 fluorometer calibrated with standard concentrations of purified CHL (Sigma Chemical). To correct for phaeopigment fluorescence, extracts were read before and after acidification with 0.2 mL of 1N HCl (Holm-Hansen et al. 1965).

Phytoplankton Biovolume

Samples for phytoplankton identification and enumeration were collected from the same water sample used

to measure photosynthesis, CHL, and nutrients. Table 5 lists the phytoplankton samples that were analyzed. Phytoplankton samples were preserved with acid-Lugol's solution (APHA 1985) immediately upon collection (final concentration = 1%). One hundred mL samples were settled for at least 5 days in Utermohl chambers and cells were identified (Seaburg et al. 1979) and enumerated using the inverted microscope technique (Lund et al. 1957).

The dimensions of the phytoplankton were measured and cell volumes were calculated using appropriate geometrical equations. The density of the cells was assumed to be the same as water, and cell volume was converted to biomass (g C) by the equation:

1) $\log_{10} C = 0.94 * (\log_{10} \text{biovolume}) - 0.60$

(Smayda 1978), where C is grams carbon and biovolume is cell volume (μm^3).

Table 5. Inventory of phytoplankton samples analyzed.

Sampling Date	Depths 4.5	6	8	10	13	15	16	17	18	20
24 Nov 1989	X	X	X	X	X	X		X	X	X
14 Dec	X	X	X	X	X	X	X	X	X	
16 Jan 1990	X	X	X	X	X	X	X	X	X	
30 Oct	X	X		X		X	X		X	
13 Nov	X	X	X	X	X	X		X		
27 Nov	X	X	X	X		X	X		X	X
11 Dec	X	X		X		X			X	
20 Dec	X	X	X	X	X			X		
3 Jan 1991	X	X		X	X		X	X	X	
22 Jan		X		X		X				

Phytoplankton Productivity: Measurement and Verification

Photosynthesis was measured in situ by the ^{14}C method. Two clear and one opaque acid washed 145 mL Pyrex glass screw-top bottles were rinsed 3 times with sample, filled gently, and placed in a darkened box until all the samples were collected. The entire sampling procedure was done under a tent and took about an hour.

An ampulated working solution of ^{14}C-CO_3^{-2} (120 μCi/mL, 1990-1991; 121 μCi/mL, 1991-92) was added to the samples as a tracer to measure photosynthesis. The tracer was injected into the samples with a Gilson P1000 Pipetman. In 1990-1991, 26.4 μCi and 84.0 μCi of ^{14}C-carbonate were added to samples from 4.5 to 10 m and 13 to 20 m, respectively. In 1991-1992, 26.6 μCi and 69.0 μCi of tracer were added to samples from 3.9 to 10 m and 13 to 20 m, respectively. On 9 September 1991, 53.2 μCi and 157.3 μCi were added to samples from 3.9 to 10 m and 13 to 20 m, respectively. To samples from 12 m, 42.4 μCi was added, during both 1990-1991 and 1991-1992. A bubble was left in the bottle to help mix the tracer into the sample especially at deeper depths where the difference in density between the ^{14}C solution and sample was greatest.

After all the samples were inoculated, samples were taken from the tent in the darkened box to the ice hole and resuspended from a nylon cord at the depth of collection for 24 hours. The nylon cord was checked twice during the

1990-1991 season and at the start of the 1991-1992 season for stretching or shrinkage; neither occurred. The ice hole was covered by a dark blanket to reduce the direct transmittance of surface light through the ice hole to where the samples were suspended. Incubations were usually started between 0600-0800 h local time when nearby mountains obstructed direct sunlight.

After the incubation, samples were again placed in the darkened box and taken to the Lake Bonney field laboratory. Samples were filtered through Whatman GF/C glass fiber filters under low vacuum (<10 mm Hg). Filtering all the samples took less than 2 hours and was done in a dark room. Filters, filtration funnels and incubation bottles were not rinsed with deionized water (to remove unassimilated ^{14}C) to avoid rupturing cells from the depths of high salinity. Instead, filters were placed in 20 mL glass scintillation vials and 0.5 mL 3 N HCl was added to volatilize unassimilated inorganic ^{14}C. Samples were then dried on a hot plate (50° C). Care was taken that the filters were completely immersed in the acid to ensure that the inorganic label would be converted to CO_2.

Dissolved inorganic carbon (DIC) in water samples was measured by infrared absorption. Samples were injected into a glass chamber filled with 6N H_2SO_4 and sparged with ultra high purity N_2 gas, which passed through an ascarite pre-column to remove any CO_2 contamination. The instrument