CRAWFORD LAKE

FACTORS INFLUENCING THE SEASONAL CHANGES IN PRIMARY PRODUCTIVITY OF THE PHOTOSYNTHETIC BACTERIA OF CRAWFORD LAKE, ONTARIO

Shawn R. T. Severn

(Submitted in partial fulfillment of the requirements for the degree of Master of Science)

BROCK UNIVERSITY

St. Catharines, Ontario

November 1981

Science seldom proceeds in the straightforward logical manner imagined by outsiders. Instead, its steps forward (and sometimes backwards) are often very human events in which personalities and cultural traditions play major roles.

The Double Helix
by James D. Watson.

Though I kept insisting that we should keep the backbone in the center, I know none of my reasons held water.

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ABSTRACT

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Analysis of the data indicated that (^{14}C) bacterial photosynthesis was potentially limited by the ambient bicarbonate concentration. Once a threshold value (of 270 mg/l) was reached a dramatic (2 to 10 fold) increase in the primary productivity of the bacteria was observed. Light intensity appeared to have very little effect on the primary productivity of the bacteria, even at times when analyses by Parkin and Brock (1980a) suggested that light intensity could be limiting (i.e., 3.0-5.0 ft. candles). Shifts in the absorption maxima at 430 nm of the bacteriochlorophyll spectrum suggested that changes in the species or strain composition of the photosynthetic bacteria had occurred during the summer months. It was speculated that these changes might reflect seasonal variation in the wavelength of light reaching the bacteria.

Chemocline erosion did not have the same effect on the population size (biomass) of the photosynthetic bacteria in Crawford Lake (this thesis) as it did in Pink Lake (Dickman, 1979). In Crawford Lake the depth of the chemocline was lowered with no apparent loss in biomass (according to bacteriochlorophyll data). A reverse

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The photosynthetic bacteria contributed a significant proportion (10-60%) of the lake's primary productivity. Direct evidence was obtained with (^{14}C) labelling of the photosynthetic bacteria, indicating that the zooplankton were grazing the photosynthetic bacteria. This indicated that some of the photosynthetic bacterial productivity was assimilated into the food chain of the lake. Therefore, it was concluded that the photosynthetic bacteria made a significant contribution to the total productivity of Crawford Lake.

ACKNOWLEDGEMENTS

There are a great many people that have in some way contributed to the final draft of this thesis. Many of these people are not mentioned below but I would like to extend my sincerest thanks to each of them for their kindness and support.

My own family has been especially encouraging. From my youngest sister Kelly, who spent her summer vacation keeping me company during the 1980 field season, to my parents who have contributed support and refuge through some very turbulent times. And to my new wife, Corinne, who kept me going as the end of the thesis approached and often had to stand my cantankerous disposition during many times of writer's block. I owe you my love and appreciation.

I have been fortunate to have some very special friends throughout my career at Brock. These people are deserving of so much for their kindness. They are Wendy Dean, Wendy Cox, Steve and Janet Brooks, Freida Nicholls, Peter Steele, Anne-Marie Calarco, Peter and Diane Gibson, Mike Cheek and Chris Earle. Several very special thank-yous are also appropriate, to my roommates Roger Paterson and Rob Carins. To George and Peggy Melvin who have read my thesis and been supportive throughout my career at Brock.

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Again Thanks.

Shawn Severn

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LITERATURE REVIEW

Crawford Lake is located on Silurian Guelph-Ambel dolomite (Karrow, 1963; Tovell, 1965; Boyko, 1973), in Halton Region.

The lake is situated 300 m above sea level and 170 m above Lake Ontario. The lake displays micro-climate conditions which are atypical of the low-lying area (Boyko, 1973). In general, the area has a 200-day growing season, experiencing light spring and summer winds followed by strong fall and winter winds (Boyko, 1973).

To date Crawford Lake has been examined for the sediment pollen distribution (Boyko, 1973), zooplankton population dynamics (Prepas and Rigler, 1978), sediment chironimid head capsule distribution (Cheek, 1979) and Chaoborus population dynamics (Sardello, pers. comm.). The photosynthetic bacterial layer was examined by Dickman (1979) and Severn (1979).

Major Classes of Photosynthetic Bacteria

To provide some background, a review of the major groups of anaerobic photoautotrophs is given below. It is in no way complete or comprehensive as the published literature concerning identification and biochemical characteristics of these organisms is both voluminous and often contradictory.

The prokaryotic autotrophs are presently divided into 5 groups: Cyanobacteria, Chlorobiaceae, Chloroflexaceae, Rhodospirillaceae and Chromatiaceae (Pfennig, 1967, 1979; Pierson and Castenholz, 1974a).

These groups are sufficiently different from one another that they can be distinguished by their spectral properties (Table 1).

The cyanobacteria are characterized by the pigments chlorophyll a and phycobiliproteins (Stanier, 1974a; Pfennig, 1979). They are the only prokaryotes possessing photosystem II (Avron, 1967). Therefore, unlike photoautotrophic bacteria, they are able to evolve O_2 as a consequence of photosynthesis. Hence, they are typically found in well oxygenated water. Oscillatoria limnetica and O. salina, found in Solar Lake, Israel, are the only cyanobacteria able to photosynthesize anaerobically (Cohen et al., 1977b). In both cases the presence of H_2S inhibits photosystem II (Cohen et al., 1975). However, the sulphide acts as an electron donor to photosystem I with no apparent decrease in productivity (Cohen et al., 1975).

There are two forms of green sulphur bacteria (Chlorobiaceae) (Stanier and Smith, 1960; Gloe et al., 1975). Both forms have BChl a (Table 2). However, the green form has one of either BChl c or BChl d and the brown form BChl e (Liaaen Jensen, 1965). The The brown form also has carotenoides isonenieratene and B-isorenieratene which tend to extend the absorption range between 480 and 550 nm (Liaaen Jensen, 1965; Trüper and Genouese, 1968; Pfennig, 1979). Both forms are non-motile, but some species are able to form gas vacuoles or consortiums with non-pigmented cells (Pfennig and Trüper, 1974). The green consortium is called Chlorochromatium and the brown Pelochromatium (Pfennig and Trüper,

Table 1: Bacteriochlorophyll of the Phototrophic Bacteria (adapted from Pfennig, 1979)

Chlorophyll		Characteristic Peak (um)				Division of Bacteria
Plant Chlorophyll				660-670		Cyanobacteria
Bchl	a	375	590	800-810	830-890	All (except Cyanobacteria
	b	400	605	835-850	1015-1035	Chromatiaceae & Rhodospirillaceae
	c	335	460	745-760	812	Chlorobiaceae & Chloroflexaceae
	d	325	450	725-745	805	Chlorobiaceae
	e	345	450-460	715-725	805	Chlorobiaceae (Brown forms)

1974; Pfennig, 1979).

All green sulphur bacteria are obligate anaerobic photoautotrophs capable of photoassimilation of CO_2 and some simple organic molecules (like acetate) depending on the concentration of both CO_2 and H_2S (Kelly, 1974).

Chloroflexus aurantiacus is the only species in the division Chloroflexaceae. These rare motile bacteria are found in hot springs with an average temperature of 50 to 60 degrees Celsius (Pierson and Castenholz, 1974a; b). C. aurantiacus is an obligate phototrophic anaerobe, resembling Chlorobium sp. (Pfennig, 1979). Unlike Chlorobium sp., C. aurantiacus can survive oxygenated environments using respiration (Pfennig, 1979).

The Rhodospirillaceae have the ability to exist facultatively as microaerophilic lithoorganotrophs or as anaerobic photoorganotrophs. These bacteria, however, are never found at high concentrations (Pfennig and Trüper, 1974).

The Chromatiaceae are obligate photolithotrophic or photoorganotrophic anaerobes. Thiocapsa roseopersicina is the only exception. This organism can exist as an organotroph under microaerophilic conditions (Pfennig and Trüper, 1974; Pfennig, 1979).

Under anaerobic conditions, Rhodospirillaceae and Chromatiaceae are distinguished by their respective inability or ability to oxidize elemental sulphur to sulfate during CO_2 photoassimilation (Pfennig and Trüper, 1974). All species of Chromatiaceae have the ability to oxidize sulphide ions to elemental sulphur and ultimately to sulphate, but none of the Rhodospirillaceae can perform the oxidation

of sulphur to sulphate. Both the Chromatiaceae and Chlorobiaceae have adenyl-sulphate reductase and therefore can reduce sulphate to elemental sulphur. The Chromatiaceae and Rhodospirillaceae both have BChl a and b (Table 2). The Chromatiaceae contain spirilloxcinthin, okeme and rhodopinal carotenoids (Pfennig and Trüper, 1974). Both have motile and non-motile forms, with or without gas vacuoles and flagella (Pfennig and Trüper, 1974). The motile forms of Chromatiaceae often undergo a diurnal migration (Sorokin, 1970).

In summary, there are several characteristics that all photosynthetic bacteria possess. They are generally greater than 0.5 um in length or width (cyanobacteria have been found as large as 10 um; Pfennig and Trüper, 1974). Coccoidal, vibroidal, and bacillus shapes are common to all the divisions (Pfennig and Trüper, 1974). Pleomorphism, different shapes under different environmental conditions, is also common to all species (Caldwell and Tiedje, 1975a). Unfortunately, these characteristics and shared similarities make species identification and ecological studies on natural populations difficult (Caldwell and Tiedje, 1975a).

A Review of the Ecology of the Photosynthetic Bacteria

The literature dealing with the ecology of photosynthetic bacteria can be roughly divided into two groups: 1) studies that attempt to demonstrate the ecological significance of the photosynthetic bacteria, and 2) studies examining parameters (such as

Table 2: Major Differences Between the Divisions of the Phototrophic Bacteria (from Pfennig, 1979)

Division	Photo pigments	Carotenoid	Life conditions
Cyanobacteria	Chl a phycobiliproteins	B-carotene & zeaxanthin	--aerobically - photo-assimilation using both photo systems --anaerobically using only photosystem I
Chlorobiaceae	Bchl a, c, d & e	isorenieratene & B-isorenieratene	--obligate photolithotrophs anaerobic
Chloroflexaceae	Bchl a & c		--obligate phototrophic anaerobes --facultative microaerophyllic heterotrophs
Chromaticaeae	Bchl a & b	spirilloxanthin okene & rhedopinal series	--photoorganotrophs & photo autotrophic --obligate anaerobes, except C. roseo-persicina which can exist as a hetero-trophic in microaerophyllic conditions
Rhodospir-illaceae	Bch a & b	spirilloxanthin, ketocarotonoids of spheradenone and rhodopinal series	--photoorganotrophs --obligate photolithotrophs --can exist heterotrophically under micro-aerophyllic conditions

light, H_2S and oxygen) that might control the primary productivity and population size of the phototrophic bacteria. Many studies attempt a combination of the two approaches. However, in the following review each will be treated separately.

The literature is replete with examples attempting to persuade readers of the importance of photosynthetic bacteria. In all cases, researchers have stressed high relative primary productivity (percent productivity) values as the parameter which is indicative of the importance of the photosynthetic bacteria (Table 3). Values as high as 85 and 91 percent have been reported for the contribution made by the anaerobic autotrophs to the total lake primary productivity (Culver and Brunskill, 1969; Cohen et al., 1977b). However, primary productivity values as low as 0.26 percent have also been demonstrated (Parkin and Brock, 1980b). This suggests that photosynthetic bacteria may be very important in some lakes and unimportant in others.

I feel it is misleading to assume a high primary productivity value represents high ecological significance. The real importance of any organism to an aquatic system should be based on its probability of affecting other trophic levels (Ricklefs, 1976). For example, if the productivity produced by the anaerobic photoautotrophs is locked up in the anaerobic zone (unavailable to the next trophic level), then they are unimportant to the system. However, if the production, by the anaerobic photoautotrophs, is used by the next trophic level, then even low levels of anaerobic

Table 3: Primary Production in Meromictic Lakes by Photosynthetic Bacteria (from Cohen et al., 1977b; Pfennig, 1979)

Name of Lake	% Contribution to Total Productivity	Reference
Peter, Paul and Murror	0.26 to 6.3	Parkin and Brock, 1980b
Belovod	40	Lyalikova (1957)
Belovod	20	Sorokin (1970)
Muliczne	24	Czeczuga (1968a)
Horuna	20	Takahashi & Ichimura (1968)
Suigestsu	45	Takahashi & Ichimura (1968)
Kisoratsu Reservoir	60	Takahashi & Ichimura (1968)
Medicine	55	Hayden (1972)
Fayetteville (N.Y.)	85	Culver & Brunskill (1969)
Solar	91	Cohen et al. (1977a,b)

This is not a complete list, but it demonstrates the extensive examination of productivity in these systems.

photoautotrophic productivity would be significant.

Indirect evidence already exists suggesting that the photosynthetic bacteria are grazed by zooplankton (Culver and Brunskill, 1969; Prepas and Rigler, 1978). However, the direct evidence is as yet poor and inconclusive (Sorokin, 1966; Takahashi and Ichimura, 1968).

Obtaining direct evidence, through ^{14}C labelling experiments or direct observation, would demonstrate conclusively the importance of anaerobic photosynthetic organisms to a meromictic lake.

The Study of Controlling Factors

The study of factors that control the population size and productivity of photosynthetic bacteria was begun under laboratory conditions in the mid-forties. Based on his laboratory experiments Van Neil (1944) predicted that the major factors influencing the population size and productivity of photosynthetic bacteria in their natural environment would be light intensity, H_2S concentration and the presence or absence of oxygen. This prediction formed the basis for virtually all the modern work on the anaerobic photoautotrophs.

Light Intensity and Quality

The intensity of light reaching the photosynthetic bacteria is usually selected as the most important factor influencing photosynthetic productivity (Takahashi and Ichimura, 1968, 1970;

Lawrence et al., 1978; Cohen et al., 1977b; Parkin and Brock, 1980b). Light has also proved to be the most difficult factor to understand. In all but one case (Parkin and Brock, 1980b) researchers have been unable to show any correlation between light intensity and productivity. Culver and Brunskill (1969) stated that light was controlling during periods of ice cover and at no other time.

Takahashi and Ichimura (1970) produced one of the most extensive pieces of work on the relationship between biomass (bacteriochlorophyll concentration) and the light intensity incident on the phototrophic bacteria. They suggested a direct logarithmic relationship between the percentage of light and photosynthetic bacterial biomass (Table 4). Their equation was based on several assumptions. Two of these will be discussed below. The first assumption, that self shading within the population would give lower values than expected, was addressed at some length. They concluded that in very dense populations the equation would tend to over-estimate the maximum bacteriochlorophyll concentration. Their conclusion was based on the assumption that at high densities self shading would tend to decrease the over-all productivity. Hence, only photosynthetically-active cells would be included in their analysis.

It was clear to me, however, that to assume that only photosynthetically-active cells were included in their measurements was faulty because they were not measuring just photosynthetically-active cells but all cells that contained bacteriochlorophyll (i.e.,

they measured bacteriochlorophyll concentration). Therefore, it did not matter if the cells were photosynthetically active as long as they were intact and contained bacteriochlorophyll. Hence, their expectation should have been to under-estimate the potential standing crop. This was probably why both Harutori and Wakunuma lakes had higher than expected bacteriochlorophyll concentrations (Table 5). The reason the other four lakes were over-estimated could be due to the second assumption.

In the second assumption, Takahashi and Ichimura (1970) claimed that a limiting nutrient would only affect the rate increase of production but not the maximal obtainable production. This may have been a reasonable assumption at that time. However, it was discovered by Kelly (1974) that low CO_2 or H_2S not only reduced the rate of production but also limited the maximum amount of growth. Hence, the under-estimation observed in Wakuike, Kisaratsu, Suigetsu and Suga Lakes (Table 5).

As I indicated above, Takahashi and Ichimura (1970) were unable to predict the maximum population size based on light intensity (Table 5). This was probably because their assumptions were faulty in the ways that have been pointed out above. However, this was one of the first attempts to tackle what has proved to be a very difficult problem.

Parkin and Brock (1980b) were the only researchers, to my knowledge, able to demonstrate a linear correlation between production and light intensity based on field data (Fig. 1). However, interpretation of the figure was difficult. Essentially, they suggested that lakes with good penetration have high product-

Table 5: Observed Bacteriochlorophyll Concentration and Calculated Bacteriochlorophyll Maxima for Six Japanese Lakes

Lake	[BChl] measured mg/m^2	[BChl] calculated
Harutori	587 d	280
Wakuike	235 c	345
Kisaratsu	352 d	370
Suigetsu	182 d	300
Suga	119 d	370
Wakunuma	492 d	330

Based on Table 2; Takahashi and Ichimura, 1970.

Figure 1: Percent production as a function of percent of surface light. The graph was originally introduced by Parkin and Brock, 1980b. The numbers represent the following lakes:

1	Mary	9	Kisaratsu
2	Fish	10	Haruna
3	Peter	11	Wadolek
4	Hiruga	12	Waldsea
5	Mirror	13	Wadolek
6	Paul	14	Green
7	Rose	15	Kisaratsu
8	Waku-Ike	16	Solar

PERCENT PRODUCTION
0.1
1
10
100
PERCENT OF SURFACE LIGHT
10
1
0.1
0.01
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

ivity. Unfortunately, the terms "percent production" and "percent surface light" were too vague. Without absolute values it was impossible to know if a low percent production value represented low algal production with even lower photosynthetic bacterial production, or if it meant high algal values with low to moderate bacterial productivity values. However, in a general way these types of relationships do tend to be useful.

In another paper, Parkin and Brock (1980a) stated that the photosynthetic bacteria are saturated at light intensities on the order of 1-10 u E/m^2s. This was a very significant point for two reasons: 1) most culture work done on the photosynthetic bacteria used light intensities in excess of 700 lux (about 70 ft. candles or 25 u E/m^2s; Pfennig, 1967; Takahashi and Ichimura, 1968, 1970); 2) that field productivity data tended to be taken at about 12:00 hours, or at times when the intensity reaching the bacteria was in excess of 1-10 u E/m^2s. Therefore, it could be concluded that, with the possible exception of cloudy days and ice cover, photosynthesis would not be limited by light intensity. Hence, Parkin and Brock's (1980b) relationship between percent light intensity and percent production might have no ecological significance. Nonetheless, these two works constitute some of the best work on light intensity and productivity of the 1970s.

Pfennig (1967) proposed that the species composition of photosynthetic bacteria at the chemocline was a result of the wavelength of light incident at the chemocline. The results of numerous

laboratory studies have lent support to this hypothesis (Matheron and Baulaigue, 1977; Herbert and Tanner, 1977; Osnitskaya, 1964; Osnitskaya and Chudina, 1964, 1977). In all cases, these works have demonstrated specific wavelengths of light absorption by different species of photosynthetic bacteria. There have been two field studies on naturally-occurring populations of phototrophic bacteria. Trüper and Genovenese (1968) predicted the presences and demonstrated the occurrence of a blue light (460-500 nm) absorbing bacteria, Chlorobium phaeobacteriodes, in Faro, Sicily. Parkin and Brock (1980a) demonstrated conclusively that the wavelength of light was a major factor controlling photosynthetic bacterial species composition in three small lakes in Wisconsin. Their conclusion was based on two observations: 1) photosynthetic bacterial species composition (based on spectral analysis) was strongly dependent on the wavelength of light incident on the photosynthetic bacteria, and 2) they were able to select the dominant species by changing the wavelength of light in laboratory cultures (Parkin and Brock, 1980a). This work was particularly beneficial as it points the direction for further questions concerning seasonal selection of bacterial species due to change in the incident wavelength of light.

Chemical Factors

Photosynthetic bacteria require light, H_2S and CO_2 in order to photoassimilate inorganic carbon (Pfennig, 1979). CO_2 is usually

iting to algae in most aquatic environments
ver, there is some evidence that this may not be
thetic bacteria (Culver and Brunskill, 1969;
and Brunskill (1969) observed a 2.5 fold
ssimilation of ^{14}C, $NaHCO_3$ after precipitation
monimolimnion of lakes. Kelly (1974)
probium could be limited by insufficient CO_2
e relieved by the addition of acetate. However,
n no careful examination of the potential
tivity due to low CO_2 concentration under

s, H_2S has been suggested as a potential,
nashi and Ichimura, 1968; Culver and
well and Tiedje, 1975a,b; Parkin and Brock,

e levels rise from zero near the chemocline to
d 4.0 mg/L in the monimolimnion along a well-
ost meromictic lakes (Caldwell and Tiedje,
Tiedje (1975b) suggested that in combination
, H_2S concentration was responsible for the
ithin the water column of species observed in
Lakes, Michigan.
uggest that the rate of H_2S production and not
tion is the limiting factor (Culver and

H_2S production is
Reduction of
pply to these organisms
lation (Culver and
$_2S$ production could be
ere not necessarily
lphide, as a limiting
ation (Parkin and Brock,
ity can vary widely
reshold there will be
d productivity
shold limitation is a relatively
. and as yet supporting
to be a prime candidate

purple photosynthetic
fennig, 1967, 1979).
would cause a mass
thetic bacteria
e process in which the
s eroded is called
is process of partial
emocline occurs twice

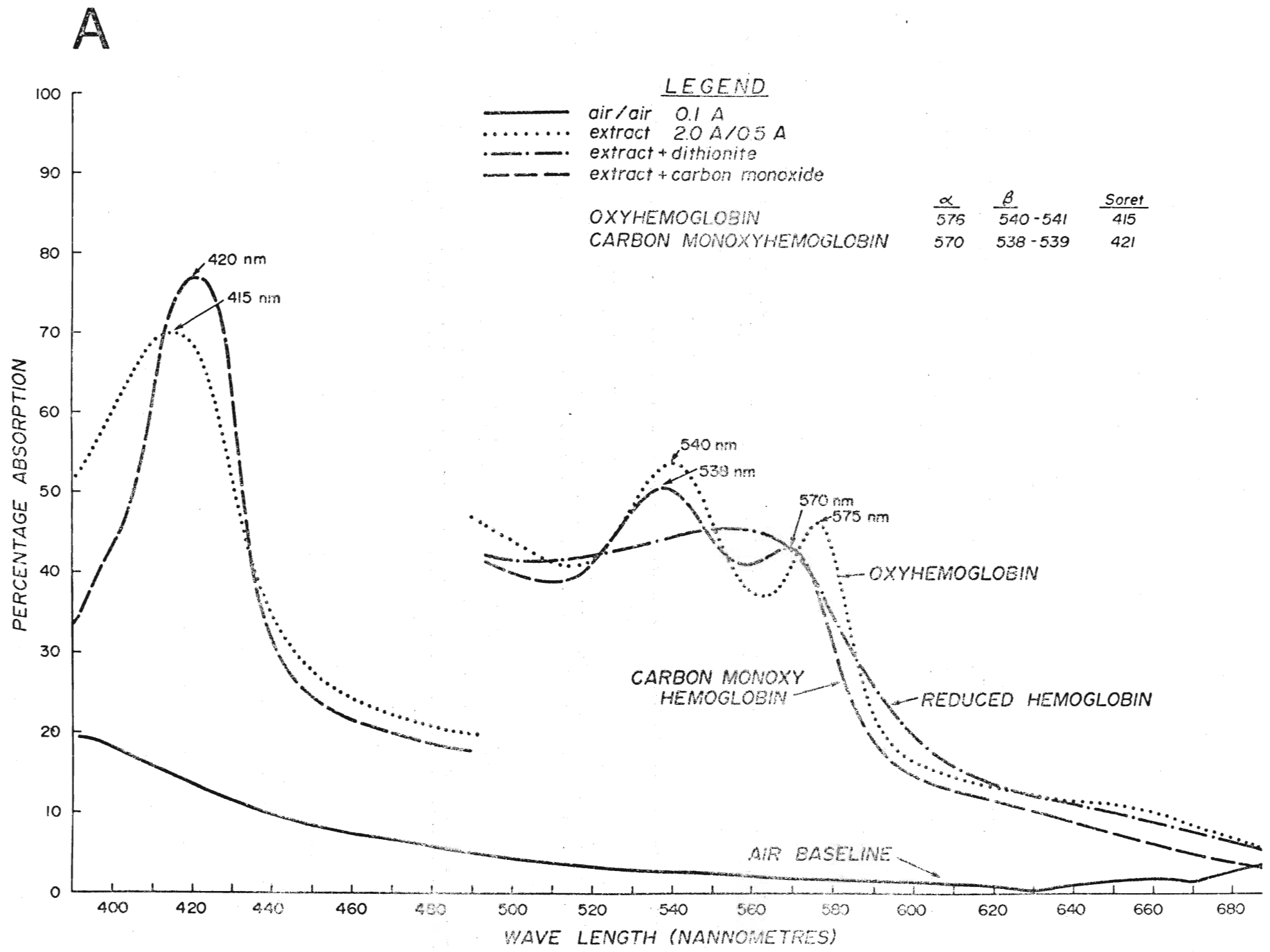
a year (Hutchinson, 1957), but has the most impact on the bacteria during the fall mixing period. In Pink Lake, oxygen is introduced several meters into the anaerobic zone, resulting in a yearly mortality of the vast majority of the photosynthetic bacteria (Dickman, 1979).

This process alone could account for a massive loss of productivity. Therefore, it has been included here as a possible controlling factor. As yet, little is known about ventilation and its effects on photosynthetic bacteria. In fact, it is not known if all lakes are affected in the same way as Pink Lake.

Zooplankton Grazing

The presence of zooplanktors with "pink guts" has been observed numerous times (Takahashi and Ichimura, 1968; Culver and Brunskill, 1969; Seki et al., 1974; Prepas and Rigler, 1975; Lawrence et al., 1977). In all cases the pink coloration in the zooplankton was attributed to the presence of photosynthetic bacteria. However, spectral analyses of the red pigments in the zooplankton by Nicholls (pers. comm.) on samples of Daphnia sp. collected by M. Dickman and R. M. Miracle from the anaerobic zone of Crawford Lake, indicated that some of the coloration of the animals was due to the presence of haemoglobin (Fig. 2). At that time no evidence was obtained that would suggest the coloration was due to the photosynthetic bacteria. From these data it was not possible to conclude that the zooplankton were grazing the photosynthetic bacteria. In

Figure 2: Absorption spectrum of haemoglobin in Daphnia sp. by Nicholls, pers. comm. The figure was obtained from M. D. Dickman.


A
LEGEND
air / air 0.1 A
extract 2.0 A / 0.5 A
extract + dithionite
extract + carbon monoxide
α β Soret
OXYHEMOGLOBIN 576 540 - 541 415
CARBON MONOXYHEMOGLOBIN 570 538 - 539 421
420 nm
415 nm
540 nm
539 nm
570 nm
575 nm
OXYHEMOGLOBIN
CARBON MONOXY HEMOGLOBIN
REDUCED HEMOGLOBIN
AIR BASELINE
PERCENTAGE ABSORPTION
WAVE LENGTH (NANNOMETRES)
100
90
80
70
60
50
40
30
20
10
0
400
420
440
460
480
500
520
540
560
580
600
620
640
660
680

fact, the presence of zooplankton in the anaerobic zone could be interpreted as a predator avoidance adaptation.

Hence, it was still necessary to carry out labelling experiments to determine if the zooplankton did graze the photosynthetic bacteria.

INTRODUCTION

In the past decade, many authors have observed that the biomass and primary productivity of the photosynthetic bacteria vary widely depending on the time of year (Takahashi and Ichimura, 1968, 1970; Culver and Brunskill, 1969; Lawrence et al., 1978; Parkin and Brock, 1980b). Most have attributed the observed variation to one of a number of physical and chemical factors (i.e., light intensity and H_2S concentration). However, in all cases the factor suggested as the major limiting factor was often the only factor examined (Culver and Brunskill, 1968; Takahashi and Ichimura, 1970). Studies that have examined a number of factors tend to use either a larger number of lakes over a single day (Parkin and Brock, 1980b), impose laboratory conditions on a natural population (Parkin and Brock, 1980a) or examine the diurnal effect of a single factor (Parkin and Brock, 1981). To my knowledge, no one has examined the seasonal variation in primary production and biomass as a possible function of several factors.

There have been many studies that attempt to convince the reader of the importance of the photosynthetic bacteria to an aquatic ecosystem (Lyalikova, 1957; Czeczuga, 1968a; Sorokin, 1970; Hayden, 1972; Cohen et al., 1977a,b). All of these studies claimed that the photosynthetic bacteria were important because they made a substantial contribution to the total inorganic carbon fixed in the lake. Recently these criteria were questioned because they

tended to reject lakes where the photosynthetic bacteria made a relatively small (< 1%) contribution (Parkin and Brock, 1980b). An alternative would be to demonstrate not only a contribution to the total inorganic carbon fixed but that some of the primary production got into the secondary level of the food web. This would involve demonstrating that some of the photosynthetic bacterial production was assimilated by a herbivore (for example a zooplankton). The cladocern zooplankton, Daphnia pulex, has been observed in the anaerobic zone (Prepas and Rigler, 1978) and contains a blood-like haemoglobin (Fig. 2) which would allow it to stay in the anaerobic zone for an extended period of time. These observations suggest that the zooplankton are able to stand what are normally toxic conditions of the anaerobic zone, at least for a short time. However, it is not known if the Daphnia sp. swim into the anaerobic zone simply to avoid predation or if they actually utilize the photosynthetic bacteria. Takahashi and Ichimura (1968) and Sorokin (1970) claimed to have demonstrated zooplankton grazing, but to my knowledge the data were never published.

The purpose of this study was to collect chemical (bicarbonate concentration) and physical (light intensity and ventilation) data that would lead to an explanation of some of the seasonal variations in the primary productivity (^{14}C uptake) and biomass (bacteriochlorophyll concentration) of the photosynthetic bacteria of Crawford Lake, Ontario.

Furthermore, it was necessary to examine the question of the importance of the photosynthetic bacteria to the total primary production of the lake, demonstrating that they fulfill both of the criteria--primary and secondary production contributions--to the lake.

SITE DESCRIPTION

Crawford Lake (43° 28'N, 79° 57'W) (Fig. 3), located near Campbellville, Ontario, is a bicarbonate-rich meromictic lake (Boyko, 1973; Dickman and Hartman, 1979). The lake has a steep-sided conical morphometry, with a mean depth of 10.5 meters, a maximum depth of 24 meters, an area of 2.1×10^4 m^2 and a volume of 2.2×10^5 m^3. The lake is protected from the wind by steep dolomite cliffs topped with a deciduous-coniferous forest.

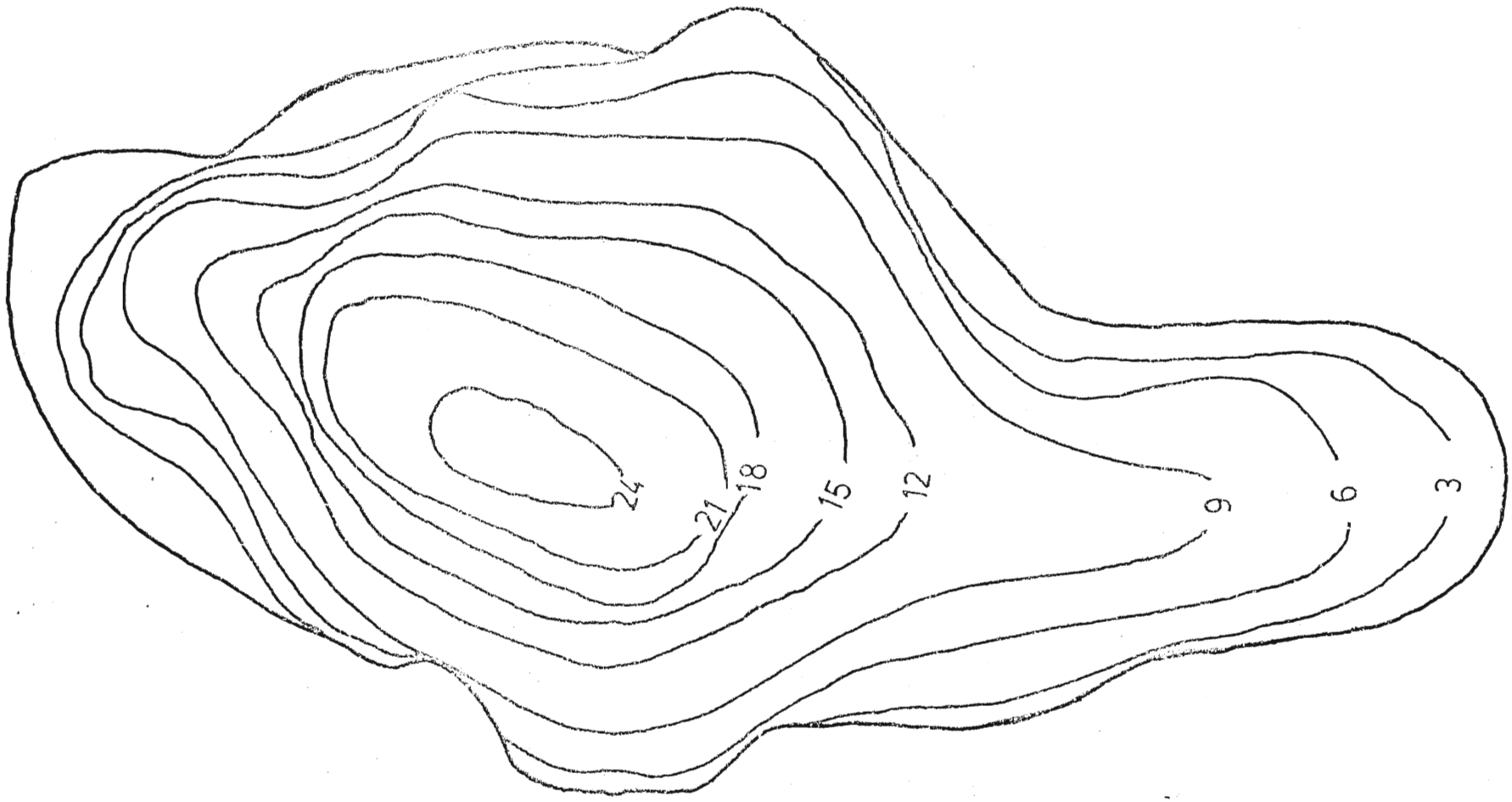
MATERIALS AND METHODS

Sampling Schedule

Crawford Lake was sampled weekly during the 1979 field season from mid-May to fall overturn at the end of November. I felt that this sampling schedule would reveal any seasonal changes. In the second year (beginning May 1980), a more intensive schedule was adopted in order to monitor day-to-day changes as well as seasonal changes. In 1980, a daily sampling schedule from the 10th to the 20th of each month was begun. In addition, there were periodic samplings made between the 10 consecutive sample periods.

In 1980, the intensive sampling program was begun in May and continued to the October overturn. Inclement weather in November (1980) forced a sampling regime of every other day from the 9th to

Figure 3: Contour map of Crawford Lake, with both area and depth in meters (after Prepas and Rigler, 1978).



Scale in meters - CRAWFORD LAKE

By the end of the month (25th of November) the lake had begun to freeze. The lake was also sampled on two days in December (5th and 7th) following a thaw period. However, after the 7th of December the ice conditions forced me to terminate my sampling program.

Conductivity, Oxygen, Temperature and Light

On each sampling date in 1979 and at a minimum of once a month in 1980, the following profiles were obtained: conductivity (umhos/cm), temperature (°C), oxygen (% saturation) and light (foot candles; starting September 1979). Conductivity, temperature and dissolved oxygen were all measured using Yellow Springs Instrument Company meters (Model 51 and Model 33, respectively). The light measurements were made using a Protomatic meter (Dexter, Michigan).

Temperature, dissolved oxygen, conductivity and light profiles were made at one meter depth intervals from the surface of the bottom of the lake (0-23 m at my mid-lake sample location) or to the zero point of the meter. Conductivity was measured at 0.25 meter intervals from 13 to 17.0 meters in late November. Measurements were also taken in reverse order on several occasions, in order to check meter readings. Light measurements were taken under direct light conditions (i.e., no cloud cover) whenever possible. On days

of partial cloud cover measurements were taken both in direct sunlight and under cloud cover.

Measurements of conductivity, temperature and dissolved oxygen are displayed in isopleth graphs similar to those presented by Wetzel (1975). Measurements of light intensity from 13 m were used as an indicator of the intensity at the chemocline, because below this depth the meter approached its lower limit of sensitivity.

Chemocline Location

In order to study the photosynthetic bacteria it was necessary to select a method that would allow me to locate the top of the chemocline with some precision. The conductivity profile from 13 through 16 meters provided a quick and easy method of locating the chemocline. The chemocline location often coincided with the dissolved oxygen minimum. However, this correlation failed when the mixolimnion developed an anaerobic zone above the chemocline in August. At these times (as was the case in August-November 1980) it was possible to check the chemocline's depth with a light meter. The rapid drop in light intensity as recorded by the underwater photocell was always correlated with the presence of a dense "cloud" of photosynthetic bacteria at the chemocline.

Water Column Transparency (Secchi Disc)

Water Column Transparency was determined using a 30 cm Secchi Disc. The disc was lowered into the water on a metered cord. The depth (in meters) that the disc disappeared from sight was recorded and is referred to as the Secchi depth. All measurements were made in the shade of the canoe.

Method for Obtaining Water Samples for Chemical Analysis, Characterization, Bacteriochlorophyll Analysis and Productivity

In 1979 and 1980, water samples were taken from the monimolimnion in order to determine depth and seasonal changes in the biomass of the photosynthetic bacteria. I used cell number as an indicator of biomass in 1979. However, since changes in cell number were significantly correlated with the changes in bacteriochlorophyll concentration, only bacteriochlorophyll concentration was used in 1980. This simplified the biomass determinations immensely.

In 1979 water samples containing the bacteria were collected with a 6 liter Van Dorn water sampler (Wildlife Supply Company) at one meter intervals from 15 to 20 meters. Typically, a single Van Dorn sample was obtained from each depth. However, multiples of three were taken at each depth from time to time in order to calculate sample variance. Photosynthetic bacteria are both light and temperature sensitive (Pfennig, 1979). For this reason, the water containing the bacteria was stored on ice in black 3 liter bottles until they could be transported back to the laboratory (usually within two hours). The laboratory procedures for characterization and bacteriochlorophyll

analysis are presented in a later section.

A twelve volt sealed aquatic bilge pump was secured to a 30 m garden hose (metered at one meter intervals). The pump was powered by a 12 volt battery located at the surface. The system was operated by lowering the pump end of the hose to the desired depth, waiting five minutes for clearing and then collecting the desired water sample. This system provided a continuous water supply from the desired depth.

In 1980, samples were taken every three meters from the top of the chemocline. The following parameters were measured: pH, alkalinity, H_2S, bacteriochlorophyll concentration and primary productivity. As well, samples were taken periodically to attempt to characterize and culture the photosynthetic bacteria. However, a viable culture was not obtained.

Chemical Analysis

pH

Three water samples were taken from each of 0, 3, 6, 9, 12 and 15 meters and top of chemocline twice a month (first and last day in the field). The pH of the water was determined in the field using a Metrohm Herisaw pH meter (model #488). During May to September a

gel-filled pH probe was used. Both were standardized using pH 4.0, 7.0 and 10.0 buffers.

Alkalinity

Replicate water samples were obtained as noted in the section on pH. The alkalinity of the water was determined using a standard titration method (Lind, 1974). Lake water was titrated with 0.02 N H_2SO_4 to an end point of pH 4.3 determined both colormetrically (bromcresol green-methyl red indicator) and with a pH meter. The following calculation was used to determine the $mgCaCO_3$/liter from the titrated volume of H_2SO_4.

$$\text{Total Alkalinity } (mgCaCO_3/l) = \frac{(A-C) \times N \times 40{,}000}{\text{mls of sample}}$$

C = titration of indicator

A = Volume of Acid to titrate to pH 4.5

N = Normality of the Acid

All titrations were done in the field. Samples from the chemocline had to be filtered first because the photosynthetic bacteria's pink color interfered with the titration.

Determination of Bacteriochlorophyll Concentration

This parameter was monitored throughout both of the field seasons. The bacteriochlorophyll concentration was used primarily in monitoring seasonal changes in photosynthetic bacterial biomass during both field seasons.

Water samples (containing the photosynthetic bacteria) were taken from the monimolimnion in the following ways. In 1979, a profile of biomass versus depth was obtained by sampling at a series of depths (15-20 meters) with the Van Dorn. In 1980, only the most concentrated zone of photosynthetic bacteria was sampled. This was always at the top of the chemocline which was at a depth of approximately 15 m. Both samples were obtained as described in the above section.

Fifty to five hundred milliliters was filtered through a Whatman GF/C filter concentrating the bacteria on the filter. This was repeated a minimum of three to a maximum of ten times for each bottle returned to the laboratory.

In 1979, samples were contained on the filter papers, lyophilized and stored at -76° in dark containers for a maximum of two months. Some filters were analyzed immediately in order to determine the loss of bacteriochlorophyll due to storage. It turned out that the loss was minimal. The water samples were filtered in the field in 1980, stored on ice in the dark and analyzed for bacteriochlorophyll within two weeks after filtering.

In both years the analysis of the bacteriochlorophyll concentration on the filter was done in the same way.

First the samples containing the photosynthetic bacteria were homogenized (using a glass homogenizer) into approximately 30 mL of an acetone:methanol:water mixture (80:15:5; Daley et al., 1973). The resulting slurry was then passed through another Whatman GF/C filter with 3 to 5 mL of Acetone-Methanol H_2O mixture. The supernatant was collected and the volume measured and analyzed spectrophotometrically (from 640 nm - 670 nm). The residue left after the first extraction was re-analyzed to determine the efficiency of the extraction procedure (about 90-95%). All the filters containing the photosynthetic bacteria were analyzed, hence a mean and standard deviations were calculated for each water sample.

The bacterioclorophyll concentration was calculated from data obtained from the spectrum according to the equation of Takahashi and Ichimura (1968):

$$\text{mg } (\text{BChl})_{654}/\text{L} = 10.2 \text{ X D X F}$$

D = Absorbance at 654 nm

$$\text{F} = (\text{Extracted Volume}) \text{ X } (\text{Filtered Volume})^{-1}$$

This method used 90% acetone; therefore, a number of samples were redone to check if this caused a difference in the concentration calculation. The concentration difference calculated was minimal as long as the acetone : methanol peak was used for the calculation. The methanol tends to shift the 654 nm peak to 657 nm.

In Situ ^{14}C Productivity Method for Determination of the Carbon Uptake by the Photosynthetic Bacteria

The productivity for both the photosynthetic bacteria and algae was measured *in situ* by the ^{14}C method (Lind, 1974; Parkin and Brock, 1980).

Field Procedures

Biological Oxygen Demand (B.O.D.) 125 mL bottles (3 light, 3 dark) were flushed with at least three volumes of water obtained from the desired depth.[1] The bottles were then inoculated with 8 uCi of sterile anaerobic NaH $^{14}CO_3$ solution per bottle and stoppered to prevent air bubble formation. After a five minute dark incubation, the bottles were resuspended to depths corresponding to the collection depths. The incubation period was 4 hours (10 AM to 2 PM). After incubation the bottles were prepared for scintillation counting.

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[1] An *In Situ* Productivity Machine was developed throughout the experimental period and used in the spring (the first prototype) and again in the late fall (the second prototype). The methods for the new device are in Appendix 1.

Laboratory Procedures

Filtering

After incubation, two 25 mL replicates of water from each B.O.D. were filtered through a 0.45 um millepore filter (HAWP 04700) collecting the photosynthetic bacteria on the filters. Each filter was then washed with 50 mls of 1% HCl and dried or fumed for 4 hours in HCl. Both methods were used as it was suggested (Parkin, pers. comm.) that the fuming method would provide better results by reducing $^{14}CO_2$ retention on the filter. In comparing the two methods I observed no difference. Hence, the 1% HCl wash method was used most often as it was easier to use and allowed me to handle more samples.

The dried and washed filter papers containing the photosynthetic bacteria, were then placed in a toluene-based scintillation cocktail (A.C.S. Amersham-Searle). The vials were stored in the dark until they were counted (at least 24 hours after the filters were placed in the scintillation cocktail).

Scintillation Counting

The vials containing the photosynthetic bacteria were counted in a Searle Liquid Scintillation counter for 20 minutes per vial. Along with the sample vials were 5 blanks each containing scintillation fluid and an unused filter paper.

The number of counts/min. and the internal to external standards used to calculate counting efficiency, were recorded.

Background

It was necessary to correct the sample vials for the artificial luminescence of the scintillation fluid-filter paper combination. This was done by subtracting the blank (cocktail and filter) from the sample vials containing the labelled bacteria, filter and scintillation cocktail.

Efficiency and Quenching

The scintillation counter's ability to count the real number of emissions from the ^{14}Carbon in the vials was dependent on two factors. The first was inherent in the machine's electronic inability to detect all the emissions from the fluid. The second was due to the loss of emissions from chemical absorption within the vial (quenching). These two factors potentially contribute to the underestimation of the actual number emissions.

Bacteriochlorophyll absorbs light, hence affects the amount of quenching. The effect of the bacteriochlorophyll on the efficiency was corrected by placing known concentrations of bacteriochlorophyll into scintillant, then adding ^{14}C hexaderane with a known D.P.M. (disintegration per minute). A series from no bacteriochlorophyll to relatively high concentrations was counted. The C.P.M. (counts per minute) to D.P.M. ratio was used to give the percent efficiency expected for any bacteriochlorophyll concentration. This was further simplified by using the ratio of the internal to external standard which was dependent on the amount of quenching. The internal to external

ratio was plotted against its respective percent efficiency. The result was a graph that for any internal to external ratio gave a corresponding percent efficiency value. Since each C.P.M. had an internal to external ratio associated with it, it was possible to correct the C.P.M. of the sample vial directly without knowing the bacteriochlorophyll concentration. This method also took into account the machine's inherent inefficiency. The final result being that both factors were corrected with one calculation.

Calculation of ^{14}Carbon Uptake

The mg Carbon/m^3.hr. uptake was calculated according to Lind (1974). The equation used was:

$$P_1,Pd = A\left(\frac{r}{R} \times C \times \frac{1}{t}\right), \text{ where}$$

P_1,Pd = light and dark productivity

A = # counts corrected for background and efficiency

$r = \frac{\text{volume of the B.O.D. bottle}}{\text{volume filtered}}$

$R = 2.22X10^6$ X uCi added

t = time (hrs.)

C = Alkalinity temperature conversion factor

Zooplankton Grazing on Photosynthetic Bacteria Determinations

General Approach

I was also interested in demonstrating by direct observation that the zooplankton (specifically Daphnia pulex) were grazing on the photosynthetic bacteria. Two methods were selected. The first

was based on being able to label zooplankton with previously labelled ^{14}C of photosynthetic bacteria. The second method consisted of spectrophotometrically analyzing their gut contents for bacteriochlorophyll.

It was hoped that the one method would confirm the other.

^{14}Carbon Labelling of the Zooplankton (Haney Trap)

This potentially quantitative method was used in a qualitative way to show photosynthetic bacterial uptake by Daphnia pulex (the dominant form present in November 1980).

A Haney Trap (supplied by Dr. P. Stokes, University of Toronto) was used to capture and label Daphnia pulex found at 15.5 and 16 meters in Crawford Lake. All the experiments were attempted in late November 1980.

Preparation of Bacterial Pellet

The trap was inoculated with a ^{14}C labelled pellet of photosynthetic bacteria prepared in the following way.

One liter of water from 15 meters (containing photosynthetic bacteria) was incubated in the presence of 100-200 uCi of Na $(^{14}C)CO_3$ low light (100 ft. candles). The sample was then concentrated by centifugation, washed with filtered sterile anaerobic water and recentrifuged. The resulting pellet was resuspended in sterile

water (approximately 15 mL) taken from 15 meters and in the dark until it was used later that day.

Incubation of the Haney Trap

The piston in the trap was inoculated with the ^{14}C labelled bacteria and closed. Then the trap was lowered to the depth of the initial sample (15.5-16 m). The outside plexiglass doors were closed with a messenger which also opened the piston containing the bacteria. The zooplankton were incubated for 0, 5, 10, 15, 20 and 25 minutes in separate tests. After the incubation the trap was pulled up and the zooplankton removed.

Preservation

After incubation, the zooplankton were collected and placed in 10 mL of carbonated water (in order to anaesthetize them, Prepas & Rigler, 1978). Then forty percent formalin was added to bring the concentration of formalin to approximately 4%. The samples were transported to the laboratory for further analysis.

Counting the ^{14}C Content of the Zooplankton

Upon returning to the laboratory the Daphnia pulex were taken from the sample bottles and washed; first with 25 mL diluted H_2O and then with 25 mL of 1% HCl. The D. pulex were then placed into scintillation bottles containing 5 mL of tissue solubilizer (N.C.S. Amersham-Searle) for 7 days. Then 10 mL of organic scintillation

cocktail was added. The vials were stored in the dark for a minimum of 72 hours. After the second storage period the bottles were placed in a scintillation counter and counted every 20 minutes for 4 hours per vial. This included a blank that held the N.C.S., O.C.S. and non-labelled Daphnia pulex. This experiment was repeated at least twice per incubation time interval and as many as 5 times for the 5 minute incubation.

Each vial was corrected for background and for the number of Daphnia pulex per sample vial in the following way:

$$\text{\# of counts/min/D. pulex} = (\text{C.P.M.} - \text{B}) \times \frac{1}{\text{D}}$$

C.P.M. = Counts per minute

B = Background

D = # of D. pulex in the vial

Spectral Analysis of the Gut Contents of Daphnia pulex

An alternate method was selected to test the observations made using the Haney Trap method. This was also carried out in late November using Daphnia pulex.

D. pulex were captured using a Birge-Juday closing net. The samples were taken by pulling the net from 17 to 15 meters and then closing the net. Samples were brought to the surface in the closed net and preserved as described in the previous section.

In the laboratory the D. pulex were washed with distilled water and homogenized in 2 mL of 90% acetone. The resulting solution was

filtered (Whatman GF/C) and washed in 3 mL of acetone. The supernatant was examined using a scanning spectrophotometer from 370 nm to 700 nm. The resulting spectrum was compared to the spectrum obtained from an acetone extracted sample of photosynthetic bacteria.

RESULTS

Physical Parameters

Monitoring Chemocline Changes

The reasons for monitoring the seasonal fluctuation in chemocline depth were two fold: 1) the peak population of photosynthetic bacteria coincides with the first major increase in conductivity at 14-15 m (Fig. 4); 2) the formation of the chemocline in the spring and deterioration in the fall can be monitored and compared to changes in photosynthetic bacterial population size (Fig. 5).

The chemocline was delimited in Crawford Lake by a steep change in conductivity (600 to 1000 umhos/cm (Fig. 5)). Specific conductance was therefore used to monitor changes in the depth of the chemocline. Chemocline depth fluctuated between 14.0 and 15.5 meters in 1979 and 13.5 to 15.0 meters in 1980. These fluctuations followed a very predictable seasonal pattern. In the spring of both years, the chemocline was located at a depth of approximately 15.0 meters. During the spring, observations made with S.C.U.B.A. indicated that the photosynthetic bacteria in the anaerobic zone had formed pink "clouds" with an upper limit of approximately 14.5 m. Gradually, the clouds formed a plate as the chemocline-mixolimnion junction became sharper during the summer. The plate was present during the summer and early fall.

The chemocline coincided with the top of the anaerobic zone during spring (Fig. 6). However, during the summer the water above the

Figure 4: The relationship between bacteriochlorophyll concentration (mg/m^3;---) and conductivity (umhos/cm; ——; corrected to 25°C) as a function of depth (meters).

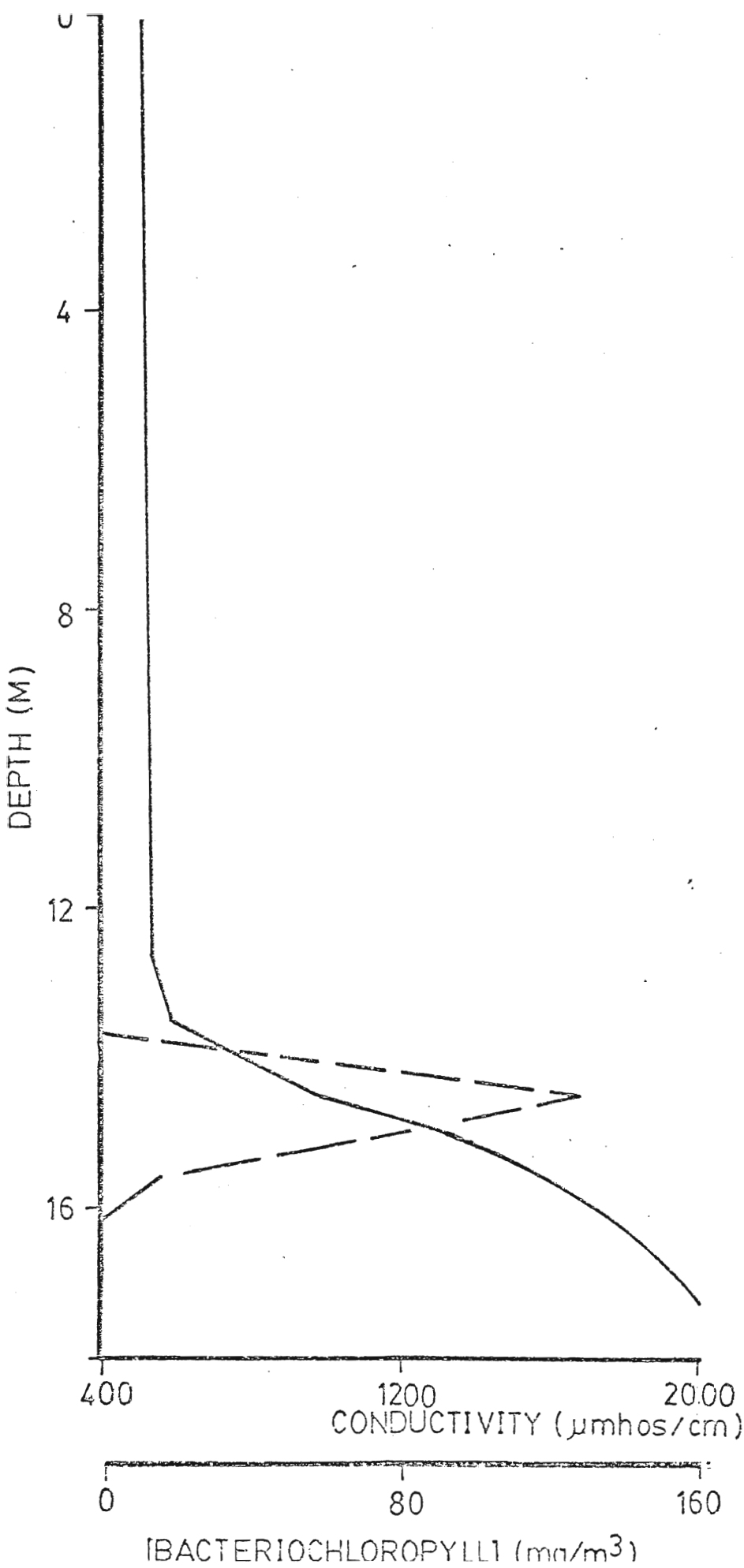

0
4
8
12
16
DEPTH (M)
400
1200
2000
CONDUCTIVITY (µmhos/cm)
0
80
160
BACTERIOCHLOROPYLL1 (mg/m3)

Figure 5: An isobar diagram of temperature corrected (25°C) specific conductivity (umhos/cm) as a function of both depth (meters) and time (months). The thickened line at zero meters extends over the period of ice cover. The thickness of the line does not represent ice thickness. Plot of raw data in Appendix 3K.

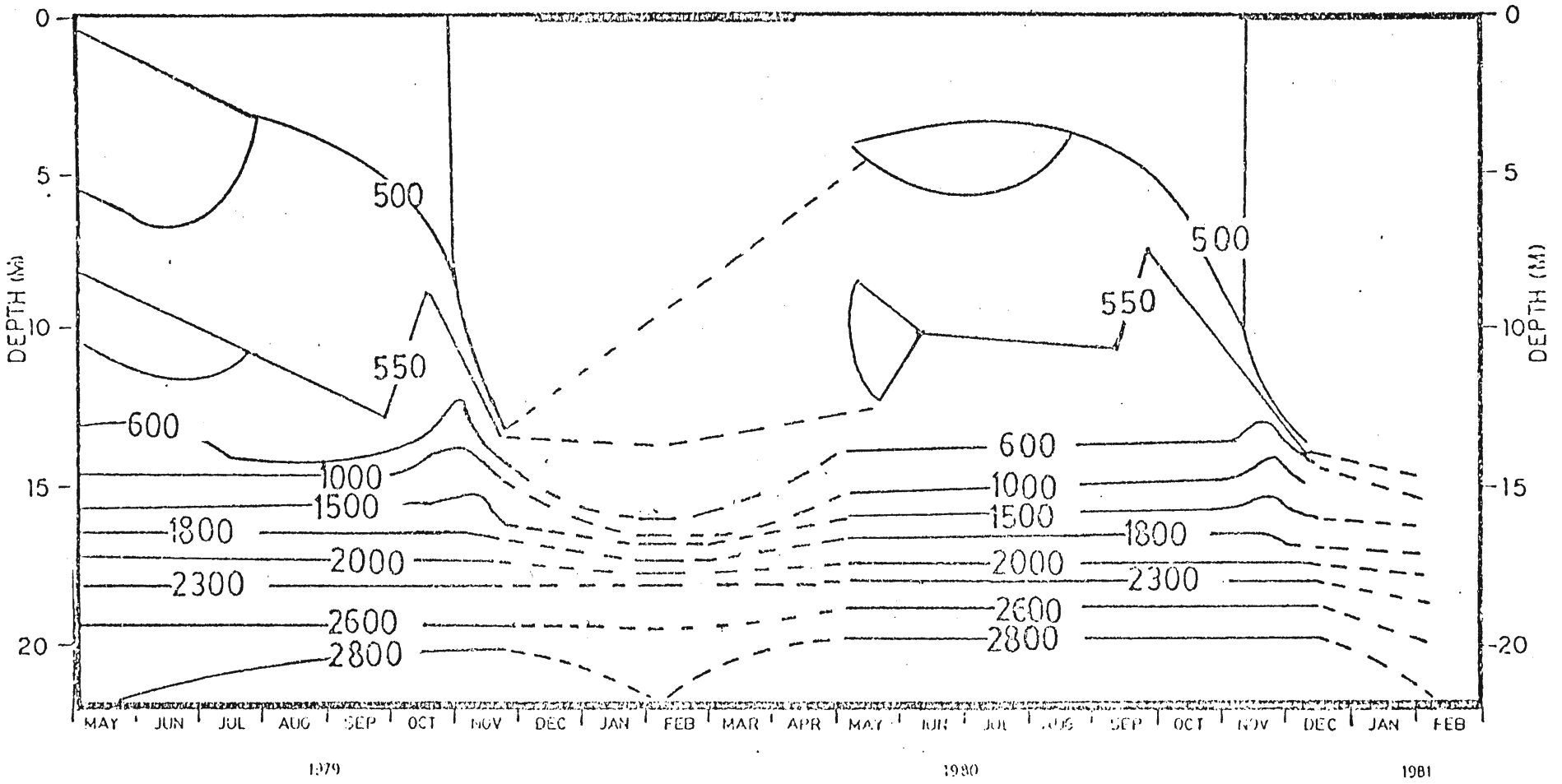
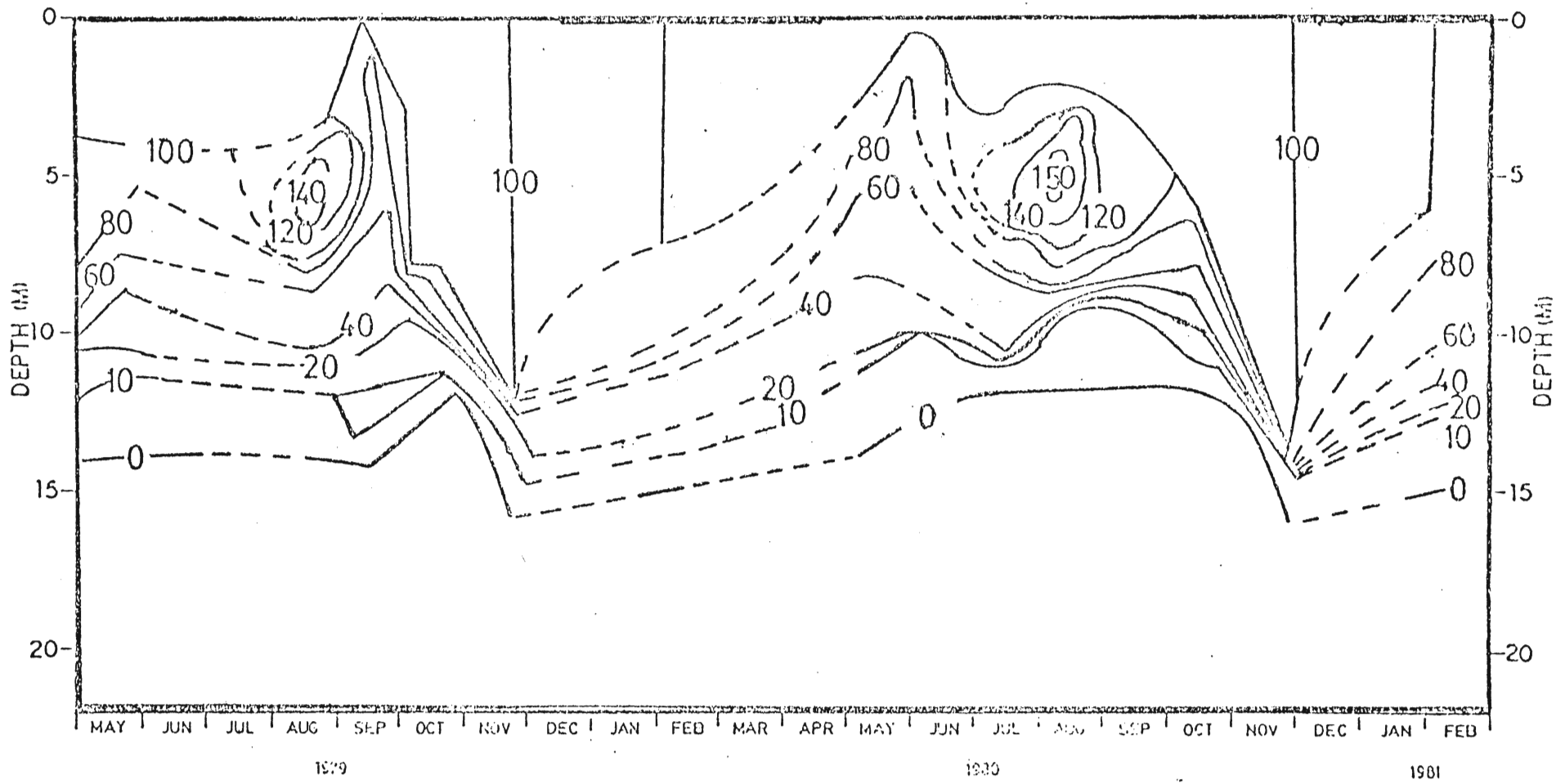

0
5
10
15
20
DEPTH (M)
500
550
600
1000
1500
1800
2000
2300
2600
2800
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
1979
1980
1981

Figure 6: An isobar diagram of dissolved oxygen (% saturation) as a function of both depth (meters) and time (months May 1979 to February 1981). The thick line at zero meters was used to represent ice cover. The thickness of the line does not represent ice thickness. Plot of raw data in Appendix 3L.


0
5
10
15
20
DEPTH (M)
100 - τ
140
120
80
60
40
20
10
0
100
150
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
1979
1980
1981

chemocline (i.e., the lower hypolimnion) became anaerobic (Fig. 6). The chemocline was thereafter defined in terms of rapid light attenuation and specific conductivity.

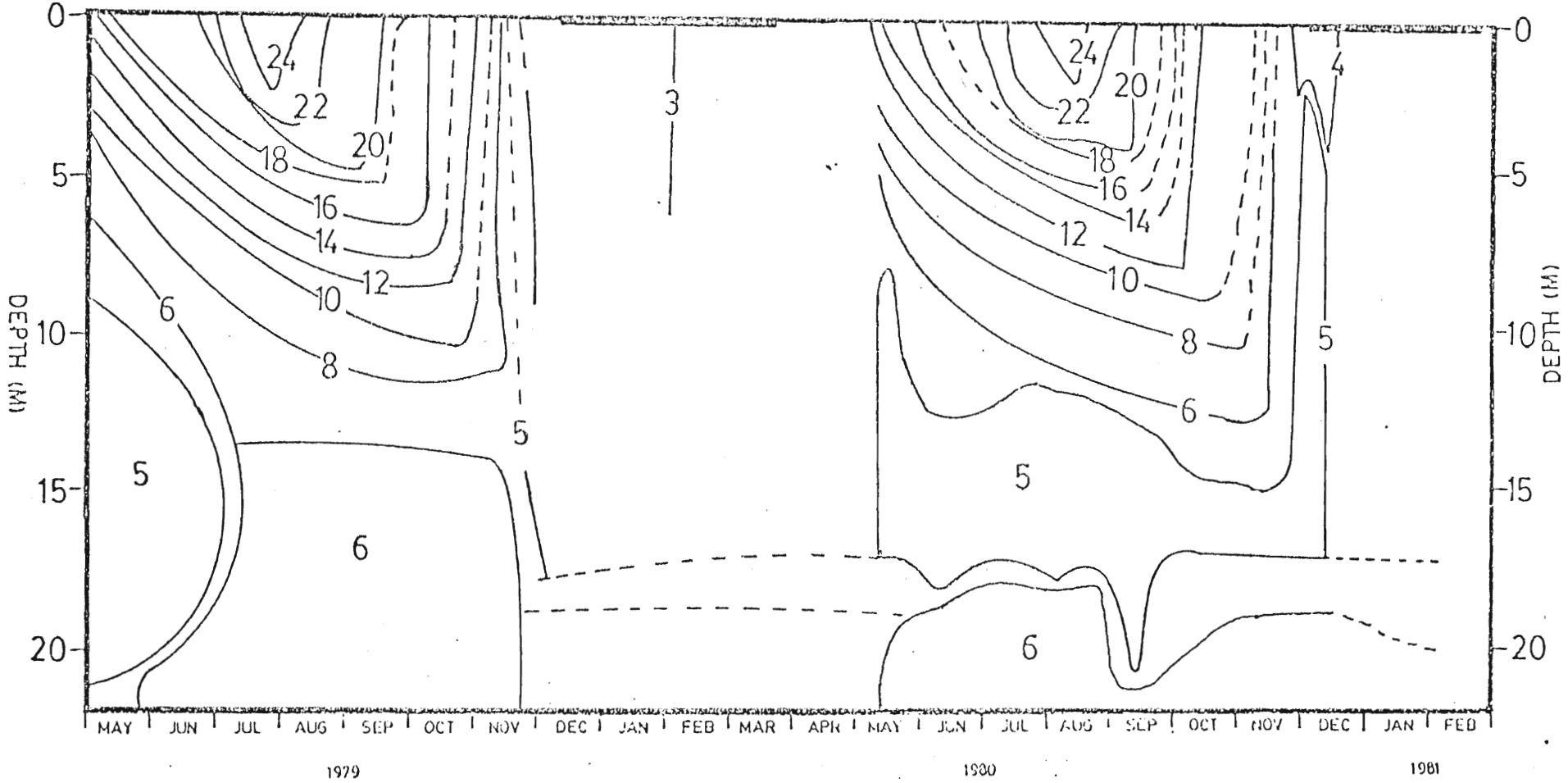
Ventilation was defined as the erosion of the top of the chemocline by wind-generated mixing. This was observed in the fall of both years (1979 and 1980). Prior to ventilation, the chemocline was observed at 14.0 meters in late October 1979 and 14.25 meters in mid-November 1980 (Fig. 5). Ventilation eroded the top of the chemocline to 15.1 and 15.25 meters in 1980. The erosion of the chemocline occurred in the first week of November 1979 and between the 17th and 21st of November, 1980.

In both years complete mixolimnetic homeothermy was observed only after ventilation had occurred (Fig. 7). This was unexpected as it was assumed that homeothermic conditions necessarily preceded ventilation. This will be described in more detail in the discussion section.

Seasonal Changes in the Chemocline Light Intensity Incident on the Chemocline

Productivity data suggested the subdivision of the summer into three periods: spring (May-June), summer (July-September) and fall (October-November). The objective for dividing the photosynthetic bacteria seasonal productivity pattern into 3 was to determine whether light intensity or light quality and photosynthetic bacterial productivity followed the same seasonal pattern. Light intensity measured at 13 meters was used to indicate the relative amount of

Figure 7: An isotherm diagram of temperature (°C) as a function of both depth (meters) and time (months, May 1979 to February 1980). The thick line at zero meters represents ice cover. The thickness of the line does not represent ice thickness. Plot of raw data in Appendix 3M.


0
5
10
15
20
DEPTH (M)
24
22
20
18
16
14
12
10
8
6
5
3
4
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
1979
1980
1981

light incident at the chemocline (Fig. 8).

There was no significant difference (U_{obs} = 62, U_{crit} = 33 at P = 0.95) between the number of sample days of high light intensity in spring as compared to summer. However, there was a significant difference (U_{obs} = 36.0, U_{crit} = 55 at P = 0.95) in the number of days with high light intensity during the summer when compared to the fall. Therefore, light intensity was high in spring and summer, low in fall while the pattern of photosynthetic bacterial productivity was low in the spring and fall and high only in summer.

Changes in pH and Alkalinity at the Chemocline

Alkalinity was measured because of its possible importance to photosynthesis (Pfennig, 1979) and because it was necessary for the determination of ^{14}C primary production (Vollenvider, 1969b).

The chemocline was monitored for changes in pH (-log[H+]) and alkalinity (expressed as mg$CaCO_3$/l) from April to May 1980.

The alkalinity at the chemocline peaked in August at 350 mg $CaCO_3$/l (Fig. 9). The lowest recorded value was 154 mg$CaCO_3$/l in December (Fig. 9).

The pH was 6.6 $\pm$ .05 throughout most of the study period, ranging from a maximum of 6.8 in August and a minimum of 6.4 in July (Fig. 10).

Figure 8: Light intensity (ft. candles) at 13 meters plotted against time (months May 1980 to December 1980). The points ● without vertical bars represent the light intensity at 1200 hours. Points ● with vertical bars represent the average of the recorded intensities over the time period 10 AM to 2 PM. The vertical bars extended from the lowest to the highest light intensity values recorded on the sample day.

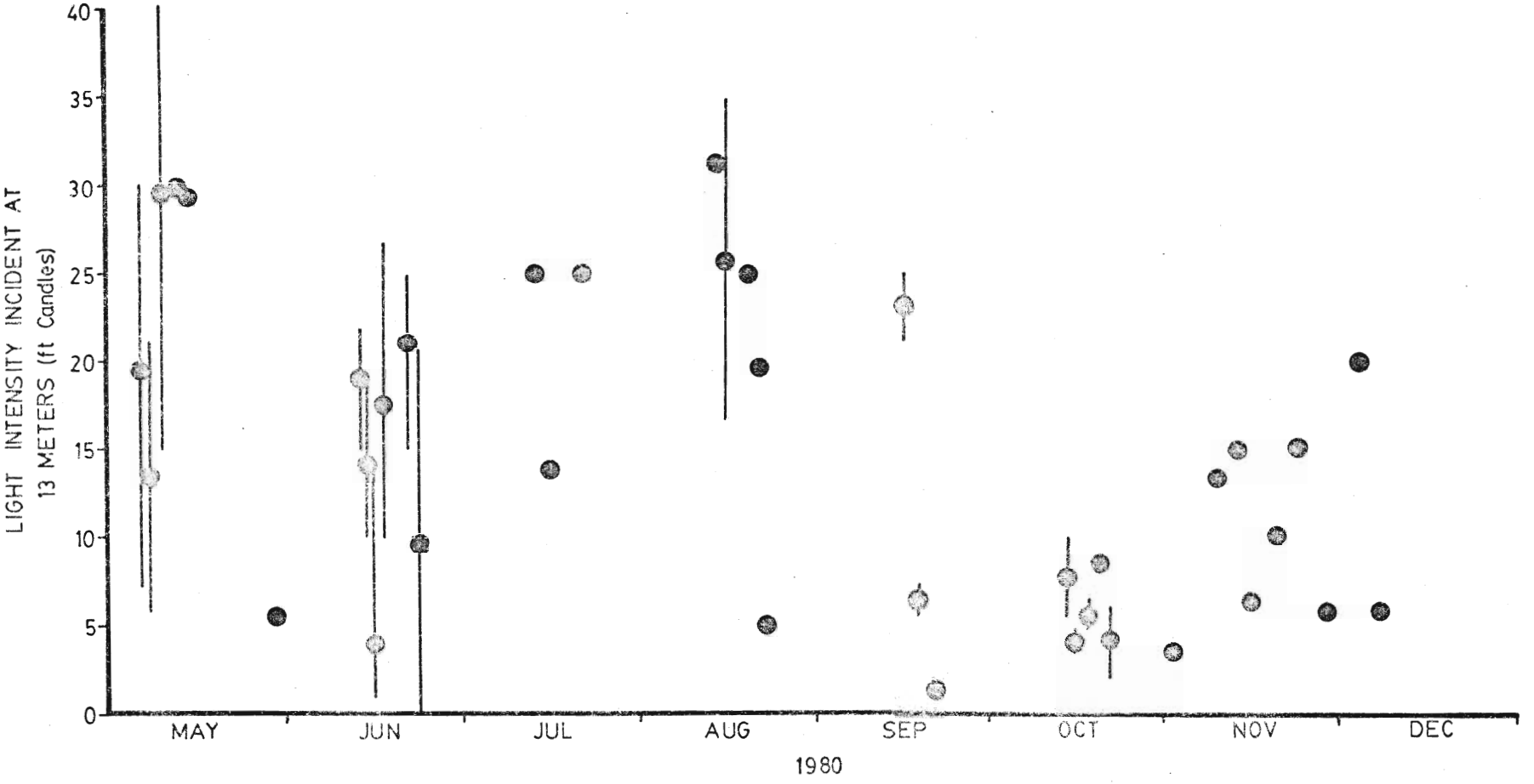

LIGHT INTENSITY INCIDENT AT
13 METERS (ft Candles)
40
35
30
25
20
15
10
5
0
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
1980

Figure 9: The change in alkalinity at chemocline ($MgCaCO_3/l$) as a function of time from May to December 1980. The maximum recorded error was $\pm$ 7.5 $mgCACO_3/l$ in August.

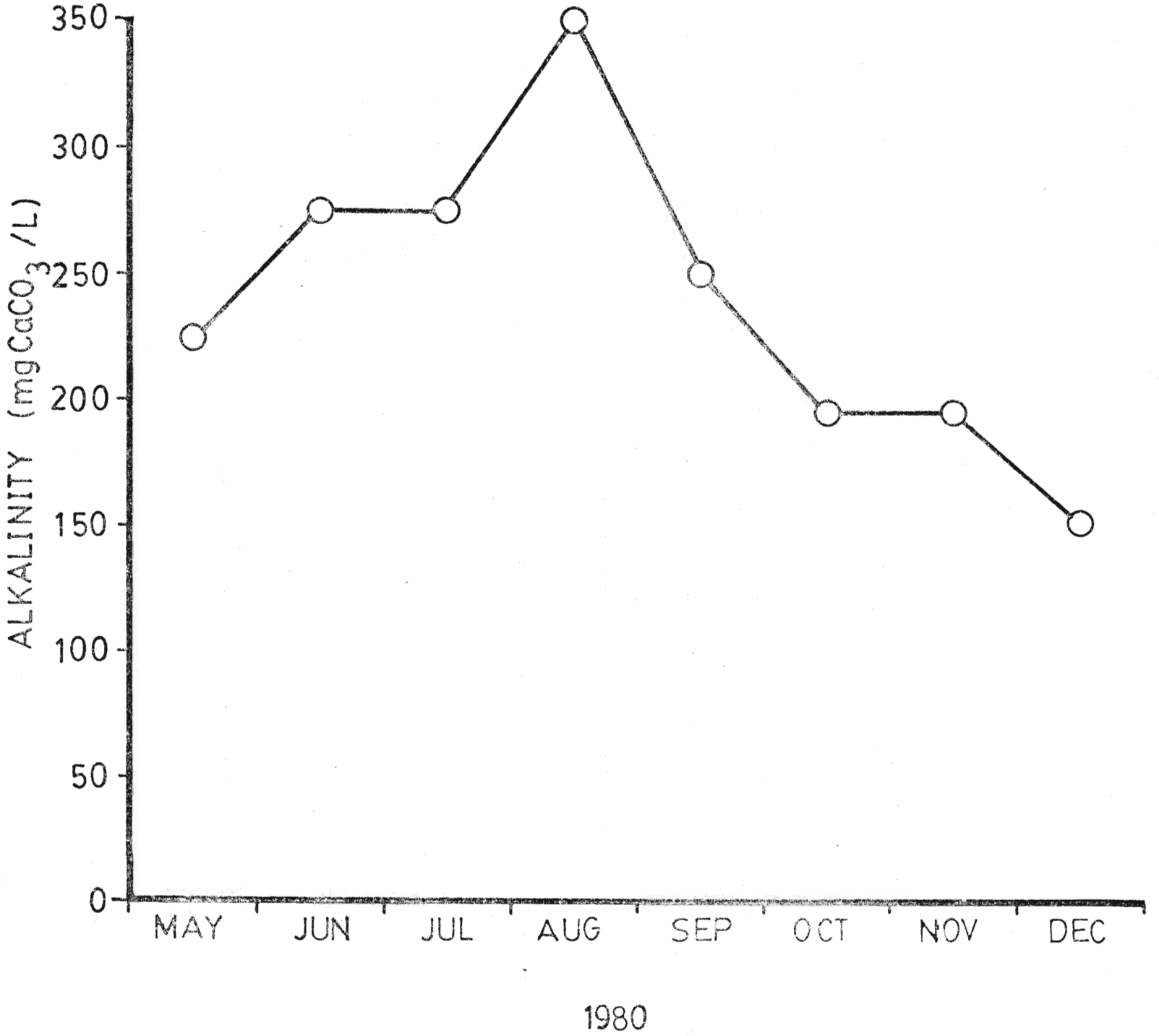
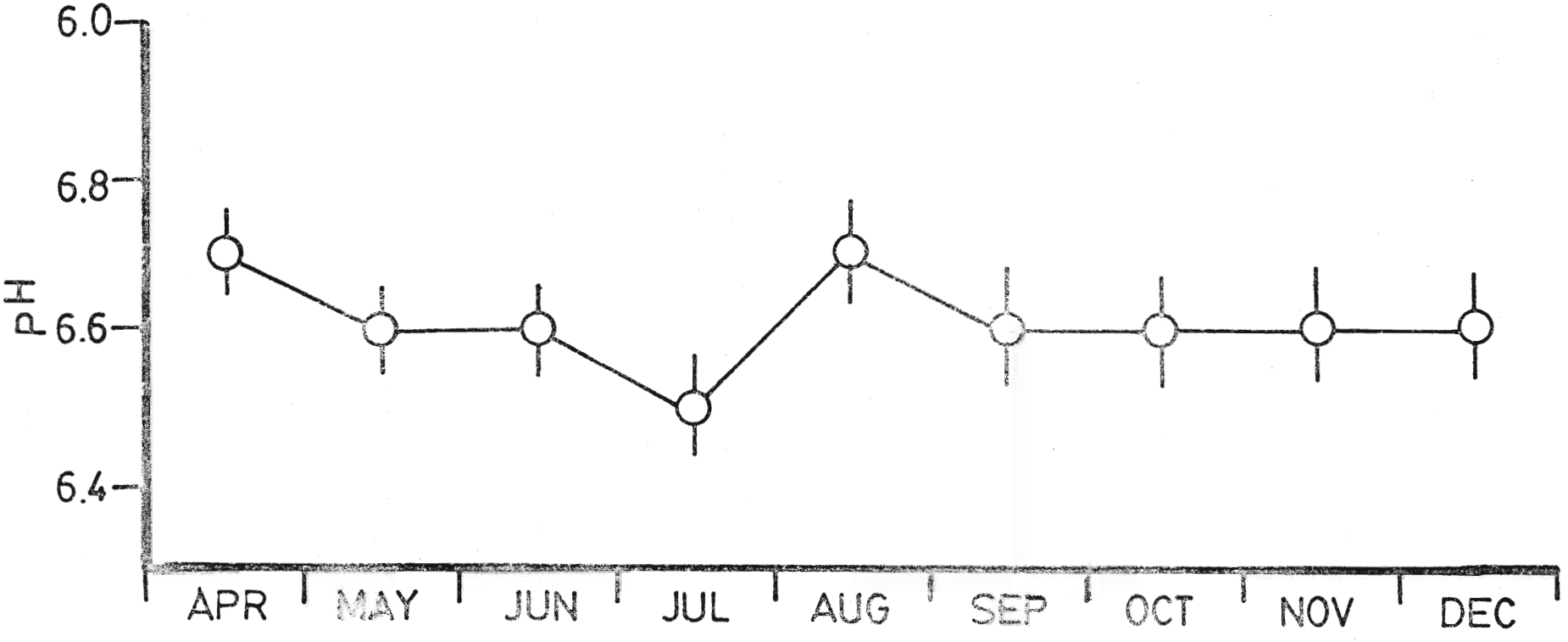

350
300
250
200
150
100
50
0
ALKALINITY (mg $CaCO_3$ /L)
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
1980

Figure 10: The pH at the chemocline recorded from April to December 1980. The vertical bars represent the potential range due to the accuracy of the pH meters used.


6.0
6.8
pH
6.6
6.4
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

Biological Parameters

Monitoring Changes in the Population Size of the Photosynthetic Bacteria

Bacteriochlorophyll concentration was used as an indicator of photosynthetic bacterial population density (Severn, 1979). I was interested in examining seasonal changes in the population size of the bacteria for patterns that could be correlated with physical and biological changes in the lake.

Analysis of the 1979 population data showed no pattern. The population size of photosynthetic bacteria decreased from a maximum in June and maintained a level approximately half that of June's for the rest of the study period (Fig. 11).

In 1980, the population size of the photosynthetic bacteria oscillated, approximating a sinusoidal curve. The sine curve was fitted by eye (Fig. 12). This curve was approximated by the equations, $y_1 = 35 \sin (36 x + 86.4) + 100$, when $x < 150$ and $y_2 = 53.2 \sin [2.5 (x-150 + 361] + 185$, when $x > 150$ and was tested using least squares analysis.

The population size of the photosynthetic bacteria peaked in August (day 100) and again in December (day 221).

Cycling was observed in the change in the wavelength of the peaks in the blue region of the acetone spectra obtained during the study period (Fig. 13). The cycling was best represented by peak #1 (Fig. 14). The change in the peak followed a sine curve (Fig. 13).

Figure 11: Bacteriochlorophyll concentration (mg/m^3) as a function of time (days and months). The straight line was fitted by regression analysis and the dotted line (---) by eye. The vertical bar on the straight line represents the 95% confidence interval. Day zero was arbitrarily set equal to May 1, 1979.

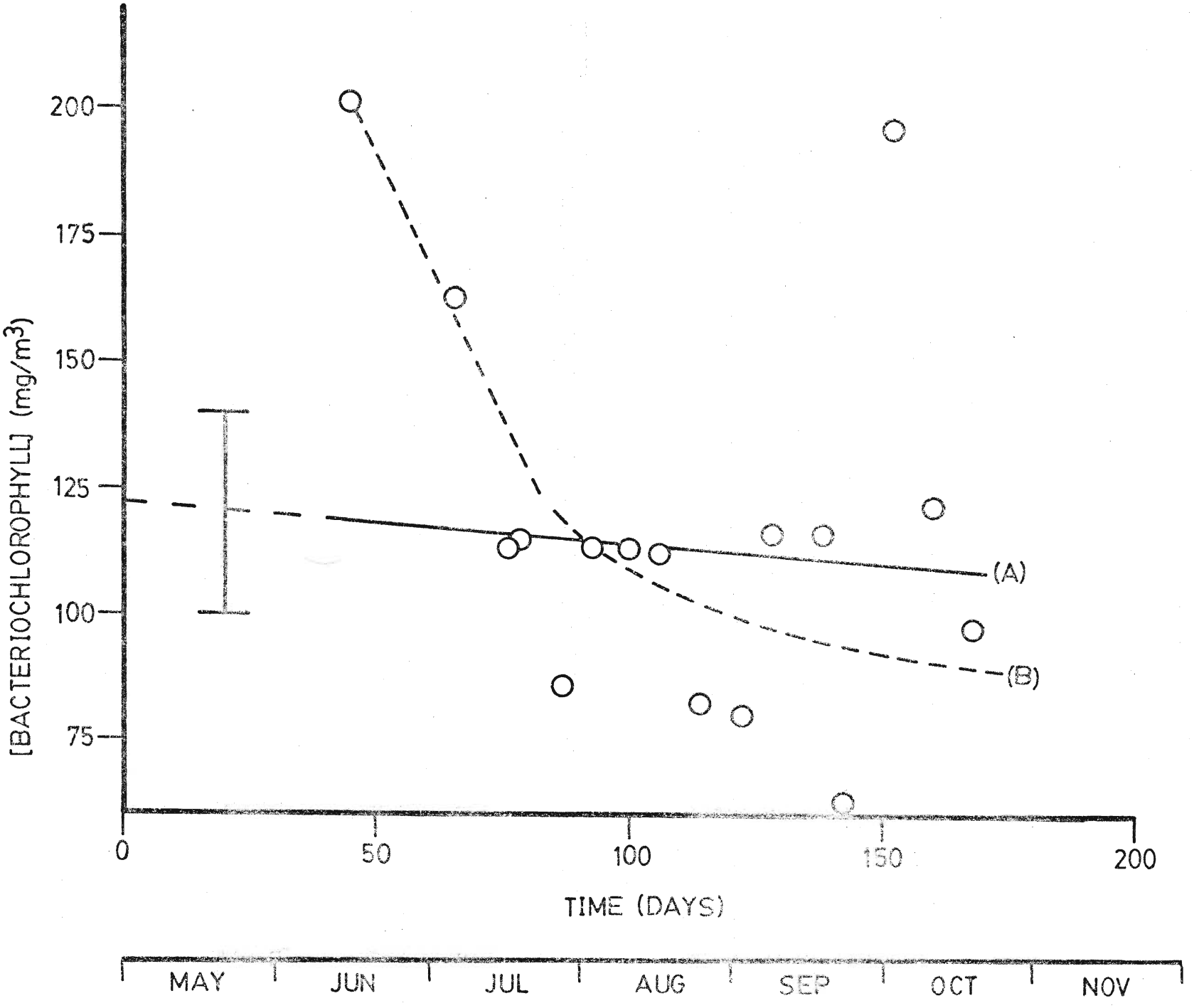

200
175
150
125
100
75
[BACTERIOCHLOROPHYLL] (mg/m3)
(A)
(B)
0
50
100
150
200
TIME (DAYS)
MAY
JUN
JUL
AUG
SEP
OCT
NOV

Figure 12: Bacteriochlorophyll concentration as a function of time (days and months). Line (A) is a sinusoidal line ($y = 35 \sin (3.6x + 86.4) + 100$, when $x \leq 150$ or $y_2 = 53.5 \sin [2.5 (x-150) + 261] + 185$, when $x \geq 150$). Line B was fitted by regression ($y = 0.33x + 62$). The vertical bar on the straight line represents the 95% confidence interval. Day zero was arbitrarily set equal to May 1, 1980.

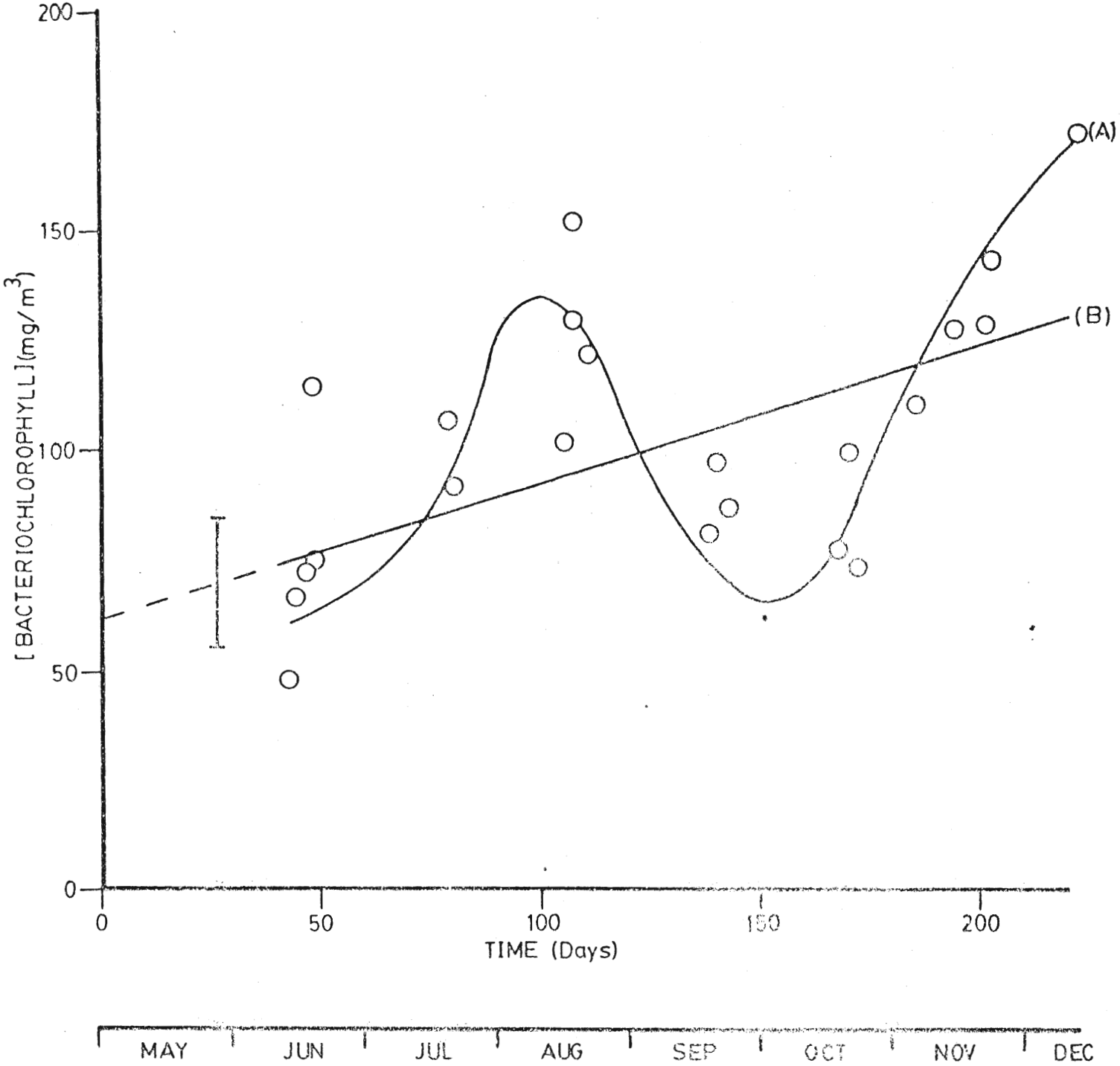

200
150
100
50
0
[BACTERIOCHLOROPHYLL](mg/m3)
(A)
(B)
0
50
100
150
200
TIME (Days)
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

Figure 13: Seasonal change in wavelength (nm) in the soret region of the bacteriochlorophyll spectrum. The line fitted through the points is approximated by the equation, $y = 5.5 \sin (1.95x + 83.67) + 339$, the vertical bar represents a range of $\pm$ .5 nm on multiple spectra taken on that day.

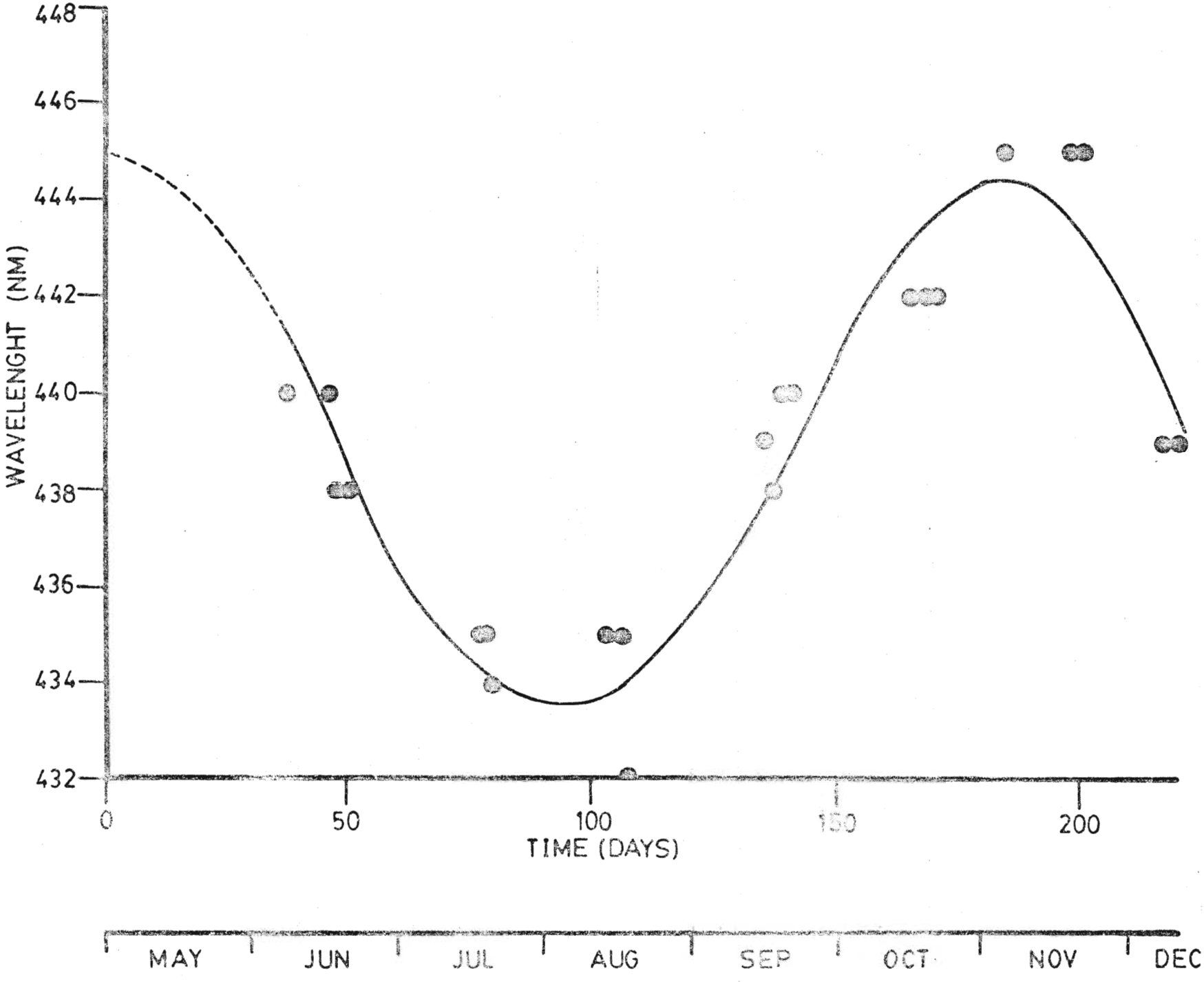
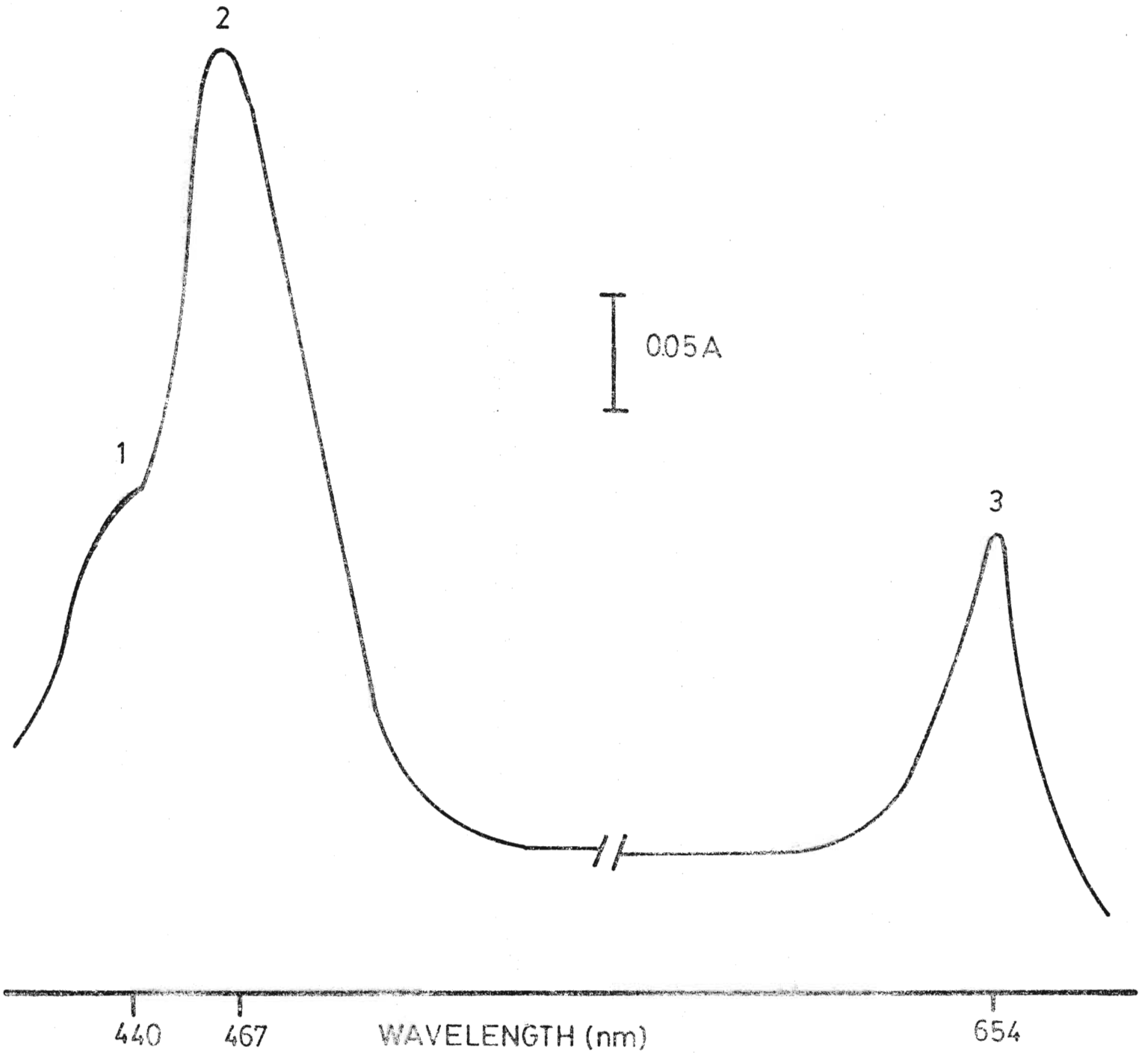

448
446
444
442
440
438
436
434
432
WAVELENGHT (NM)
0
50
100
150
200
TIME (DAYS)
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

Figure 14: Acetone extracted spectrum of photosynthetic bacterial pigments. The vertical bar represents 0.05 absorbance units and the wavelength was measured in nanometers.


2
1
3
0.05A
440
467
WAVELENGTH (nm)
654

A trough occurred in August and a peak was observed in early to mid-November (day 190).

Monitoring Changes in the Population Size as a Result of Ventilation

The objective was to collect data that would indicate the effect of ventilation on the population size of photosynthetic bacteria. Samples were collected at 0.25 meter intervals through the chemocline (Fig. 15). The peak bacteriochlorophyll concentration was observed at 14.25 meters on November 17th. Bacteriochlorophyll concentration maxima were observed at 15.25 meters on November 21st and December 9th. Hence, from November 17th to 21st the chemocline depth decreased by one meter.

Seasonal Changes in Primary Productivity

The seasonal changes in primary productivity of the photosynthetic bacteria were examined in order to determine whether a seasonal pattern existed. Only one general pattern emerged. I observed that the study period could be subdivided into three main periods (spring, summer and fall) based on the analysis of the photosynthetic bacterial primary productivity. Spring (May and June) and fall (October to December) were characterized by relatively low primary productivity (1.8 to 25 $mgC/m^3/hr$; (Fig. 16). This period also demonstrated a range of primary productivity values over a wide range of bacteriochlorophyll concentrations (Fig. 17). The summer period (July to September) was characterized by higher productivity values (25 to

Figure 15: Bacteriochlorophyll concentration (mg/m^3) as a function of depth (meters), for November 17 (——), November 21 (---) and December 9 (- · -). The horizontal bars represent 95% confidence intervals.

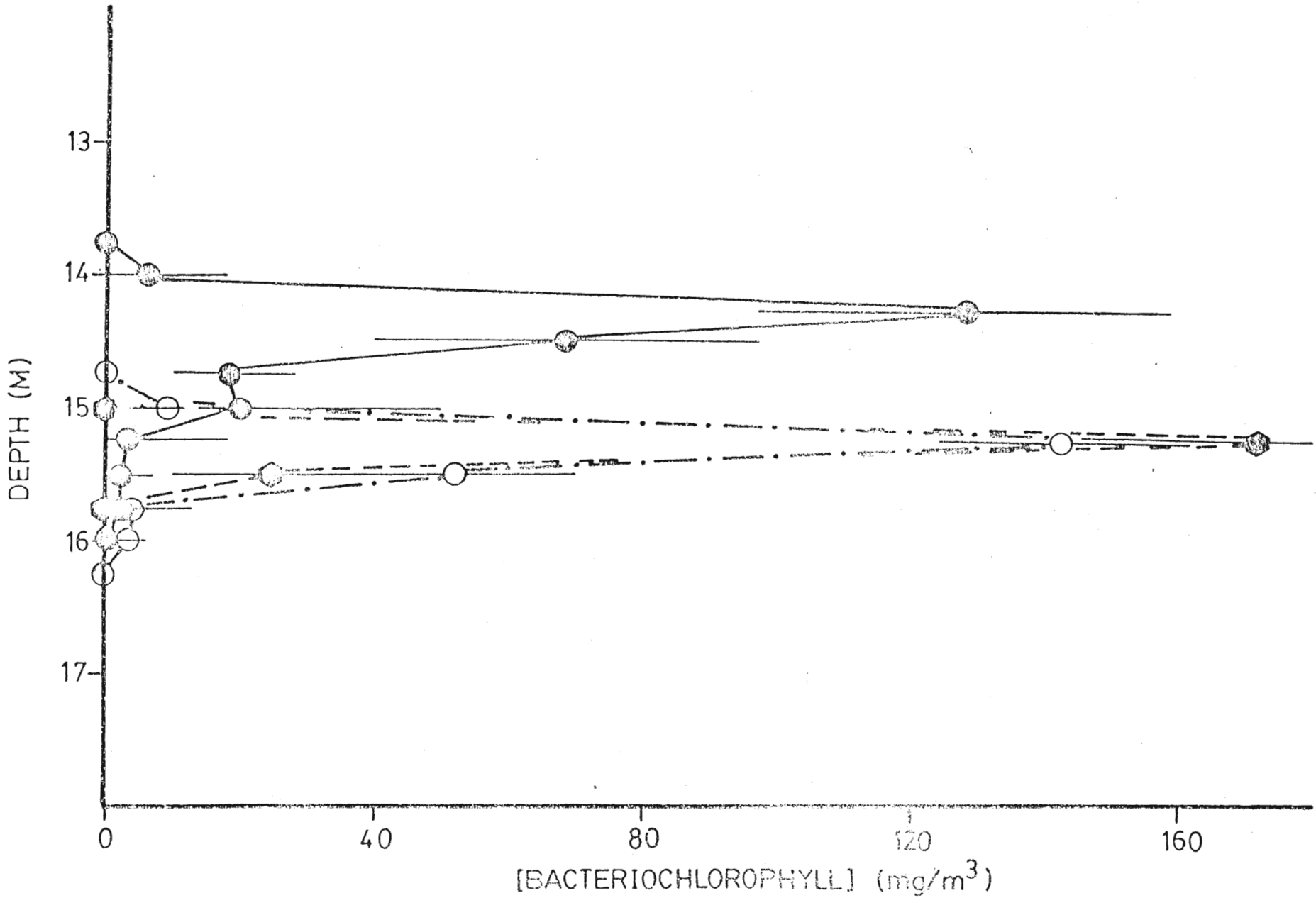

DEPTH (M)
13
14
15
16
17
0
40
80
120
160
[BACTERIOCHLOROPHYLL] (mg/m3)

Figure 16: The primary productivity (Pl-Pd equals ^{14}C uptake in light bottle - ^{14}C uptake in dark bottle; mgCarbon m^{-3}. hr^{-1}) as a function of time (May to December 1980 ◯). The vertical bars (⊕) represent the mean net productivity and 95% confidence interval for each month. The line (——) joins the mean net productivity values of each month. For clarity purposes the mean ● and 95% confidence values were off-set to the right of the mid-point of each month.

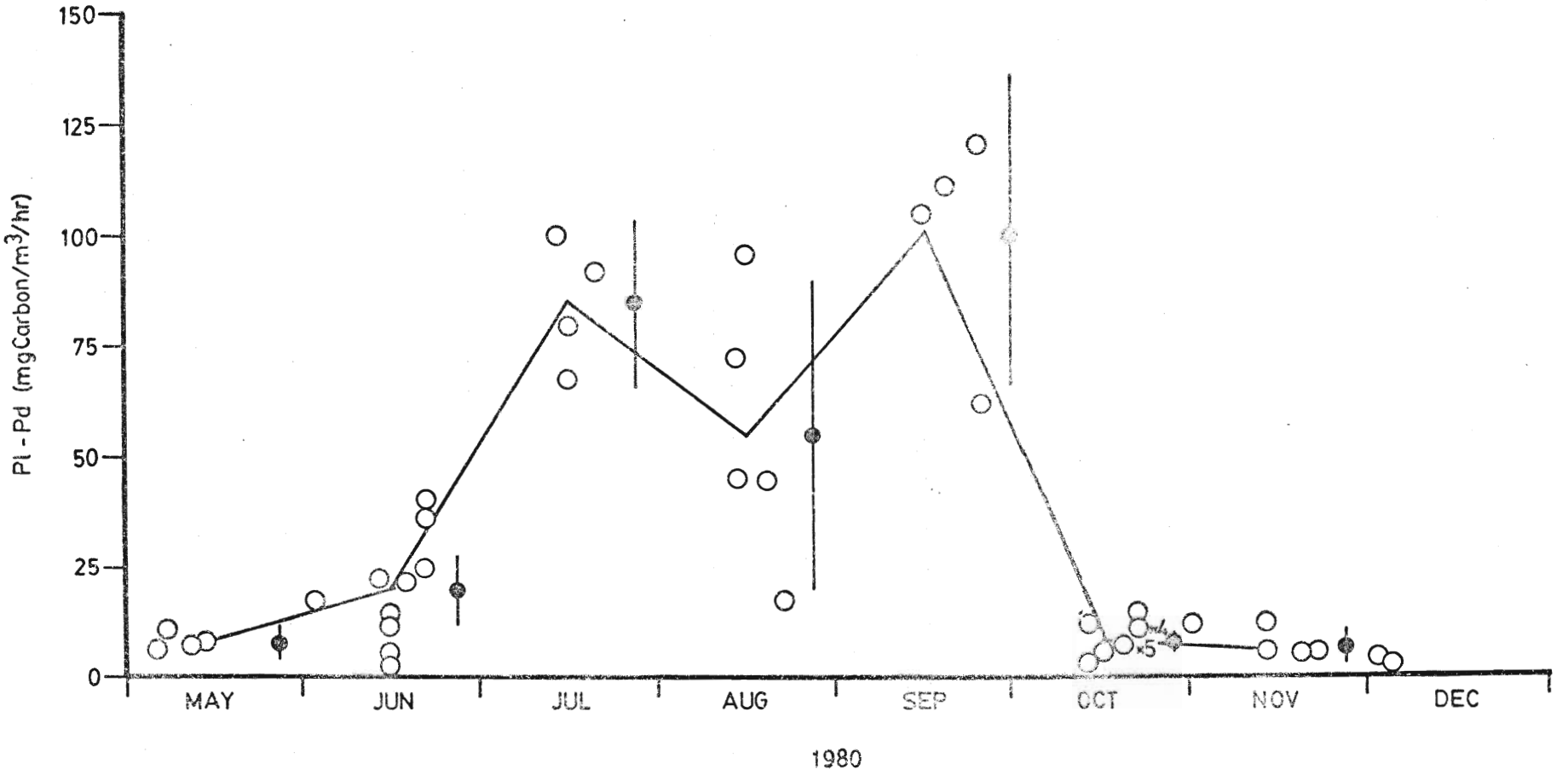
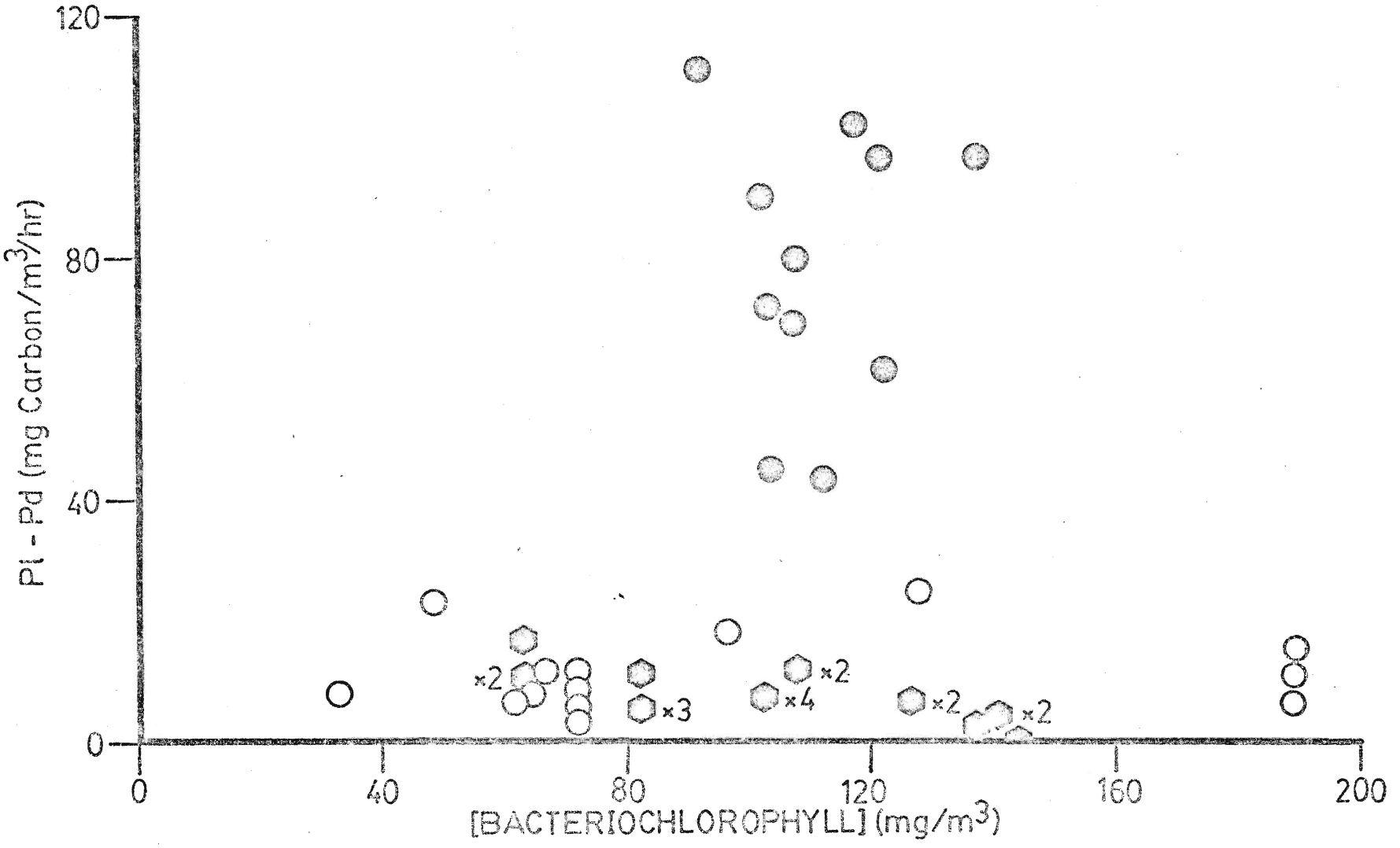

150
125
100
75
50
25
0
Pl - Pd (mgCarbon/m3/hr)
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
1980

Figure 17: Primary productivity (Pl-Pd mg Carbon x m^{-3} x hr^{-1}) plotted against bacteriochlorophyll concentration ([bacteriochlorophyll] mg/m^3). The numbers (for example X2) represent number of values identified by that particular x and y.

May and June
July, August and September
October, November and December


120
80
40
0
Pt - Pd (mg Carbon/m3/hr)
0
40
80
120
160
200
[BACTERIOCHLOROPHYLL] (mg/m3)
×2
×3
×4
×2
×2
×2
×2

125 mgC/m^3/hr; Fig. 12), which were distributed over a narrower range of bacteriochlorophyll concentrations (Fig. 17). In all subsequent analyses this set of subdivisions will be used to simplify the description of the time period May to November, 1980.

Comparisons Between Several Selected Factors and Productivity

Lake transparency, light intensity and alkalinity, were monitored and compared to corresponding changes in photosynthetic bacterial primary productivity during the 1980 field season. The effect (if any) that these changes had on primary productivity of the photosynthetic bacteria was examined in this section.

Lake Transparency

Primary productivity as a function of lake transparency (Secchi depth) yielded no significant (meaningful) relationship (Fig. 18). Correcting for population size (by dividing net productivity by bacteriochlorophyll concentration) did not improve the scatter of the points or yield any indication that lake transparency affected primary productivity (Fig. 19).

The Effect of Light Intensity at 13 Meters

No positive correlation was found between light intensity and bacterial primary productivity for the spring (Fig. 20), summer (Fig. 22) or fall (Fig. 24). Nor was there any correlation found

Figure 18: Primary productivity (Pl-Pd) as a function of Secchi depth (meters). The data were divided by season, Spring ◉, Summer ○ and Fall ⬡ for reasons given in text.

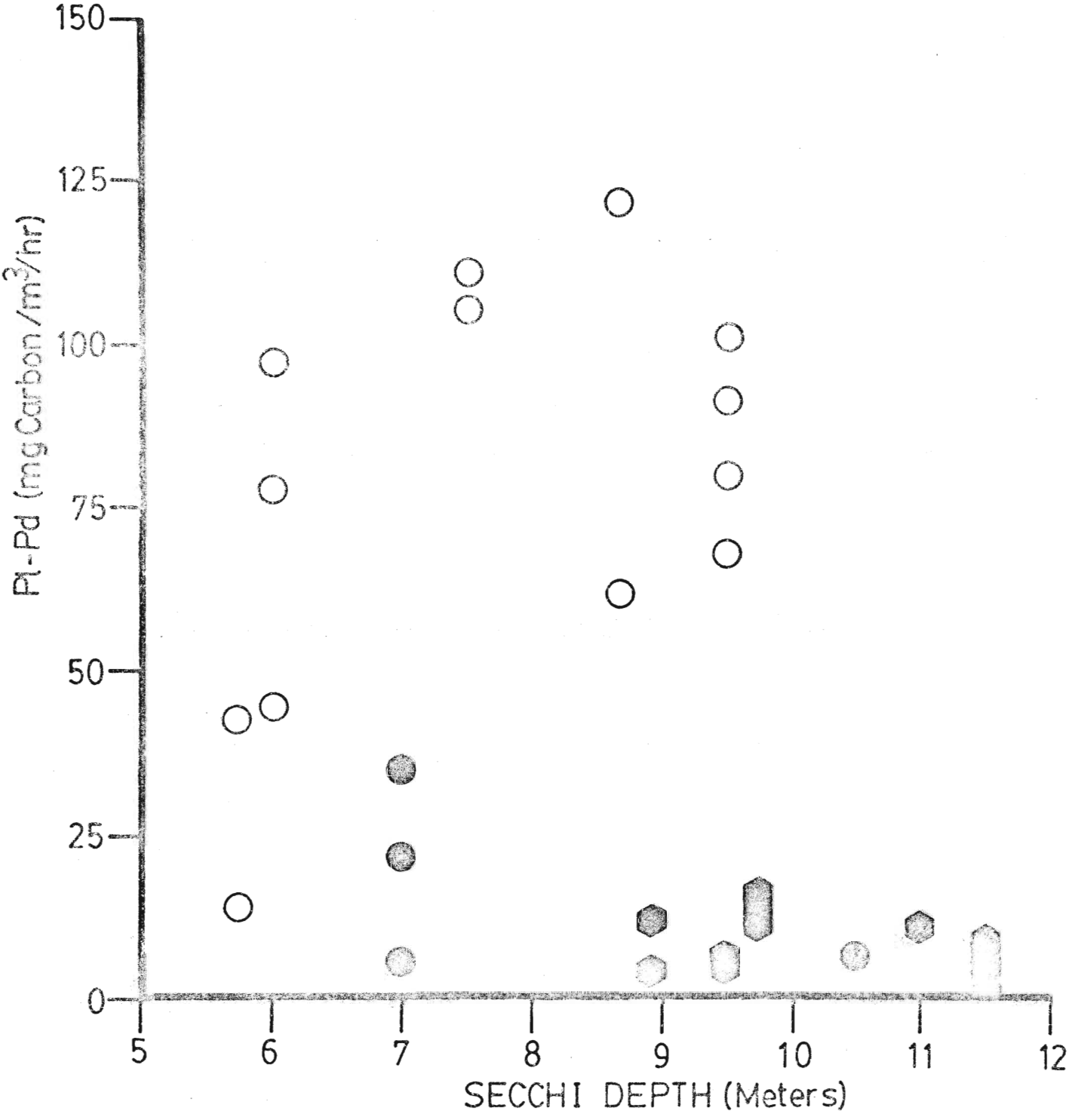

150
125
100
75
50
25
0
Pt-Pd (mg Carbon/m³/hr)
5
6
7
8
9
10
11
12
SECCHI DEPTH (Meters)

Figure 19: Population corrected productivity (Pl-Pd/[BChl] as explained in the text was plotted against Secchi depth (meters). The data were divided into 3 seasons (Spring ◐, Summer ○ and Fall ⬡) for reasons given in the text.

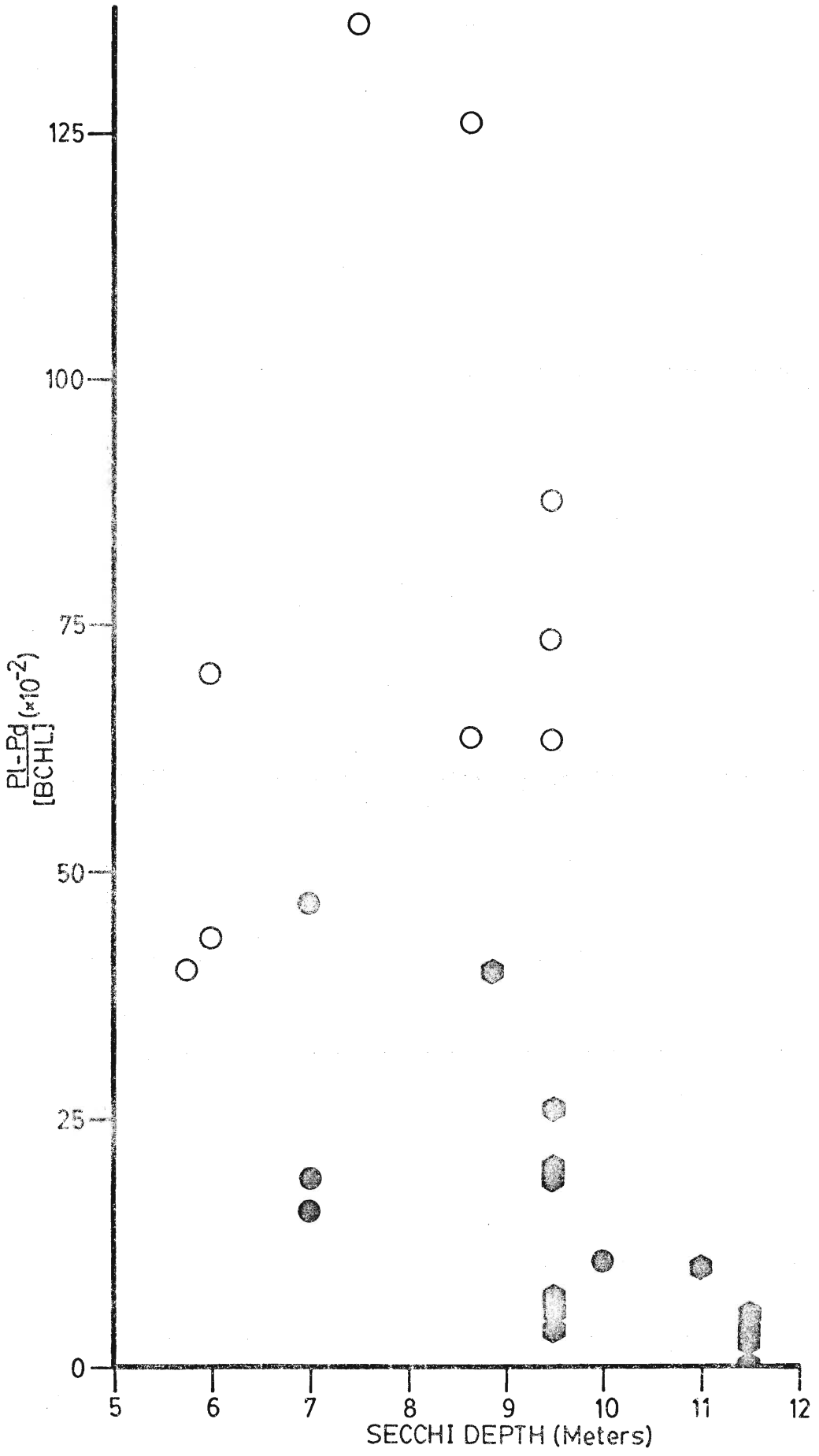

125
100
75
50
25
0
PL-Pd / [BCHL] (×10⁻²)
5
6
7
8
9
10
11
12
SECCHI DEPTH (Meters)

Figure 20: Primary productivity (Pl-Pd [mg Carbon/m^3/m]) as a function of light intensity incident on 13 meters (ft. candles) for the months of May ○ and June ◎.

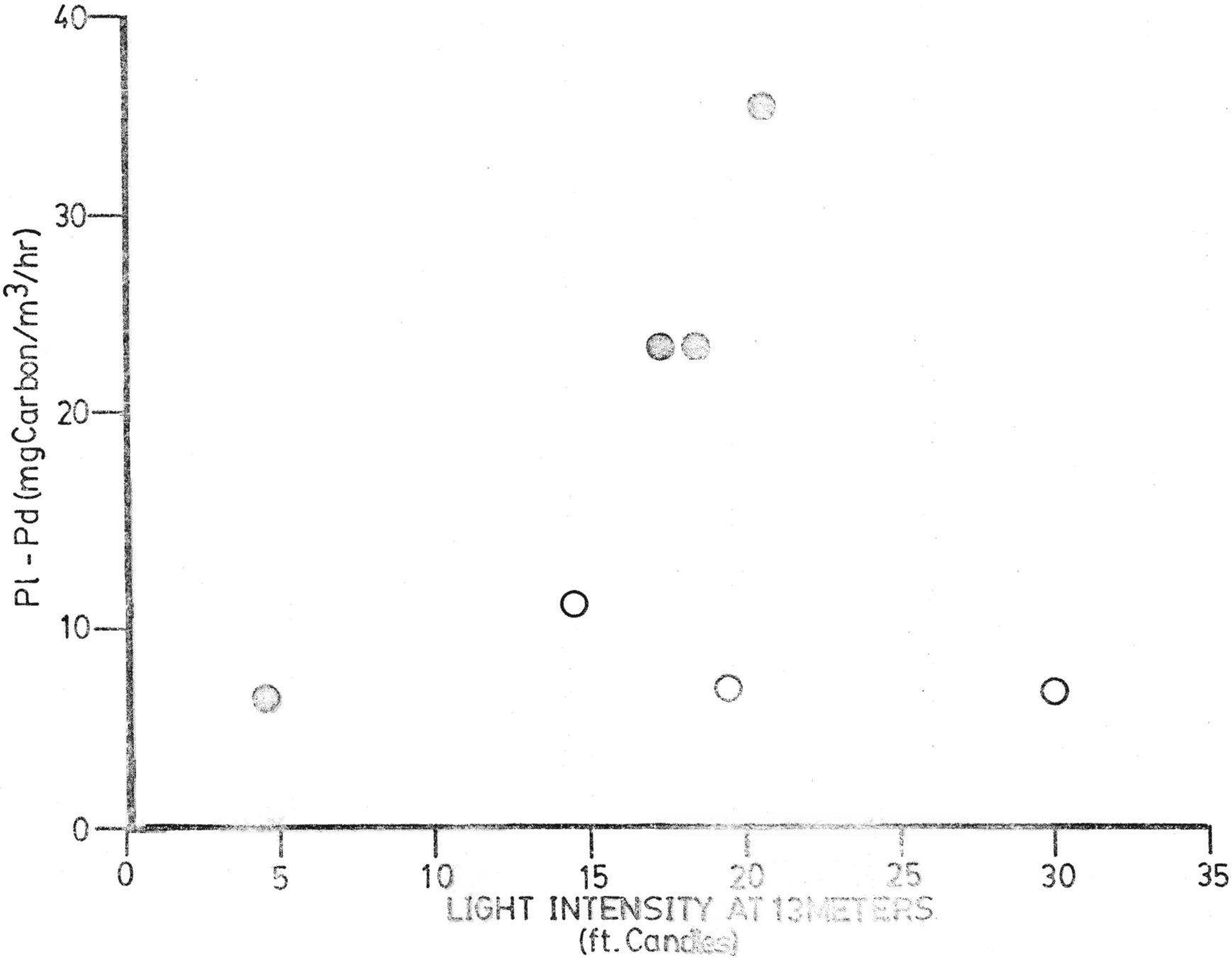

40
30
20
10
0
Pl - Pd (mgCarbon/m3/hr)
0
5
10
15
20
25
30
35
LIGHT INTENSITY AT 13METERS
(ft. Candles)

Figure 21: Primary productivity (Pl-Pd/[Bchl]) corrected for the population size of photosynthetic bacteria as a function of light intensity incident on 13 meter (ft. candles) for May ○ and June ◍.

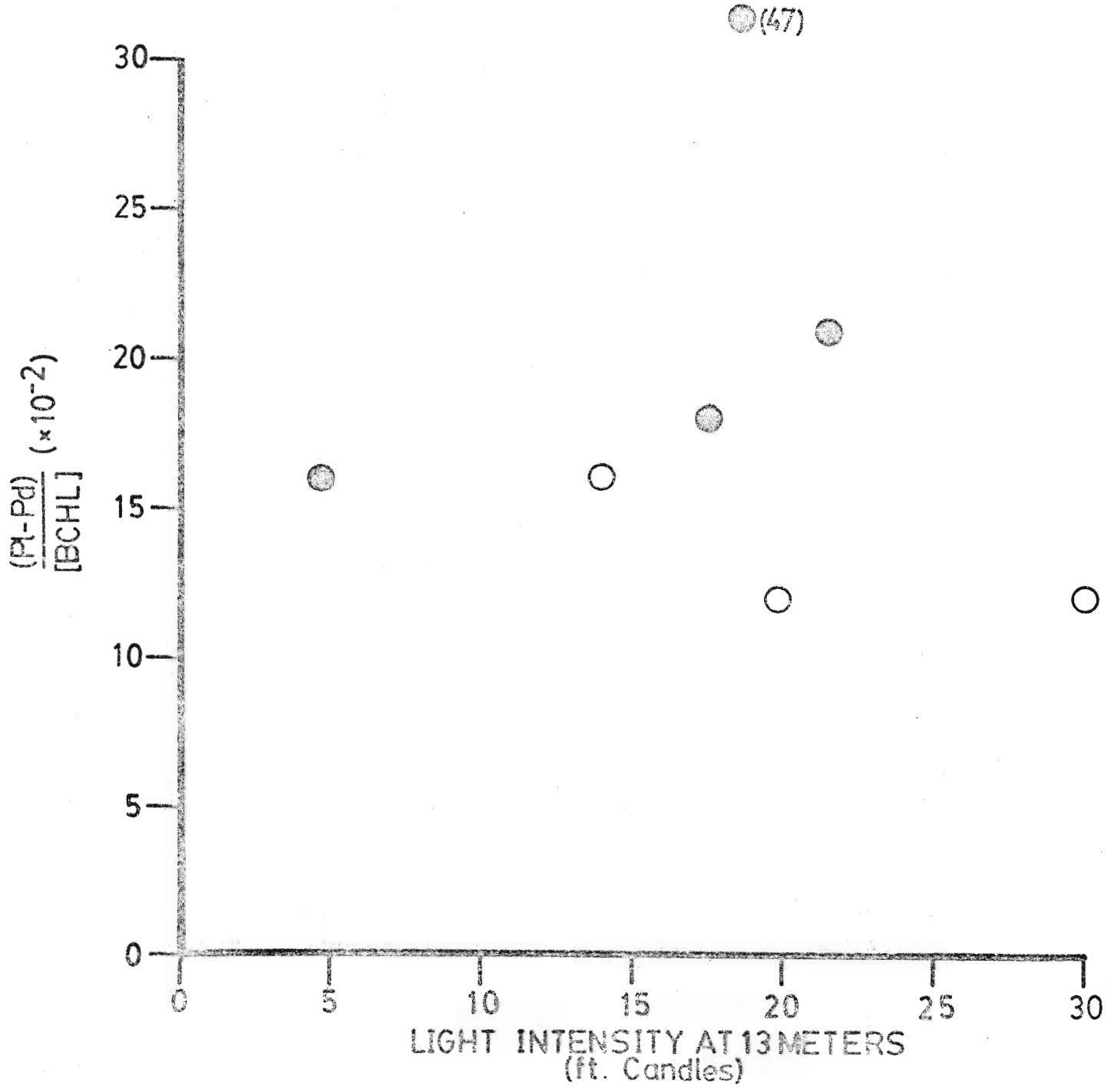

(47)
30
25
20
15
10
5
0
(Pt-Pd)/[BCHL] (×10⁻²)
0
5
10
15
20
25
30
LIGHT INTENSITY AT 13 METERS
(ft. Candles)

Figure 22: Primary productivity (Pl-Pd) as a function of light intensity incident on 13 m (ft. candles) for the months of July ○ , August ⬡ and September ◍ .

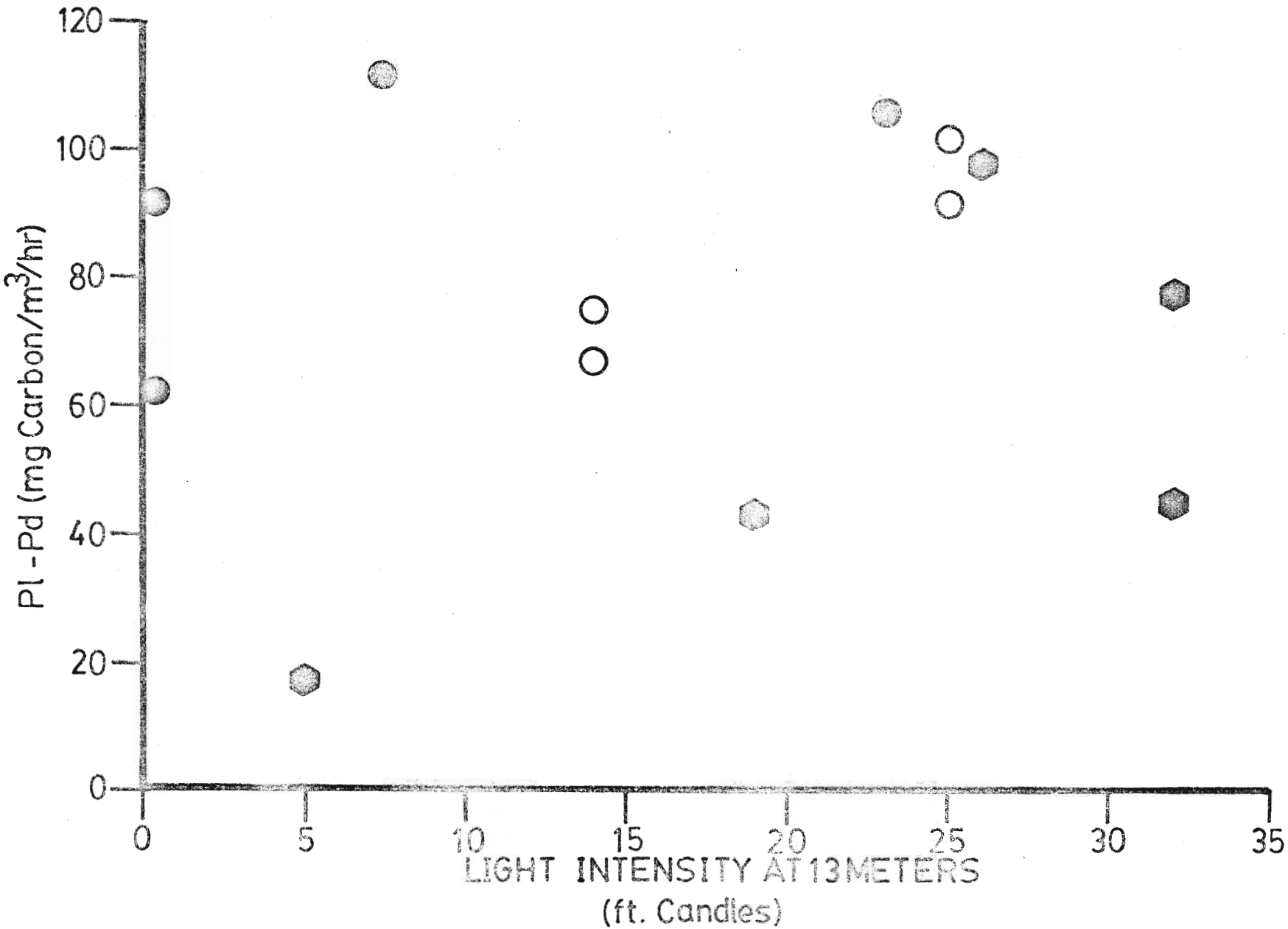

120
100
80
60
40
20
0
Pl - Pd (mg Carbon/m3/hr)
0
5
10
15
20
25
30
35
LIGHT INTENSITY AT 13 METERS
(ft. Candles)

Figure 23: Productivity (Pl-Pd/[Bchl]) corrected for population size as a function of light intensity incident on 13 meters (ft. candles) for July ○ , August ⬡ and September ◍ .

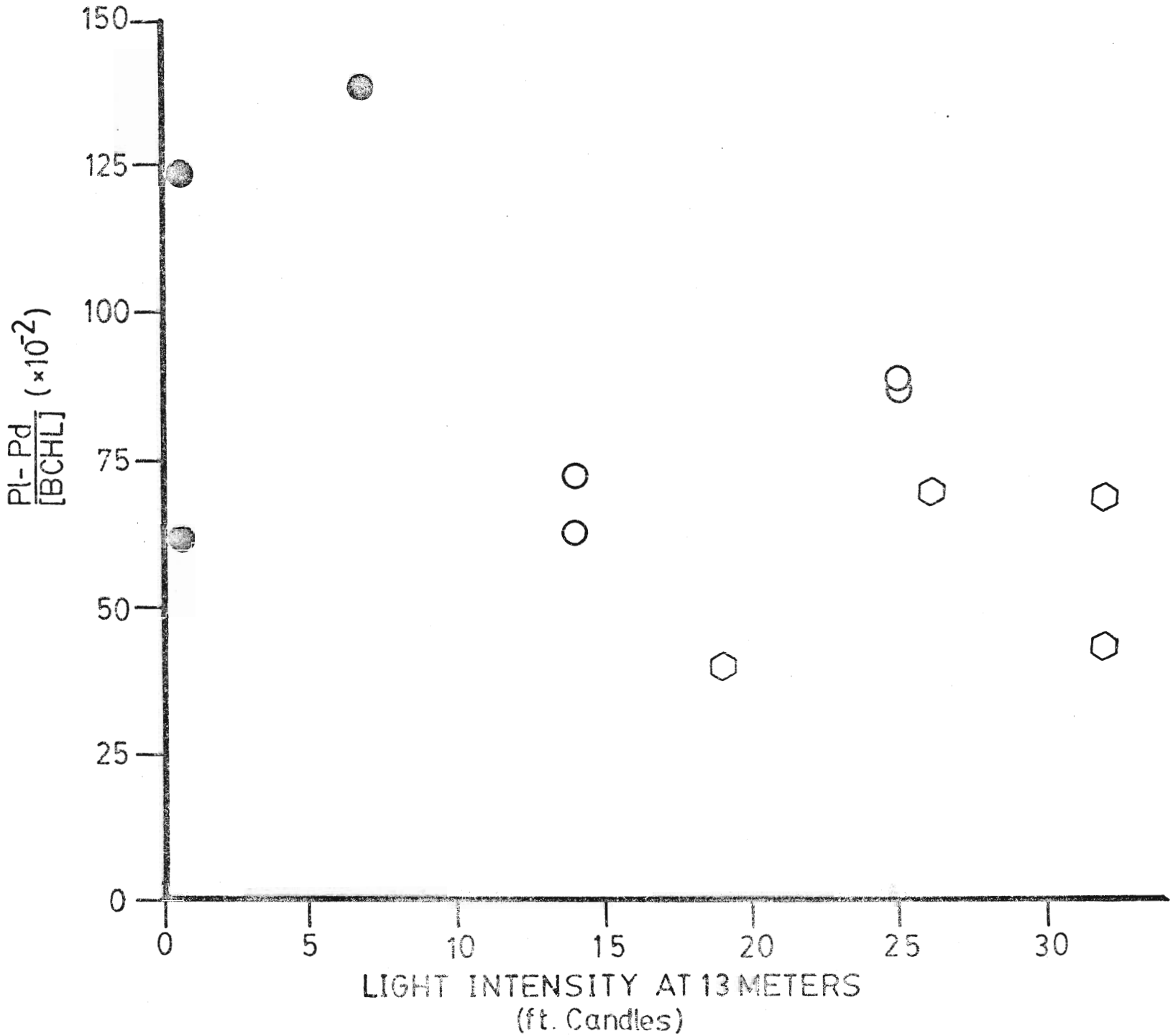

150
125
100
75
50
25
0
$\frac{Pl-Pd}{[BCHL]}$ ($\times 10^{-2}$)
0
5
10
15
20
25
30
LIGHT INTENSITY AT 13 METERS
(ft. Candles)

Figure 24: Primary productivity(Pl-Pd)as a function of the light intensity incident on 13 meters (ft. candles) for the months October ●, November ○ and December △.

between light intensity and primary production corrected for the size of the photosynthetic bacterial population for spring (Fig. 21), summer (Fig. 23) or fall (Fig. 25). However, if May was excluded from the spring analysis, then a significant ($r = 0.99$, $p \geq 0.95$) positive correlation between both primary productivity and population size corrected productivity and light intensity occurred (Figs. 20 and 21).

Alkalinity

Alkalinity as a function of both primary productivity and population size corrected productivity approximated a hyperbolic curve. This indicated that high productivity values would only be obtained after the alkalinity (predominantly HCO_3^-) reached a plateau value (270 $mgCaCO_3/l$; Figs. 26 and 27).

Zooplankton Grazing

The objective of this portion of the thesis study was to obtain direct evidence that the zooplankton were grazing the photosynthetic bacteria. Two methods were used: 1) acetone spectral analysis of the gut contents of D. pulex, and 2) isotope studies as described in the methods section.

The wavelength of the absorbance peaks of the gut contents of the Daphnia pulex and filtered samples of photosynthetic bacteria obtained from 15 meters coincided (Fig. 28). This indicated the presence of photosynthetic bacterial pigments in the guts of the

Figure 25: Productivity (Pl-Pd/[BChl]) corrected for population size of the photosynthetic bacteria as a function of light intensity incident on 13 meters for October ●, November ○, and December △.

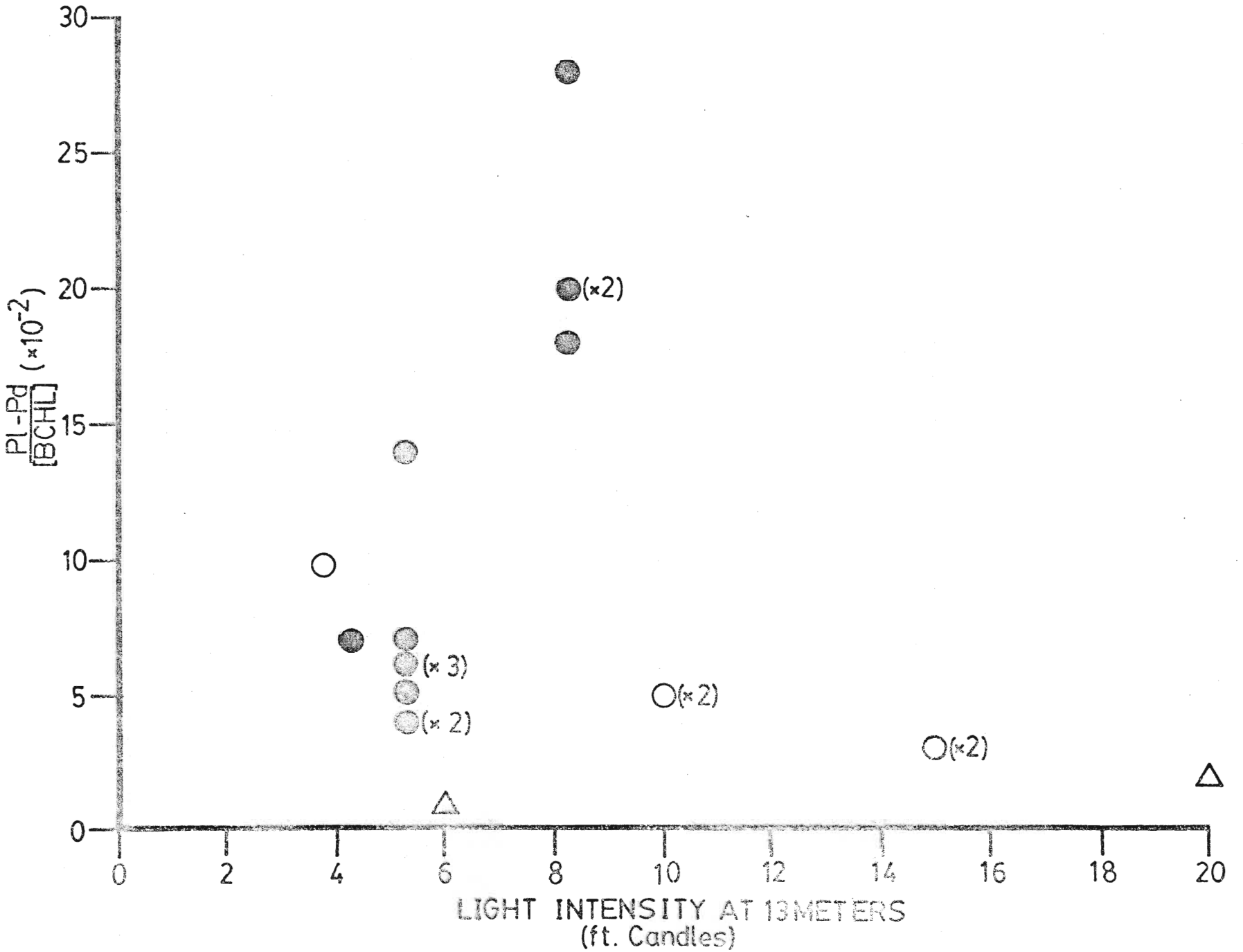

30
25
20
15
10
5
0
(×2)
(× 3)
(× 2)
O(× 2)
O(×2)
0
2
4
6
8
10
12
14
16
18
20
LIGHT INTENSITY AT 13 METERS
(ft. Candles)

Figure 26: Alkalinity ($mgCaCO_3/l$) as a function of productivity (Pl-Pd). The horizontal bars represent the 95% confidence intervals for the primary production.

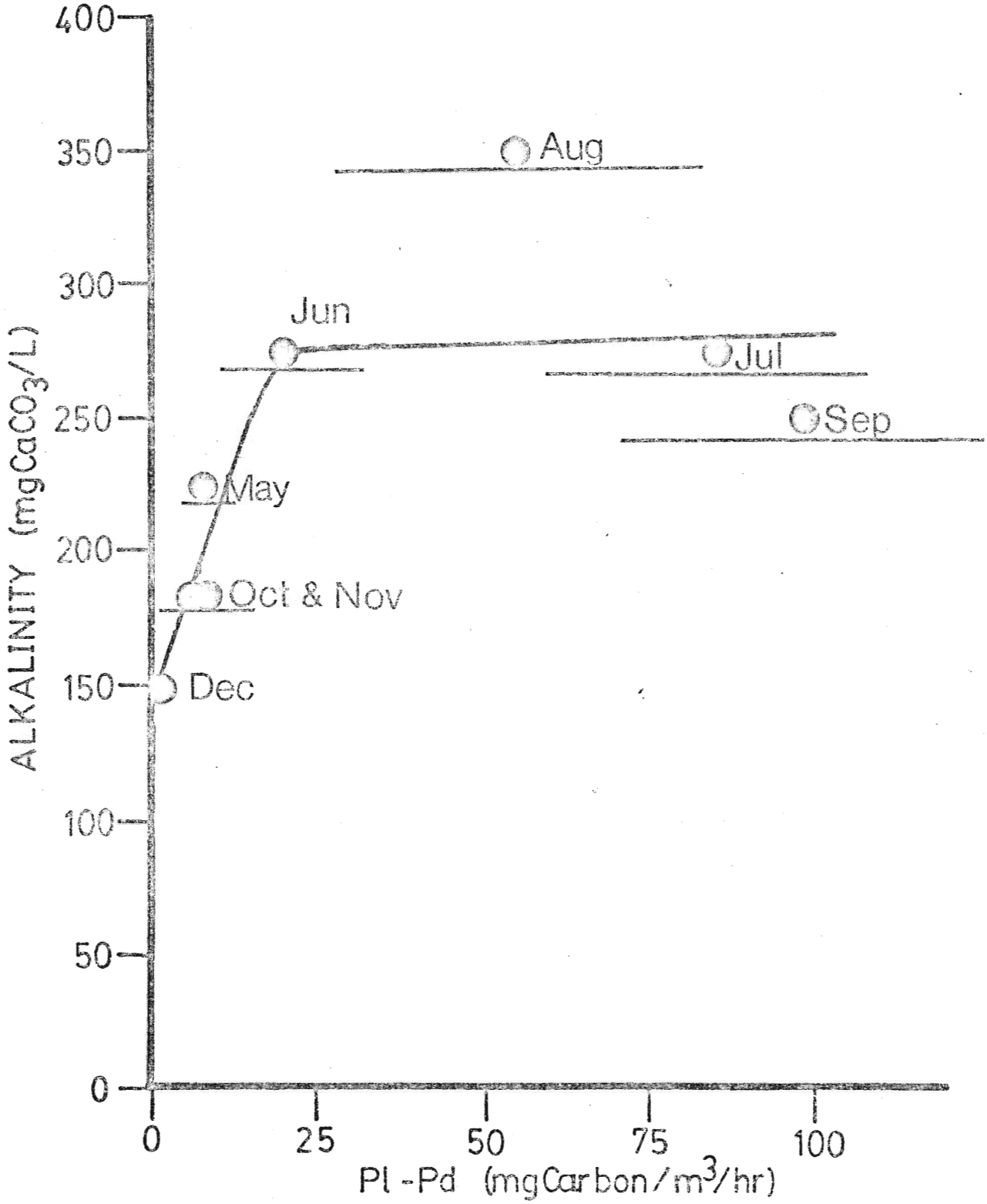

400
350
300
250
200
150
100
50
0
ALKALINITY (mgCaCO3/L)
Aug
Jun
Jul
Sep
May
Oct & Nov
Dec
0
25
50
75
100
Pl-Pd (mgCarbon/m3/hr)

Figure 27: Alkalinity ($mgCaCO_3/l$) as a function of productivity (Pl-Pd) corrected for the population size of the photosynthetic bacteria.

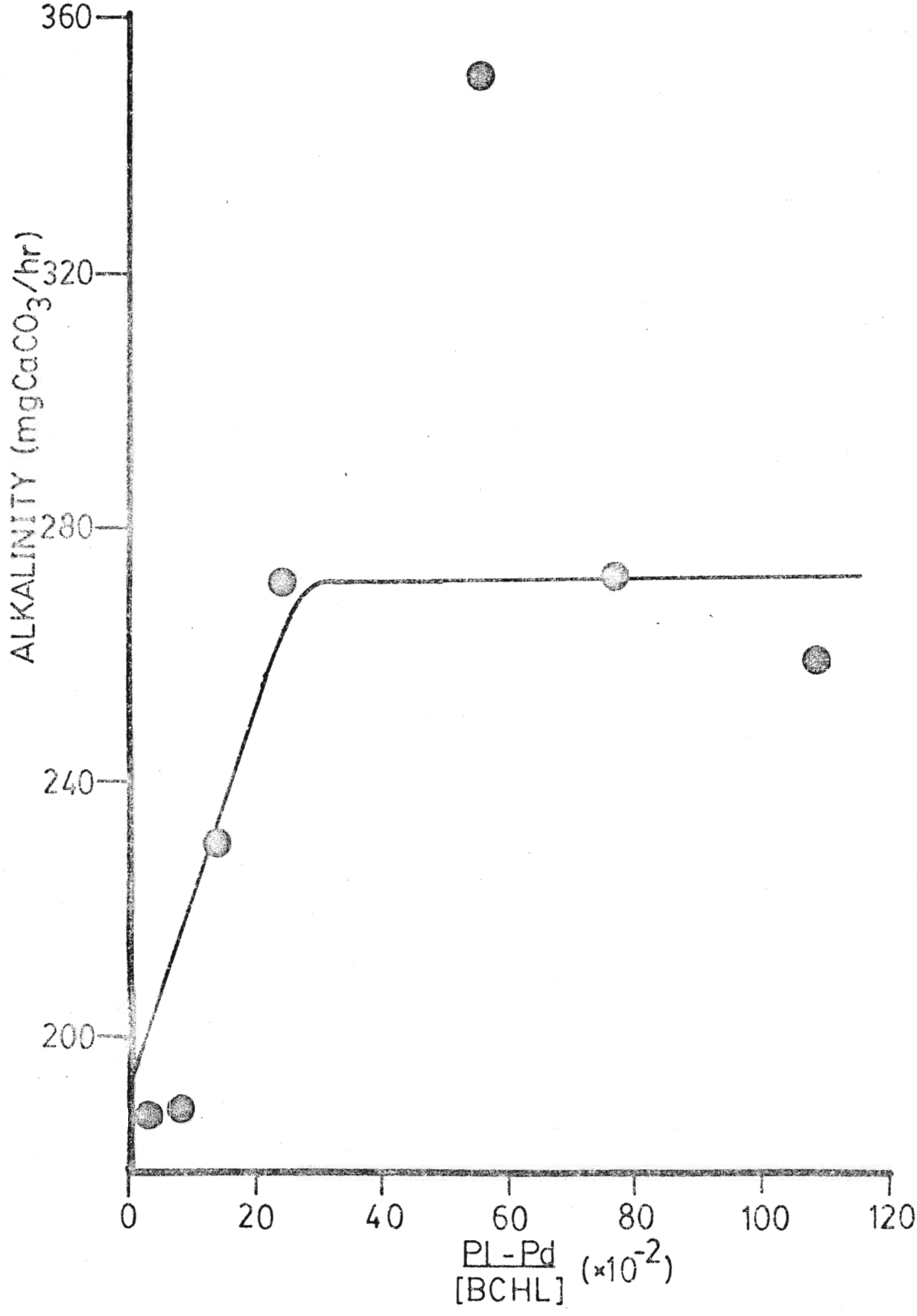
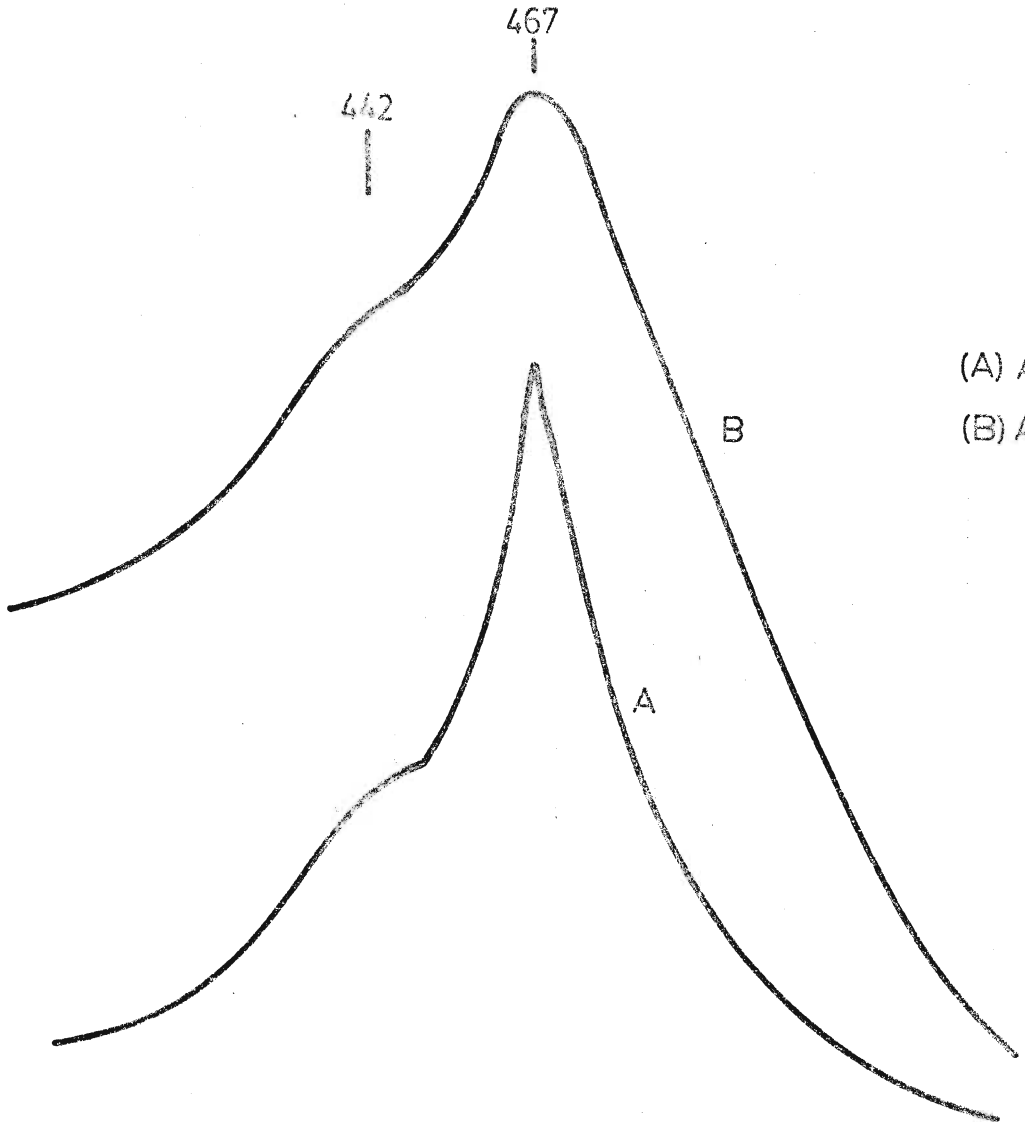
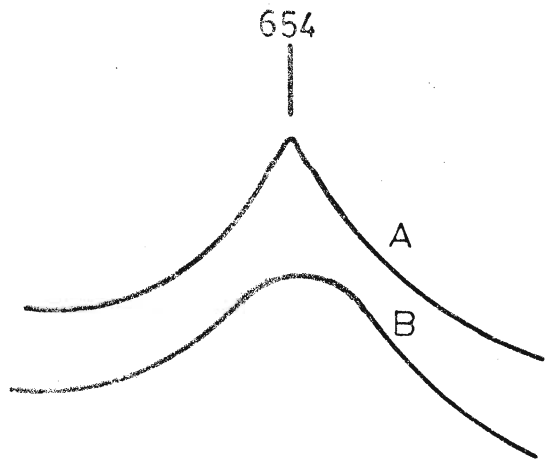

360
320
280
240
200
ALKALINITY (mgCaCO3/hr)
0
20
40
60
80
100
120
Pl - Pd / [BCHL] (×10-2)

Figure 28: The acetone extracted spectrum of the pigments obtained from a filtered sample of photosynthetic bacteria and the gut contents of *Daphnia sp.* obtained from the chemocline of the Crawford Lake.



(A) Acetone Spectrum of Photosynthetic Bacteria

(B) Acetone Spectrum of the guts of 22 Daphia sp



zooplankton.

Analysis of the data from labelling experiments showed increasing uptake of labelled material over time (Fig. 29). This indicated that the zooplankton were taking up and retaining radioactive material during the incubation period. Only a five minute incubation was required to show a significant ($p \leq 0.05$) uptake over unlabelled (control) samples (Fig. 29).

Water Column Productivity

The primary productivity of the entire water column was monitored from June to September, 1980 (Fig. 30), in order that the relative monthly contributions made by the photosynthetic bacteria could be compared to the algae (Fig. 30).

Both the algal and bacterial photosynthetic production showed the same trend (Fig. 30), i.e., low in the spring (< 10 mgC/m^2/hr) increasing to a peak in August (> 45 mgC/m^2/hr) and then decreasing in the fall (< 10 mgC/m^2/hr). The percent contribution of the bacteria was highest in June and July (45-60%), decreasing to less than 10% in October (Fig. 30). The average percent contribution for those months was 32.

Figure 29: The counts per minute (CPM) per Daphnia sp. as a function of time for Daphnia fed labelled photosynthetic bacteria ○ ($y = 2.0x + 2.9$; $r = .97$) and those fed non-labelled photosynthetic bacteria ■.

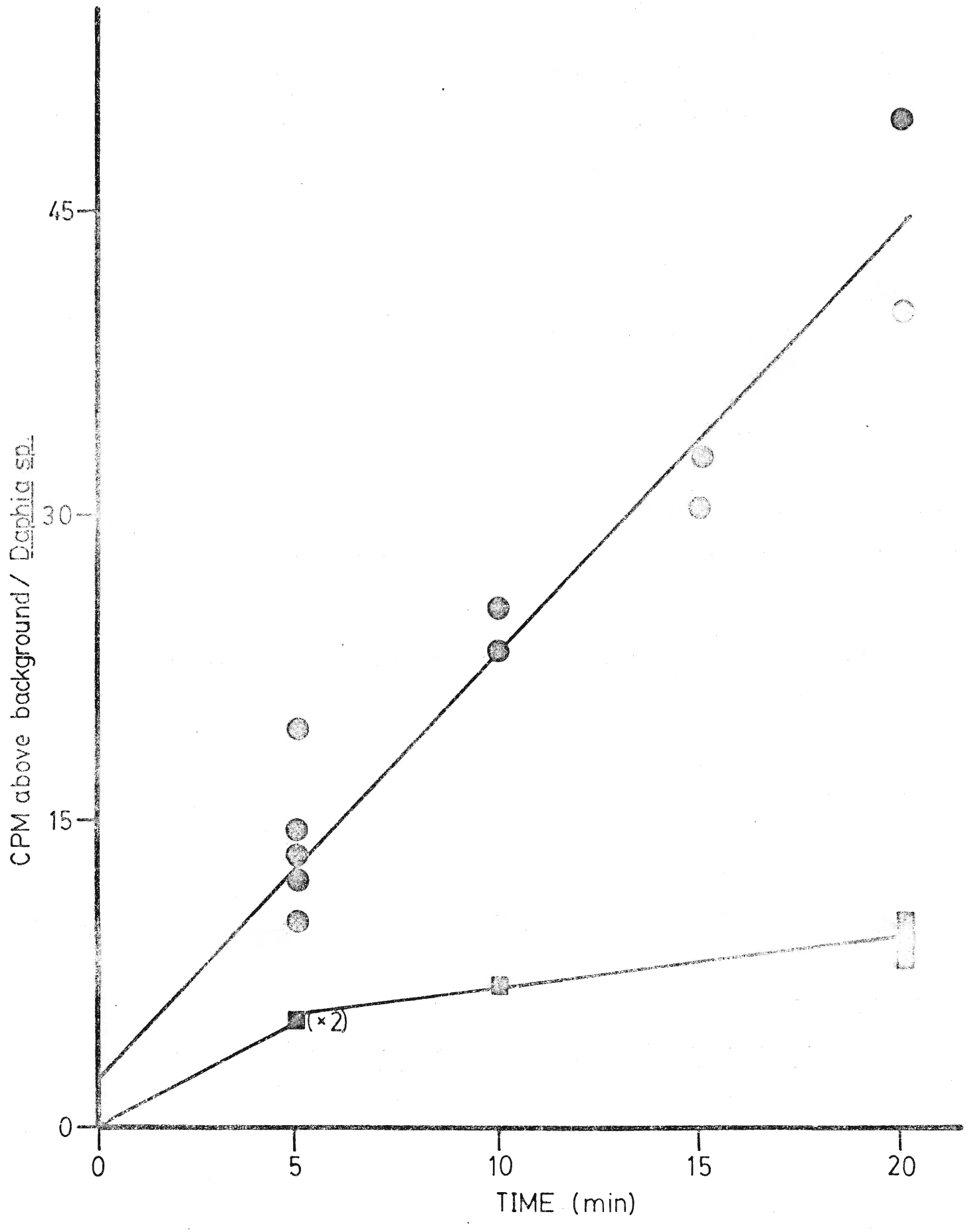

CPM above background / Daphia sp.
45
30
15
0
0
5
10
15
20
TIME (min)
(×2)

Figure 30: Seasonal changes in the total water column primary productivity ($mgC/m^2.hr$) of the algae and photosynthetic bacteria (June to October 1980). The non-stippled area represents the ^{14}Carbon uptake by both the algae and the bacteria. The stippled area represents uptake by only the photosynthetic bacteria.

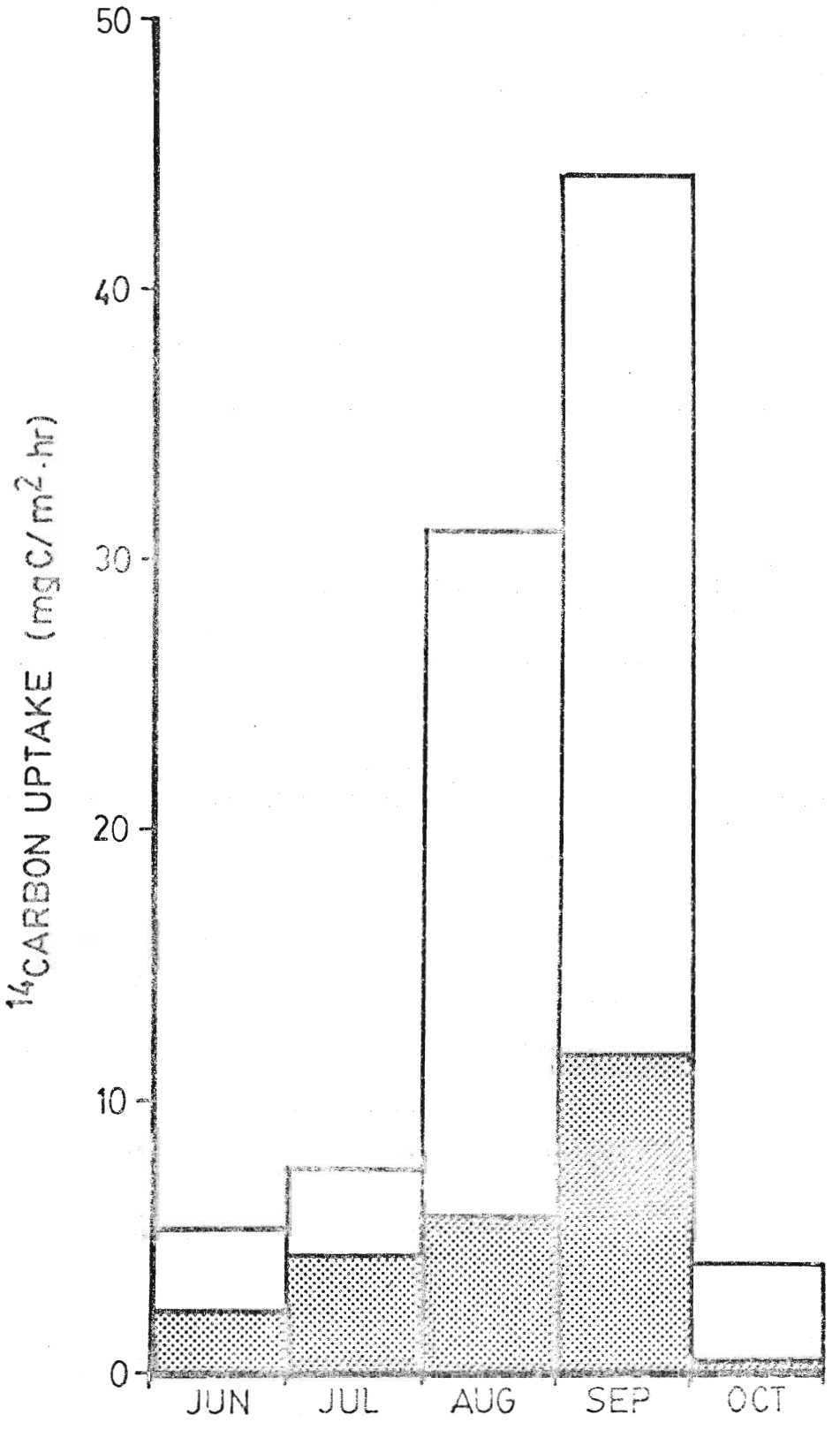
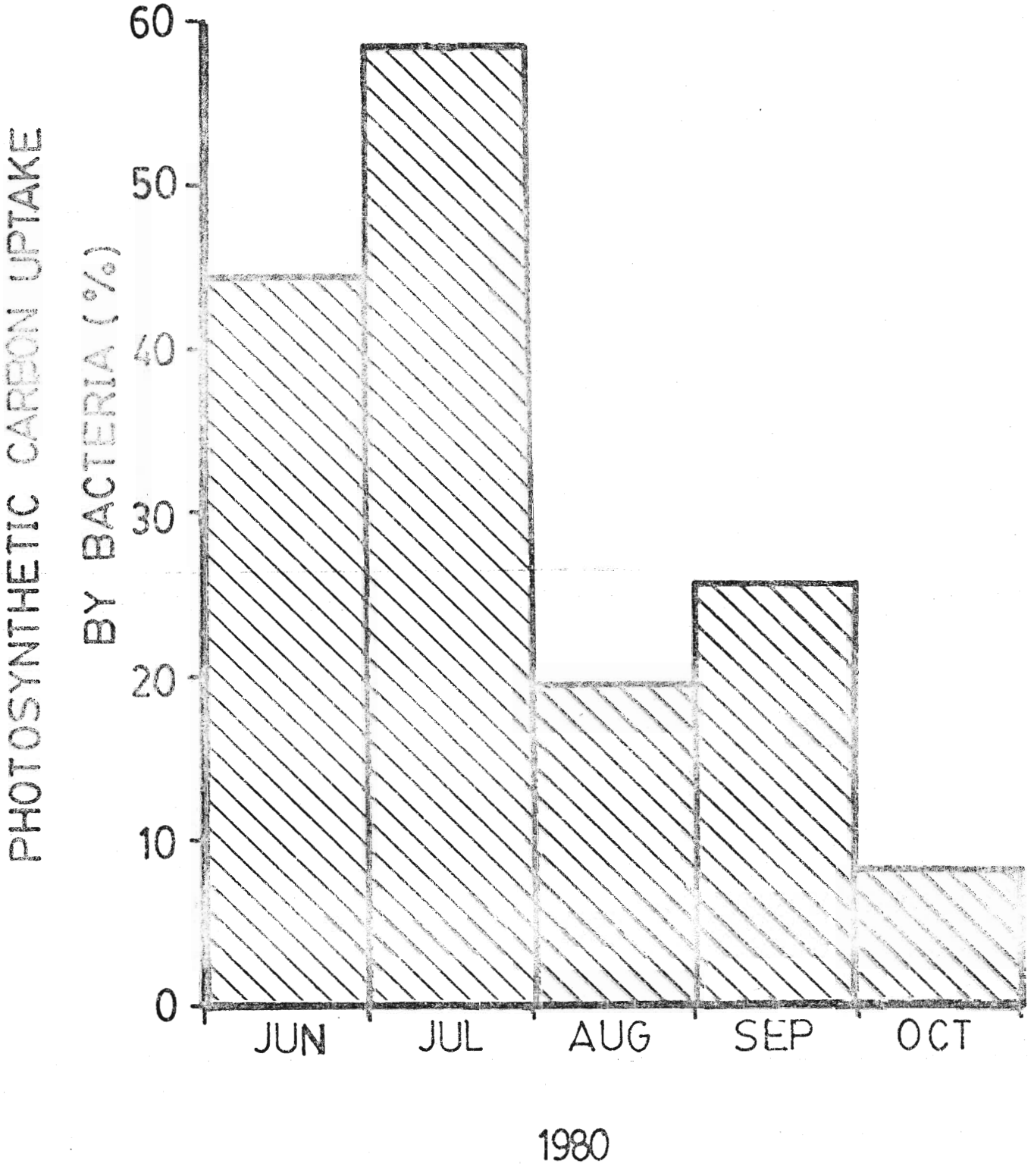

50
40
30
20
10
0
^{14}CARBON UPTAKE (mgC/m^2.hr)
JUN
JUL
AUG
SEP
OCT
1980

Figure 31: Monthly (June to October 1980) changes in the percent primary production by the photosynthetic bacteria of the total primary production.


PHOTOSYNTHETIC CARBON UPTAKE
BY BACTERIA (%)
60
50
40
30
20
10
0
JUN
JUL
AUG
SEP
OCT
1980

DISCUSSION

The first goal of this thesis was to collect chemical and physical data that would lead to an explanation of some of the seasonal variations in the primary productivity (measured as ^{14}C uptake) and biomass (bacteriochlorophyll concentration).

The study concentrated on three factors: bicarbonate concentration, light intensity and ventilation. Changes in bicarbonate concentration and light intensity at the chemocline were examined with respect to changes in primary productivity, whereas the process of ventilation was used to explain variations in the biomass of the photosynthetic bacteria in the late fall. Furthermore, some data were collected examining seasonal variation in the spectral location of the carotinoid peaks. These kinds of data suggested there may have been changes in the quality or wavelength of light reaching the photosynthetic bacteria. Alternatively, it may only reflect changes resulting from other variations in the lake.

The second goal of the thesis was to determine the importance of the photosynthetic bacteria to the aquatic ecosystem of Crawford Lake. In order to accomplish this it was necessary to demonstrate two criteria. The first was that the photosynthetic bacteria make a substantial contribution to the primary production of the lake. As indicated in the literature review, this has been demonstrated many times in other lakes, but never in Crawford Lake. The second criterion suggested was to demonstrate that the photosynthetic

bacteria made a contribution to the secondary production of the lake (i.e., zooplankton). This could only be accomplished by demonstrating that the zooplankton ingested the bacteria, which to date has not been demonstrated.

This was the first study on the photosynthetic bacteria in Crawford Lake to monitor seasonal variation in both the biomass and primary productivity. Many of the technical problems encountered could not be foreseen from other studies. Therefore, this study was treated as a beginning, often asking more questions than it answers.

Parameters Influencing Primary Production and Biomass

Light Intensity and Bicarbonate Concentration

Primary production ($H(^{14}C)CO_3$ uptake) was monitored from May to December 1980 (Fig. 16). During that period a very distinct pattern emerged. During the spring (May and June), the productivity was low on the order of 2-10 mg C/hr/m^3, increasing in the summer (July-September) to between 25 and 150 mg C/hr/m^3 and decreasing in the fall (October to December) to values similar in magnitude to those found in the spring. This seasonal variation has been observed numerous times in other meromictic lakes (Huron Lake, Takahashi and Ichimura, 1968; Fayettesville Green Lake, Culver and

Brunskill, 1969; Waldsea Lake, Lawrence et al., 1978). There are two explanations that are typically used to explain the seasonal variation. Takahashi and Ichimura (1968, 1970) suggested that the pattern was due to seasonal variation in light intensity incident on the photosynthetic bacteria. Alternatively, Culver and Brunskill (1969) suggested that the pattern was due to the organic material associated with the precipitation of $CaCO_3$ from the mixolimnion, which in turn stimulated H_2S production. The hypothesis suggesting that an insufficient concentration of H_2S could limit productivity has recently received support from work done by Parkin and Brock (1981). They demonstrated that there was a nocturnal build-up of H_2S, which was utilized by the phototrophic bacteria the next day. However, there has been no further evidence to suggest that the precipitation of $CaCO_3$ stimulates H_2S production.

If the observed variation in primary productivity could be attributed to the seasonal variation in the intensity of light reaching the bacteria, then a similar seasonal pattern in light intensity variation should be predicted. However, it is possible that the light intensity did not affect the whole study period, that is, it was saturating during some part of the season. In this case a positive correlation comparing light intensity and primary production might be observed during months that light intensity was lower.

Changes in light intensity incident on the phototrophic

bacteria of Crawford Lake were monitored from May to December 1980 (Fig. 8). Throughout both the spring and summer the light intensity maintained an average mid-day value of between 15 and 20 ft. candles. However, as the vertical bars indicate, there was an enormous amount of daily variation depending on weather conditions. In October there was a decrease in the intensity of the light reaching the bacteria to between 5-10 ft. candles, which increased slightly in November. Unlike the spring and summer, the light intensity reaching the bacteria during the incubation period (10.00 a.m. to 2.00 p.m.) had less daily variation (at least for the few points which could be obtained). These data suggest that light intensity probably was not responsible for the spring to summer variation, but might have contributed to decreased primary productivity in the fall. However, even the light intensity in the fall may not have been limiting. Parkin and Brock (1980a) demonstrated that light intensities of roughly 1-10 μein/m^2s (roughtly 3-10 ft. candles) saturated the photosynthetic mechanism of laboratory and naturally-occurring populations of photosynthetic bacteria, suggesting that even in the fall, light intensities exceeded limiting values.

Analyses of the monthly primary production light intensity comparisons (including those corrected for seasonal variations in biomass) suggested no positive relationship (Figs. 20 to 25), the possible exception occurring in June (Fig. 20). However, there may be too few points to attribute much significance to the relationship

(Fig. 20). As no positive relationship was observed in October or November, at a time when a relationship might be predicted based on the overall seasonal pattern (Fig. 8) and the lower average light intensities, it was inferred that some other factor may be exerting a greater limitation on the primary production. This was further supported using an alternate relative measure of the clarity of the water, Secchi depth (Figs. 18 and 19). These data suggested that there was no relationship between the clarity of the water and primary production in either the spring or fall. However, there was a positive non-significant relationship ($p \geq 0.05$) between Secchi depth and primary production during the summer. This appears to contradict the light intensity data; however, the contradiction may not exist if a re-evaluation of what these (light intensity and Secchi depth) parameters really measure is considered. Since Secchi depth variation reflects changes in the color and particulate material (such as algae) in the mixolimnion it may reflect a potential variation in the wavelength of light reaching the bacteria, as opposed to the light intensity measurements which reflect the intensity of light reaching the bacteria over a broad range of wavelengths. This is speculation, but if it were true, then the Secchi depth might reflect a change in the quality (wavelength) of light reaching the bacteria because it reflects the concentration of materials in the water that absorb light; for example, algae and possibly some humic material from the coniferous forest surrounding the lake, whereas measuring the light intensity

indicated something of the magnitude or quantity of light reaching the bacteria over the range of the phototubes' sensitivity.

The preceding explanation is highly speculative. However, there is some literature that suggests that certain groups of photosynthetic bacteria are selected by the wavelength of light incident on the photosynthetic bacterial layer (Trüper and Genouese, 1968; Parkin and Brock, 1980a, Pfennig, 1979). This is especially true for those forms that are found in deeper lakes (Trüper and Genouese, 1968). If it were true for Crawford Lake, then a shift in the wavelengths reaching the bacteria or in the internal components of the bacterial layer might be observed. The equipment to measure the wavelength of light incident on the phototrophic bacteria was unavailable during this study. Alternatively, variations in the location (nm) of one of the two carotinoid peaks (peak 1, Fig. 14), obtained from acetone extractions, was used because this peak is associated with the light harvesting material of the photosynethic bacteria (Pfennig, 1979). Therefore, it was hypothesized that these pigments might reflect seasonal variation in the quality of the light incident on the bacteria. A plot of the wavelength (nm) of the peak maximum against time (days and months) produced a sigmoidal curve (Fig. 13). This curve suggested that during the study period there was a continuous oscillation in the species, strain and/or intracellar components of the photosynethic bacterial layer. Further, the majority of the

curve falls through the summer (Fig. 13) which tends to suggest that a shift in the layer occurred predominantly during the summer. This tends to support earlier speculation concerning the relationship between the Secchi depth and primary productivity during the summer (i.e., the Secchi depth reflects light quality reaching the bacteria). Initially, it does not lead to an explanation of the observed variation in primary production from spring to summer and summer to fall; however, it does tend to support arguments made later in the discussion. Therefore, another factor was considered which might lead to an explanation of this phenomenon.

Seasonal variations in the alkalinity were chosen as alternate factors which might be associated with the seasonal changes in the primary production (Fig. 9). Crawford Lake experiences a $CaCO_3$ precipiation from the mixolimnion to the monimolimnion, similar to the one observed in Fayettesville Green Lake by Culver and Brunskill (1969). However, unlike the Fayettesville Green Lake study, the gradual loss of the bicarbonate (decrease in alkalinity) was observed in Crawford Lake (Fig. 10). The increase and eventual loss of the bicarbonate to the top of the chemocline coincided with the increase and decrease in the primary production of the photosynthetic bacterial layer (Fig. 16). This suggested that the increase of bicarbonate might have stimulated photosynthetic H (^{14}C) CO_3 uptake. Next, the monthly average primary production was compared to variations in the alkalinity (Fig. 26). May,

October, November and December, all had relatively low bicarbonate concentrations and low primary production. However, August, which had the highest alkalinity, had a primary productivity intermediate between June, which had low primary productivity (20 mg C/m^3hr) but intermediate alkalinity (270 mg $CaCO_3$/l) and July and September, which had an alkalinity similar to June's but had very high primary productivity values (80-100 mg C/m^3hr). These data tend to support the hypothesis that the bicarbonate stimulates primary production; however, the results were not completely expected. The hypothesis would predict that July and September's productivity would be intermediate between June and August. In the following few paragraphs a possible explanation for the unexpected results was attempted.

Once the concentration of bicarbonate reaches a value of between 260 and 270 mg $CaCO_3$/l, the potential for primary production became very high (i.e., values reached in July and September). However, further increases in the bicarbonate concentration would not stimulate further productivity because the photosynthetic mechanism has been saturated for the present population size (i.e., no increase in August). Hence, only a further increase in the photosynthetically-active cells at the top of the chemocline could produce more productivity. This type of explanation was used to explain the saturating effects of light and H_2S on laboratory populations (Parkin and Brock, 1980a). This

could be taken a step further, to suggest that increases in biomass (there was a slight increase in August) as the result of an increase in productivity (Fig. 12) would cause further self-shading, ultimately reducing the measured photosynthesis (i.e., higher biomass but lower photosynthesis in August). This does not contradict earlier statements because only an increase in cells that see the light would increase production. If this is already maximized, then further increases in cell number would just be adding to the biomass and not to primary production. The higher productivity values would continue until the bicarbonate concentration fell below the saturating value, which it did between September and October. However, this does not explain why June did not have higher productivity. To explain this, it was necessary to re-examine the light quality data (Fig. 13). Between June and July the largest shift of the carotenoid peaks occurred. As suggested earlier, this could indicate a change in the strain, species and/or cellular components of the photosynthetic bacterial layer. Therefore, it is possible that there was a shift in the photosynthetic bacteria from those that could use an organic carbon source to those that use the increased concentration of inorganic carbon more efficiently. This appeared to contradict earlier statements claiming that the shift was due to changes in the wavelength of light reaching the bacteria. At this time it is not possible to separate the two possibilities.

The suggestion that the photosynthetic bacteria could switch from organic to inorganic photoassimilation was documented by Kelly (1974) on laboratory populations. However, it has never been attempted on a naturally-occurring population.

To this point it has been hypothesized that not only did the increase in alkalinity (HCO_3) increase photosynthesis, but that the photosynthetic bacteria could shift from utilizing an organic carbon source to using an inorganic carbon source. Further support was obtained for this hypothesis from an unexpected result comparing primary productivity and biomass (bacteriochlorophyll).

It was hypothesized that since the bacteriochlorophyll concentration represented both the population size (Severn, 1979) and the amount of photosynthetic apparatus available for photosynthesis, that there would be a correlative relationship between the amount of bacteriochlorophyll present and the primary production (Fig. 17). However, when these two parameters were compared, there was no apparent relationship. The amount of bacteriochlorophyll appeared to have varied independently from the amount of photosynthesis. Furthermore, a seasonal plot of the bacteriochlorophyll concentration tended towards a sigmoidal curve, which was unlike the seasonal plot of the primary productivity curve (Fig. 13). These observations suggested that the amount, and more importantly the times, of the inorganic carbon assimilation, were insufficient to account for the spring and late fall population size. This inconsistency suggested that the method of measuring total

($H(^{14}C)CO_3$) inorganic carbon photoassimilation did not measure the total carbon, both inorganic and organic, taken up by the photosynthetic bacteria. Therefore, it was concluded that the photosynthetic bacteria could switch carbon sources.

Thus far in the discussion of bicarbonate as a chemical factor that might stimulate summer primary production, it has been assumed that the stimulation was the direct cause of the increase of the bicarbonate. However, Culver and Brunskill (1969) suggested that the organic material associated with the $CaCO_3$ precipitation stimulated H_2S production by the sulphate-reducing bacteria which, in turn, stimulated photosynthetic bacterial primary production. At this point in time it is not possible, with data collected as part of this thesis, to discount this possibility. However, it was possible to suggest how this problem could be examined in the future and what kinds of data would be necessary to substantiate or disprove the effects of the H_2S and/or HCO_3.

In order to substantiate the claim that H_2S stimulated photosynthesis, it would be necessary to demonstrate that the addition of H_2S stimulated primary production. This would probably not be difficult as it has already been demonstrated that diurnal changes in H_2S do stimulate or limit photosynthesis (Parkin and Brock, 1981). It would then be necessary to demonstrate that there was an increase in H_2S as a result of the $CaCO_3$ precipitation which occurs in the spring. This might be extremely difficult because if the bacteria were limited by the concentration of H_2S, any new H_2S

production should be utilized immediately. It might be sufficient to demonstrate an increase in SO_4 reduction occurring during the same period as the $CaCO_3$ precipitation. Lastly, it would be necessary to develop an alternate explanation as to the lack of correlation between the population size and primary production. This would be necessary because if H_2S was limiting, then photo-organotrophic assimilation would also be limited. If any of these criteria could not be demonstrated this would tend to disprove the effects of H_2S as a factor that influenced the summer increase in primary production.

It would be useful to demonstrate that the addition of bicarbonate does directly stimulate photosynthesis. This was recently done as part of my Ph.D thesis studies (Appendix 3J). Furthermore, it would be useful to demonstrate either that there was no organic material associated with the $CaCO_3$ precipitation, which would also tend to disprove the H_2S hypothesis, or that it did not directly stimulate photosynthesis. It is unlikely that organic material stimulated photosynthesis because if it had the results would have showed an increase in biomass but no increase in primary production (measured as $H(^{14}C)CO_3$), because the cell would have continued to use the organic material; that is, assuming there was a switch for photoorganotrophic to photo-lithotrophic bacteria.

Ventilation

Ventilation,or chemocline erosion, was hypothesized by Dickman and Artuz (1979) to be responsible for the elimination of bacteria from the chemocline in the Black Sea. Further, Dickman (1979) suggested it was responsible for the loss of a significant proportion of the bacterial biomass of the chemocline in Pink Lake. He hypothesized the following sequence of events to explain the observations. During fall mixing the combination of oxygen toxic (to the phototrophic bacteria), aerobic water and scraping action of mixing resulted in the death and removal of the bacteria from the chemocline. This hypothesis predicted both a decrease in the top of the chemocline and the loss of photosynthetic bacteria from that zone (i.e., the observations that gave rise to the hypothesis).

The photosynthetic bacteria in Crawford Lake were also associated with the top of the chemocline (Fig. 4). During the autumn of 1979, the conductivity data suggested that the top of the chemocline was lowered approximately one meter (Fig. 5). As well, there had been aerobic water introduced into the anaerobic zone to 15 meters (Fig. 6). This indicated that the bacteria would have been in contact with oxygenated water during mixing. These data suggested that mixing had occurred in Crawford Lake; however, in 1979 changes in biomass after ventilation were not monitored, therefore it was not known if a loss in the photosynthetic bacterial biomass had occurred as previously predicted.

(approximately 50-100 times their cell diameter/minute) in order to avoid the oxygen entrainment. Since the Chlorobiceae are unflagellated (Pfennig, 1979), it would be very unlikely that they could move themselves at this rate. Therefore, it seemed reasonable to examine only those explanations that assumed that the bacteria were moved.

Homeothermy had been thought to occur during or before ventilation. This is assumed by the original hypothesis because the depth of mixing was felt to be restricted by the thermocline. It was not until after the breakdown of the temperature barrier that ventilation of the chemically more dense water could occur (Edmondson and Anderson, 1965; Hutchinson, 1957). In Crawford Lake homeothermy (samples taken every few days) did not appear to have occurred until after ventilation (Fig. 8). In 1980, homeothermy did not appear to occur until 2-5 days after ventilation. This suggested that the main mixing force (Fig. 32) did not ventilate the chemocline directly. Instead, possibly a secondary mixing current occurred as a result of the first (Edmondson and Anderson, 1965). This current would be generated as the result of some of the main current's energy generating a current going in the opposite direction under the thermocline (Fig. 33). This new current would have less magnitude and be restricted between the top of the chemocline and bottom of the thermocline. In both 1979 and 1980, the thermocline just prior to ventilation appeared to be between 10 and

Figure 32: The pre-ventilation mixing pattern.

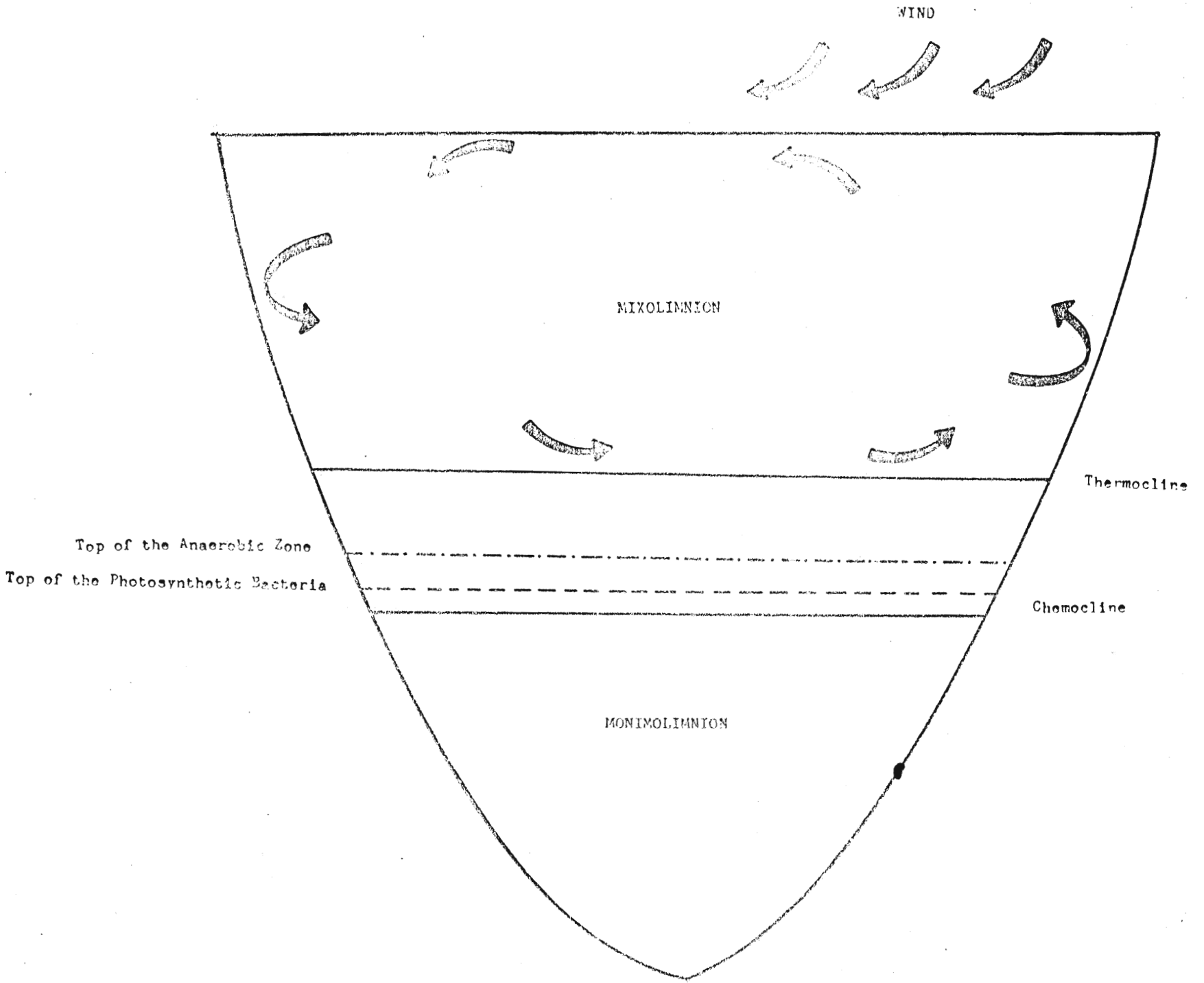
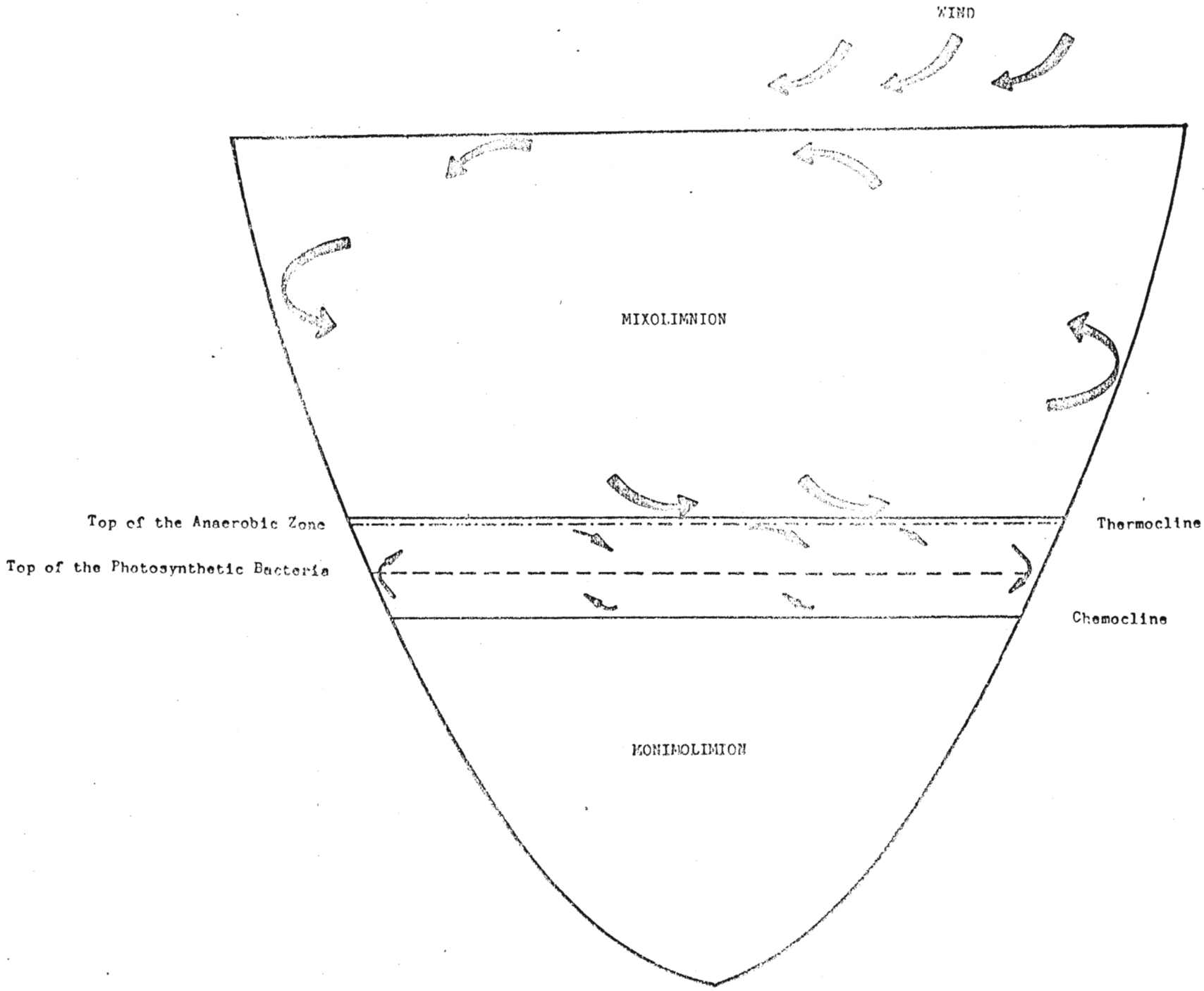

WIND
MIXOLIMNION
Thermocline
Top of the Anaerobic Zone
Top of the Photosynthetic Bacteria
Chemocline
MONIMOLIMNION

Figure 33: The mixing pattern during ventilation counter current.


WIND
MIXOLIMNION
Top of the Anaerobic Zone
Thermocline
Top of the Photosynthetic Bacteria
Chemocline
MONIMOLIMION

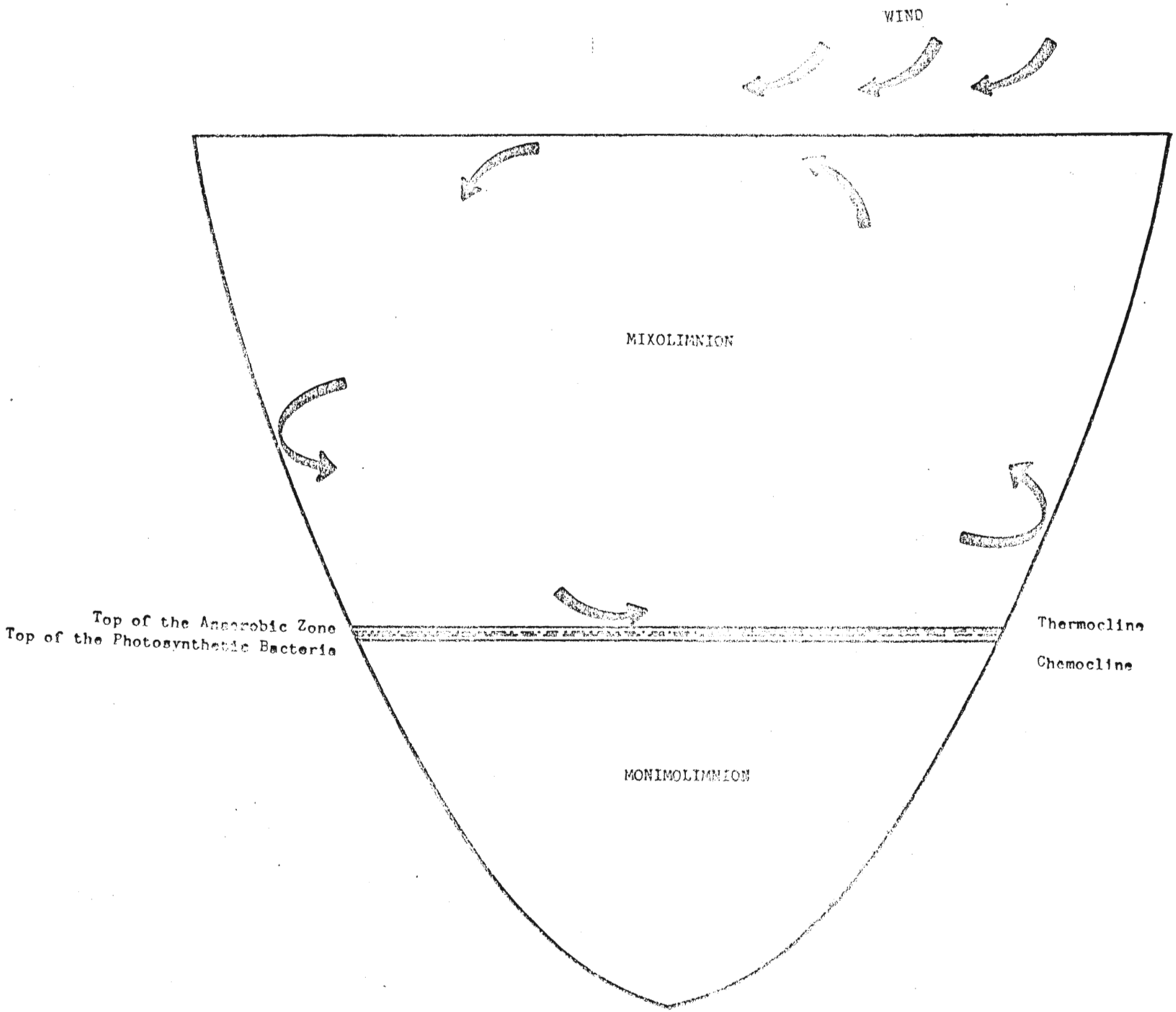
12 meters (Fig. 8). This would have to approximate the time that the secondary current was generated. Therefore, in 1979, oxygenated water would have been introduced into the bacterial layer, whereas in 1980 the secondary current would have been completely anaerobic. Therefore, in 1979, the data would predict that the bacterial layer may have been ventilated and reduced due to the infusion of oxygen. However, in 1980, the bacteria that were ventilated and relayered on to the new chemocline were still viable (Fig. 34). The main mixing current would be still pushing down, but the steep conical morphometry, denser water and the secondary current would probably resist further mixing. Furthermore, ice cover occurred in mid-November 1980, which would nullify further wind input into the major mixing current.

This explanation predicts that years with a large mixolimnetic anaerobic zone overlying the permanently anaerobic monimolimnion would generate the results observed in 1980. However, years when the anaerobic zone does not extend as far into the mixolimnion would generate results consistent with the original hypothesis.

The Photosynthetic Bacterial Contribution to Crawford Lake

The literature is replete with examples attempting to persuade the reader of the importance of the photosynthetic bacteria to an aquatic system. However, as explained in the literature review, very few have really accomplished their goal. The tendency has been

Figure 34: Post-ventilation and the formation of the strong barrier to further ventilation.


WIND
MIXOLIMNION
Top of the Anaerobic Zone
Top of the Photosynthetic Bacteria
Thermocline
Chemocline
MONIMOLIMNION

to show that the photosynthetic bacteria contribute a significant proportion of the total fixed carbon to the lake, concluding from this that the bacteria were important contributors to the aquatic ecosystem. However, this analysis left the question of how much (if any) is actually utilized by the other trophic levels in the system. Therefore, two criteria have been suggested that must be demonstrated before it can be concluded that the bacteria make a significant contribution to the ecosystem of the lake. First, it was necessary to demonstrate that the phototrophic bacteria made a contribution to the primary production of the lake. Secondly, and most important, that some of this production be contributed to the diets of the secondary producers of the food web.

In Crawford Lake, the photosynthetic bacteria contributed between 10-60% of the total inorganic carbon fixed during the summer (Fig. 31), with the greatest relative contribution occurring in June and July, although the greatest contribution in absolute terms (mg $C/m^3/hr$) occurred in September (Fig. 30). Therefore, it appeared that the photosynthetic bacteria did make a substantial contribution to the over-all production of the lake. This did not include considering the role the bacteria would play if they were recirculating organic carbon into the lake.

The second problem was to demonstrate that some of this production was available to another trophic level. Zooplankton have been observed in the anaerobic zone of other lakes numerous times

(Takahashi and Ichimura, 1968; Culver and Brunskill, 1969; Lawrence et al., 1978). Daphnia pulex have been reported in the anaerobic zone of Crawford Lake (Prepas and Rigler, 1978). Typically, they are reported to have a red carapace, typically thought to be due to carotinoids associated with the photosynthetic bacteria. However, a study done by Dickman and Nicholls (Fig. 2) indicated that at least some of the red pigment was haemoglobin. Samples taken from Crawford Lake about the same time as Nicholls and Dickman's study and extracted with 90% acetone, did not show any of the characteristic bacteriochlorophyll peaks which would be expected if the Daphnia were grazing the bacteria. Therefore, before the study began there was evidence to suggest that the zooplankton were present in the anaerobic zone but may not be grazing the bacteria. This supported the hypothesis that the zooplankton were avoiding predation during the day by staying in the anaerobic zone. Therefore, in order to support or disprove the importance of the photosynthetic bacteria to the lake (the original problem) it was necessary to address the question of whether or not the Daphnia were grazing the photosynthetic bacteria.

The first experiment tried was to modify the method in which the zooplankton were preserved and extracted with acetone. The presence of the bacteriochlorophyll in the zooplankton would suggest that the bacteria were grazed. As stated earlier, when originally tried, this method did not indicate the presence of bacterio-

chlorophyll. When the procedure was retried (fall 1980), before preserving in 4% formalin the samples were first placed in carbonated water. This anaesthetized them and, as a secondary result, prevented them from dumping their gut contents. When spectra were taken of these samples, the characteristic bacteriochlorophyll peaks were observed (Fig. 28). This suggested that not only was there bacteriochlorophyll in the Daphnia but that it was located in their guts. To further test the grazing hypothesis a Haney trap was used to incubate a radio-labelled pellet of photosynthetic bacteria in a sample of Daphnia trapped in the monimolimnion. In this case, analysis of the Daphnia indicated that some of the radioactivity was being taken up (Fig. 29). Another observation made at the time was that the Daphnia were still swimming about the trap after a twenty minute incubation. These data convincingly demonstrate not only that the Daphnia graze the bacteria but they appear to be able to stay in the zone for some time. This was probably the result of the haemoglobin present in the Daphnia.

These data fill both criteria for demonstrating the importance of the photosynthetic bacteria to a lake's food web. Furthermore, it suggests questions concerning what proportion of a Daphnia's diet is filled by the photosynthetic bacteria. Do the Daphnia migrate at night to graze the algae or are they feeding exclusively on the bacteria? Alternatively, it may be possible to explain some of the variations in the population size

(biomass) as a result of seasonal variation in feeding stress. These types of questions are now being addressed by Mr. Mazumder as a master's thesis at Brock University.

CONCLUSIONS

The following are some conclusions made as a result of the analysis of the data collected as part of this thesis.

1. That at least some part of the seasonal variation in the photosynthetic bacterial primary production appeared to be explained by seasonal variation in the bicarbonate concentration. However, it is still not known if the bicarbonate was directly or indirectly (i.e., H_2S stimulation) responsible for the summer increase in primary production.
2. That no relationship was observed between light intensity and primary production. It was inferred from this observation that changes in light intensity could not explain any of the seasonal variations in the primary production.
3. Shifts in the soret region (blue) of the absorption spectrum of acetone extracted samples were indicative of a pigment shift resulting from a species or intracellular rearrangement of the bacterial layer. The possible causes were either a change in the wavelength of light incident on the bacteria or a change in the trophic nature of the bacterial layer (i.e., switching from photo litho to organotrophic).
4. Ventilation did not eliminate the photosynthetic bacteria as predicted. The original hypothesis was modified to explain these results. The modified hypothesis predicted a secondary

mixing current, which was responsible for the observed ventilation. It also predicted that years with a large mixolimnetic anaerobic zone ($>$ 10 meters) should not ventilate the photosynthetic bacteria. However, years that have a lesser anaerobic zone would show ventilation of the bacteria.

5. The photosynthetic bacteria contributed a substantial proportion of the primary production of the lake (10-60%). Furthermore, the Daphnia were not only equipped to stay in the anaerobic zone because of the presence of haemoglobin but were able to graze the photosynthetic bacteria.

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Personal Communications

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APPENDIX 1

The In-Situ Productivity ^{14}C Incubator

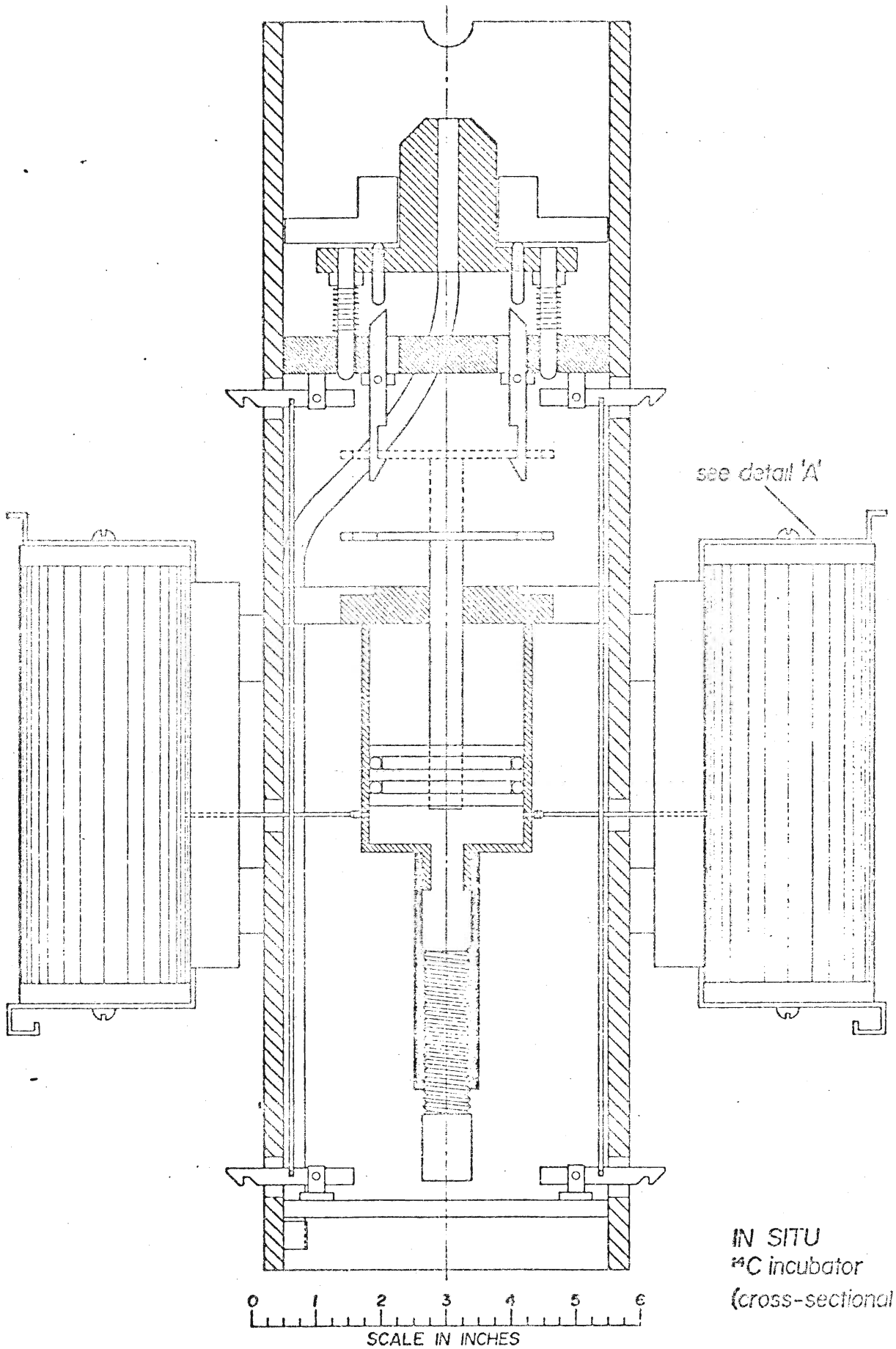
As well as using the light-dark bottle technique (method section) a new device was developed and used (using B.O.D. bottles) to measure ^{14}C uptake by the photosynthetic bacteria. A new technique had to be developed because I was unable to assure myself that the bacteria were not affected by oxygen, light, temperature and change in pressure that cells would experience in the B.O.D. bottle technique.

During the course of the experiments two working models were developed (Figs. A1 and A2). However, the first model was eventually rejected. I was unable to assure myself that the ^{14}C was being consistently evenly distributed over all the chambers. The second model was designed to have six separate loading chambers each holding 10 mls (averaged 9.9 to 10.1). This guaranteed even distribution.

Loading the Chamber

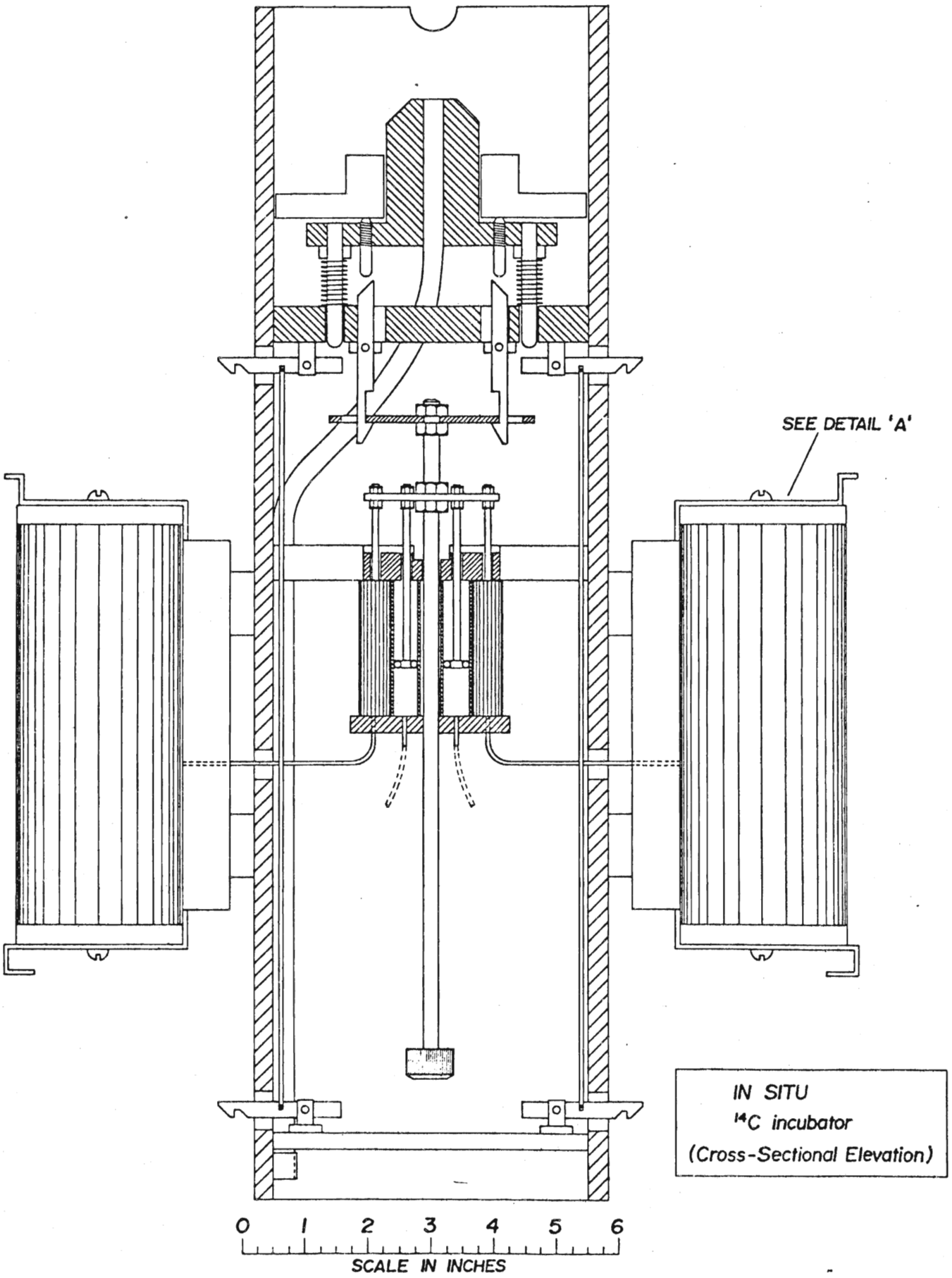
Essentially, the In situ productivity incubator (I.S.P.I.) consisted of 6 chambers (3 light and 3 dark) that could hold 300 mls of H_2O each. The lids and piston mechanism (in the last model there were 6 pistons, one for each ^{14}C container) were fixed to a release mechanism (Fig. A3). A rod inserted into the apparatus from the bottom was used to charge the piston (Figs. A1 and A2). Next, the bottom doors were opened in Model 1 or the top doors in Model 2. The (^{14}C) $NaHCO_3$ was then added at the bottom in Model 1 or through the septums

Figure A1: *In Situ* productivity ^{14}C incubator, Model 1. M. Dickman and M. Benkel were principal contributors to the formation of both models.



IN SITU
^{14}C incubator
(cross-sectional elevation)

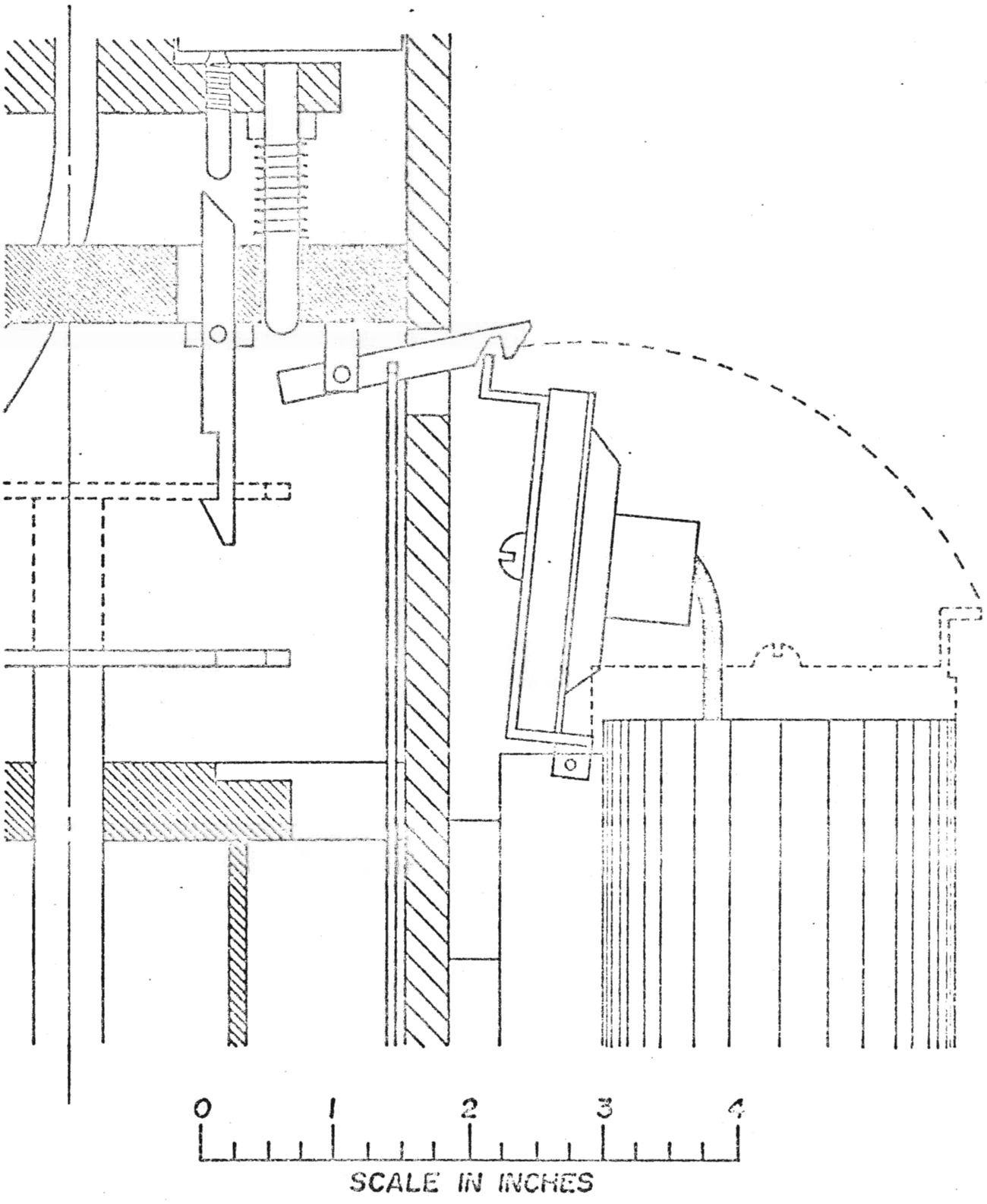
Figure A2: *In Situ* productivity ^{14}C incubator, Model 2



IN SITU
^{14}C incubator
(Cross-Sectional Elevation)

Figure A3: *In situ* productivity ^{14}C incubator, showing door and piston mechanism.

IN SITU
^{14}C incubator (detail A)



in Model 2. The septums in Model 2 allowed me to keep the ^{14}C solution anaerobic until it was used. In both cases the bicarbonate was loaded until the clear vinyl-plastic tubes filled with the solution. The top or bottom doors were then opened and the machine was rinsed with distilled H_2O into a catch basin.

The ISPI was then taken to the incubation buoys and lowered to the incubation depth. A messanger released from the surface closed the doors and released the ^{14}C into the tubes, in that order.

At the end of the incubation, the ISPI was returned to the surface. Each tube was emptied into a small bucket. The bucket was emptied into dark bottles (1-6) until the sample could be analyzed.

The ISPI had three major technical drawbacks. 1) It was too heavy when it was being removed from the water; hence, it was prone to damage. 2) Removing the incubated water from the tubes was messy; however, this could be solved if a top and bottom pour spout was added. 3) The tubes were too long; hence there was both photosynthetically-active and non-active cells present in the sample. Therefore, high dark uptake values were a constant problem. This was also a problem with the light-dark B.O.D. bottles.

The ISPI had several advantages. 1) It was compact for Model 2, only the machine, a syringe, bucket ^{14}C $NaHCO_3$ and 6 dark bottles were required for incubation. 2) I could guarantee that the photosynthetic bacteria experienced no light, temperature (Note ^{14}C $HaHCO_3$ was kept cold), or oxygen shock. Hence it gave a truer picture of the photosynthetic capacity of the photosynthetic bacteria.

3) Although no difference was found in the mean of the ^{14}C uptake, between the BOD battle and model 2 of the ISPI, this may be due to the extremely low uptake. There were indications that there was much less variance associated with the measurements (Table 1A). Therefore, this could be a very useful device with the suggestions I have made previously.

Table 1A: An Example Comparison of Mean Productivity Values Produced by the ISPI(2) Machine and B.O.D.

Methods (done Oct. 18, 1980)			
		$mgC/m^4/hr$	
		Light Bottles	Dark Bottles
		15.79	9.27
		14.63	7.32
		14.895	9.56
	Mean	15.11 (.607)	8.72 (1.23)
ISPI		14.38	10.77
Method		15.80	10.49
		15.40	9.17
	Mean	15.19 (.732)	10.15 (.862)
		14.93	9.75
		15.21	9.65
		14.77	9.50
	Mean	14.97 (.223)	9.63 (.126)
B.O.D.		13.15	11.35
Method		19.73	11.58
		21.64	11.05
	Mean	18.17 (4.45)	11.33 (.276)

( ) = S.T.D. to convert to $\pm$ confidence interval multiply by 1.66.

Figure 2A: The spectrum obtained from a homogenized sample of the photosynthetic bacteria of Crawford Lake.

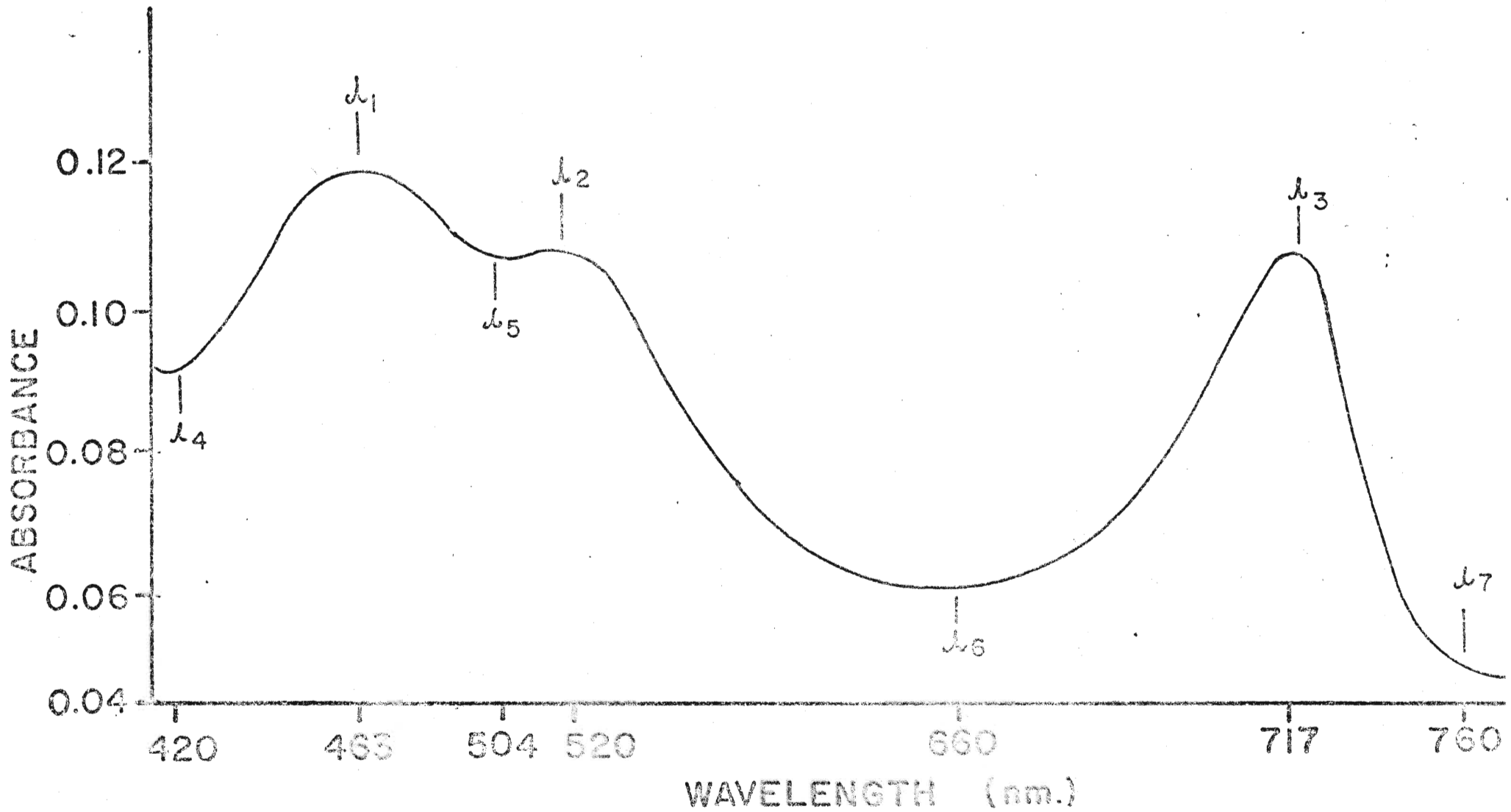

ABSORBANCE
0.12
0.10
0.08
0.06
0.04
λ1
λ2
λ3
λ4
λ5
λ6
λ7
420
463
504
520
660
717
760
WAVELENGTH (nm.)

Figure 2B: A scanning electron micrograph of a cell taken from 15 meters in Crawford Lake. The picture was taken by H. Melleville of the Geology Department, Brock University. The line represents one μm.

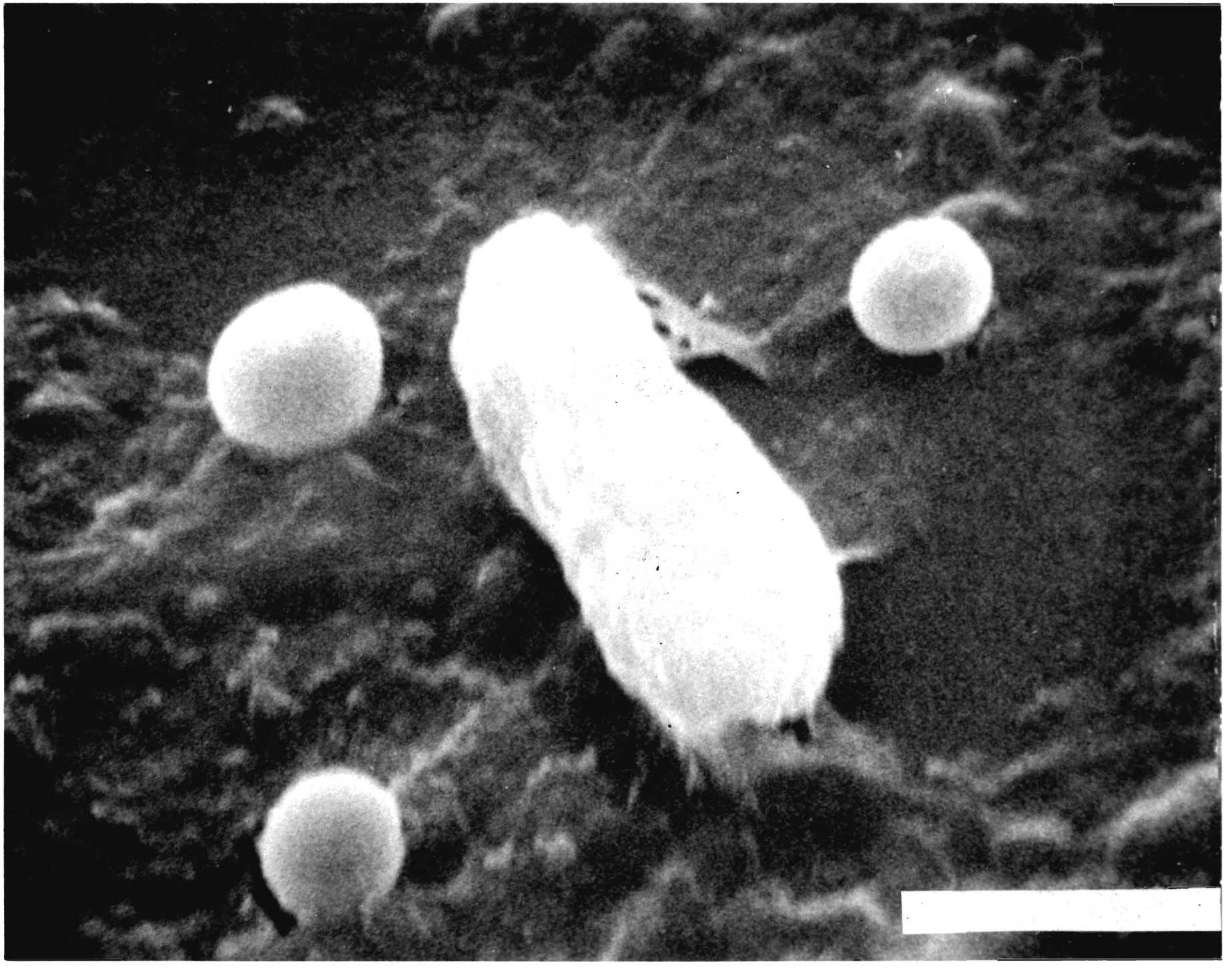
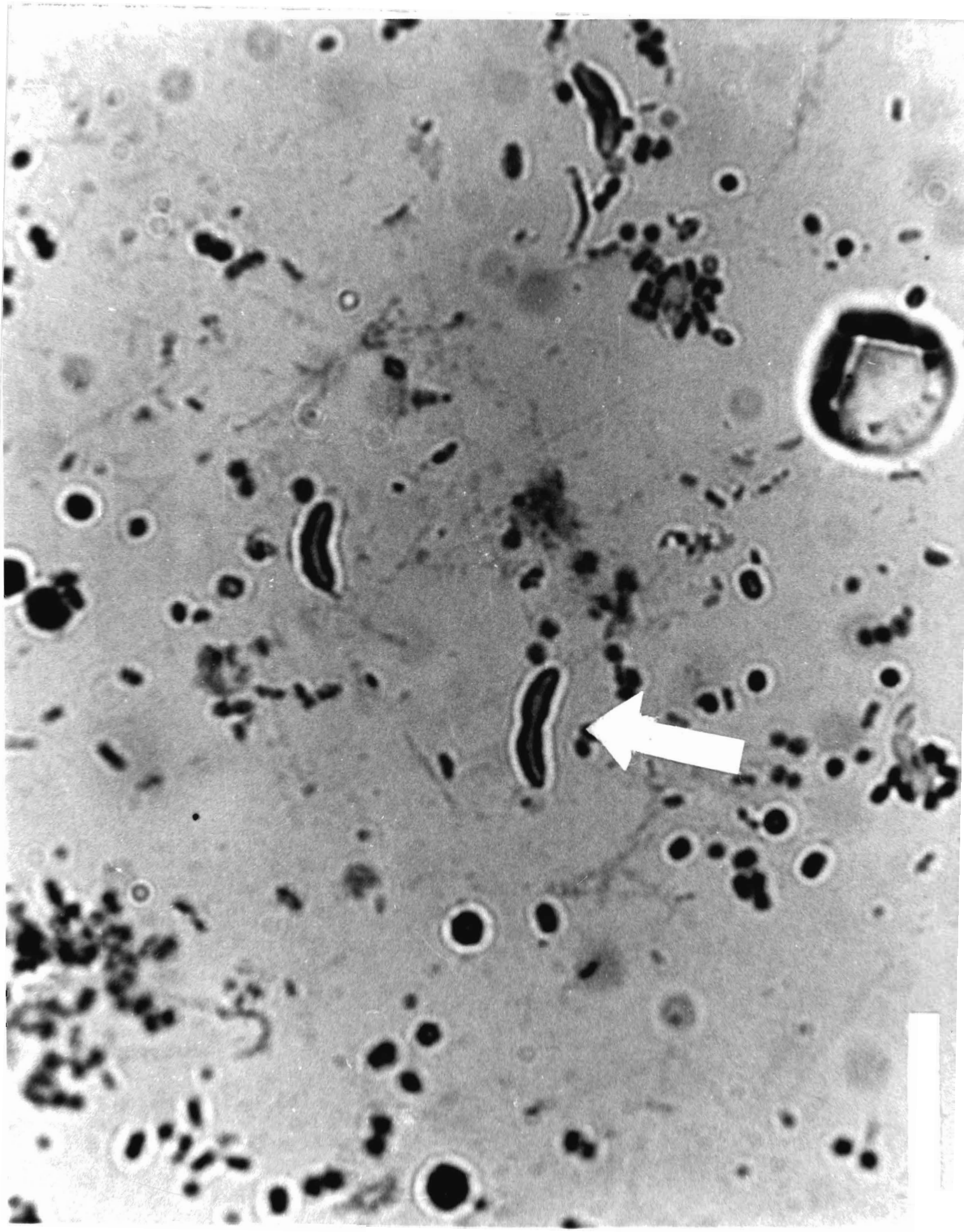


Figure 2C: A light microscope picture of cells taken from the chemocline in Crawford Lake. Line represents 5 μm.



APPENDIX 3

Extra Data

In this section I have placed data that were not necessarily used as part of the thesis, but collected during the 1979 and 1980 field seasons. I have included these data as useful reference material for those who may study Crawford Lake in the future. This may be important as Crawford Lake is protected by the Halton Region Conservation Authority. Therefore, future studies are highly probable. I have made no attempt to analyze these data, only compile and record them. I am indebted to Dr. M. Ouellette for the Fe, M_n, O_2 C_{total}, $C_{inorganic}$, pH, SO_4, Ca and Mg profiles that he made.

Figure 3A: Light intensity (at 0.1 m, taken at 12:00 noon) as a function of time from May to December 1980. The vertical bars represent the range of values recorded during other times in the day. Those days with no bars represent days when the precipitation was sufficiently intense to cause damage to the machine if repeated measurements were made.

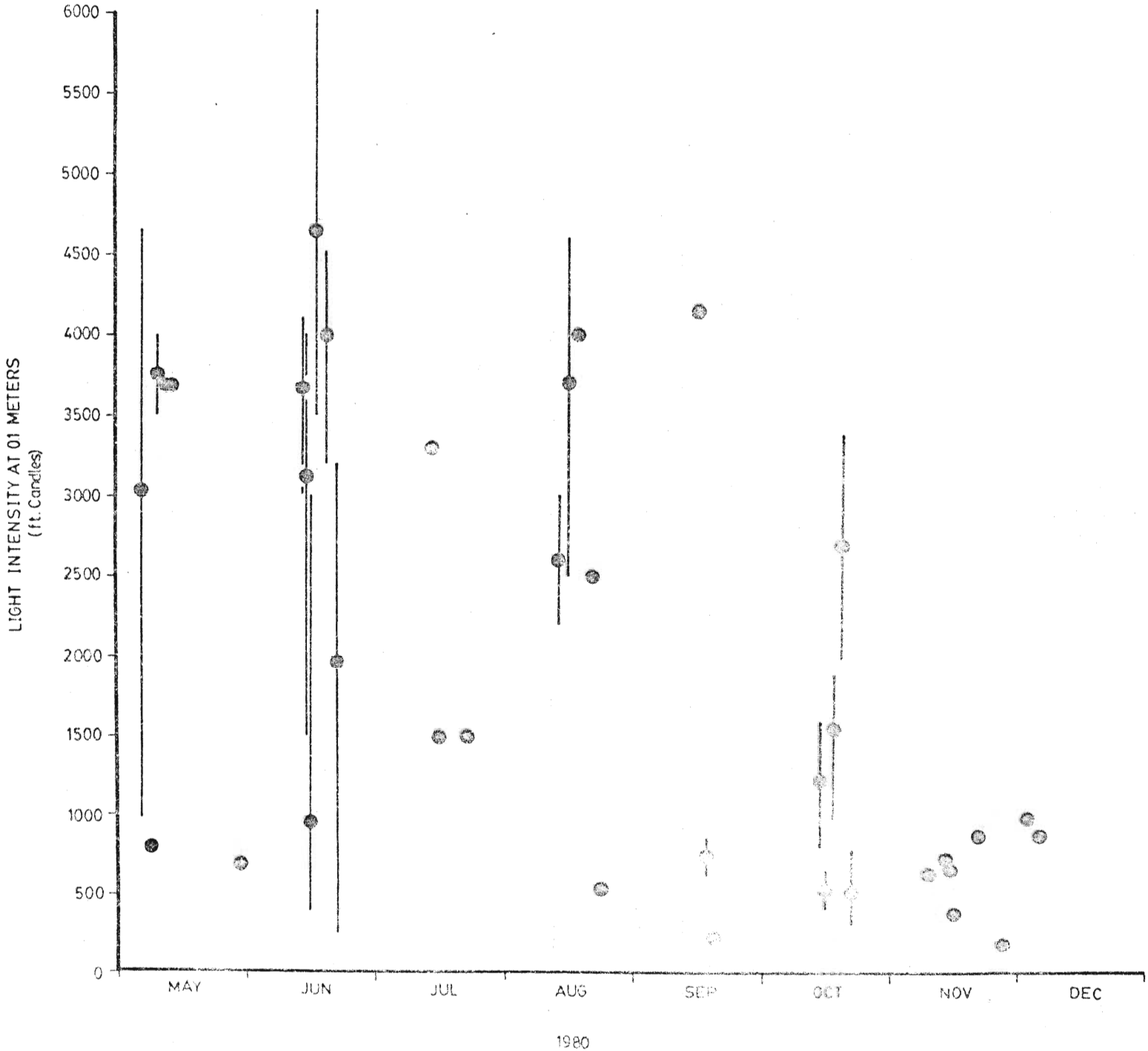

LIGHT INTENSITY AT 01 METERS
(ft. Candles)
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
1980

Figure 3B: Alkalinity ($mgCaCO_3/l$) depth (meters) profiles recorded from June to December 1980. The values represent the mean value of 25 samples taken over 7 days in approximately the middle of each month. The maximum recorded error was ± 7.5 $mgCaCO_3$/liter. The symbols represent:

- June
- July
- August
- September
- October
- November
- December

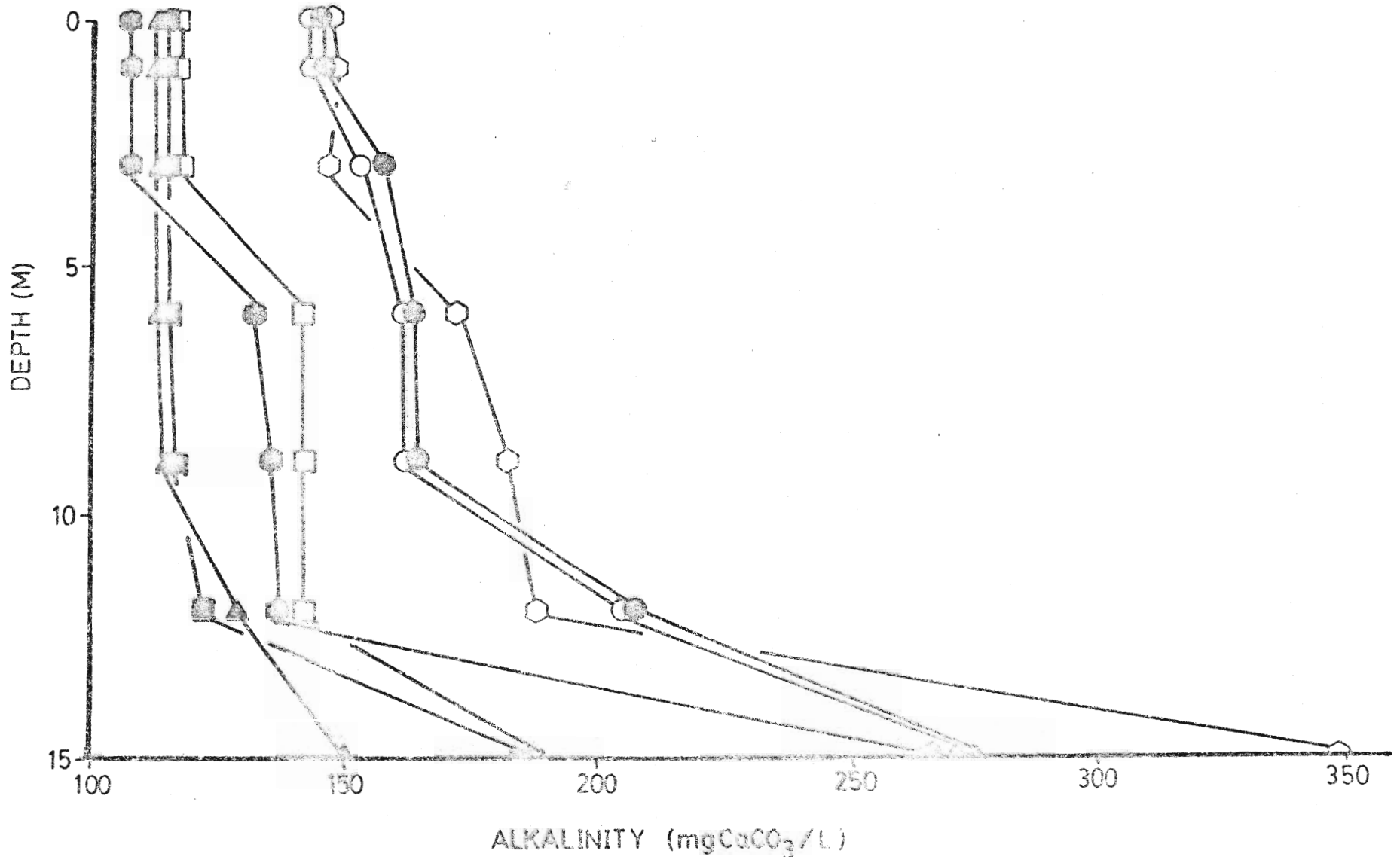

0
5
10
15
DEPTH (M)
100
150
200
250
300
350
ALKALINITY (mgCaCO3/L)

Figure 3C: pH profiles recorded monthly from June to December 1980. The figure is characterized by the following symbols:

All units are (-log[H+])

△ June
▲ July
▲ June & July
□ August
◪ August & June
⬟ September
▣ October
● November
○ December
◍ December & November

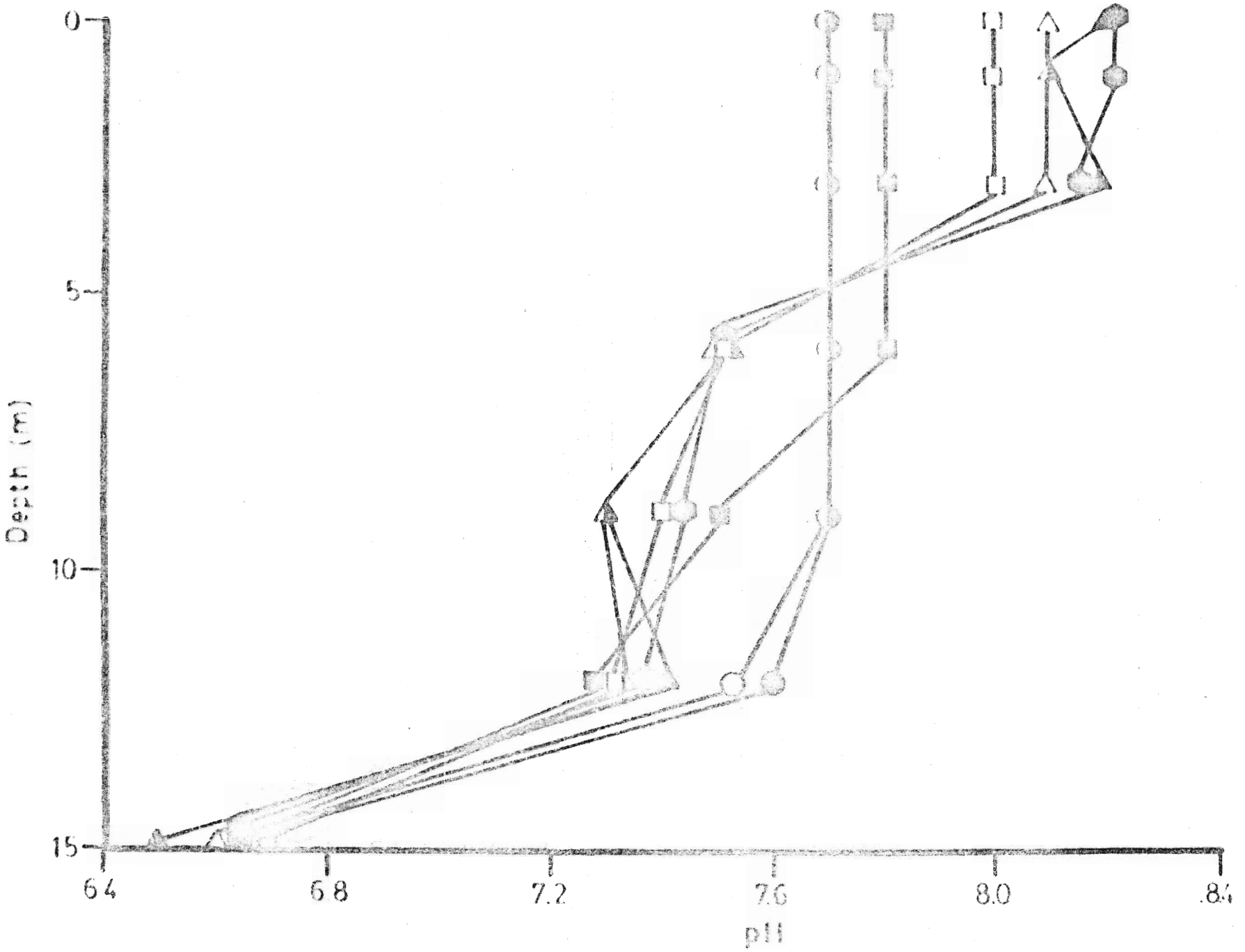

0
5
10
15
Depth (m)
6.4
6.8
7.2
7.6
8.0
.84
pH

Figure 3D: Dissolved ions concentration as a function of depth. Iron, Manganese, Oxygen, Total Carbon, Inorganic Carbon, pH, Sulphate, Calcium and Magnesium profiles. Done by Dr. M. Ouellette.

Figure 3E: Light attenuation (●), Secchi depth (○), and depth of 1% surface light (◍), as a function of time (months).

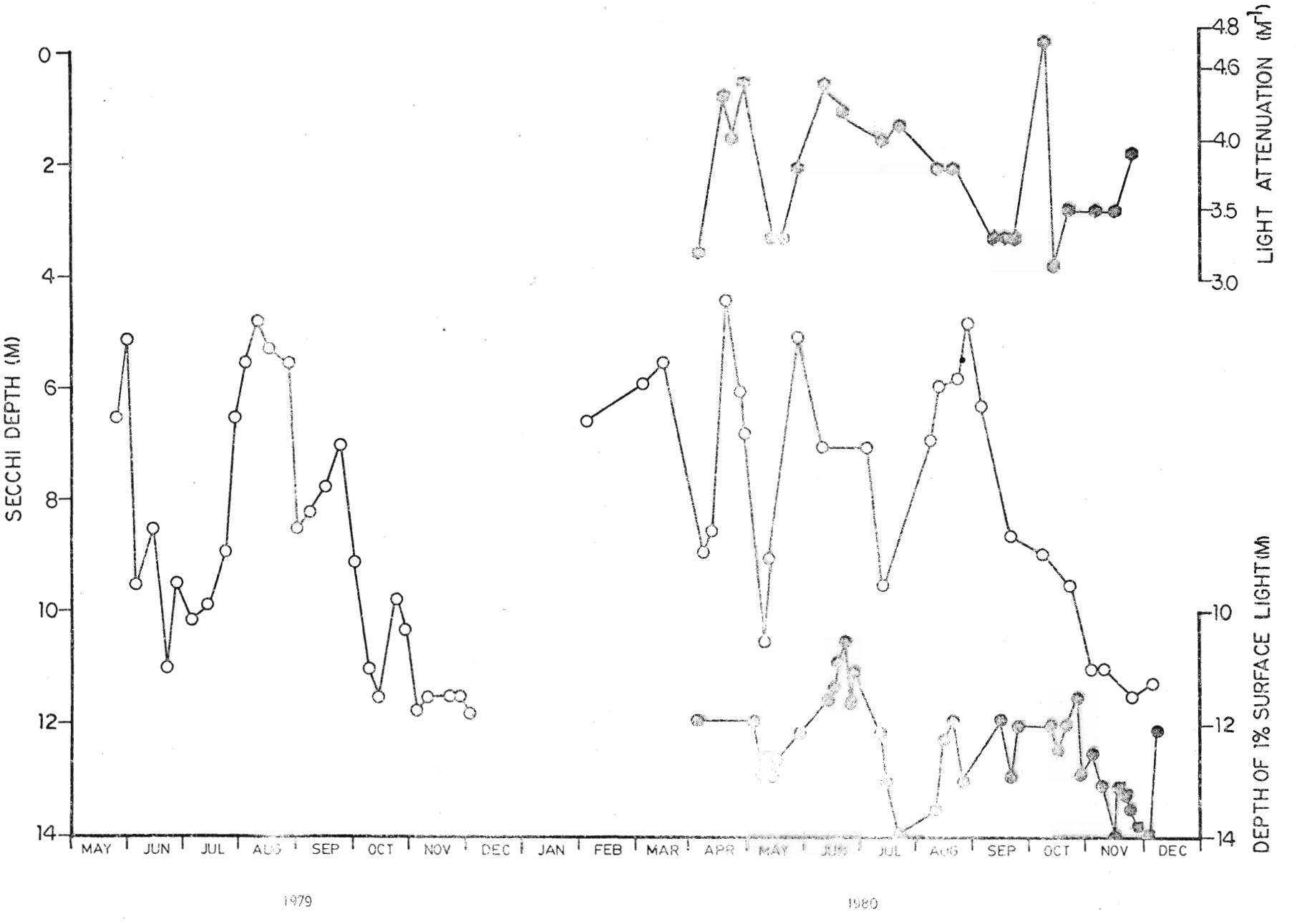
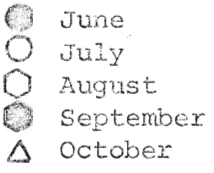

SECCHI DEPTH (M)
0
2
4
6
8
10
12
14
LIGHT ATTENUATION (M^{-1})
4.8
4.6
4.0
3.5
3.0
DEPTH OF 1% SURFACE LIGHT (M)
10
12
14
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
1979
1980

Figure 3F: The seasonal change in primary productivity (Pl-Pd; mg c/m/hr) as a function of depth (m).



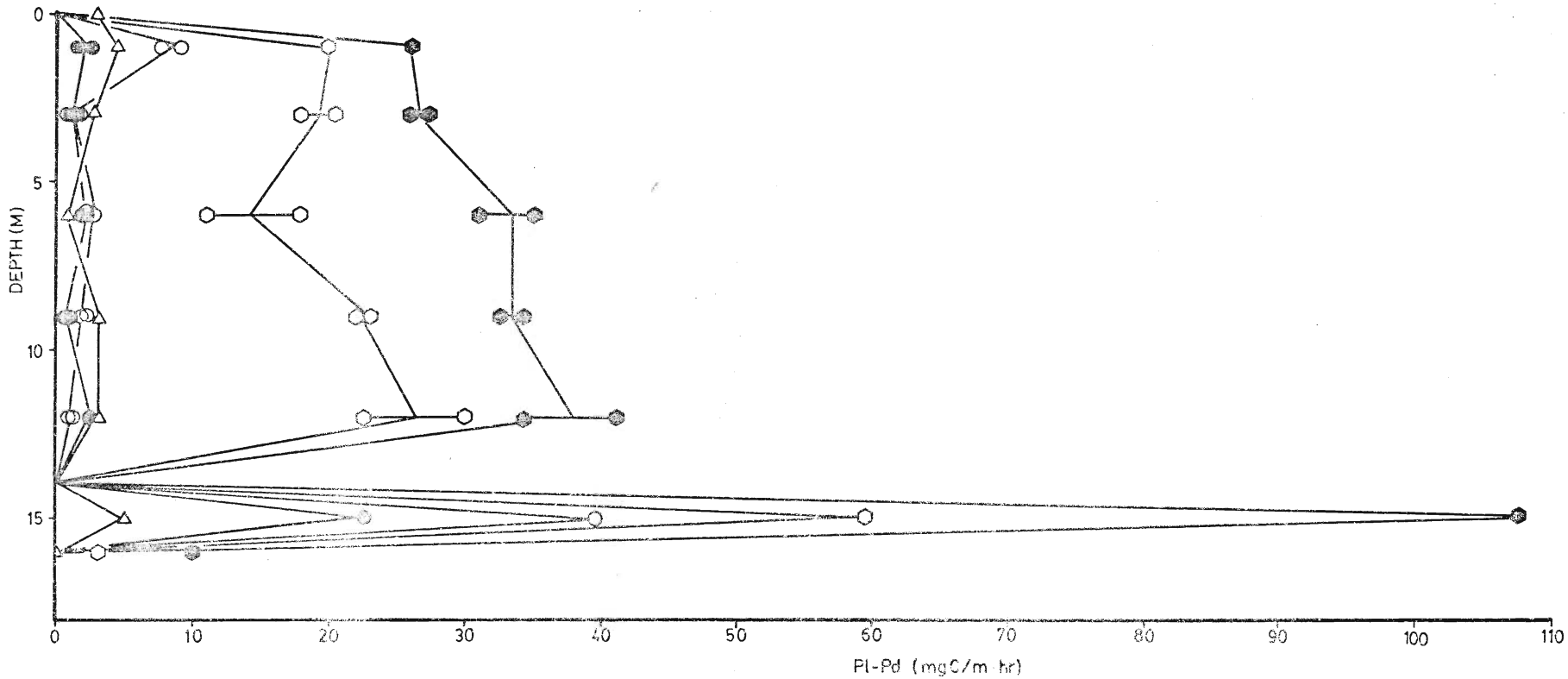

DEPTH (M)
0
5
10
15
0
10
20
30
40
50
60
70
80
90
100
110
Pl-Pd (mgC/m hr)

Figure 3G: Bacteriochlorophyll concentration (mg/m^3) as a function of time, for both light and dark (stippled) incubated bottles. The vertical bar represents 95% confidence interval.

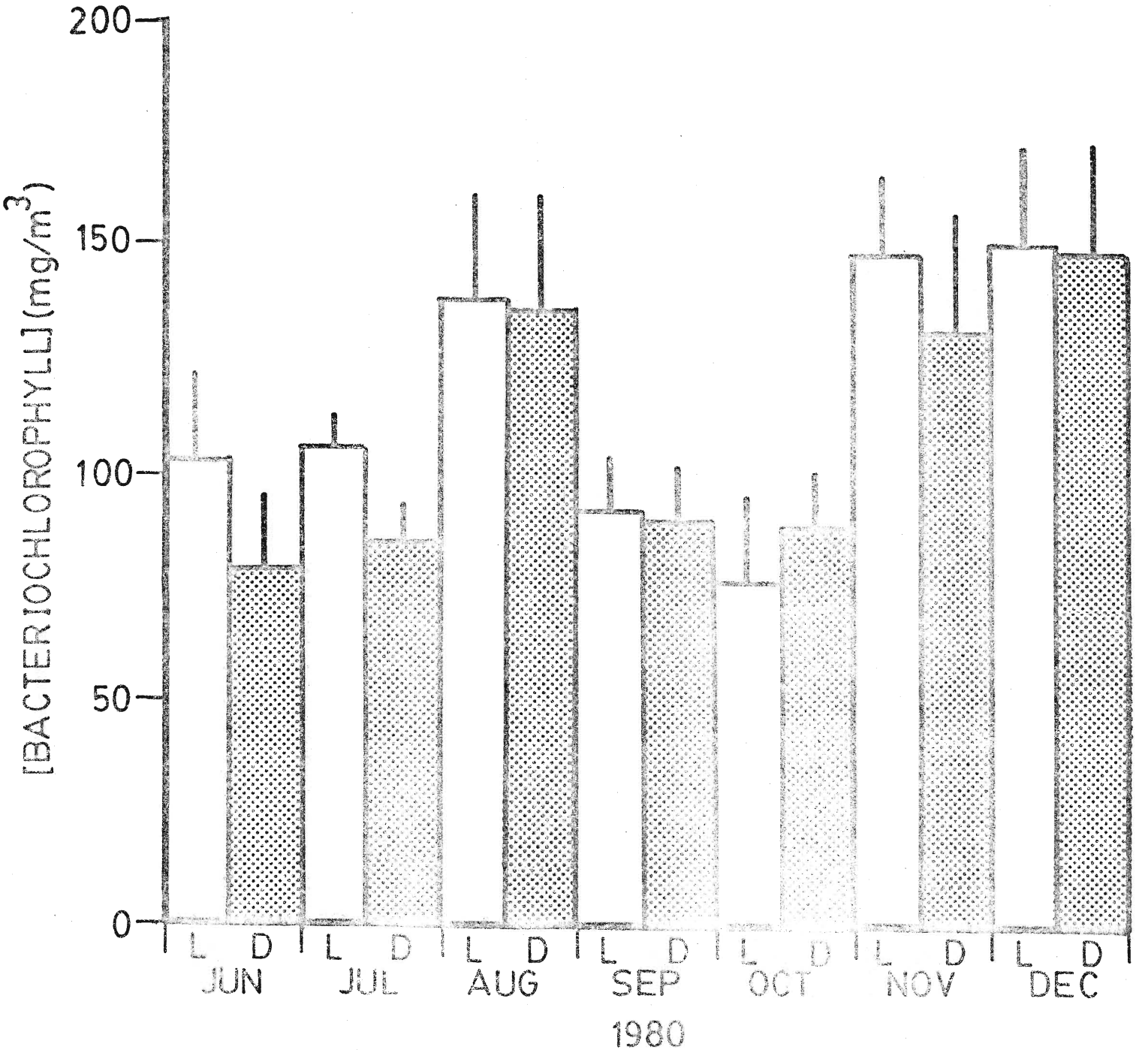
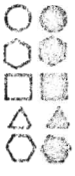

200
150
100
50
0
[BACTERIOCHLOROPHYLL] (mg/m3)
L D L D L D L D L D L D L D
JUN JUL AUG SEP OCT NOV DEC
1980

Figure 3H: Bacteriochlorophyll concentration (mg/m^3) as function of depth (m).



All determinations made in 1979.

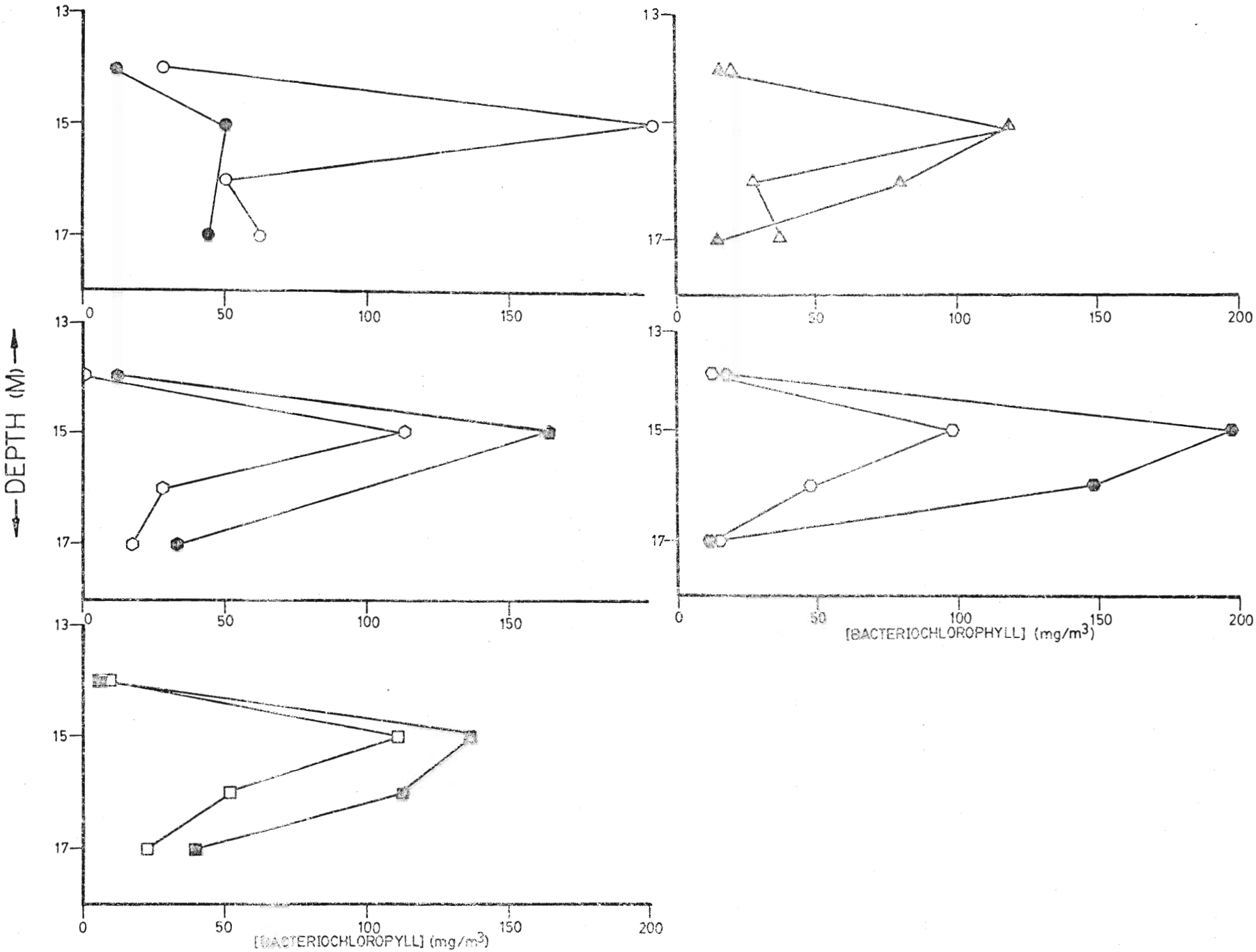

DEPTH (M)
13
15
17
0
50
100
150
200
[BACTERIOCHLOROPYLL] (mg/m3)
[BACTERIOCHLOROPHYLL] (mg/m3)

Figure 3I: The depth (meters) and concentration (#/m^3) of Daphnia pulex, keratella quadrata and D. rosea as a function of time (June to August, 1979). The samples were collected by Lucy Sardella and counted by S. Severn.

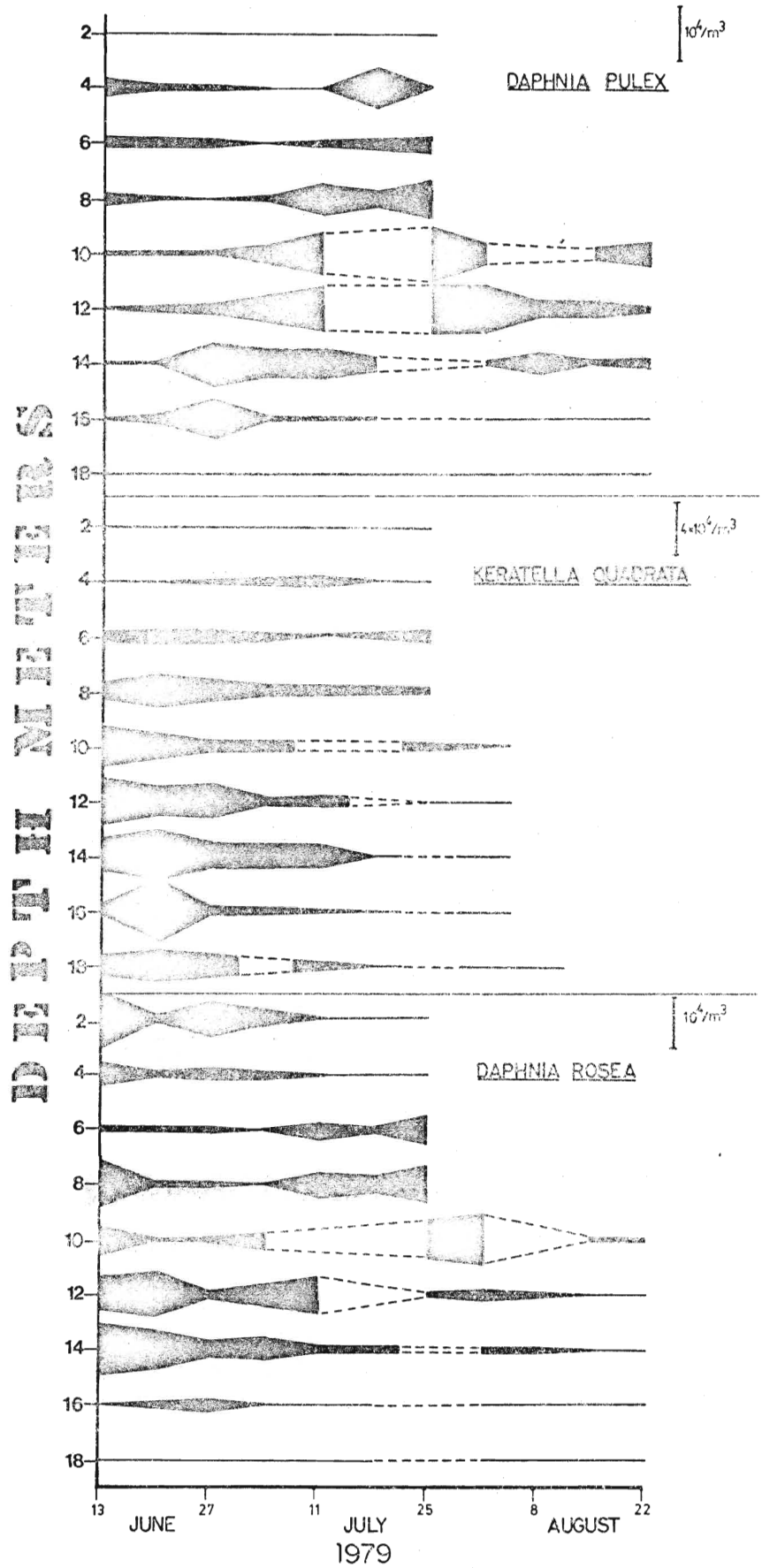

DEPTH IN METERS
$10^4/m^3$
DAPHNIA PULEX
$4\times10^4/m^3$
KERATELLA QUADRATA
$10^4/m^3$
DAPHNIA ROSEA
2
4
6
8
10
12
14
15
18
13
JUNE
27
11
JULY
25
8
AUGUST
22
1979

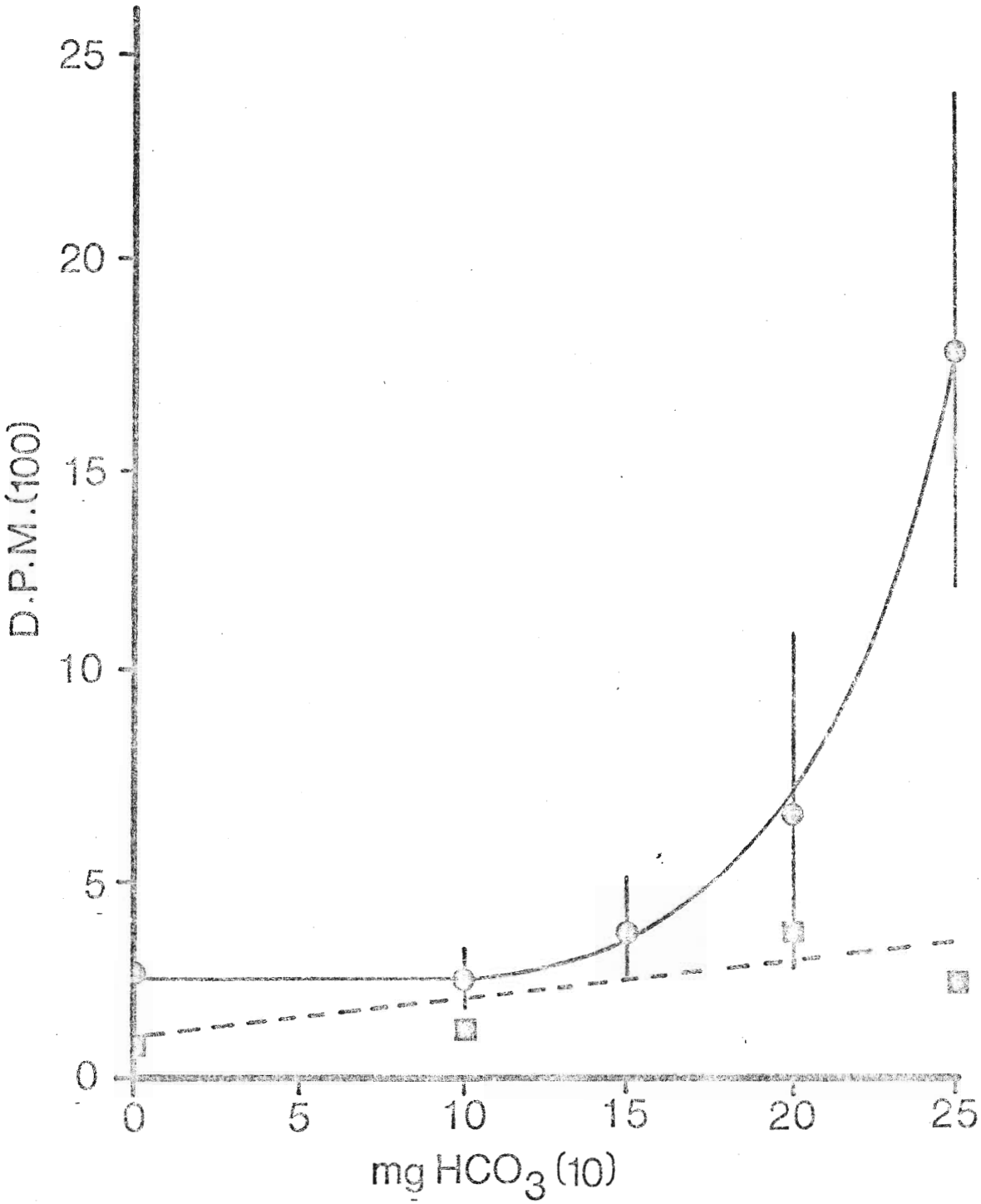
Figure 3J: The disintegration per minute of H (^{14}C) HCO_3^- labelled photosynthetic bacteria were monitored as a function of increased addition of HCO_3^- to the ambient concentrations of HCO_3^- present in Crawford Lake water. The circles represent the mean of 5 replicates of bottles incubated at 25 μein/m^2/s. The vertical bars represent the 95% confidence interval about the mean. The squares mean represented samples incubated in the dark. Three replicates at each HCO_3^- were used to calculate the mean.

Figure 3K: Temperature corrected (25°C) Specific Conductivity (umhos/cm) as a function of both depth (meters) and time (months). Conductivity values are represented by the following symbols (all umhos/cm corrected to 25°C).

The thickened line at zero meters extend over the period of ice cover. The thickness of the line does not represent ice thickness.

Figure 3K: Temperature corrected (25°C) Specific Conductivity (umhos/cm) as a function of both depth (meters) and time (months). Conductivity values are represented by the following symbols (all umhos/cm corrected to 25°C).

The thickened line at zero meters extend over the period of ice cover. The thickness of the line does not represent ice thickness.


25
20
15
10
5
0
D.P.M.(100)
0
5
10
15
20
25
mg HCO_3 (10)

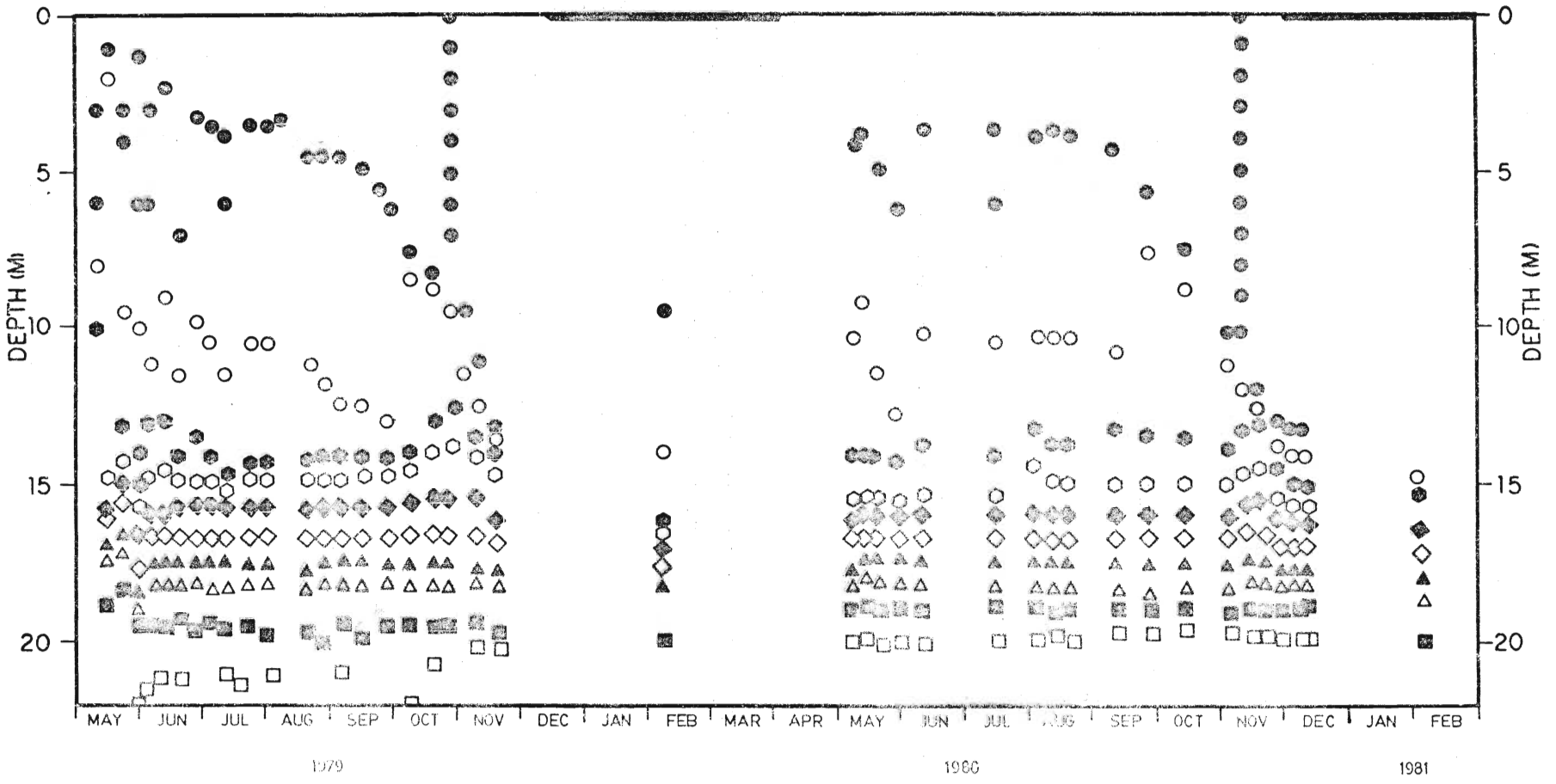
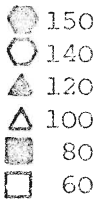
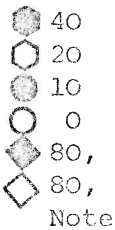

0
5
10
15
20
DEPTH (M)
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
1979
1980
1981

Figure 3L: Dissolved oxygen (% saturation) as a function of both depth (meters) and time (months May 1979 to February 1981) graph. The symbols represent the following dissolved oxygen values:

(% Saturation)



150
140
120
100
80
60



40
20
10
0
80, 60, 40, 20, 10
80, 60, 40, 20

Note: these values were too close together to plot separately. Hence, alternative symbols were used to represent the collective values.

The thick line at zero meters was used to represent ice cover. The thickness of the line does not represent ice thickness.

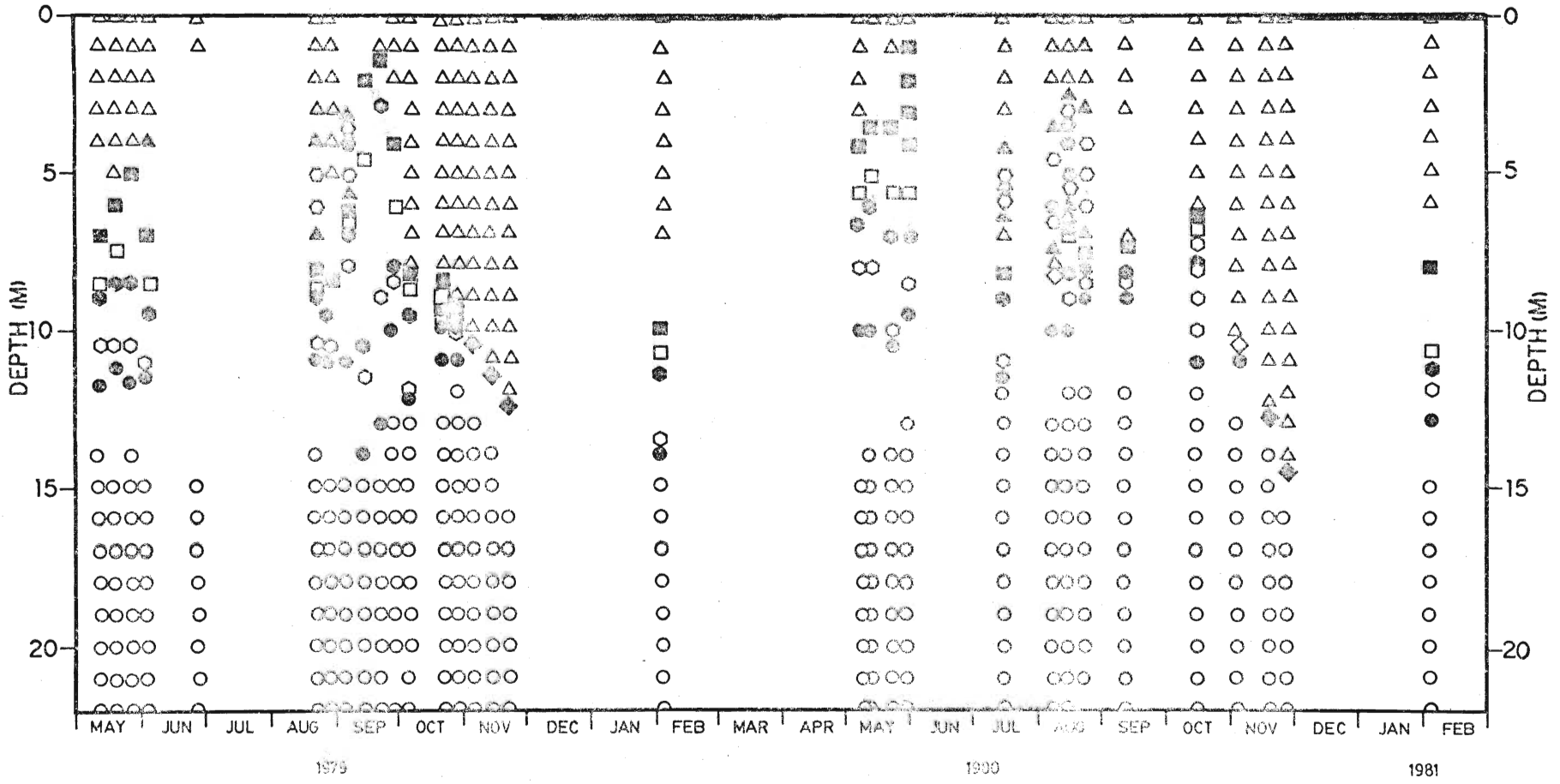
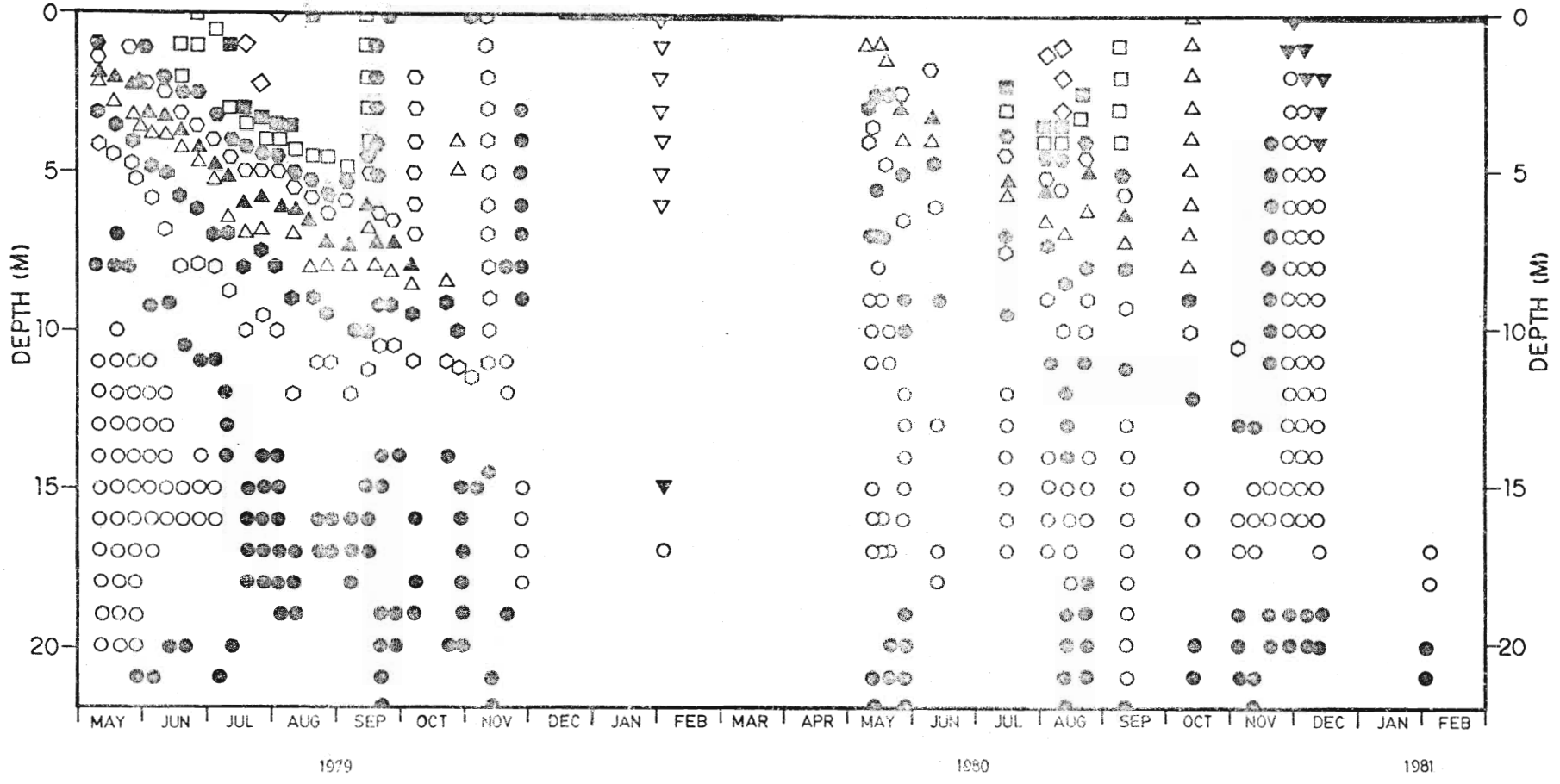

0
5
10
15
20
DEPTH (M)
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
1979
1980
1981

Figure 3M: Temperature (°C) as a function of both depth (meters) and time (months, May 1979 to February 1980). The symbols represent the following temperatures (°C)

The thick line at zero meters represents ice cover. The thickness of the line does not represent ice thickness.


0
5
10
15
20
DEPTH (M)
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
1979
1980
1981

APPENDIX 4

Program Used to Calculate Primary Productivity Values

This is a photocopy of the program used to calculate the primary productivity values of the photosynthetic bacteria. Note that the 1.06 x multiplication factor was not used for the production calculation for the bacteria as it was not known if it applied. However, it was used for the algal primary production calculation (Lind, 1924).

PROGRAM FOR PHYSICS WANG

```
 10 REM THIS PRODUCTIVITY 'HOT PROD'
20 A$="RAW COUNTS" B$="QUENCH CORRECTED":C$="MG C/M^3":D$="RATE":E$="KINETICS"
:G$="TOTAL WATER COLUMN"
30 PRINT "INPUT EXPERIMENTAL DATE (E$), AND TYPE OF EXPERIMENT:(1) FOR KINETIC
S, (2) FOR TOTAL WATER COLUMN" INPUT E$,K:IF K=1THEN 40:IF K=2THEN 50
40 SELECT PRINT 211(95):PRINT E$, E$:GOTO 60
50 SELECT PRINT 211(95):PRINT E$, G$
60 PRINT :PRINT A$, B$, C$, D$
70 SELECT PRINT 005(64):PRINT "REQUEST DATA INPUT"
80 PRINT ;TAB(24);"BACKGROUND (B)=";:INPUT B
90 PRINT ;TAB(24);"TUBE VOLUME (V)=";:INPUT V
100 PRINT ;TAB(24);"FILTER VOLUME (U)=";:INPUT U
110 PRINT ;TAB(24);"ALKALINITY (Z)=";:INPUT Z
120 PRINT ;TAB(24);"ALKANITY CORRECTION (D)=";:INPUT D
130 PRINT :PRINT "REQUEST ADDITIONAL DATA"
140 PRINT ;TAB(24);"NUMBER OF COUNTS (A)=":INPUT A
150 PRINT ;TAB(24);"QUENCH (F)=";:INPUT F
160 PRINT ;TAB(24);"TOTAL MICROCURRIES (M)=":INPUT M
170 PRINT "DATA CHECK : IF DATA CORRECT PUNCH CONTINUE EXECUTE"
180 PRINT "F=";F;TAB(20);"A=";A
190 PRINT "B=";B;TAB(20);"V=";V
200 PRINT "U=";U;TAB(20);"Z=";Z
210 PRINT "D=";D;TAB(20);"M=";M:STOP
220 J=(A-B):L=(-27.98*F)+96.58:N=(1-(L/100))*J:G=N*J
230 I=G*D*(V/U)*Z:P=I/((2.22*10^3)*M)
240 PRINT "REQUEST TIME (T) VALUE FOR THIS RUN":INPUT T:Q=P/T
250 SELECT PRINT 211(95)
260 PRINTUSING 270, A, G, P, Q
270 ######.##
280 SELECT PRINT 005(64):PRINT "IF SERIES CALCULATIONS ENTER 1":INPUT Y:IF Y=1
THEN 130
290 PRINT "DATA DO YOU WISH TO BEGIN A NEW SERIES; IF YES ENTER 2":INPUT Y:IF
Y=2THEN 20
300 RESTORE :END
```

PROGRAM FOR BIOLOGY WANG

```
10 REM THIS PRODUCTIVITY 'HOT PROD'
20C$="MG C/M!3":D$="RATE":F$="KINETICS":G$="TOTAL WATER COLUMN"
30 PRINT "INPUT EXPERIMENTAL DATE (E$), AND TYPE OF EXPERIMENT:(1) FOR KINETIC
S, (2) FOR TOTAL WATER COLUMN":INPUT E$,K:IF K=1THEN 40:IF K=2THEN 50
40 SELECT PRINT 215(40):PRINT E$, F$:GOTO 60
50 SELECT PRINT 215(40):PRINT E$, G$
60 PRINT :PRINT  C$, D$
70 SELECT PRINT 005(64):PRINT "REQUEST DATA INPUT"
80 PRINT ;TAB(24);"BACKGROUND (B)=";:INPUT B
90 PRINT ;TAB(24);"TUBE VOLUME (V)=";:INPUT V
100 PRINT ;TAB(24);"FILTER VOLUME (W)=";:INPUT W
110 PRINT ;TAB(24);"ALKALINITY (Z)=";:INPUT Z
120 PRINT ;TAB(24);"ALKANITY CORRECTION (D)=";:INPUT D
130 PRINT :PRINT "REQUEST ADDITIONAL DATA"
140 PRINT ;TAB(24);"NUMBER OF COUNTS (A)=";:INPUT A
150 PRINT ;TAB(24);"QUENCH (F)=";:INPUT F
160 PRINT ;TAB(24);"TOTAL MICROCURRIES (U)=";:INPUT U
170 PRINT "DATA CHECK : IF DATA CORRECT PUNCH CONTINUE EXECUTE"
180 PRINT "F=";F;TAB(20);"A=";A
190 PRINT "B=";B;TAB(20);"V=";V
200 PRINT "U=";U;TAB(20);"Z=";Z
210 PRINT "D=";D;TAB(20);"W=";W:STOP
220 J=(A-B):L=(-27.98*F)+96.58:N=(1-(L/100))*J:G=N+J
230 I=G*D*(V/W)*Z:P=I/((2.22*10!3)*U)
240 PRINT "REQUEST TIME (T) VALUE FOR THIS RUN":INPUT T:O=P/T
250 SELECT PRINT 215(40)
260 PRINTUSING 270,  P; O
270 %######.##
280 SELECT PRINT 005(64):PRINT "IF SERIES CALCULATIONS ENTER 1":INPUT X:IF X=1
THEN 130
290 PRINT "DATA DO YOU WISH TO BEGIN A NEW SERIES; IF YES ENTER 2":INPUT Y:IF
Y=2THEN 20
300 RESTORE :END
```