CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

Canada

40

0-315-14426-2

National Library of Canada / Bibliothèque nationale du Canada

CANADIAN THESES ON MICROFICHE / THÈSES CANADIENNES SUR MICROFICHE

62335

NAME OF AUTHOR / *NOM DE L'AUTEUR* Ronald Gordon Smith

TITLE OF THESIS / *TITRE DE LA THÈSE* Photosynthetic and light independent carbon fixation in Macrocystis integrifolia

UNIVERSITY / *UNIVERSITÉ* Simon Fraser University

DEGREE FOR WHICH THESIS WAS PRESENTED / *GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* Master of Science

YEAR THIS DEGREE CONFERRED / *ANNÉE D'OBTENTION DE CE GRADE* 1983

NAME OF SUPERVISOR / *NOM DU DIRECTEUR DE THÈSE* Professor L. M. Srivastava

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

DATED / *DATÉ* April 19, 1983 SIGNED / *SIGNÉ* [redacted]

PERMANENT ADDRESS / *RÉSIDENCE FIXÉ* [redacted]

CL-9 3-74

PHOTOSYNTHETIC AND LIGHT INDEPENDENT CARBON FIXATION IN *MACROCYSTIS INTEGRIFOLIA*

by

Ronald G. Smith

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

1983

Approval

Name: Ronald Gordon Smith

Degree: Master of Science

Title of Thesis: Photosynthetic and light independent carbon fixation in Macrocystis integrifolia

Examining Committee

Chairman: Dr. R. W. Mathewes

Dr. L. M. Srivastava, Senior Supervisor

Dr. L. D. Druehl

Dr. C. L. Kemp

Dr. G. R. Lister

Dr. M. E. Vidaver, Professor, Biological Sciences, Public Examiner

Date approved 83/04/18

PARTIAL COPYRIGHT LICENSE

I hereby grant to Simon Fraser University the right to lend my thesis, project or extended essay (the title of which is shown below) to users of the Simon Fraser University Library, and to make partial or single copies only for such users or in response to a request from the library of any other university, or other educational institution, on its own behalf or for one of its users. I further agree that permission for multiple copying of this work for scholarly purposes may be granted by me or the Dean of Graduate Studies. It is understood that copying or publication of this work for financial gain shall not be allowed without my written permission.

Title of Thesis/~~Project/Extended Essay~~

Photosynthetic and light independent carbon fixation in Macrocystis integrifolia

Author: _____
(signature)

Ron Smith
(name)

April 19, 1987
(date)

ABSTRACT

The seasonal photosynthetic performance of three age class blades of Macrocystis integrifolia was studied by determining their photosynthetic rate versus irradiance (P vs I) curves and pigment contents over 15 months. All blade types were irradiance-saturated at values ranging from 25 to 70 $\mu E/m^2/sec$. Young and mature blade tissues had higher photosynthetic maxima and initial slopes on an area basis than the older blade tissue, even though the latter had pigment concentrations similar to those in the mature blade tissue. All these parameters varied on a seasonal basis. The photosynthetic maxima ranged from 0.1-0.8 $\mu mol\ C/cm^2/h$, and showed two peaks, one in late summer-early fall and the other in late winter. Changes in the initial slope of the P vs I curve and pigment concentrations in the blade tissues suggest that changes in the size of or efficiency of electron transfer in the photosynthetic unit occur. These data are discussed in relation to changes in seawater temperature and nitrate concentrations.

The light independent carbon fixation by M. integrifolia and the role mannitol plays in this process was determined by incubating young or mature blade discs in ^{14}C-carbonate with or without added mannitol. The light independent carbon fixation rates of young M. integrifolia blade discs were greater than those of mature blade discs — in young blade discs these rates ranged from 6.0-14.0% of the carbon fixed in the light. Most of the ^{14}C-activity in blade discs incubated in ^{14}C-carbonate was contained in the organic acid, amino acid, sugar and ethanol-insoluble fractions. The proportions of ^{14}C-label in these fractions changed with duration of incubation. ^{14}C- as well as unlabelled mannitol was taken up by M. integrifolia blade discs. The uptake varied between February and May and seemed to be greater in light than in dark. The distribution of

^{14}C-labelled components from discs incubated in ^{14}C-mannitol was similar to that found in discs incubated in ^{14}C-carbonate except that the activity in the ethanol-insoluble fraction was increased. Blade discs which were previously depleted of their mannitol reserves were incubated in ^{14}C-carbonate with added mannitol. Mannitol increased the light independent carbon fixation rate and slightly altered the distribution of ^{14}C-labelled components. From the data presented it is apparent that mannitol taken up or synthesized by blade discs enters the light independent carbon fixation pathway after being broken down to phosphoenol pyruvate, and thus contributes to anaplerotic and/or respiratory pathways.

ACKNOWLEDGEMENTS

I would like to thank several people whose help made this thesis a reality.

Dr. Lalit M. Srivastava provided me with the opportunity and means to carry out this project and deserves credit for his patience and the help he provided throughout the study.

The other members of my committee, Drs. L. Druehl, C.L. Kemp and G. Lister, were always available when the call went out for help.

Drs. K. Rosell and W. Wheeler helped in the experimental design and interpretation of the data.

The director and staff of the Bamfield Marine Station and A. Cifarelli and M. Yang at Simon Fraser University aided me in several general and technical aspects of the study.

Finally, severals others, most notably D. Gill and J. Watson, helped in the collection of the plants.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

A. INTRODUCTION

The purpose of this study was twofold. Firstly, to determine the ambient seasonal photosynthetic performance of three age class blades of the brown alga, Macrocystis integrifolia Bory (Order Laminariales). Secondly, to determine how the light independent carbon fixation process of M. integrifolia is affected by extended periods of darkness and to determine what role mannitol has in this process.

Seasonal Photosynthetic Performance

The photosynthesis versus irradiance (P vs I) curves of algae are potentially very informative (Beardal and Morris, 1976; Prezelin, 1981; Talling, 1957). Changes in the irradiance-saturated photosynthetic rate (P_{max}) provide clues to the overall activities of the carbon fixation enzymes (Steeman Nielsen, 1975) and factors which affect those enzymes such as temperature and nutrient supply. Changes in the initial slope reflect on changes in the light harvesting capabilities of a tissue subjected to subsaturating light conditions and thus on changes in the size and/or numbers of photosynthetic units (PSU) and/or the efficiency of electron transfer in the PSU (Prezelin, 1981). P vs I curves have been used widely in studies on phytoplankton to deduce adaptive changes under varying environmental conditions (see e.g., Beardall and Morris, 1976; Platt and Jassby, 1976; Prezelin, 1981; Welshmeyer and Lorenzen, 1981; Yentsch and Lee, 1966) but their use in estimating the photosynthetic performance of marine algal macrophytes has been limited.

Lüning (1971) reported that the P_{max} levels of Laminaria hyperborea fronds were higher in the spring-summer than in the winter months. These

data were subsequently verified for L. longicruris (Hatcher et al., 1977) and several other marine macrophytes (King and Schramm, 1976). Several authors have reported similar data for M. pyrifera (Clendenning, 1971) and other marine algal macrophytes (Brinkhuis, 1977a, 1977b; Littler et al., 1979; Yokohama, 1973; Zavodnik, 1973) although they did not produce P vs I curves. These studies have indicated that the photosynthetic capacity of marine algal macrophytes is affected by seasonal light, temperature and/or nutrient conditions. Tissue age has also been reported to affect the photosynthetic capacity of kelp, with mature tissues generally having the higher carbon fixation rates (Clendenning, 1971; Johnston et al., 1977; Küppers and Kremer, 1978; Lüning, 1971; Wheeler, 1980; Willenbrink et al., 1979).

The lesser giant kelp, Macrocystis integrifolia Bory in British Columbia, grows from ca. 4 m below low tide to the surface; therefore, each plant may simultaneously experience photosynthetically saturating and subsaturating irradiances. The photosynthetic performance of M. pyrifera and M. integrifolia blades and their P vs I curves in relation to age have been recorded previously (Clendenning, 1971; Wheeler, 1980; Willenbrink et al., 1979). The present study reports on the seasonal changes in P_{max} and initial slopes of three age class blades of M. integrifolia. Data on pigment concentration and environmental parameters, such as nitrate concentration, temperature and irradiance, are included. The results show marked differences in the photosynthetic performance of different age class blades and pronounced seasonal variations in P_{max} as well as the initial slope of photosynthesis. These results provide background data for a study of photosynthetic enzyme levels and analysis of pigment-pigment, pigment-protein and electron transport interactions in the PSU.

Light Independent Carbon Fixation

Brown algae, including M. integrifolia, fix carbon mainly through the reductive pentose phosphate pathway, utilizing the enzyme ribulose bisphosphate carboxylase (RuBPC), although appreciable amounts of carbon can be fixed in young tissue through β-carboxylation of phosphoenol pyruvate (PEP) utilizing the enzyme phosphoenol pyruvate carboxykinase (PEPCK) (Akagawa, 1972a, 1972b, 1972c; Kremer and Küppers, 1977; Kremer, 1979, 1981; Willenbrink et al., 1979). The initial products of the β-carboxylation process are ATP and oxaloacetate which in turn characteristically yields, among other products, amino acids (aspartate, glutamate and alanine) and TCA cycle organic acids (Akagawa et al., 1972a; Craigie, 1963; Kremer, 1979). PEP utilized in light independent carbon fixation is thought to be derived from mannitol in the dark (Johnston et al., 1977; Lüning, 1981; Yamaguchi et al., 1966) or by the products of photosynthesis in the light (Kremer, 1981). Competition for PEP by pyruvate kinase, the enzyme responsible for converting PEP to pyruvate, may be a regulatory step of the β-carboxylation process (Kremer, 1981).

Since β-carboxylation is a light independent process, Willenbrink et al. (1979) hypothesized that it may be important for growth of young tissue living in low light environments. Kremer (1981) hypothesized that β-carboxylation may be a means by which fast growing brown algal tissues produce additional energy plus their required metabolic intermediates.

Previous light independent carbon fixation studies have characteristically only lasted for short time periods (Akagawa et al., 1972a, 1972b; Craigie, 1963; Kremer, 1979, 1981; Willenbrink et al., 1979). The present study reports on the longer term light independent carbon fixation rates and the products of kelp discs and on the role of mannitol

in this process. The results, plus a review of the energetics involved in the light independent carbon fixation process as compared to electron transport, suggest that the above hypotheses are not entirely correct.

B. MATERIALS AND METHODS

Seasonal Photosynthetic Performance

Sampling

Fronds of *Macrocystis integrifolia* Bory were collected monthly, January 1981 through March 1982, from a kelp bed situated in front of Bamfield Marine Station (BMS), Vancouver Island, B.C., Canada. At each sampling time, 5 mature fronds were harvested by SCUBA diving and maintained in the sea at the BMS float. Three blades were cut from each frond. These were: the first free blade below the apical scimitar (young tissue); the blade closest to the holdfast which still had enough tissue for experimentation (old tissue); an intermediate blade on or near the water surface which could be considered fully mature (mature tissue). The excised blades were kept in running seawater at the BMS for the duration of the experiments. Concurrent environmental data on nitrate concentrations, water temperatures and light levels at the surface and 4 m depth were kindly supplied by Dr. L.D. Druehl.

Photosynthetic Performance

Preliminary results indicated that the carbon fixation rates of discs (standardized to μmol carbon fixed/cm^2/h) were constant through the ranges of tested incubation times (5-20 min), disc sizes (2-15 cm^2) and 'wound

healing times' (see below, 0-120 min) and were suboptimal when the preincubation times were less than 10 min (data not shown). Thus, for photosynthetic measurements the following procedure was adopted (see also Kremer, 1978). Two 4 cm^2 discs (both sides of the disc were used in the surface area calculation) were punched from each blade, scraped clean of all visible epiphytes, left in seawater for 60 min in low light (wound healing time) during which they stopped exuding mucilage, and then preincubated for 10 min under the particular conditions of an experiment. The discs were then placed in an incubation chamber containing 1.65 l of filtered sea water (Millipore, 0.45 um) and approximately 300 µCi of ^{14}C-sodium carbonate (spec. activity 59 mCi/mmol, Atomic Energy Commission Ltd., Canada). The incubations lasted for 10 min. The incubation chamber was illuminated from both sides. High intensity movie flood lamps (G.E. 375 watt R-30 lamps) were used for the 480 and 240 $\mu E/m^2/sec$ irradiances while G.E. 150 watt reflector flood lamps were used for the 10-120 $\mu E/m^2/sec$ irradiances (irradiances were measured with a Li-cor model 185a or b quanta meter, Lincoln, NB, USA). A change in the light source was necessary due to the heat output and short lifetime of the high intensity lamps. Irradiances were varied by changing the lamps' distance from the incubation chamber as well as by placing neutral density screening on an external cooling bath. Incubation temperatures approximated the ambient temperatures (see Fig. 2) in the kelp bed and were maintained with a controlled temperature unit (Haake model F3, Berlin, Germany). A flattened plastic rod fitted into a Dremel drill (Moto-Tool, Rachine Wi., USA) provided continuous stirring in the incubation chamber. Following incubation, the discs were killed in heated 80% ethanol.

Analytical Procedures

Most of the ^{14}C-activity in the fed discs was present in the ethanol-soluble fraction (see Table I), presumably due to the short incubation time used in the experiments. Therefore, the ^{14}C-activity in the ethanol-insoluble fraction was ignored for the determination of the P vs I curves. A 0.5 ml volume of 50% acetic acid was added to a 0.5 ml aliquot of the ethanol-soluble fraction to drive off all unfixed labelled carbon. Subsequently 7-10 ml of Aquasol II (New England Nuclear) were added and the samples counted in a liquid scintillation counter (Beckman LS 8000).

The ^{14}C levels in the incubation medium were monitored before and after each experiment. Five 0.25 ml aliquots of the incubation medium were added to solutions containing 10 ml of Aquasol II and 1 ml of 0.1 N NaOH (see Kobayashi and Harris, 1978) which were subsequently counted. Total carbonate levels in the incubation medium were determined using the pH technique described by Strickland and Parsons (1972).

The carbon fixation rate of each disc was calculated and standardized using a modified formula (equation 1 listed below) presented by Strickland and Parsons (1972).

Equation 1: CFR=disc DPMs*C*K*(1/inc DPM)

where:

CFR--carbon fixation rate in μmol C/cm^2/hr

disc DPMs--total ethanol-soluble DPMs per sample disc

C--amount of total CO_2 in the incubation bath

K--unit correction constant

inc DPM--total DPMs in the incubation bath

Pigment Analysis

In order to see how pigment concentrations affected the intial slope of the P vs I curve of M. integrifolia blade discs, chlorophyll$_a$ (chl$_a$), chlorophyll$_c$ (chl$_c$) and fucoxanthin concentrations were determined throughout the study. These analyses were carried out on freshly collected blades using the DMSO method (Seely et al., 1972) as modified by Wheeler (1980). The method involved soaking two 2 cm^2 discs (both sides of the disc were used in the surface area calculation) from each blade in DMSO for 10 min, pouring off the DMSO fraction, and adding acetone to the discs. Water was added in a 4:1 (DMSO:H_2O) ratio to the DMSO fraction and the solutions' absorbances were then read at 480, 582, 631 and 665 nm. The acetone fraction was refrigerated for several hours. When the discs appeared pigmentless, water and methanol were added in a 3:1:1 (acetone: H_2O:MeOH) ratio and the solutions' absorbances were recorded at 470, 581, 631 and 664 nm. The chl$_a$, chl$_c$ and fucoxanthin concentrations were determined using the equations of Seely et al. (1972).

Ethanol-insoluble Fraction

In order to determine whether appreciable amounts of ^{14}C label entered the blade discs during the 10 min incubation period, the ^{14}C-activities present in the ethanol-insoluble and -soluble fractions of young, mature and old M. integrifolia blade discs were compared at saturating light irradiances (see Fig. 12). The activity in the ethanol-soluble fraction was determined as above. The ethanol-insoluble fraction was ground with a mortar and pestle,

centrifuged at 400 X g for 10 min, washed, recentrifuged, soaked for 10 min with 50% acetic acid, filtered, combusted on a Packard Tricarb B306 combustor, and counted. The activity in the ethanol-insoluble fraction was expressed as a percentage of the total ^{14}C-labelled carbon fixed.

Effect of Nitrate on P_{max}

The seasonal dip in the P_{max} of M. integrifolia (see Fig. 9) may partially be caused by nutrient deficiencies. To test this hypothesis, experiments were carried out with young blade discs in June and mature blade discs in July, 1981. Each month the top 1-2 m of two fronds were conditioned for 5 days in either nitrate-rich seawater (deep water from the BMS seawater system) or nitrate-poor seawater (surface water). For each of five days discs were punched from both fronds and incubated in ^{14}C-carbonate medium at a saturating light irradiance of 120 $\mu E/m^2/sec$ (see Fig. 12). The samples were analyzed for their ethanol-soluble and -insoluble fractions. In July, the pigment concentrations of both fronds were also monitored over the five day period. Wheeler and Srivastava (1983) monitored, in conjunction with this study, the nitrate concentrations of the kelp's internal nitrate pools and the conditioning medium.

Light Independent Carbon Fixation

^{14}C-carbonate Fixation

The ability of M. integrifolia discs to fix ^{14}C-carbonate over extended periods of time was determined by incubating discs in Erlynmeyer flasks

containing ^{14}C-carbonate (1.0 mCi) for up to 22 h. For these experiments young and mature discs (25.5 cm^2) were incubated in light and dark. Agitation of these discs, and those of subsequent experiments, was accomplished by either; bubbling with air, magnetic stirring bars, or a shaker table.

Further analyses of the ^{14}C-labelled components of similar discs incubated in ^{14}C-carbonate were also carried out. For these experiments, young and mature blade discs (a total surface area of 70.7 cm^2) were incubated in 250 ml of seawater containing 250 µCi of ^{14}C-carbonate. The incubations lasted for 4 or 12 h in the dark. A further description of the methods used for these analyses are given elsewhere.

Effects of Incubation and Preincubation Conditions

The effects of extended dark incubations and preincubations and light pretreatment on the light independent carbon fixation rates of kelp tissues have not been reported thus far. In this study these were determined by incubating discs in ^{14}C-carbonate while changing the dark preconditioning time, dark incubation time or the predark incubation light treatment. Freshly collected young blades were used for these experiments because of their reported ability to fix carbon in the dark (Kremer, 1979; Willenbrink et al., 1979). The standard procedure for these dark fixation experiments included a 30 min light treatment at a saturating light irradiance of 120 $\mu E/m^2/sec$ (see Fig. 12), a 10 min dark preincubation period and a 10 min incubation in ^{14}C-carbonate enriched seawater. These experiments were carried out using the P vs I apparatus. Depending on the treatments, variations on the standard technique were adopted.

Mannitol Uptake

To test whether mannitol was taken up and utilized by M. integrifolia blade discs, two experiments were carried out. In the first of these experiments young and mature discs were kept in the dark for 48 h, which depleted the internal mannitol reserves, and then placed in media containing mannitol in light or dark for up to 12 h. Further details of this experiment are given in the 'Effect of Added Mannitol' section. To determine the mannitol level in the discs, the ethanol-soluble fraction of ground discs was mixed with 2.7-7.0 mg of sorbitol, an internal standard; dried; acetylated with a pyridine/acetic anhydride mixture (1:1, v/v); dried; dissolved in chloroform; and analysed by gas chromatography (Hewlett-Packard model 5880, gas cappillary column OV225; Dr. K. Rosell, personal communication).

In the second experiment, young and mature blade discs (14.1-25.5 cm^2) were incubated in ^{14}C-mannitol media (0.63 µCi; spec. activity 50 mCi/mmol, Amersham) for up to 48 h in light or dark. The ^{14}C-labelled components of similar discs (a total surface area of 70.7 cm^2) incubated in ^{14}C-mannitol media (250 µCi in 250 ml of seawater) for 4 or 12 h in the dark were determined using techniques described elsewhere.

Effect of Added Mannitol on ^{14}C-fixation

The effect of exogenously supplied mannnitol on a disc's light independent carbon fixation rate was determined in March and May, 1982. Young and mature discs (14.1 cm^2) were darkened for 48 h in 1 l of seawater (to deplete the discs of their mannitol reserves), the water being changed every day. After this time the discs were placed in either ^{14}C-carbonate, ^{14}C-carbonate plus mannitol or mannitol media for up to 24 hours in dark

(March and May) and light (May only). Mannitol and ^{14}C-carbonate were added to the appropriate flasks in 3-4 gm and 1.1 mCi quantities, respectively. Carbon fixation rates of the first two treatments were compared to the mannitol concentrations found within the discs from the third treatment. The ethanol-soluble fraction of those discs which were incubated for 12 h in May were further partitioned using techniques described below.

Respiration of ^{14}C-mannitol in the Dark

The amount of absorbed mannitol used in the dark respiration of young or mature blade discs was determined by incubating 5 young or mature blade discs (a total surface area of 70.7 cm^2) in darkened sealed flasks containing approximately 30 μCi of ^{14}C-mannitol for 4 h and then injecting, sequentially, 10 ml of a saturated KOH solution into a vial suspended within the incubation flask and 30 ml of 50% acetic acid into the sea water medium. It was assumed that the acetic acid would drive off all the respired ^{14}C-CO_2 which would then be absorbed by the KOH solution. Flasks were opened 10 min after the acetic acid injection. The disc's ethanol-soluble and -insoluble fractions were analysed using standard techniques, while the respired fraction was analysed by extracting a 0.5 ml aliquot from the KOH solution and adding 7 ml of Aquasol II in preparation for liquid scintillation counting. The low levels of ^{14}C-activity in these samples precluded the further partitioning of the ethanol-soluble and -insoluble fractions.

^{14}C-mannitol Chase Period

To determine the fate of ^{14}C-mannitol incorporated by young discs in the dark, five sets of five discs (total surface area, 70.7 cm^2) were incubated for 4 h in 250 ml of sea water containing 250 µCi of ^{14}C-mannitol. After this period, all discs were kept in 'cold' sea water containing 3 gm of added 'cold' mannitol. Five discs were then sampled after 0, 15, 30, 60 and 120 min chase periods. The ethanol-soluble and -insoluble fractions of these samples were further fractionated using the techniques described below.

Ethanol-soluble and -insoluble Fractionation

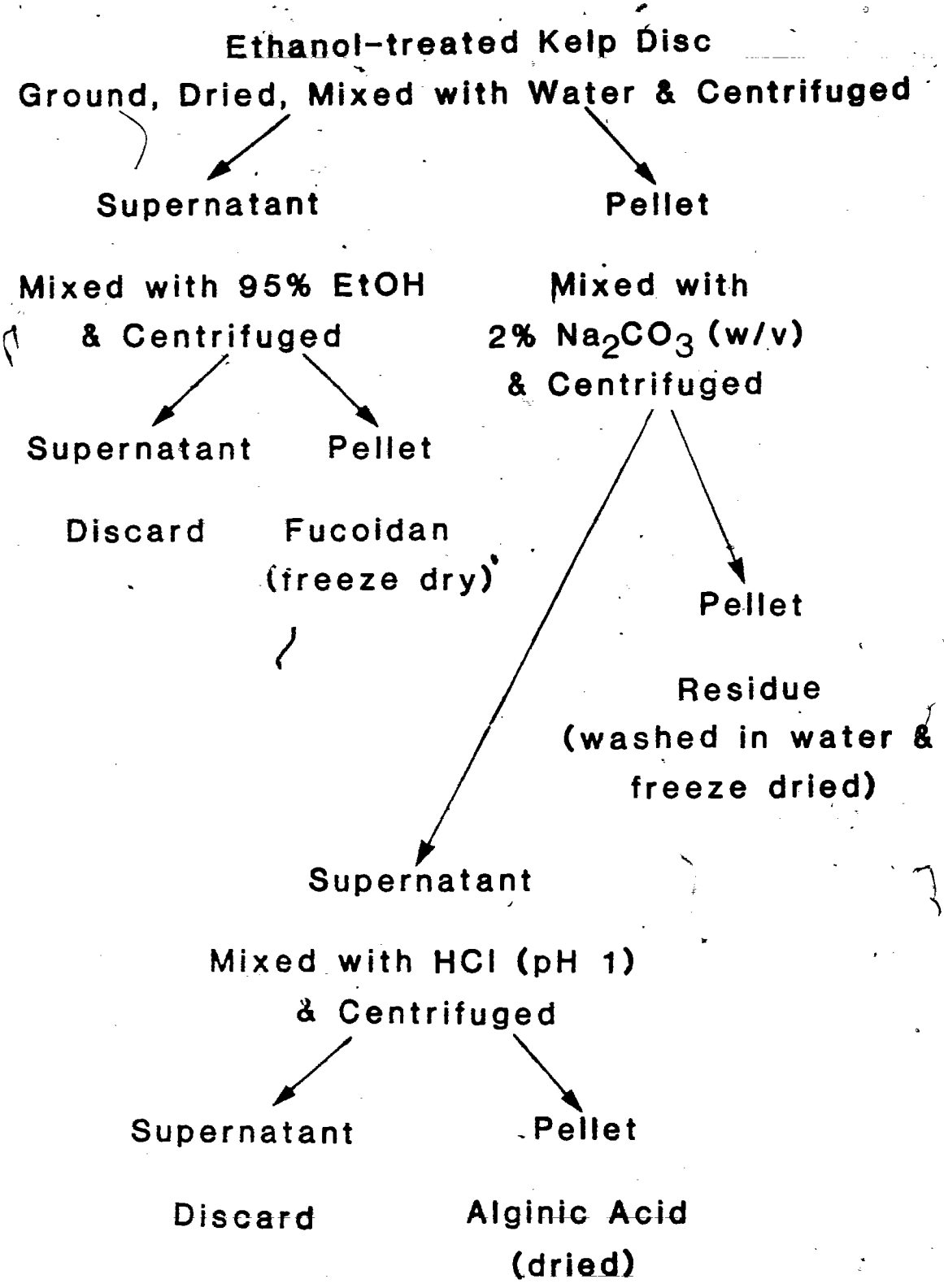
Where applicable, the ethanol-soluble and -insoluble fractions were analyzed using standard techniques. Several of the above samples also underwent further analysis. The ethanol-soluble fraction of these samples were dried and redissolved in 2 ml of 0.2 N citrate buffer. An aliquot of each sample was then separated into sugar (including mannitol), amino acid, organic acid, and phosphate ester fractions using ion-exchange chromatography (Sephadex QAE-A-25 and SP-C-25, Pharmacia, see Redgwell, 1980), with the ^{14}C-activity of each fraction being determined by scintillation counting. The efficiency of separation was determined by evaporating the various fractions of one sample to dryness, redissolving them in 4 ml of 50% ethanol, spotting an aliquot of each on filter paper, and then treating them with the following reagents: ninhydrin for amino acid detection; bromophenol blue for organic acid detection; acid molybdate for phosphate ester detection; and periodate-silver nitrate for sugar detection (see Stahl, 1969). It was found that the sugar fraction contained

considerable amounts of ninhydrin positive materials while the sugars themselves were undetectable. Further analysis of the sugar fraction by gas chromatography (Hewlett-Packard model 5790A; cross linked dimethyl silcon column, 12.5 m long, 0.2 mm diameter) did, however, show that mannitol and/or sugars were present in this fraction. The remaining fractions were relatively contamination free.

The identities of the total and ^{14}C-labelled amino acids present in the citrate buffer were determined with an amino acid analyzer (Beckman model 119), with 0.04 μM of N-leucine being added as an internal standard. In the case of the ^{14}C-labelled amino acids, each sample was run through the amino acid analyzer's column with 0.9 ml fractions being collected, mixed with Aquasol II, and counted on the scintillation counter.

The ^{14}C-activities in the total ethanol-insoluble, fucoidan, alginic acid and residue fractions of the above samples were also determined. The ground and dried kelp discs were divided into two portions: the first being analysed for its total ethanol-insoluble ^{14}C-activity using previously described techniques; and the second, used to determine the ^{14}C-activity in the fucoidan, alginic acid and residue fractions using the procedure outlined in figure 1.

Figure 1. The methodology for extracting fucoidan, alginic acid and the residue from the ethanol-insoluble fractions of ground and dried Macrocystis integrifolia blade discs (Dr. K. Rosell, personal communication).


Ethanol-treated Kelp Disc
Ground, Dried, Mixed with Water & Centrifuged
Supernatant
Pellet
Mixed with 95% EtOH
& Centrifuged
Mixed with
2% Na_2CO_3 (w/v)
& Centrifuged
Supernatant
Pellet
Discard
Fucoidan
(freeze dry)
Pellet
Residue
(washed in water &
freeze dried)
Supernatant
Mixed with HCl (pH 1)
& Centrifuged
Supernatant
Pellet
Discard
Alginic Acid
(dried)

C. RESULTS

Seasonal Photosynthetic Performance

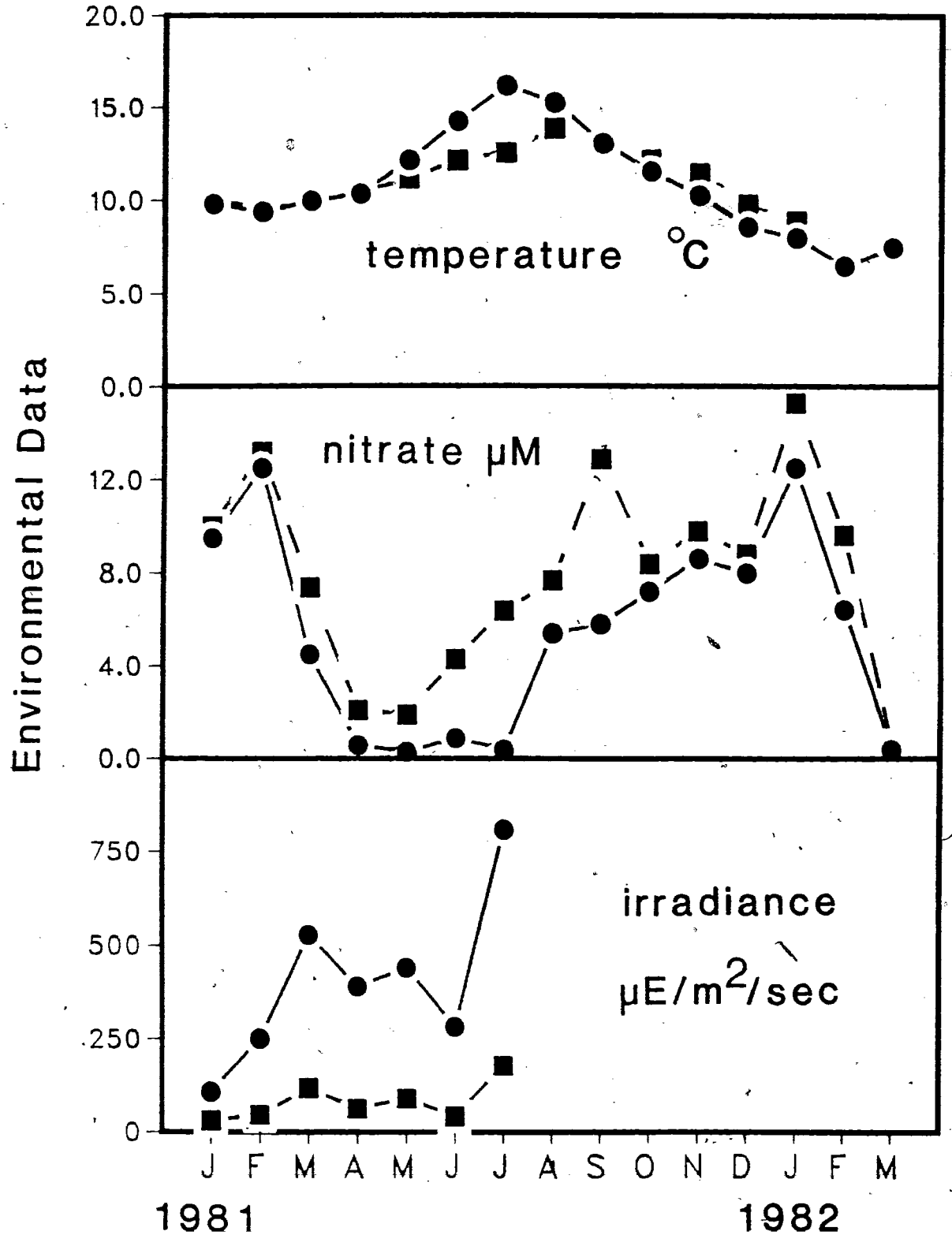
Ambient Temperature, Nitrate and Irradiance Levels

The environmental data are summarized in figure 2. Surface water temperatures were low in winter (6-10° C) and high in summer (15-16° C); at 4 m depth the seasonal range was less, appproximately 8-10° C in winter and 12-14° C in summer. Nitrate concentrations in surface waters were high in winter (Jan-Feb 1981), underwent a precipitate decline to reach near zero levels from March to July, then rose again to reach high levels in winter. Nitrate concentrations at 4 m depth followed a similar trend but were noticeably higher than nitrate levels in the surface water. Irradiance data were only available for January to July, 1981. These limited data, collected once a week in open water at midday, indicate that surface irradiances were consistently higher than the photosynthetically saturating irradiances of 50-70 $\mu E/m^2/sec$ for *M. integrifolia* blade discs (see Fig. 12). Also, the irradiances at 4 m depth were considerably lower than those at the surface and may be photosynthetically subsaturating for parts of the year.

Pigment Analysis

The pigment data are summarized in figures 3-5. Chl_a, fucoxanthin and chl_c

Figure 2. Temperature, nitrate levels and irradiance at surface water (circles) and 4 m depth (squares) in Bamfield. Each parameter was measured weekly and these values combined to give monthly means. The nitrate levels were determined by using an Autoanalyzer (Technicon, FL, USA), whereas the irradiances were taken with a quanta meter (Li-cor model 185, Lincoln, NB, USA).


Environmental Data
20.0
15.0
10.0
5.0
0.0
temperature °C
12.0
8.0
4.0
0.0
nitrate µM
750
500
250
0
irradiance
µE/m²/sec
J F M A M J J A S O N D J F M
1981
1982

concentrations were generally higher in mature than in young blade tissues; pigment levels in the old tissue fluctuated between those in the young and mature blade tissues. The chl_a and fucoxanthin levels of all three tissue types were high in the winter, declined in spring-summer, and then rose in fall to winter levels. Chl_c concentrations showed a similar seasonal pattern but its decline and subsequent rise lagged slightly behind those of chl_a and fucoxanthin. The molar ratios of fucoxanthin to chl_a and chl_c to chl_a were similar for the 3 age class blades (Figs. 6-7). For most of the year, the fucoxanthin to chl_a ratio stayed at about 0.8 but there was a rise in March and a still larger rise in August-September. The molar ratios of chl_c to chl_a fluctuated around 0.25 with pronounced peaks in March and May-June and a depression in September-October. The March peaks in chl_c to chl_a and fucoxanthin to chl_a ratios were more pronounced for young than for mature or old blade tissues.

Photosynthetic Performance

Figure 8 shows typical P vs I curves for M. integrifolia blade discs from the three age classes. The seasonal variations in P_{max} are shown in figure 9. Mature and young blade tissues generally showed much higher photosynthetic rates than the old blade tissues (Figs. 8,9). The P_{max} of young and mature blade tissues showed a marked seasonality with minima in early spring and early winter and maxima in late summer-early fall and late winter (Fig. 9). The seasonal variation in P_{max} of old blade tissues was not as large as in the young and mature blade tissues but it seemed to show the same seasonal trend.

The initial slopes, calculated from linear regression analysis of the carbon fixation rates of blade discs incubated under subsaturating

Figure 3. Seasonal changes in the chlorophyll$_a$ concentrations of young, mature and old Macrocystis integrifolia blade discs (when larger than the symbols, the 95% confidence levels are represented by vertical bars; n=10; 2 discs/blade X 5 blades).

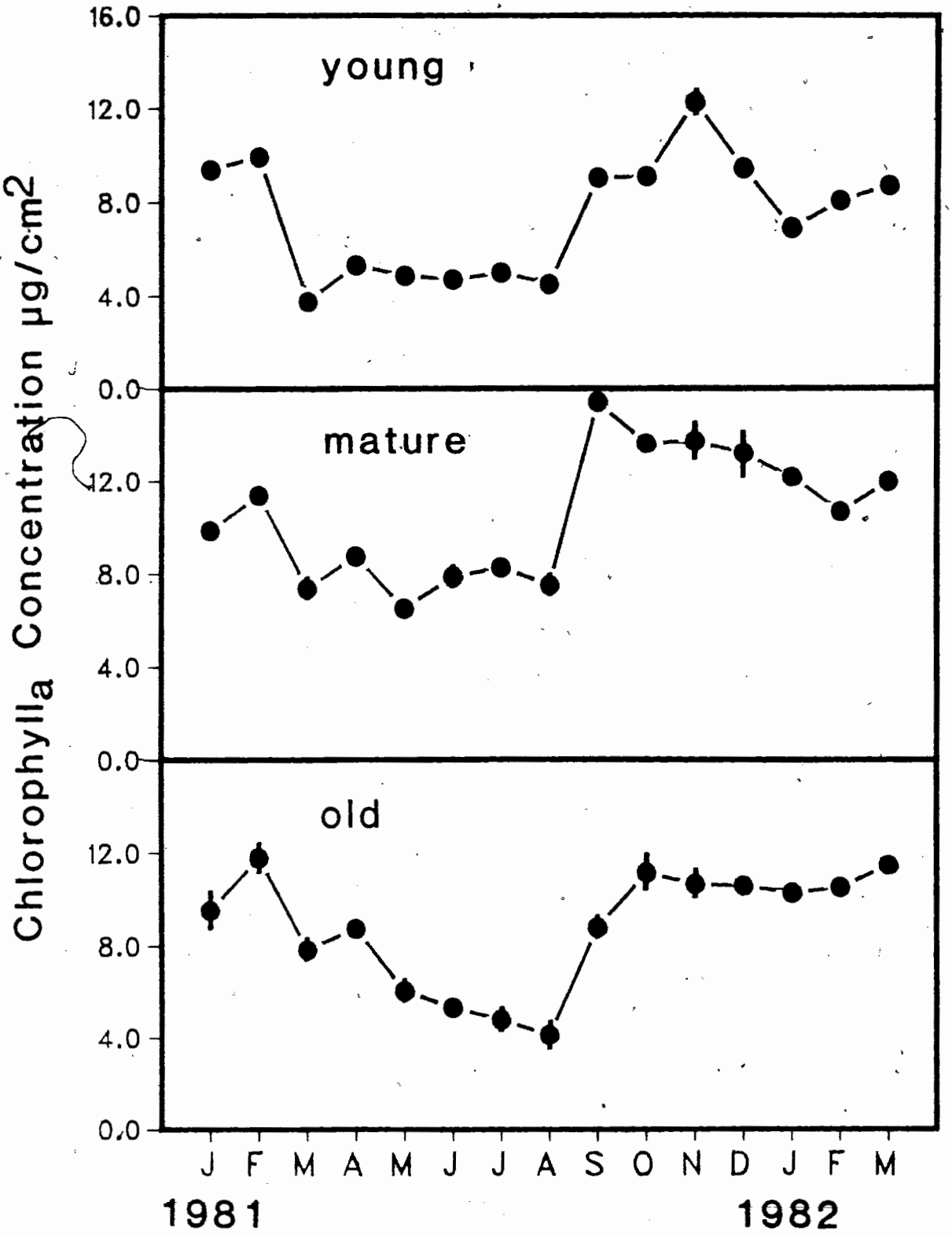

young
mature
old
Chlorophyll a Concentration µg/cm2
16.0
12.0
8.0
4.0
0.0
J F M A M J J A S O N D J F M
1981
1982

Figure 4. Seasonal changes in the chlorophyll$_c$ concentrations of young, mature and old Macrocystis integrifolia blade discs (when larger than the symbols, the 95% confidence levels are represented by vertical bars; n=10; 2 discs/blade X 5 blades).

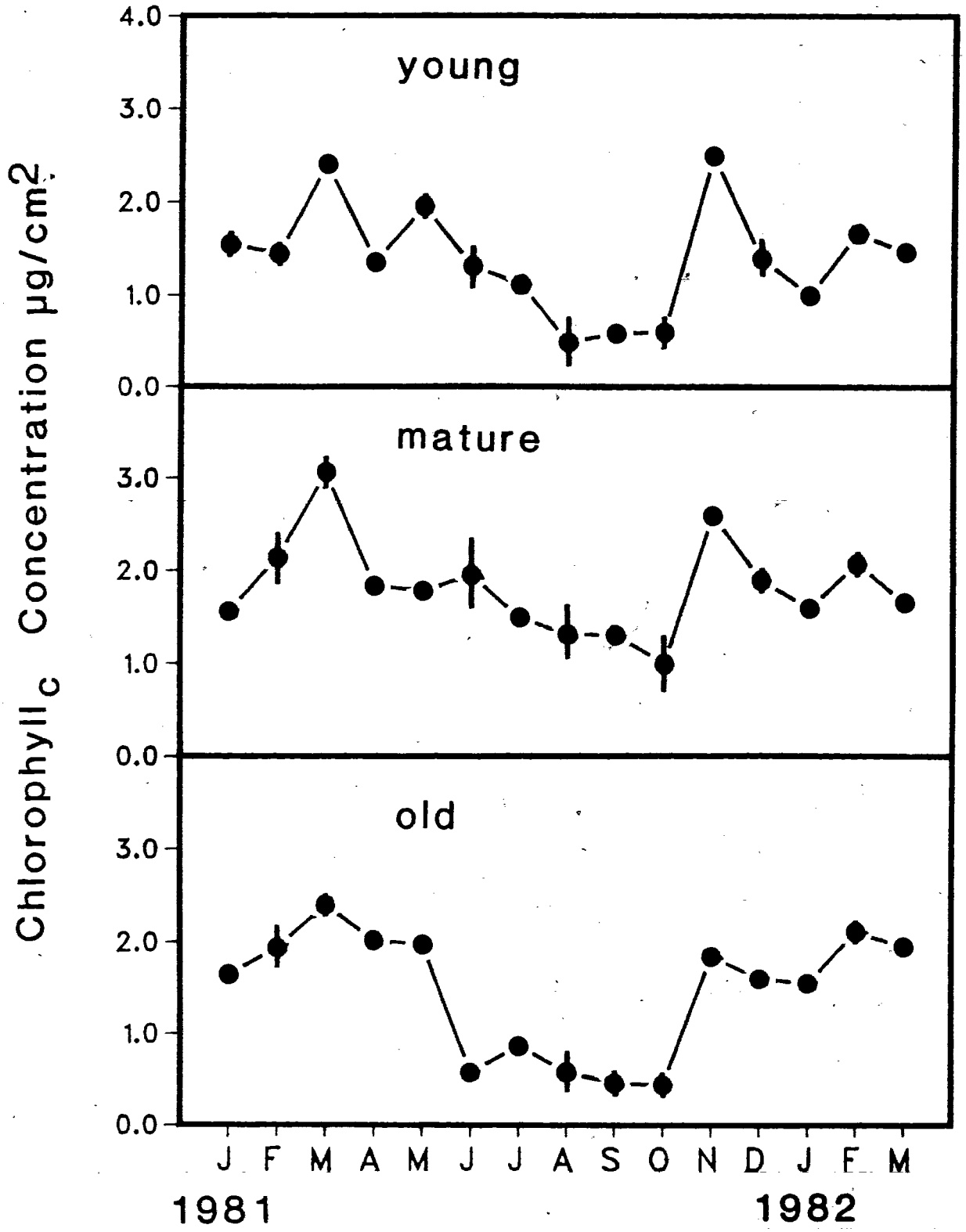

young
mature
old
Chlorophyll c Concentration µg/cm2
4.0
3.0
2.0
1.0
0.0
J F M A M J J A S O N D J F M
1981
1982

Figure 5. Seasonal changes in the fucoxanthin concentrations of young, mature and old Macrocystis integrifolia blade discs (when larger than the symbols, the 95% confidence levels are represented by vertical bars; n=10; 2 discs/blade X 5 blades).

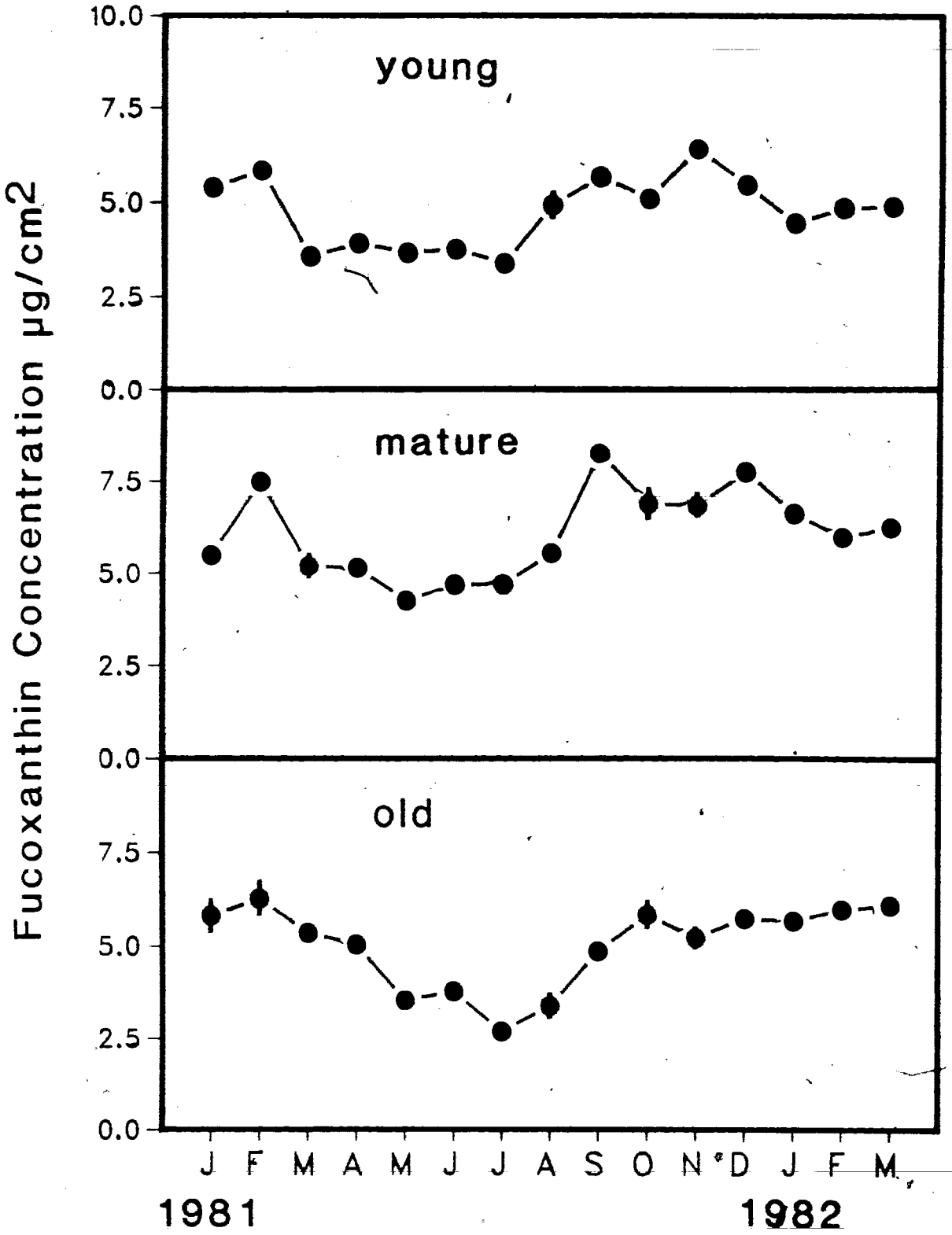

Fucoxanthin Concentration µg/cm2
young
mature
old
10.0
7.5
5.0
2.5
0.0
J F M A M J J A S O N D J F M
1981
1982

Figure 6. Seasonal changes in the chlorophyll$_c$ to chlorophyll$_a$ molar ratios for young, mature and old *Macrocystis integrifolia* blade discs. The data points represent the ratios of the mean pigment concentrations.

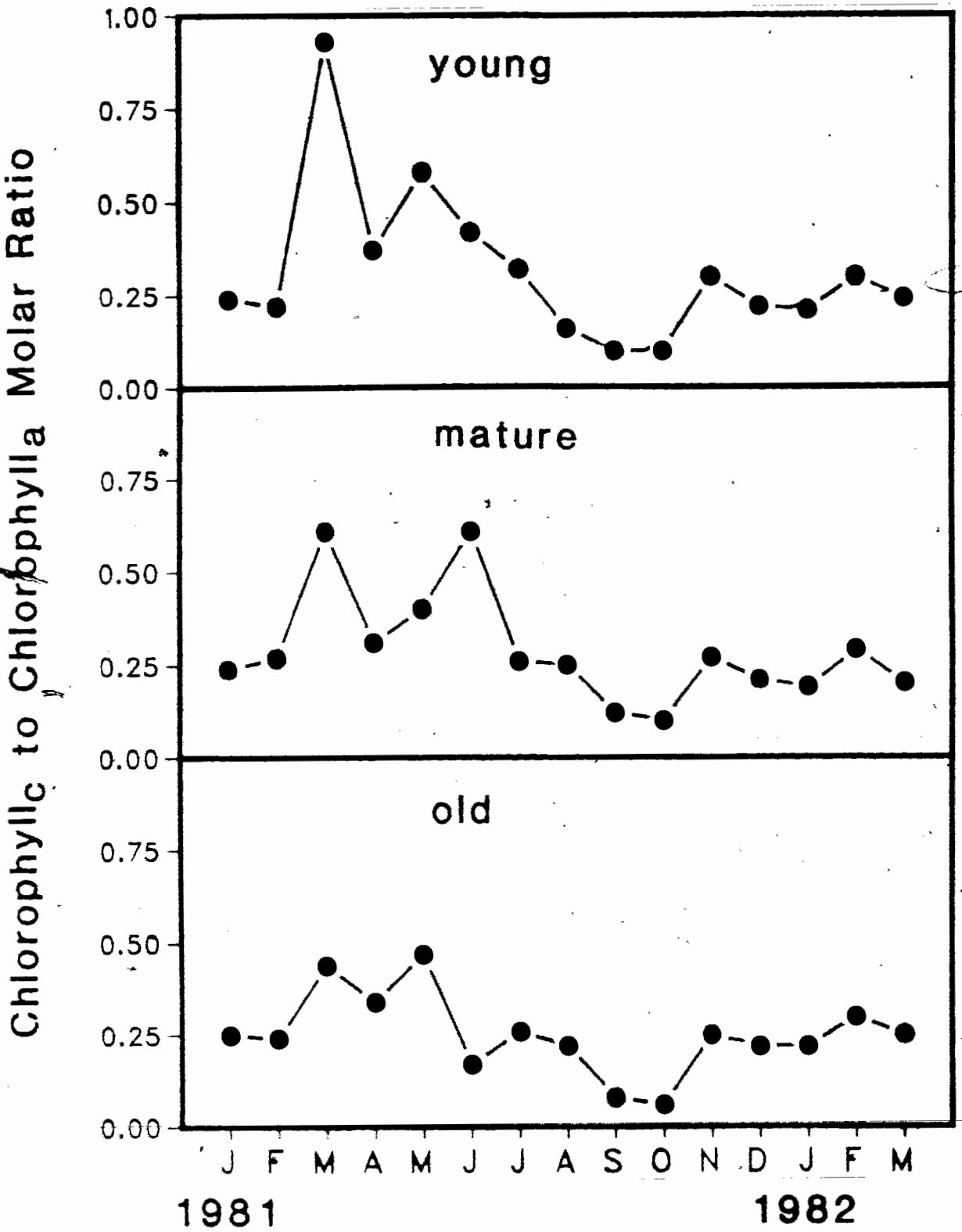

Chlorophyll$_c$ to Chlorophyll$_a$ Molar Ratio
1.00
0.75
0.50
0.25
0.00
young
mature
old
J F M A M J J A S O N D J F M
1981
1982

Figure 7. Seasonal changes in the fucoxanthin to chlorophyll$_a$ molar ratios for young, mature and old Macrocystis integrifolia blade discs. The data points represent the ratios of the mean pigment concentrations.

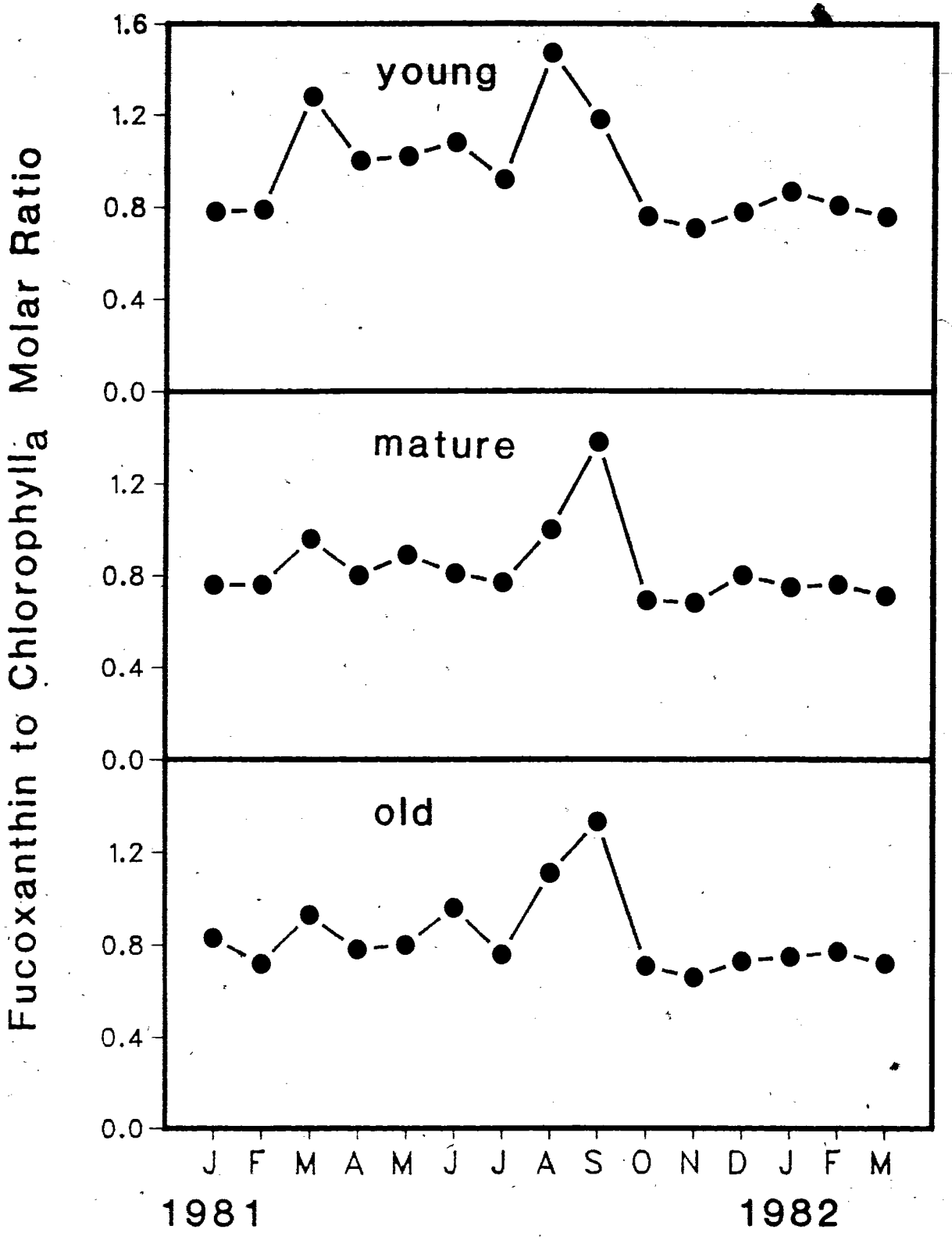

Fucoxanthin to Chlorophyll a Molar Ratio
young
mature
old
1.6
1.2
0.8
0.4
0.0
J F M A M J J A S O N D J F M
1981
1982

Figure 8. Photosynthetic rate versus irradiance curves of young, mature and old Macrocystis integrifolia blade discs for September, 1981. The data were derived from the ^{14}C-activity in the ethanol-soluble fractions of discs incubated in ^{14}C-carbonate for 10 min at the ambient seawater temperature (for more details see text; n=10; when larger than the symbols, the 95% confidence levels are represented by vertical bars).

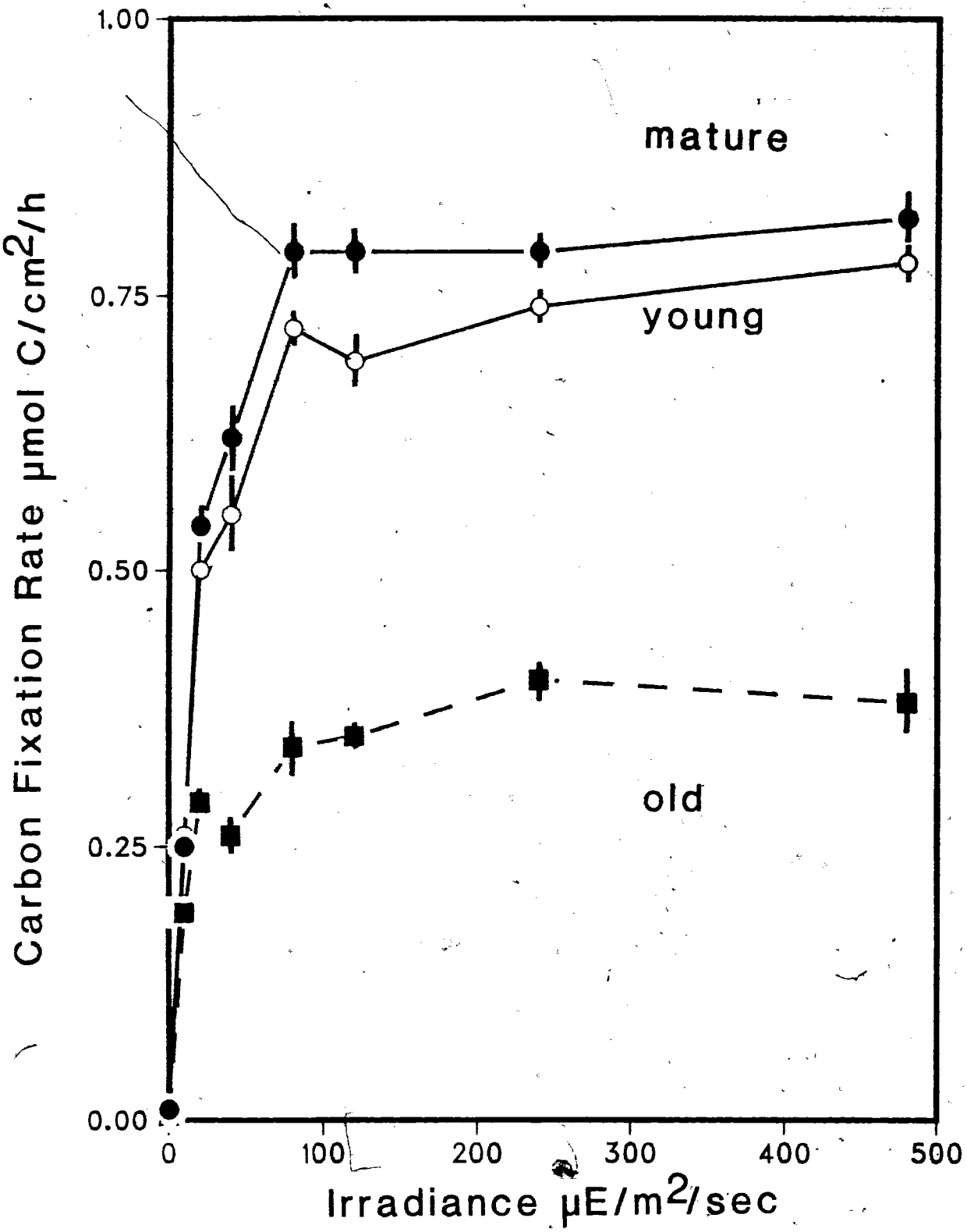

1.00
0.75
0.50
0.25
0.00
0
100
200
300
400
500
mature
young
old
Carbon Fixation Rate µmol C/cm2/h
Irradiance µE/m2/sec

Figure 9. Seasonal changes in the irradiance-saturated photosynthetic rate (P_{max}) of young, mature and old Macrocystis integrifolia blade discs. The data presented are calculated from the carbon fixation rates at 80-480 $\mu E/m^2/sec$ irradiances for each month of the study (when larger than the symbols, the 95% confidence levels are represented by vertical bars; n=40).

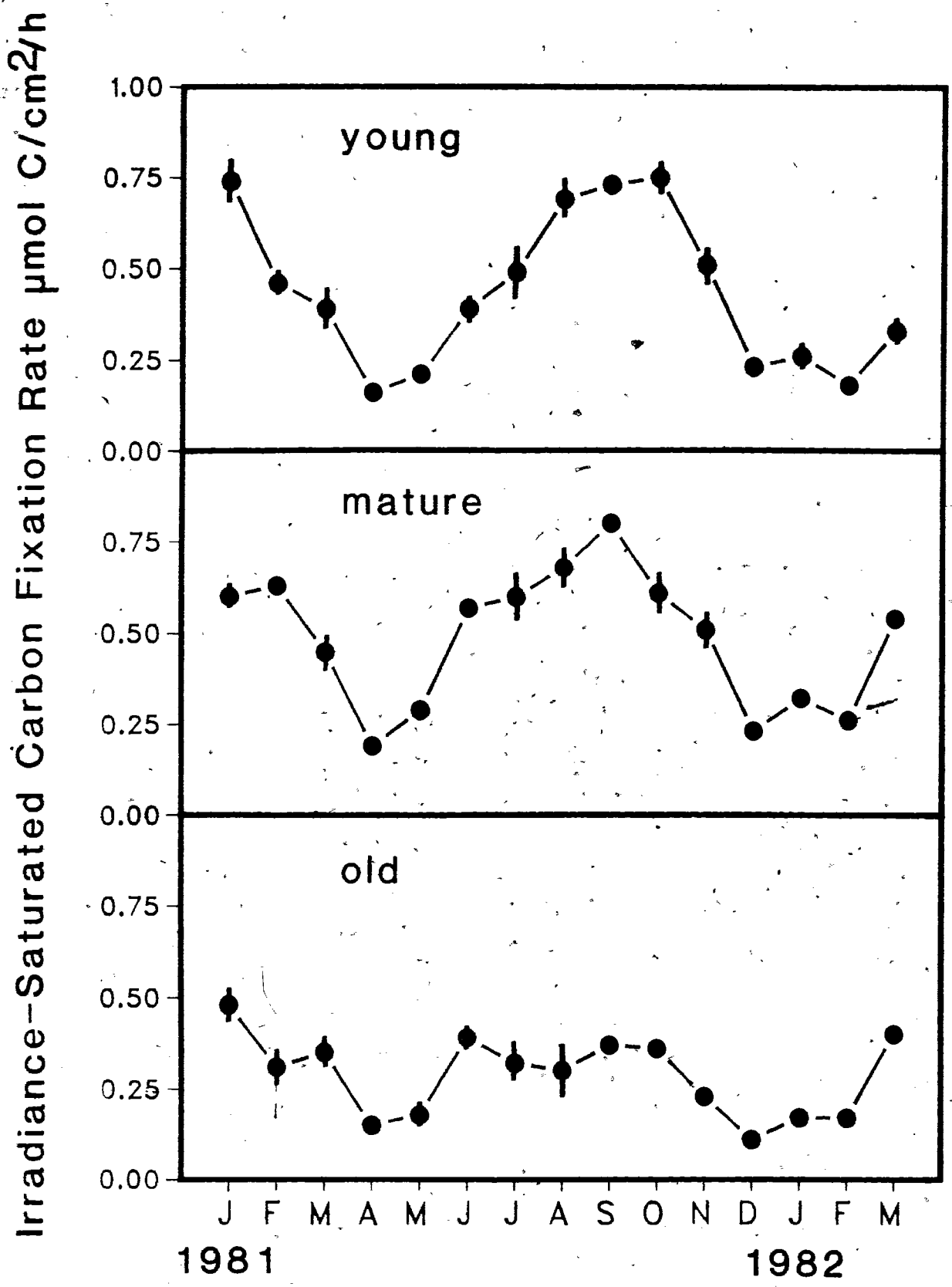

Irradiance-Saturated Carbon Fixation Rate µmol C/cm2/h
young
mature
old
1.00
0.75
0.50
0.25
0.00
J F M A M J J A S O N D J F M
1981
1982

Figure 10. Seasonal changes in the initial slope of the photosynthetic rate versus irradiance curve of young, mature and old Macrocystis integrifolia blade discs on an area (μmol C cm^{-2} $h^{-1}/\mu E$ m^{-2} sec^{-1}) basis. These data are derived from linear regression analysis of the 0-40 $\mu E/m^2/sec$ irradiance parts of the P vs I curves (n=40).

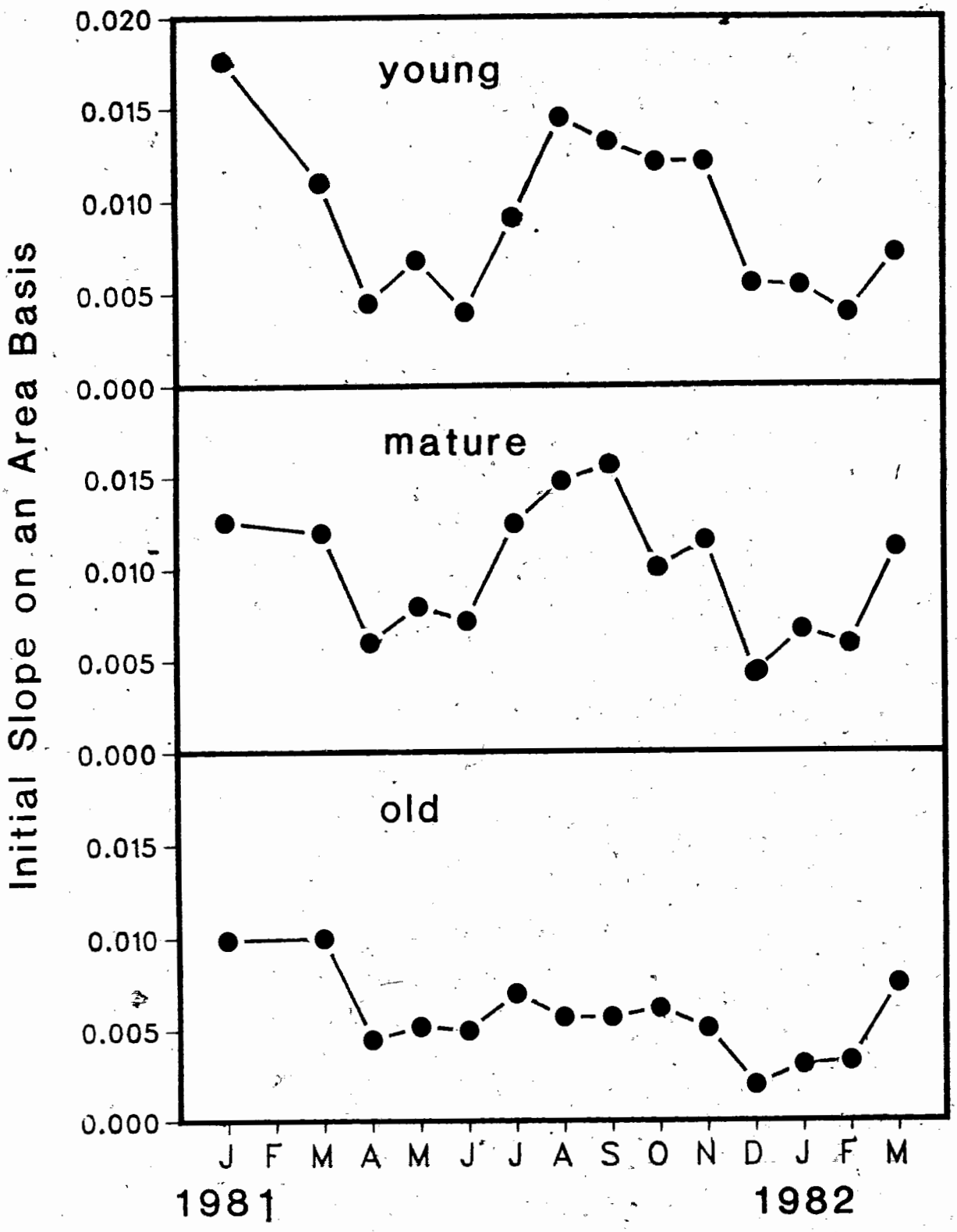

young
mature
old
Initial Slope on an Area Basis
0.020
0.015
0.010
0.005
0.000
J F M A M J J A S O N D J F M
1981
1982

Figure 11. Seasonal changes in the initial slope of the photosynthetic rate versus irradiance curve of young, mature and old Macrocystis integrifolia blade discs on a total pigment (μmol C μmol pigment^{-1} h^{-1}/μE m^{-2} sec^{-1}) basis. These data are derived from linear regression analysis of the 0-40 μE/m^2/sec irradiance parts of the P vs I curves (n=40).

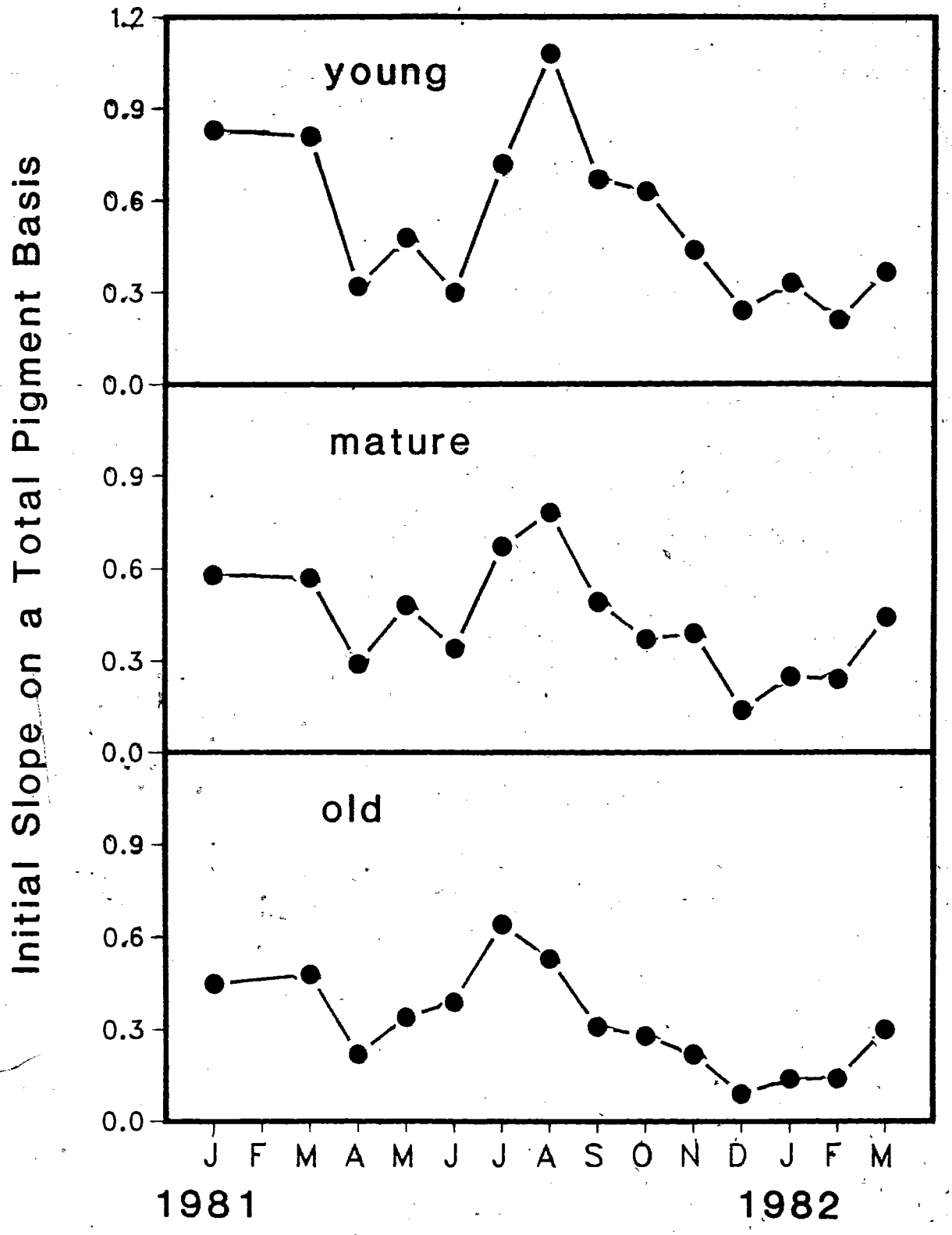
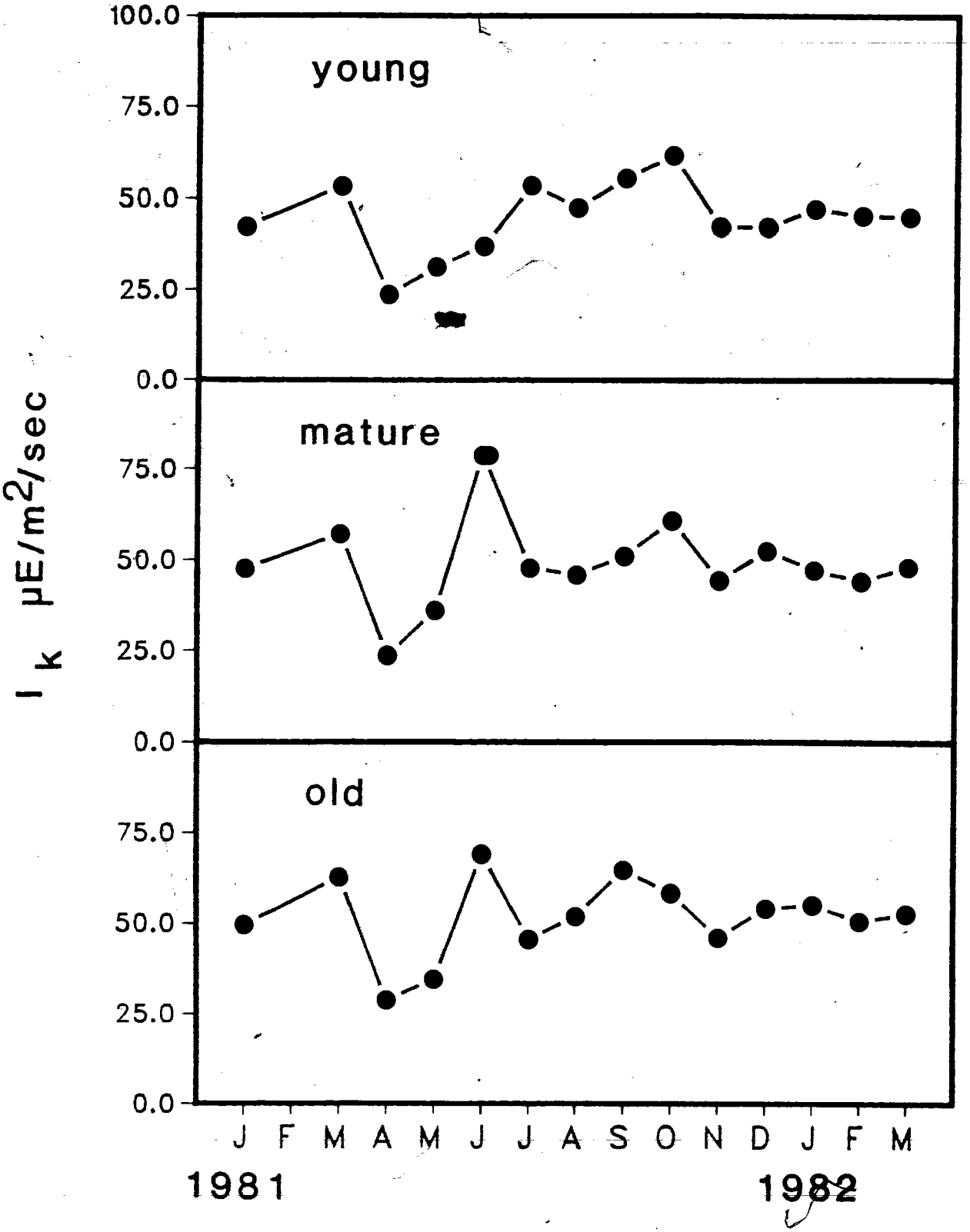

young
mature
old
Initial Slope on a Total Pigment Basis
1.2
0.9
0.6
0.3
0.0
J F M A M J J A S O N D J F M
1981
1982

Figure 12. Seasonal changes in the saturating irradiance (I_k) values, derived by taking the P_{max} to initial slope ratio, of young, mature and old Macrocystis integrifolia blade discs.


young
mature
old
100.0
75.0
50.0
25.0
0.0
I_k μE/m²/sec
J F M A M J J A S O N D J F M
1981
1982

irradiances, generally paralleled the P_{max} curves; here also the old blade tissues showed lower initial slopes and less seasonal variation than the young and mature blade tissues (Fig. 10). Initial slopes on a molar pigment basis (Fig. 11) showed similar seasonal trends except that young blade tissues had the highest initial slopes followed by mature and then old blade tissues.

The saturating irradiance of photosynthesis (I_k) was derived by taking the P_{max} to initial slope ratio. The I_k irradiance did not seem to vary between the 3 age classes (Fig. 12). Saturation occurred at about 50 $\mu E/m^2/sec$, except that in April-May significantly lower values, ca. 25 $\mu E/m^2/sec$, were recorded for all age classes and higher values, ca. 70 $\mu E/m^2/sec$, were recorded for mature and old blade tissues in June (Fig. 12).

Ethanol-insoluble Fraction

The ^{14}C-activity in the ethanol-insoluble fraction, as a percentage of the total ^{14}C fixed, is shown in Table I. Discs from older blades generally showed a higher proportion of ^{14}C-activity in the ethanol-insoluble fraction than did the discs from young and mature blades (see also Brinkhuis (1977b) for Fucus vesiculosus). There was also a higher ^{14}C-activity in the ethanol-insoluble fraction in winter than in spring-summer for all age class discs (see also Brinkhuis (1977a) for Ascophyllum nodosum). Despite a range of 6.6 to 22.0%, the ^{14}C activity in the ethanol-insoluble fraction did not alter the age-related or seasonal P_{max} trends as determined from the ethanol-soluble fraction.

Table I. Percentage of the total ^{14}C fixed in the ethanol-insoluble fraction of the three age class blade discs (values represent the mean of 3-10 measurements with the numbers in parentheses their 95% confidence levels).

	Ethanol-Insoluble Fraction % of Total		
Month	young	mature	old
May 1981	7.0 (1.0)	15.5 (3.1)	11.9 (3.4)
Jul 1981	7.3 (0.5)	8.4 (0.5)	13.9 (1.1)
Sep 1981	7.0 (0.6)	6.6 (0.5)	13.7 (0.7)
Oct 1981	10.6 (0.6)	12.3 (1.5)	17.1 (0.7)
Nov 1981	8.8 (2.2)	11.6 (1.9)	22.0 (0.9)
Jan 1982	10.8 (1.2)	12.1 (1.5)	19.5 (4.1)
Feb 1982	19.8 (2.5)	10.6 (1.8)	20.8 (5.3)
Mar 1982	12.6 (0.8)	10.1 (0.9)	9.2 (0.3)

Figure 13. The photosynthetic capacity of young (June) or mature (July) Macrocystis integrifolia blade discs conditioned in nitrate-poor (surface seawater, squares) or nitrate-rich (deep seawater, circles) flow-through seawater tanks. The data were derived from the ^{14}C-activities in the ethanol-soluble fractions of blade discs incubated for 10 min in ^{14}C-carbonate at an irradiance of 120 $\mu E/m^2/h$. The discs were punched 1 h before their incubations (when larger than the symbols, the 95% confidence levels are represented by vertical bars; n=10).

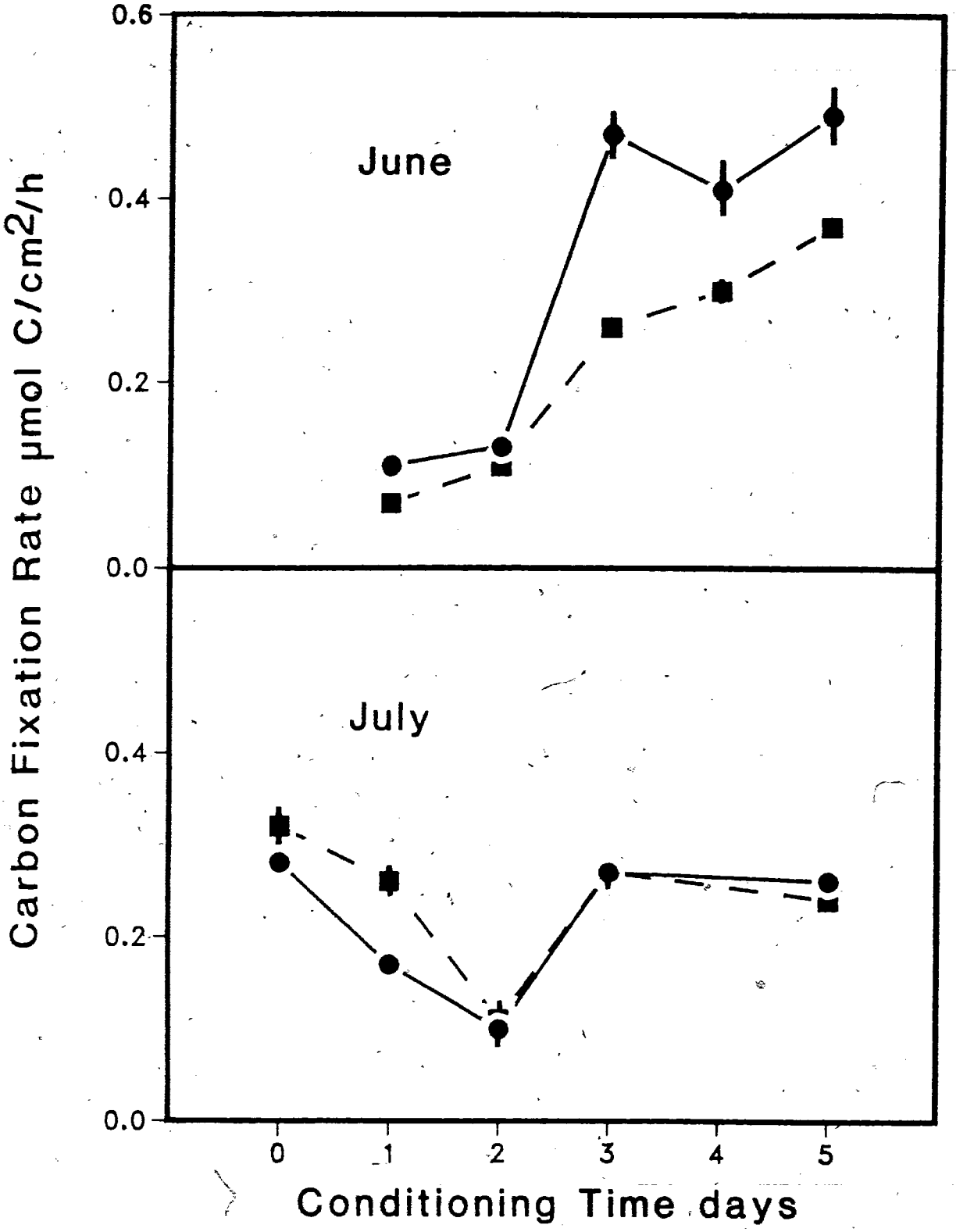

0.6
0.4
0.2
0.0
June
July
Carbon Fixation Rate µmol C/cm2/h
0
1
2
3
4
5
Conditioning Time days

Table II. Pigment concentration and ^{14}C-activity in the ethanol-insoluble fraction of Macrocystis integrifolia blade discs conditioned in nitrate-rich or nitrate-poor seawater for 5 days (n=5, the numbers in parentheses represent the values' 95% confidence levels).

conditioning treatment	day	pigment concentrations chlorophyll$_a$	chlorophyll$_c$	fucoxanthin	ethanol-insoluble fraction
		$\mu g/cm^2$			$DPM/cm^2/h$
June:					
nitrate-rich young blades	1	-[a]	-	-	2443 (2069)
	3	-	-	-	10412 (907)
	5	-	-	-	8280 (1306)
nitrate-poor young blades	1	-	-	-	2401 (327)
	3	-	-	-	5680 (1143)
	5	-	-	-	5529 (490)
July:					
nitrate-rich mature blades	0	7.99 (0.66)	1.98 (0.17)	5.14 (0.30)	6643 (780)
	1	7.28 (0.46)	1.98 (0.10)	4.95 (0.25)	-
	2	7.45 (0.38)	1.52 (0.20)	4.86 (0.15)	3433 (688)
	3	7.20 (1.03)	1.40 (0.67)	5.13 (0.35)	-
	5	7.76 (0.43)	1.44 (0.45)	5.10 (0.27)	3987 (449)
nitrate-poor mature blades	0	6.86 (1.42)	1.68 (0.35)	4.46 (0.16)	7517 (1157)
	1	6.72 (0.40)	1.73 (0.19)	4.59 (0.26)	-
	2	7.22 (0.21)	1.35 (0.27)	4.64 (0.16)	2251 (286)
	3	3.72 (0.26)	1.45 (0.25)	3.68 (0.13)	-
	5	5.62 (0.16)	0.63 (0.24)	4.02 (0.23)	5634 (531)

a - no data

Effect of Nitrate on P_{max}

Figure 13 shows the rates of ^{14}C incorporation in the ethanol-soluble fractions of fronds kept in nitrate-rich or nitrate-poor seawater. In June the P_{max} level of young blade discs punched from the frond kept in nitrate-rich seawater were increased relative to those punched from the frond kept in nitrate-poor seawater after 2 days. In July, however, no differences were detected between the P_{max} levels of mature blade discs punched from fronds conditioned in nitrate-rich or nitrate-poor seawater. The ethanol-insoluble data, of young blade discs (June), show the same trends while the nitrate treatment did not affect the ethanol-insoluble fixation rates and pigment concentrations of mature discs over the course of the July experiment (Table II).

Light Independent Carbon Fixation

^{14}Carbonate Uptake

Figure 14 shows that young blade discs incorporate far more ^{14}C into the ethanol-soluble fraction in the dark than do the mature blade discs. The opposite is true for discs incubated in the light.

Table III shows the distribution of ^{14}C-label in various fractions following incubation in the dark. The ^{14}C-activity was greater in young than in mature blade discs; in both cases the activity was greater after 12 h as compared to the 4 h incubation with proportionally more of the ^{14}C-activity being found in the ethanol-insoluble fraction. The data on various fractions are somewhat ambiguous, but show that most of the ^{14}C-activity in these discs was present in the ethanol-soluble fractions

Figure 14. ^{14}C fixed by young (circles) or mature (squares) Macrocystis integrifolia blade discs in light or dark in February. The data were derived from the ^{14}C-activities in the ethanol-soluble fraction of blade discs incubated in ^{14}C-carbonate for various periods of time. The data points represent single measurements.

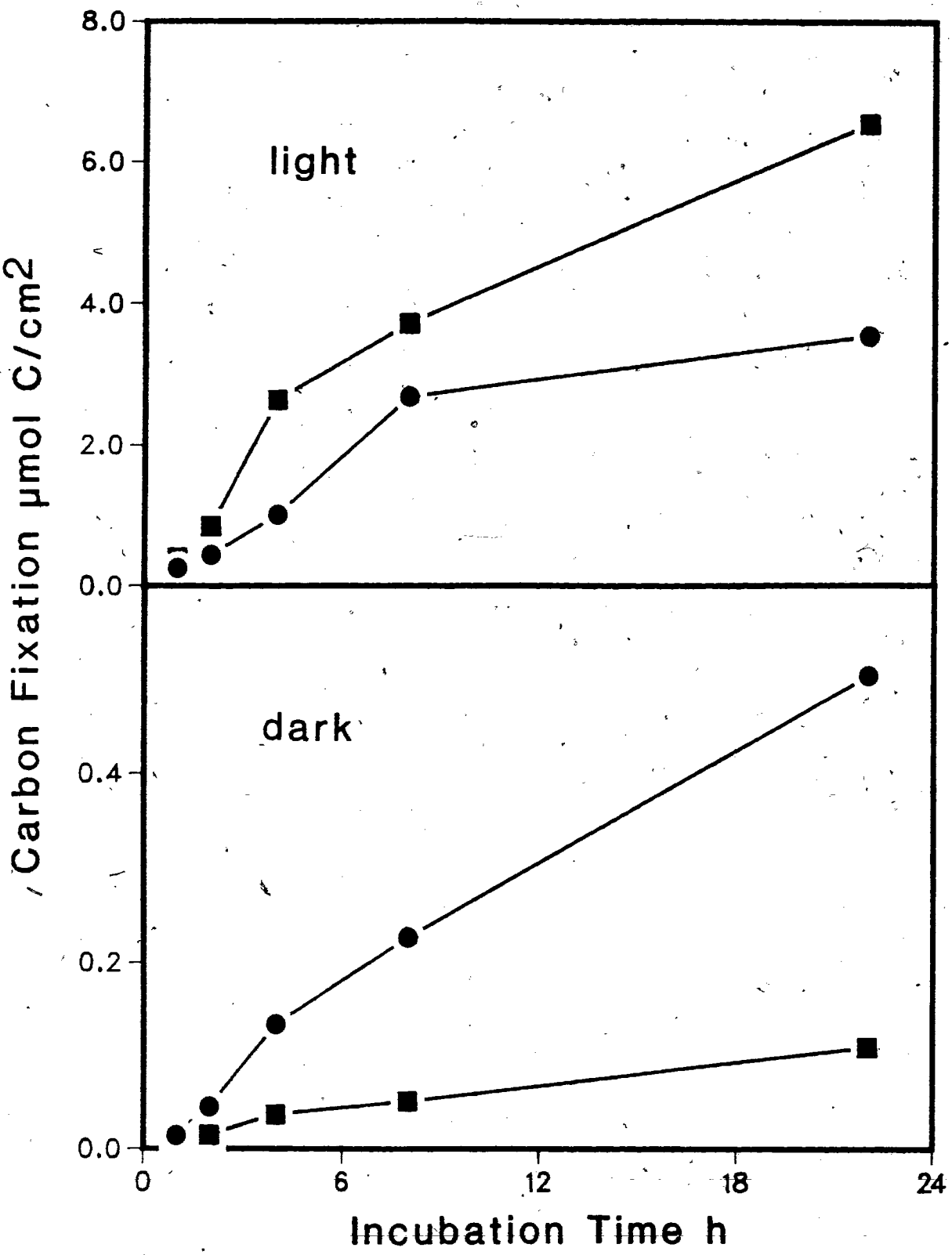

8.0
6.0
4.0
2.0
0.0
light
dark
0.4
0.2
0.0
0
6
12
18
24
Incubation Time h
Carbon Fixation µmol C/cm2

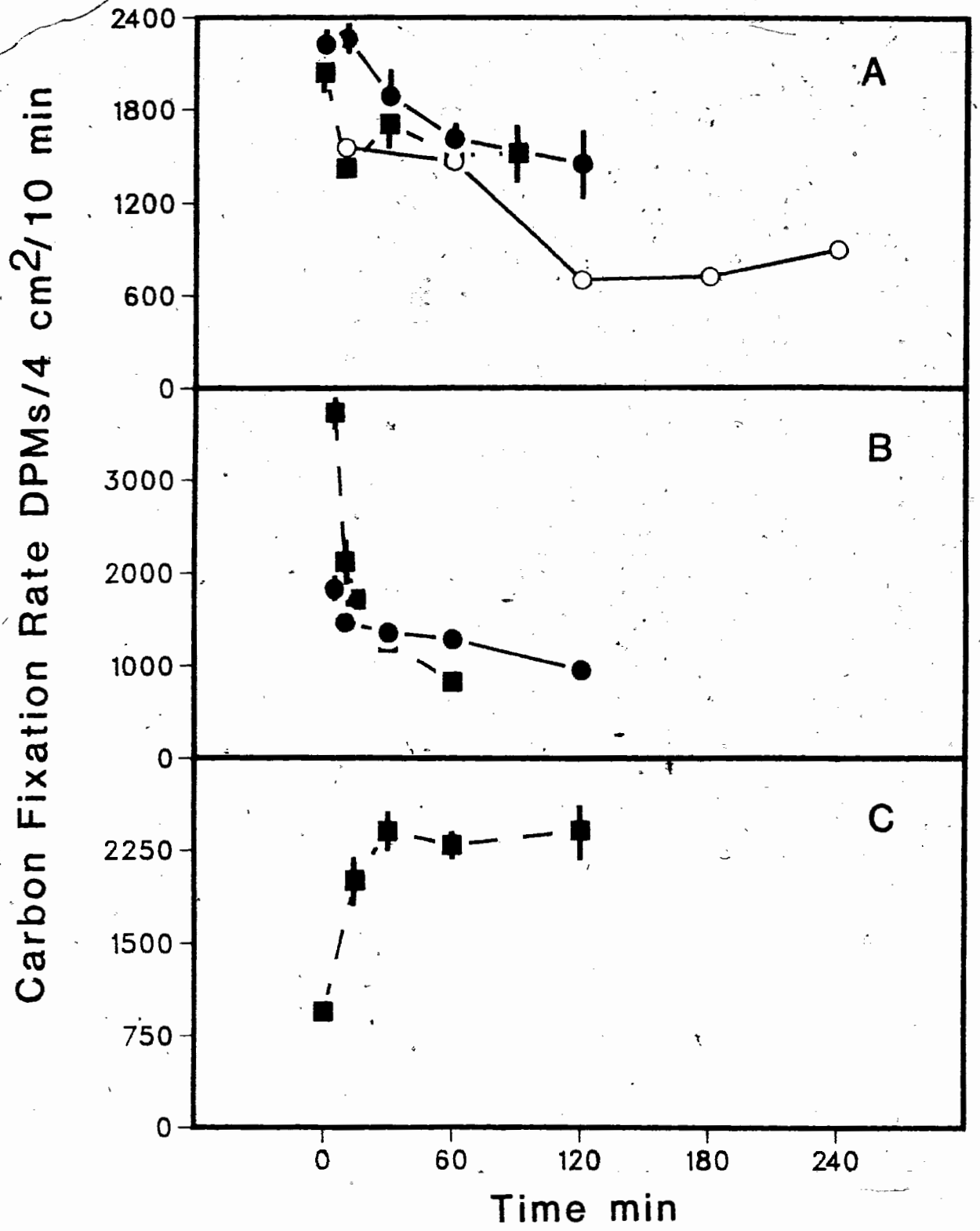
Table III. Proportions of ^{14}C-labelled components in different fractions from young and mature Macrocystis integrifolia blade discs (70.7 cm^2) incubated in ^{14}C-carbonate for 4 or 12 h in the dark.

	total ^{14}C activity	ethanol-insoluble	ethanol-soluble	amino acids	organic acids	sugars	phosphate esters
	DPM x 10^{-6}	% of total		% of ethanol-soluble			
young 4 h	4.53	21.0	79.0	36.1	49.1	9.8	5.0
mature 4 h	2.90	23.0	77.0	13.3	76.0	6.7	4.0
young 12 h	7.13	30.0	70.0	11.6	73.5	11.8	3.1
mature 12 h	4.99	41.0	59.0	7.0	64.0	25.3	3.7

Table IV. The ^{14}C-labelled and total amino acid concentrations of young and mature Macrocystis integrifolia blade discs (70.7 cm^2) incubated in ^{14}C-carbonate for 4 or 12 h in the dark.

	total concentration		alanine		aspartate		glutamate		remainder	
	hot	total	hot	total	hot	total	hot	total	hot	total
	DPM x 10^{-6}	mg	% of total concentration							
young 4 h	0.447	1.80	51.9	53.5	4.7	4.9	34.2	15.5	9.2	26.1
mature 4 h	3.338	1.04	20.8	35.1	43.8	13.2	26.8	28.5	8.6	23.3
young 12 h	2.773	0.62	78.3	66.8	11.2	7.7	9.7	11.5	0.8	14.1
mature 12 h	1.315	0.25	63.9	63.3	19.0	8.5	16.7	16.1	0.5	12.0

Figure 15. The light independent carbon fixation rates of young Macrocystis integrifolia blade discs A; preincubated in the dark for various times; B, incubated in the dark for various times and standardized to a 10 min incubation time; C, treated with light for various times before the dark incubation. Experiments were carried out in May (closed squares), July (closed circles) and August (open circles; n=6; when larger than the symbols, the 95% confidence levels are represented by vertical bars).


Carbon Fixation Rate DPMs/4 cm2/10 min
2400
1800
1200
600
0
A
3000
2000
1000
0
B
2250
1500
750
0
C
0
60
120
180
240
Time min

of which the organic acids were the major contributors. Substantial ^{14}C activity was also present in the amino acid fraction of young blade discs incubated for 4 h.

Table IV shows the further partitioning of ^{14}C label into amino acids. Curiously, the ^{14}C-activity present in the amino acid fraction was greater in mature than young blade discs after 4 h whereas the reverse was true after 12 h. Total amino acid concentrations, however, were higher in the young than in mature blade discs after 4 or 12 h. Alanine was the predominant amino acid in the young and mature blade discs both on the basis of ^{14}C incorporation and total concentration, but mature blade discs contained relatively greater proportions of ^{14}C-labelled glutamate and aspartate and total glutamate. When the incubation time was increased from 4 to 12 h, the ^{14}C-activity of young blade discs increased while the ^{14}C-activities of mature blade discs and the total amino acid concentrations of young and mature blade discs decreased. Also, with the increase in incubation time, alanine became proportionally more important whereas the proportions of the other amino acids generally decreased.

Effects of Incubation and Preincubation Conditions

The light independent carbon fixation rates of M. integrifolia blade discs were reduced when their dark preincubation times (Fig. 15A) and dark incubation times (Fig. 15B) were increased. Futhermore, it appears that the light independent carbon fixation rate reached a maximum level if the discs received approximately 30 min of 120 $\mu E/m^2/sec$ light prior to the 10 min dark incubation (Fig. 15C).

Mannitol Uptake

The mannitol levels of young and mature blade discs, as determined by gas chromatography, were depleted when the discs were kept in the dark for 48 h (Table V). When subsequently placed in mannitol-enriched media (3 gm/l) the mannitol levels of these discs were partially restored when incubated in the dark and exceeded the initial levels when incubated in the light.

The uptake of ^{14}C-mannitol by young and mature discs incubated in light and dark during 3 separate months, as determined by the ^{14}C-activity in the ethanol-soluble fractions, are shown in figure 16. In February and March, 1982, the uptake of ^{14}C-mannitol was higher in young than in mature discs. Further, the light treatment did not affect the ^{14}C-mannitol uptake of these discs with the exception of the young blade discs in March which had higher uptake rates in light than in dark. In May the ^{14}C-mannitol uptake by light-treated discs was considerably higher than that of dark-treated discs and mature discs showed higher uptake levels than young discs.

Table VI shows the distribution of ^{14}C-activity in discs incubated in ^{14}C-mannitol foe 4 or 12 h in the dark. At 4 h most of the ^{14}C label was present in the ethanol-soluble fraction; furthermore, in mature discs most of this activity was in the sugar fraction (including mannitol), although some activity was found in the amino acid and organic acid fractions as well. At 12 h, however, most of the ^{14}C-activity shifted into the ethanol-insoluble fraction, and the ^{14}C in the ethanol-soluble fraction consisted mostly of organic acids and amino acids. For 12 h incubations, there were no apparent differences between the ^{14}C-labelling patterns of young or mature blade discs.

Table V. Mannitol levels of young and mature Macrocystis integrifolia blade discs (14.1 cm^2) that were kept in the dark for 48 h and then placed in mannitol-enriched seawater (3 gm/l) for various periods of time in light or dark. The initial level represents mannitol concentrations at the time of collection, and 0 h represents mannitol concentrations after 48 h in the dark. Subsequent values represent mannitol concentrations after the blade discs had been put in mannitol-enriched seawater.

	mannitol level (µg)	
	young	mature
March - dark		
initial	1.59	1.19
0 h	0.26	0.72
1 h	0.04	0.10
2 h	0.66	0.25
24 h	0.62	0.73
May - dark		
initial	0.40	1.20
0 h	1.00	0.75
1 h	0.32	0.59
2 h	0.60	1.56
4 h	0.44	0.50
12 h	0.85	0.56
24 h	0.44	0.44
May - light		
initial	0.93	4.21
0 h	0.07	4.28
1 h	0.63	3.51
2 h	0.69	4.46
4 h	1.91	6.21
12 h	2.57	7.14
24 h	2.41	7.15

Figure 16. The ^{14}C-mannitol taken up by young (circles) or mature (squares) Macrocystis integrifolia blade discs in the light (open symbols) or dark (dark symbols). The data are derived from the ^{14}C-activity in the ethanol-soluble fraction of blade discs incubated in ^{14}C-mannitol for various periods of time. Note the change of scale between the February and March curves and those of May. The data points represent single measurements.

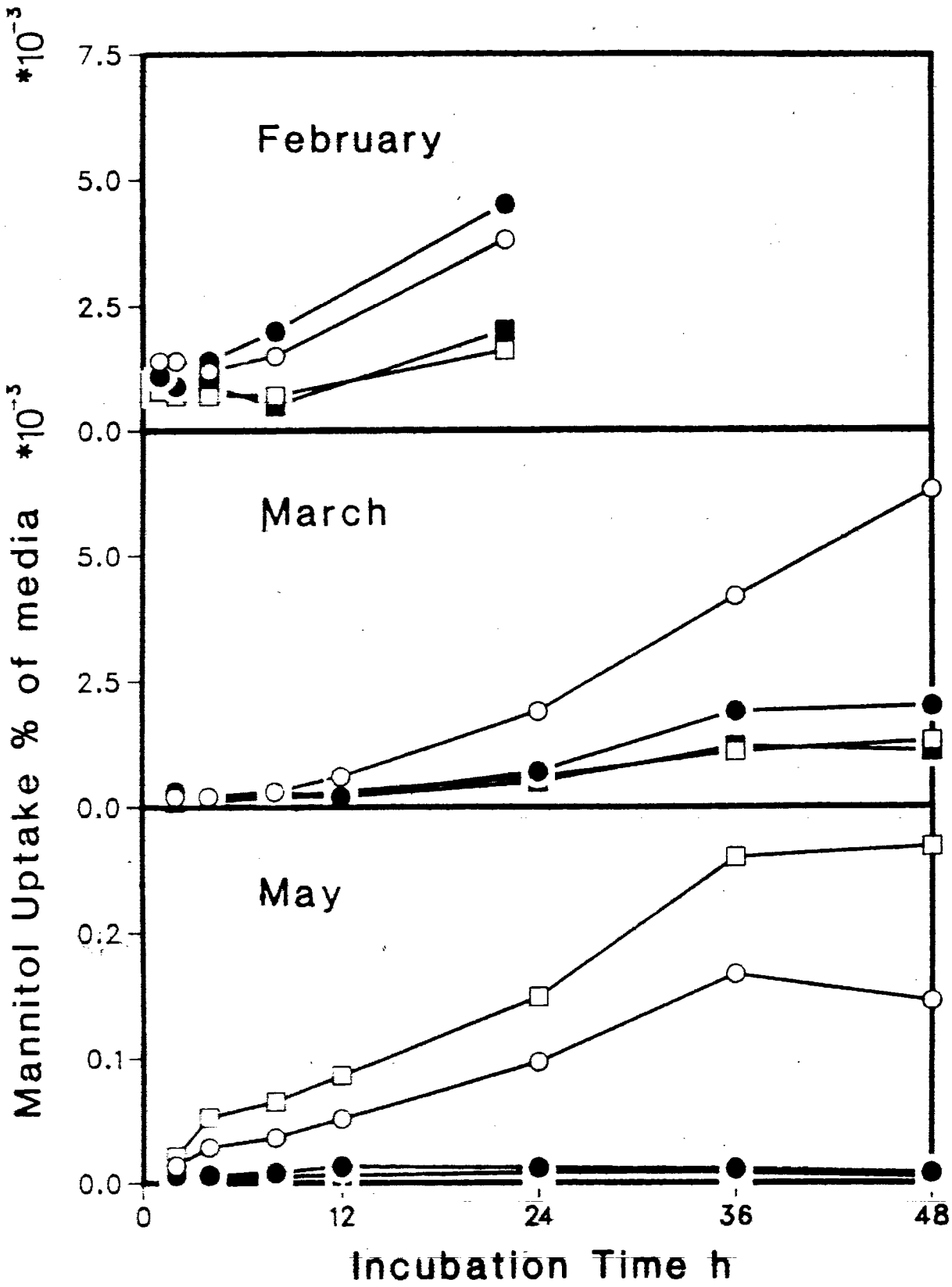

Mannitol Uptake % of media *10⁻³
7.5
5.0
2.5
0.0
February
March
May
0.2
0.1
0.0
0
12
24
36
48
Incubation Time h

Table VI. Proportions of ^{14}C-labelled components in different fractions from young and mature Macrocystis integrifolia blade discs (70.7 cm^2) incubated in ^{14}C-mannitol for 4 or 12 h in the dark.

	total ^{14}C activity	ethanol-insoluble	ethanol-soluble	amino acids	organic acids	sugars	phosphate esters
	DPM x 10^{-6}	% of total		% of ethanol-soluble			
young 4 h	0.77	16.0	84.0	-[a]	-	-	-
mature 4 h	0.37	43.0	57.0	9.1	11.3	76.4	3.2
young 12 h	4.88	76.0	24.0	26.1	56.8	10.6	6.5
mature 12 h	4.97	73.0	27.0	22.4	61.2	12.1	4.3

a-no data

Table VII. The ^{14}C-labelled and total amino acid concentrations of young and mature Macrocystis integrifolia blade discs (70.7 cm^2) incubated in ^{14}C-mannitol for 4 or 12 h in the dark.

	total concentration		alanine		aspartate		glutamate		remainder	
	hot	total	hot	total	hot	total	hot	total	hot	total
	DPM x 10^{-6}	mg	% of total concentration							
young 4 h	-[a]	1.88	-	49.0	-	8.5	-	16.2	-	26.2
mature 4 h	0.003	0.64	0.0	39.5	100.0	24.4	0.0	8.7	0.0	27.4
young 12 h	0.174	0.33	56.3	75.4	11.5	3.3	32.2	6.4	0.0	14.8
mature 12 h	0.060	0.59	0.0	62.6	100.0	4.6	0.0	13.5	0.0	17.3

a-no data

Table VII shows the further partitioning of ^{14}C label in amino acids. Even though the total amino acid concentrations decreased from 4 to 12 h, the ^{14}C-activity in the amino acid fraction of mature blade discs was higher after a 12 h, than after a 4 h, incubation (see also Table VI). Young blade discs contained ^{14}C-labelled alanine, aspartate and glutamate, while mature blade discs contained only ^{14}C-labelled aspartate. On a total concentration basis, alanine was the dominant amino acid. Furthermore, when the incubation time was increased from 4 to 12 h the proportion of alanine in the amino acid pool increased in both young and mature blade discs while that of the other amino acids, except for glutamate in mature blade discs, decreased.

Effects of Added Mannitol on ^{14}C Fixation

Figure 17 shows the effect of added mannitol on the carbon fixation capabilities of discs which were pretreated in dark for 48 h before being incubated in ^{14}C-carbonate. Young discs had higher carbon fixation capabilities than mature discs in the dark, while the reverse was true in the light. The addition of mannitol enhanced the carbon fixation capabilities of both young and mature discs in the dark (young discs showed the greater enhancement) and young discs incubated in the light for up to 12 h and seemed to lower the carbon fixation capability of mature discs incubated in light for 24 h.

The distribution of ^{14}C label in the ethanol-soluble and -insoluble fractions of blade discs kept in the dark for 48 h and then incubated in ^{14}C-carbonate with or without added mannitol for 12 h are shown in Table VIII. The trends for mature blade discs were similar to those reported earlier. In the light far more ^{14}C label went into sugars (including

Figure 17. The ^{14}C fixed by young (circles) or mature (squares) blade discs preconditioned in dark for 48 h and then incubated in ^{14}C-carbonate with (solid line) or without (dashed line) added mannitol. The data were derived from the ^{14}C-activities in the disc's ethanol-soluble fraction. The data points represent single measurements.

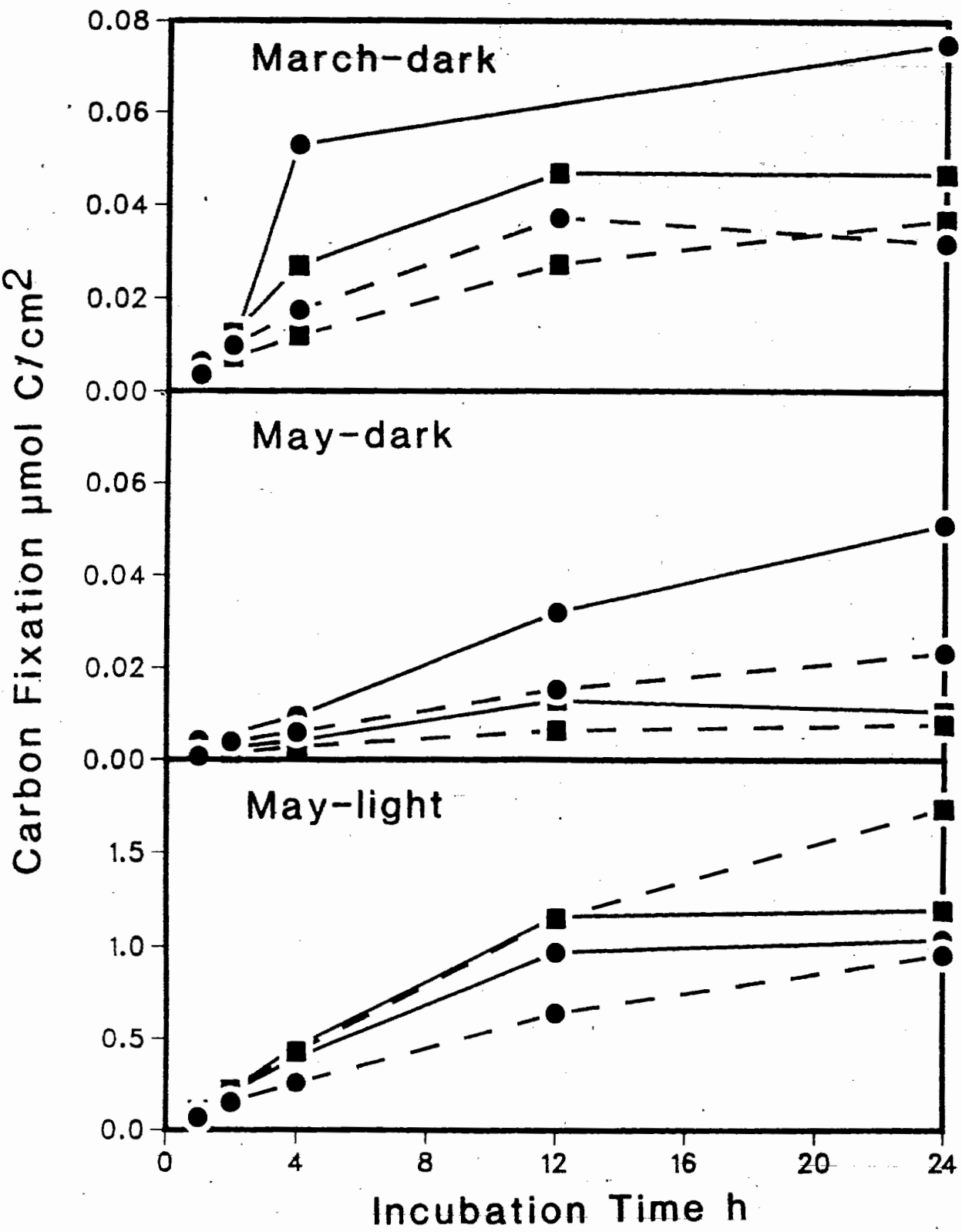

March-dark
May-dark
May-light
Carbon Fixation µmol C/cm2
Incubation Time h
0.08
0.06
0.04
0.02
0.00
0.06
0.04
0.02
0.00
1.5
1.0
0.5
0.0
0
4
8
12
16
20
24

Table VIII. Proportions of ^{14}C-labelled components in different fractions from young and mature Macrocystis integrifolia blade discs (14.1 cm^2). The discs were kept in the dark for 48 h before being incubated in ^{14}C-carbonate with or without added mannitol for 12 h in light or dark.

	total ^{14}C activity	ethanol-insoluble	ethanol-soluble	amino acids	organic acids	sugars	phosphate esters
	DPM x 10^{-6}	% of total		% of ethanol-soluble			
without mannitol:							
young - light	127.1	4.0	96.0	7.5	9.5	79.5	3.5
mature - light	281.8	23.0	77.0	6.6	8.9	82.8	1.7
young - dark	3.9	18.0	82.0	37.7	26.0	33.3	3.0
mature - dark	1.5	14.0	86.0	23.5	55.2	17.0	4.2
with mannitol:							
young - light	185.0	8.0	92.0	8.7	5.9	83.6	1.8
mature - light	197.1	7.0	93.0	7.5	3.8	87.7	1.0
young - dark	7.2	21.0	79.0	6.8	31.1	57.8	4.3
mature - dark	2.9	22.0	78.0	31.7	47.1	19.9	1.0

Table IX. The ^{14}C-labelled and total amino acid concentrations of young and mature Macrocystis integrifolia blade discs (14.1 cm^2) that were incubated in ^{14}C-carbonate with or without added mannitol for 12 h. The discs were kept in the dark for 48 h before the light (l) or dark (d) incubations.

	total concentration		alanine		aspartate		glutamate		remainder	
	hot	total	hot	total	hot	total	hot	total	hot	total
	DPM x 10^{-6}	mg	% of total concentration							
without mannitol:										
young l	6.81	0.31	24.8	23.0	31.4	21.6	5.4	6.6	38.5	48.8
mature l	7.64	0.28	32.5	26.4	41.5	24.8	6.0	6.1	20.1	42.5
young d	0.61	0.56	39.2	64.6	11.9	2.3	42.5	8.4	6.4	24.6
mature d	0.51	0.28	25.1	59.1	36.9	13.8	6.4	11.6	31.5	15.2
with mannitol:										
young l	10.93	0.30	30.6	28.1	17.7	12.4	11.3	15.1	40.4	50.8
mature l	11.97	0.32	44.5	40.1	42.1	20.7	8.8	13.2	4.0	21.3
young d	0.30	0.43	40.8	50.5	18.7	1.9	34.9	13.1	3.1	25.3
mature d	0.96	0.39	26.2	60.2	45.7	14.9	21.9	9.0	5.4	15.0

mannitol) than in amino acids and organic acids, while the reverse was true in the dark. Young discs also incorporated far more ^{14}C label in sugars than in amino acids and organic acids in the light, whereas in the dark reletively more label was present in the amino acids and organic acids. The addition of mannitol did not seem to change the distributional patterns of ^{14}C-labelling in discs kept in light or dark.

Table IX shows that the total ^{14}C-activity in the amino acid fraction of these blade discs was generally greater in: mature rather than young blade discs; discs incubated in the light rather than those incubated in the dark; and, discs incubated with mannitol rather than those incubated without mannitol. In most cases, the total amino acid concentrations did not seem to be affected by disc age, light or mannitol treatment. The relative proportions of the ^{14}C-labelled and total amino acids did not seem to be affected by the addition of mannitol whereas the effects of light treatment and disc age are unclear. It seems that alanine, aspartate and glutamate concentrations, on a total concentration basis, were higher in the dark than in the light. The ^{14}C-activities of alanine were also greater in dark than in light.

^{14}C-mannitol Chase Period

To determine the fate of ^{14}C-labelled compounds, following incubation in ^{14}C-mannitol, blade discs were placed in a 'cold' mannitol solution for varying periods of time. The distribution of ^{14}C label in young blade discs is shown in Table X. As the chase period became longer, the total ^{14}C-fixed in the samples generally decreased, the relative proportion of ^{14}C-activity present in the ethanol-insoluble and amino acid fractions increased, and that in the sugar and organic acid fractions decreased.

Table X. Proportions of ^{14}C-labelled components in different fractions from young and mature Macrocystis integrifolia blade discs (70.7 cm^2) incubated in ^{14}C-mannitol for 4 h in the dark and then incubated in mannitol-enriched seawater (3 gm/250 ml) in the dark for various periods of time.

	total ^{14}C activity	ethanol-insoluble	ethanol-soluble	amino acids	organic acids	sugars	phosphate esters
	DPM x 10^{-6}	% of total		% of ethanol-soluble			
0 min	0.23	46.0	54.0	25.8	52.4	21.0	0.8
15 min	0.12	46.0	54.0	36.2	38.4	25.4	0.0
30 min	0.16	68.0	32.0	51.4	39.0	9.6	0.0
60 min	0.11	60.0	40.0	61.4	19.8	16.8	2.0
120 min	0.07	53.0	47.0	-[a]	-	-	-

a-no data

Table XI. The ^{14}C-labelled and total amino acid concentrations of young Macrocystis integrifolia blade discs (70.7 cm^2) incubated in ^{14}C-mannitol for 4 h in the dark and then incubated in mannitol-enriched seawater (3 gm/250 ml) in the dark for various periods of time.

	total concentration		alanine		aspartate		glutamate		remainder	
	hot	total	hot	total	hot	total	hot	total	hot	total
	DPM x 10^{-6}	mg	% of total concentration							
0 min	0.012	1.12	27.5	24.1	12.1	3.4	60.4	13.2	0.0	59.2
15 min	0.015	0.65	33.8	48.3	13.9	6.2	53.2	23.4	0.0	22.1
30 min	0.014	0.75	33.5	51.0	11.2	5.0	55.3	24.4	0.0	18.4
60 min	0.013	0.95	24.3	64.1	29.4	3.6	46.2	15.9	0.0	16.4
120 min	0.005	0.89	34.2	60.1	12.4	2.1	53.4	25.1	0.0	13.7

Table XI shows that the total ^{14}C-activities and amino acid concentrations, of the blade discs treated as above, generally decreased as the chase period became longer. In these blade discs, the ^{14}C-activity was highest in the glutamate fraction followed by the alanine and then aspartate fractions with no other ^{14}C-labelled amino acids being present (Table XI). The total amino acids, however, were dominated by alanine. Increasing the chase period had no effect on the ^{14}C-labelled amino acid distributions whereas the total proportions of alanine increased and those of the remaining amino acids decreased.

Respiration of ^{14}C-mannitol Taken Up in Dark

Table XII shows that a large proportion (56-75%) of mannitol taken up in the dark is respired and that only 25-44% is retained in the ethanol-soluble and -insoluble fractions. Furthermore, the ^{14}C-activity present in the respired fraction was higher in young than in mature blade discs while that in the ethanol-insoluble fraction was higher in mature than in young blade discs.

Ethanol-insoluble Fraction

Further analysis of the ethanol-insoluble fractions of discs incubated in ^{14}C-carbonate or ^{14}C-mannitol with or without a chase period showed that these discs contained ^{14}C-labelled fucoidan, alginic acid, and a residue fraction. Unfortunately these results were not quantitative. The yield on a weight basis varied from 83 to 117%, while only 28 to 90% of the ^{14}C-acvtivity was recovered. This indicates that some of the

^{14}C may have been exchanged with ^{12}C, possibly due to the extraction procedures. The proportions of fucoidan, alginic acid and residue fractions on a weight basis were similar in young and mature blade discs, in discs incubated in ^{14}C-carbonate or ^{14}C-mannitol, and in discs incubated for 4 or 12 h in the dark. By weight, the percentage in the residue fraction ranged from 47.8 to 66.0 %, that in the alginic acid fraction from 24.6 to 35.5 % and that in fucoidan from 0.0 to 15.6%. Table XIII shows the ethanol-insoluble components of discs from the ^{14}C-mannitol chase experiment. The data show that the freeze-dried weights of blade discs were somewhat lower after longer chase periods. Furthermore, the relative proportion of fucoidan, alginic acid and residue did not change in any significant manner following chase periods of 0-120 min.

Table XII. Proportions of ^{14}C-labelled products of young and mature Macrocystis integrifolia blade discs (70.7 cm^2) incubated in ^{14}C-mannitol for 4 h in the dark. The values are from two replicates.

	total ^{14}C activity	ethanol-insoluble	ethanol-soluble	respired
	DPM x 10^{-6}	% of total		
young	0.45	14.9	10.1	75.0
young	0.51	18.8	11.0	70.2
mature	0.47	24.6	12.1	63.3
mature	0.55	34.3	9.3	56.4

Table XIII. Proportions of the total ethanol-insoluble components of young Macrocystis integrifolia blade discs (70.7 cm^2) incubated in ^{14}C-mannitol for 4 h in the dark and then incubated in mannitol-enriched seawater (3%m/250 ml) in the dark for various periods of time.

	total dry weight	fucoidan	alginic acid	residue	% yield
	mg		% of total		%
0 min	91.3	15.2	30.6	54.2	100.0
15 min	78.9	16.0	28.4	47.8	92.2
30 min	89.3	43.7	7.7	66.0	117.4
60 min	87.6	15.3	24.8	57.0	97.1
120 min	76.5	15.5	32.9	53.9	102.3

D. DISCUSSION

Seasonal Photosynthetic Performance

The rates of light saturated photosynthesis in this study varied between 0.1 and 0.8 μmol C/cm^2/h (Fig. 9). These rates are similar to those reported for M. integrifolia (0.6-1.0 μmol C/cm^2/h, Willenbrink et al., (1979)) and M. pyrifera (0.3-1.5 μmol C/cm^2/h, Wheeler, (1980); 0.5 μmol C/cm^2/h, Littler and Murray, (1974))[1]. They are also comparable to rates for several species of Laminaria (Hatcher et al., 1977; Johnston et al., 1977; Lüning, 1971), Nereocystis (Willenbrink et al., 1979) and other brown algae (Brinkhuis, 1977; King and Schramm, 1976; Littler and Murray, 1973).

This study shows that for most months young and mature blade discs had similar P_{max} values, and these values were generally much higher than those for old tissues (Fig. 9). Since both young and mature blades of M. integrifolia studied here were collected from the water surface under similar light, temperature, and nitrate conditions, any differences in P_{max} between these two blade types are probably age related. Age-related differences in P_{max} have been reported previously for blades along a frond of M. pyrifera (Wheeler, 1980) and different parts of Laminaria and Fucus thalli (Brinkhuis, 1977b; Johnston et al., 1977; Küppers and Kremer, 1978). Old blades of M. integrifolia collected at 3 to 4 m depth in this study

---

[1] For these calculations, conversion ratios of 1.2 moles O_2/mole CO_2 for the photosynthetic quotient (Strickland and Parsons, 1972), 1 g dry wt/10 g wet wt (personal observation), and 0.02 g wet wt/cm^2 (personal observation) were used.

were subjected to lower and possibly subsaturating irradiances throughout the study and lower temperatures and higher nitrate concentrations during the spring-summer period than the young and mature blades at the surface. The fact that these blades showed much lower P_{max} values than the mature and young blades, under identical experimental conditions (Fig. 9), suggests that long-lasting changes occur in the photosynthetic machinery of these blades. These changes may be due to reduced activities of the carbon fixation enzymes as a result of lower irradiances and temperatures, or greater pressures at 4 m, or tissue age. Wheeler and Srivastava (1983) have shown that old blades have relatively low nitrate uptake rates and hence the higher nitrate concentrations at 4 m depth may not really be an advantage.

Seasonal variations in P_{max} (Fig. 9) seem to be dependent on several parameters. For young and mature blade discs, the drop in P_{max} from January to April, 1981, correlates well ($r=.83$, $n=8$) with the precipitate decline in nitrate concentration in the seawater (Fig. 2). The greater carbon fixation rates of young blade discs punched from fronds conditioned in nitrate-rich as compared to nitrate-poor seawater in June (Fig. 13; see also Wheeler and Srivastava (1983) for similar data) supports this hypothesis. Also, this is in agreement with the data reported by Chapman *et al.* (1978) which show a positive correlation between the P_{max} of *Laminaria* sporophytes and the nitrate concentrations at which they were grown. Wheeler and Weidner (1983) show a similar positive correlation between the photosynthetic enzyme activities of *Laminaria* sporophytes and nitrate concentrations. The rise in P_{max} for young blade discs during spring 1981 and the second P_{max} depression in late fall-early winter (Fig. 4) both correlate with temperature ($r=.92$, $n=8$; $r=.95$, $n=12$, respectively) (see also Hatcher *et al.*, (1977) and Luning (1971) for *Laminaria* and Kanwisher

(1966) and Yokohama (1973) for other brown algae). Furthermore, the data reported by Smith et al. (1983) on the seasonal P_{max} of M. integrifolia discs at a constant temperature (15° C) suggest that there are seasonal changes in activities of the photosynthetic enzymes. These changes which may include absolute amounts and/or rates of enzymes are likely to be influenced both by availability of nitrate (Wheeler and Weidner, 1983; or other N sources, see Wheeler and Srivastava, 1983) and seawater temperature (Patterson, 1980).

The numerical values for the initial slopes in this study (Fig. 10) ranged from 0.004 to 0.018 μmol C cm^{-2} h^{-1}/μE m^{-2} sec^{-1}, which are comparable to values of 0.005 and 0.012-0.019 μmol C cm^{-2} h^{-1}/μE m^{-2} sec^{-1} computed from published data for M. integrifolia (Willenbrink et al., 1979) and M. pyrifera, (Wheeler, 1980), respectively. It is also of interest that these values compare favourably with the values of 0.015 μmol C cm^{-2} h^{-1}/μE m^{-2} sec^{-1} for shade-adapted higher plants (Böhning and Burnside, 1956).

The data on initial slopes, on an area and pigment basis, and molar ratios of pigments show several trends (Figs. 10 and 11). The initial slopes, on an area basis, of young and mature blade discs were similar, whereas the initial slopes on a pigment basis of young blade discs were generally higher than those of mature blade discs. This suggests that the mature blade discs contained pigments that did not further enhance their initial slopes. That chl_a can be accumulated in surplus is indicated by data of Chapman et al. (1978, see Figs. 4,5) who showed that, when Laminaria sporophytes were grown in media containing increasing nitrate concentrations, photosynthesis saturated while the chl_a concentrations continued to rise. The late summer rise in the initial slope of young and mature blade discs coincided with the late summer peak in the molar ratios

of fucoxanthin to chl_a, whereas the relatively high chl_c to chl_a ratios in March coincided with the high initial slopes at that time. These data suggest that changes in the size of PSU and/or the efficiency of electron transport in the PSU may occur in young and mature blade discs (Prezelin, 1981), but more precise information on pigment protein complexes is needed. It should be noted that in the present study the chl_a levels in all three blade types generally parallel the nitrate levels in the seawater (cf. Figs. 2 and 3). This seems consistent with the idea that the synthesis of chlorophyll molecules, which contain nitrogen, would be depressed when the nitrogen supplies are low.

Calculated I_k values for M. integrifolia in this study ranged from 25 to 70 $\mu E/m^2/sec$ (Fig. 12). These values are comparable to those for Macrocystis spp. (Wheeler, 1980; Willenbrink et al., 1979), other Laminariales (Johnston et al., 1977; Lüning, 1971; Willenbrink et al., 1979) and for various green algae (Arnold and Murray, 1980). They are lower than those for higher plants, which have I_k values that range from 100 $\mu E/m^2/sec$ in shade-adapted plants to over 1600 $\mu E/m^2/sec$ in sun-adapted plants (Larcher, 1975).

The present study identifies several parameters which influence the photosynthetic performance of M. integrifolia plants. Additional studies, on the photosynthetic enzymes and pigment-pigment, pigment-protein and electron transfer interactions in the PSU, should give us a better understanding of how these parameters influence the photosynthetic performance of kelps.

Light Independent Carbon Fixation

The present study confirmed the well known observation that mature blade discs fix more carbon than young blade discs in the light while the reverse is true in the dark (Fig. 14; see also Kremer, 1979; Willenbrink et al., 1979). Moreover, the dark CO_2 fixation rates of young blade discs ranged from 6-14 % of their CO_2 fixation rates in the light. These data are comparable to those reported previously by several authors for a variety of brown algae (Akagawa et al., 1972b; Kremer and Küppers, 1975; Kremer, 1979, 1981; Kremer and Küppers, 1977; Willenbrink et al., 1979). Amino acids and organic acids are the initial products of β-carboxylation of PEP in kelps in the dark (Akagawa et al., 1972a; Craigie, 1963; Kremer, 1979). In this study, most of the ^{14}C-labelled metabolites following ^{14}C-carbonate incubations in the dark were contained in the above fractions; however, considerable ^{14}C-activities were also found in the ethanol-insoluble and sugar fractions. (It is possible that some of the ^{14}C-activity in the sugar fraction was due to contamination by the amino acids.) The ^{14}C-activities in the ethanol-insoluble and sugar fractions increased after a 12 h incubation period (Table III). This indicates that amino acids and organic acids, the initial products of light independent carbon fixation, are actively metabolized in the dark.

Mannitol is taken up by M. integrifolia blade discs (Fig. 16) and apparently the rates of uptake vary with tissue age, season and light or dark. In February and March 1982, young blade discs had higher ^{14}C-mannitol uptake rates than mature blade discs. In May, light-treated blade discs had higher ^{14}C-mannitol uptake rates than dark-treated blade discs (Fig. 16). The effect of light on the uptake of mannitol by blade discs in May was confirmed by the 'cold' mannitol uptake experiments (Table

V). These data are also in agreement with those of Weidner and Kuppers (1982) which show that light enhances the ^{14}C-mannitol uptake of Laminaria hyperborea discs. The ^{14}C-mannitol uptake rates of the light-treated discs in May were comparable to those reported by Bidwell and Ghosh (1962a) for dark-treated slices of Fucus, 0.33% and 0.50% as compared to 0.53% of the supplied mannitol/g/h, respectively (assuming that the uptake by M. integrifolia blade discs is linear for 2 h and that a disc with a surface area of 1 cm^2 has a wet wt of 0.02 g, personal observation).

Interpretation of these data are difficult. The mannitol uptake rates as measured by ^{14}C-mannitol or 'cold' mannitol are underestimates since once mannitol is taken up it may be respired or converted to other products. The differences in the mannitol uptake rates between February and March and May seen here and those between M. integrifolia and Fucus (Bidwell and Ghosh, 1962a) may be due to physiological differences between these tissues. In May, mannitol concentrations, growth, translocation and net photosynthesis in M. integrifolia tissues are typically at their highest levels (Dr. K. Rosell, personal communication; Lobban, 1978a, 1978b; Tuominen, 1981; respectively). Since mannitol is a major metabolite of kelp (Kremer, 1981) its turnover, and possibly its uptake, should be highest during the fast growth periods of kelp. In February and March, by contrast, uptake rates may be sluggish because of the generally poor growth rates at this time.

Once taken up by discs, mannitol seems to be used in a variety of metabolic pathways in M. integrifolia. The addition of mannitol enhanced the light independent carbon fixation rates of young and mature blade discs which had been kept in dark for 48 h prior to their dark incubation (Fig. 17). Addition of mannitol also enhanced the ^{14}C fixation rates of young blade discs in light which had been predarkened for 48 h (Fig. 17). These

data are in agreement with those reported by Kremer (1981) that indicated that the light dependent and light independent carbon fixation pathways can work simultaneously and that mannitol acts as a substrate for the light-independent carbon fixation process.

The potential for light independent carbon fixation of a disc seems to be partially dependent on its substrate availability. The light independent carbon fixation rates were increased when added mannitol was supplied to discs (Fig. 17) and when the discs were preirradiated before the dark incubation (Fig. 15C); the rate decreased when the period of dark preincubation or incubation times were extented (Figs. 15A, B and Table X). The declines of the total ethanol-insoluble components (Table XIII), total amino acid concentrations (Tables IV, VI and XI) and ^{14}C-activity in the ^{14}C-mannitol chase experiments (Table X) indicate that once the mannitol reserves of a disc are low, other components may act as substrates for the light independent carbon fixation and/or respiratory pathways.

Further, Table XII shows that after 4 h the ^{14}C-labelled products of mannitol metabolism are present in the respired, ethanol-soluble and -insoluble fractions. In this study an average of 66% of the ^{14}C activity entering the blade discs was respired after 4 h and, as expected, the younger blade discs had higher respiratory rates than did the mature blade discs (see also Kremer, 1981). The above value is considerably higher than the respiration rates of 15.4 and 12.6% reported for ^{14}C-mannitol- and ^{14}C-glucose-treated Fucus tissues, respectively (Bidwell and Ghosh, 1962a, b). These descrepancies may be due to basic differences between the two plants, their physiologies and/or the experimental designs.

The ethanol-soluble fraction of discs incubated in ^{14}C-mannitol contained ^{14}C-labelled sugars (part of which was probably the ^{14}C-mannitol from the incubation media), amino acids, organic acids and

phosphate esters. The amino acids and organic acids most likely result from the conversion of mannitol to mannitol-1-P (utilizing the enzyme mannitol-1-phosphate dehydrogenase) and further to glycolytic pathway intermediates and PEP (Kremer, 1981) which can be channeled into either: the TCA cycle, yielding ^{14}C-labelled CO_2, amino acids and organic acids; or the light independant carbon fixation process, yielding amino acids and organic acids. The respiration data from Table XII and the enhanced ^{14}C-carbonate fixation rates by discs incubated in media containing added mannitol (Fig. 17) suggest that both of these pathways are operating in M. integrifolia blade discs. The ^{14}C-labelled phosphate esters could possibly be derived directly from mannitol (eg. mannitol-1-P) or produced from glycolytic pathway intermediates. Because phosphate esters are important in glycolysis as well as the synthesis of polysaccharides their relatively low ^{14}C-activities (Tables III, VI, VIII and X) suggest that they have high turnover rates.

The amino acid data reported in this study (Tables IV, VII, IX and XI) were derived from single samples. The variations between the treatments and the blade discs themselves makes the analysis of the data difficult. However, several trends are evident. Alanine had the highest total concentration and was also one of the major ^{14}C-labelled amino acids. These data are in agreement with those reported by Wheeler and Srivastava (1983) and Akagawa et al. (1972a, b), Craigie (1963) and Kremer (1979); respectively. It should also be noted that the proportions of total and ^{14}C-labelled alanine were more predominant in blade discs that had been incubated in the dark for extended periods of time (with the exception of the ^{14}C-labelled alanine of Table XI). The total amino acid concentrations of these discs decreased over time, therefore, it seems possible that alanine was being stored while glutamate, aspartate and the remaining amino

acids were acting as substrates for various metabolic pathways. The high proportions of ^{14}C-labelled alanine from blade discs incubated for extended periods of time was possibly a result of the above and the relatively complicated biosynthetic pathway of alanine in discs incubated with ^{14}C-carbonate as compared to the other amino acids. (The β-carboxylation of PEP bypasses pyruvate, the precursor to alanine, therefore the ^{14}C-labelled alanine must be derived from the ^{14}C-labelled organic acids of the TCA cycle (Saltman et al., 1957)).

The ethanol-insoluble fractions of discs incubated in ^{14}C-carbonate or -mannitol were analysed and found to contain (although not quantitatively) ^{14}C-labelled alginic acid, fucoidan and residue (Table XIII). Neither alginic acid nor fucoidan are synthesized directly from mannitol (Bidwell and Ghosh, 1962a; Percival, 1979), therefore the introduced ^{14}C-carbonate or -mannitol was probably converted to mannose-1-P, a precursor of alginic acid synthesis (Hassid, 1970) or other compounds which are precursors of fucoidan biosynthesis. The ^{14}C-labelled components of the residue fraction possibly include other polysaccharides, besides fucoidan and alginic acid, and protein.

In the present study the light independent carbon fixation data were obtained from blade discs which were incubated for long periods of time. Therefore, care must be taken when these results are used to explain the light independent carbon fixation process in naturally occurring M. integrifolia plants. Tissues from intact plants translocate while blade discs possibly do not. The metabolic regulation in mature blade discs may be affected by the build up of some of their products that are normally translocated. Young blade discs not receiving any translocate may be forced to use alternate metabolic pathways to fulfill their requirements. Also, Hatcher (1977) found that the respiration rates of L. longicruris tissue

slices were greater than those of intact plants. It is not known to what extent these phenomena have affected the results of the present study.

At this point the usefulness of light independent carbon fixation in kelp is still a matter of conjecture. There are two major hypotheses: one states that light independent carbon fixation is a means by which young kelp sporophytes, growing in low light environments, can fix carbon thus having an ecological advantage over other aquatic plants (Willenbrink et al., 1979). The second hypothesis states that C fixation in the dark is a way in which the young fast growing kelp tissues produce their required metabolic intermediates (Kremer, 1981). Neither of these hypotheses seems completely correct. Young tissues generally contain less mannitol and have a greater demand for mannitol than older tissues (Kremer, 1981). Thus, in the case of young sporophytes (where there is no influx of translocated materials), light independent carbon fixation would possibly depend on other substrates besides mannitol (Johnston et al., 1977). This seems to be contradictory since in a fast growing young sporophyte the demand would be for increasing the cellular components not metabolizing them away. Energetically, the light independent carbon fixation process is a relatively poor means of producing metabolic intermediates. For example, one molecule of PEP being converted to aspartate (a common end product of the light independent carbon fixation process) yields 16 ATP equivilents when metabolized through the TCA cycle but only 1 ATP equivilent when metabolized through the light independent carbon fixation pathway. Perhaps it is the replenishment of the TCA cycle intermediates, which would allow for greater ATP or reducing power production, that makes light independent carbon fixation important to young kelp tissues (see also Weidner and Kuppers, 1982). The carboxylation of pyruvate (utilizing the enzyme pyruvate carboxylase) yielding oxaloacetate has also been reported to carry

out this function (Stryer, 1981).

Appendix I

The following text gives a brief description of three experiments which produced data that were not useful to the present study.

Oxygen Electrode P vs I Curves

Initially, the P vs I experiments were to be carried out using a closed system oxygen evolution technique (see Jassby, 1978). The appartatus consisted of: a sealable plexiglas chamber (100 X 30 X 10 cm) connected to a submersible pump and an oxygen electrode and meter (YSI model 5739 and 57, respectively); a cooling tank using flow through seawater; and a light source, either sunlight with or without neutral density screens or several fluorescent light bulbs (Sylvannia Cool White) mounted on a moveable light bank. The temperatures used were those of the ambient surface seawater. The water pump created a current which reduced the boundary layers over the kelp blade and oxygen electrode surfaces as well as mixed the water in the incubation chamber.

Young or mature M. integrifolia blades, from the surface or 7-10 m depth, were attached to an elevated plexiglass support and placed in the chamber. Each blade was progressively illuminated with a decreasing series of irradiances ranging from 1000 to 0 $\mu E/m^2/sec$. The blades were preincubated and incubated for 15 min at each of the irradiances used. The net photosynthetic rate of a kelp blade incubated under a particular set of conditions was determined by subtracting the dissolved oxygen change in the control chamber (a similar chamber that did not contain a kelp blade) from the dissolved oxygen change in the experimental chamber. These rates were standardized to the surface area and dry weight of the kelp blade.

The results (not shown) indicate that the kelp blades were not irradiance-saturated at up to 1000 $\mu E/m^2/sec$ and that the photosynthetic rates at these irradiances roughly corresponded to 1 $\mu mol\ O_2/cm^2/h$. This photosynthetic rate is comparable to the others derived in this study (see Fig. 9) and those reported by Willenbrink et al. (1979) and Wheeler (1980) for M. integrifolia and M. pyrifera, respectively. The saturation irradiance (I_k), however, is considerably higher than those reported in the above studies. Two factors, among others, may account for this phenomenon. One, the water in the incubation chamber may not have been mixed thoroughly enough to accurately measure the average dissolved oxygen content in the chamber during the 15 min incubation. Two, the blades were only irradiated from one side. Thus, it is possible that the upper surface was irradiance-saturated while the lower surface remained irradiance-subsaturated. The oxygen evolution rate of the upper surface would not be affected by an increase in the irradiance while that of the lower surface would continue to rise.

Photosynthetic Performance of Young Sporophytes

The photosynthetic capacity displayed by Macrocystis is partially dependent on the sample's tissue age and water depth (Wheeler, 1980). These factors cannot be separated from each other when dealing with naturally growing mature sporophytes. An attempt was made to transplant 10 young sporophytes from the shallow water in which they were found to deeper water. As the young sporophytes grew towards the surface, their photosynthetic performances could have been compared to the photosynthetic performances of similar aged tissues, from the mature sporophyte, growing at different depths. Unfortunately, these comparisons, which would help separate the

effects of tissue age and water depth on the photosynthetic performance of kelp tissue, could not be made because the transplanted sporophytes died.

P vs I Curves of Fucus and Ulva

P vs I curves for Fucus and Ulva were also determined. This was done so a comparison could be made between the data reported here and published data obtained by various authors using different experimental techniques. Standard ^{14}C-carbonate incubation procedures were followed. Discs were ground before extracting the aliquots to facilitate the release of all the ethanol-soluble fraction from the tissue.

The P vs I curves were not conclusive. Ulva had too low a photosynthetic rate due perhaps to the small amount of tissue that was used while the Fucus data had large degrees of variability. Despite this problem it appeared that Fucus exhibited a low light saturation level.

Literature Cited

Akagawa, H., Ikawa, T. and Nisizawa, K., 1972a. Initial pathway of dark $^{14}CO_2$-fixation in brown algae. Bot. Mar. 15:119-125.

Akagawa, H., Ikawa, T. and Nisizawa, K., 1972b. $^{14}CO_2$-fixation in marine algae with special reference to the dark-fixation in brown algae. Bot. Mar. 15:126-132.

Akagawa, H., Ikawa, T. and Nisizawa, K., 1972c. The enzyme system for the entrance of $^{14}CO_2$ in the dark CO_2-fixation of brown algae. Plant & Cell Physiol. 13:999-1016.

Arnold, K.E., and Murray, S.N., 1980. Relationships between irradiance and photosynthesis for various benthic green algae (Chlorophyta) of differing morphologies. J. exp. mar. Biol. Ecol. 43:183-192.

Beardall, J. and Morris, I., 1976. The concept of light intensity adaptation in marine phytoplankton: some experiments with Phaeodactylum tricornutum. Mar. Biol. 37:377-387.

Bidwell, R.G.S. and Ghosh, N.R., 1962a. Photosynthesis and metabolism of marine algae IV. The fate of C^{14}-mannitol in Fucus vesiculosus. Can. J. Bot. 40:803-807.

Bidwell, R.G.S. and Ghosh, N.R., 1962b. Photosynthesis and metabolism of marine algae V. Respiration and metabolism of C^{14}-labelled glucose and organic acids supplied to Fucus vesiculosus. Can. J. Bot. 41:155-229.

Böhning, R.H. and Burnside, C.A., 1956. The effect of light intensity on the rate of apparent photosynthesis in leaves of sun and shade plants. Amer. J. Bot. 43:557-561.

Brinkhaus, B.H., 1977a. Seasonal variations in salt-marsh macroalgae photosynthesis. I. Ascophyllum nodosum ecads. Mar. Biol. 44:165-175.

Brinkhaus, B.H., 1977b. Seasonal variations in salt-marsh macroalgae photosynthesis. II. Fucus vesiculosus and Ulva lactuca. Mar. Biol. 44:176-186.

Chapman, A.R.O., Markham, J.W. and Luning, K., 1978. Effects of nitrate concentration on the growth and physiology of Laminaria saccharina (Phaeophyta) in culture. J. Phycol. 14:195-198.

Clendenning, K.A., 1971. Photosynthesis and general developement in Macrocystis. In:The Biology of Giant Kelp Beds (Macrocystis) in California (Ed. by W.J. North). Nova Hedwigia 32:169-188.

Craigie, J.S., 1963. Dark fixation of C^{14}-bicarbonate by marine algae. Can. J. Bot. 41:317-325.

Hassid, W.Z., 1970. Biosynthesis of sugars and polysaccharides. In: The Carbohydrates: Chemistry and Biochemistry (Ed. by W. Pigman and D. Horton). Academic Press, New York, pp 301-373.

Hatcher, B.G., 1977. An apparatus for measuring photosynthesis and respiration of large intact marine algae and comparison of results with those from experiments with tissue segments. Mar. Biol. 43:381-385.

Hatcher, B.G., Chapman, A.R.O. and Mann, K.H., 1977. An annual carbon budget for the kelp Laminaria longicruris . Mar. Biol. 44:85-96.

Jassby, A.D., 1978. Polargraphic measurements of photosynthesis and respiration. In:Handbook of Phycological Methods, Physiological and Biochemical Methods (Ed. by J.A. Hellebust and J.S. Craigie), Cambridge University Press, New York.

Johnston, C.S., Jones, R.G. and Hunt, R.D., 1977. A seasonal carbon budget for a laminarian population in a Scottish sea-loch. Helgoländer wiss. Meeresunters, 30:527-545.

Kanwisher, J.W., 1966. Photosynthesis and respiration in some seaweeds. In: Some Contemporary Studies in Marine Science (Ed. by H. Barnes). pp. 407-420.

King, R.J. and Schramm, W., 1976. Determination of photosynthetic rates for the marine algae Fucus vesiculosus and Laminaria digitata. Mar. Biol. 37:209-213.

Kobayashi, Y. and Harris, W.G., 1978. LSC Application Notes 1-30. New England Nuclear, Lachine, Quebec.

Kremer, B.P., 1978. Determination of photosynthetic rates and ^{14}C photoassimilatory products of brown algae. In: Handbook of Phycological Methods, Physiological and Biochemical Methods (Ed. by J.A. Hellebust and J.S. Craigie), Cambridge University Press, New York.

Kremer, B.P., 1979. Light independent carbon fixation by marine macroalgae. J. Phycol. 15:244-247.

Kremer, B.P., 1981. Metabolic implications of non-photosynthetic carbon fixation in brown algae. Phycologia 20:242-250.

Kremer, B.P. and Küppers, U., 1977. Carboxylating enzymes and pathway of photosynthetic carbon assimilation in different marine algae - evidence for the C_4-pathway. Planta 133:191-196.

Küppers, U. and Kremer, B.P., 1978. Longitudinal profiles of carbon dioxide fixation capacities in marine macroalgae. Plant. Physiol. 62:49-53.

Larcher, W., 1975. Physiological Plant Ecology. Springer-Verlag, New York, 252 p.

Littler, M.M. and Murray, S.N., 1974. The primary productivity of marine macrophytes from a rocky intertidal community. Mar. Biol. 27:131-135.

Littler, M.M., Murray, S.N. and Arnold, K.E., 1979. Seasonal variations in net photosynthetic performance and cover of intertidal macrophytes. Aquat. Bot. 7:35-46.

Lobban, C.S., 1978a. Growth of Macrocystis integrifolia in Barkley Sound, Vancouver Island, B.C.. Can. J. Bot. 56:2707-2711.

Lobban, C.S., 1978b. Translocation of ^{14}C in Macrocystis integrifolia (Phaeophyceae). J. Phycol. 14:178-182.

Ludlow, M.M. and Wilson, G.L., 1971. Photosynthesis of tropical plants I. Illuminance, carbon dioxide concentration, leaf temperature and leaf-air vapour pressure difference. Aust. J. Biol. Sci. 24:449-470.

Lüning, K., 1971. Seasonal growth of Laminaria hyperborea under recorded

underwater light conditions near Helgoland. In: Fourth European Marine Biology Symposium (Ed. by D.J. Crisp). Cambridge Univ. Press, pp. 347-361.

Patterson, D.T., 1980. Light and temperature adaptation. In: Predicting Photosynthesis for Ecosystem Models (Ed. by J.D. Hesketh and J.W. Jones), Vol. 1. CRC Press, Boca Raton, Florida, pp 205-235.

Platt, T. and Jassby, A.D., 1976. The relationships between photosynthesis and light for natural assemblages of coastal marine phytoplankton. J. Phycol. 12:421-430.

Prezelin, B.B., 1981. Light reactions in photosynthesis. In: Physiological Basis of Phytoplankton Ecology (Ed. by T. Platt). Can. Bull. Fish. Aquat. Sci. 210:1-43.

Redgwell, R.J., 1980. Fractionation of plant extracts using ion-exchange Sephadex. Anal. Biochem. 107:44-50.

Saltman, P., Lynch, V.H., Kunitake, G.M., Stitt, C. and Spolter, H., 1957. The dark fixation of CO_2 by succulent leaves: metabolic changes subsequent to initial fixation. Plant Physiol. 32:197-200.

Seely, G.R., Duncan, M.J., and Vidaver, W.E., 1972. Preparative and analytical extraction of pigments from brown algae with dimethyl sulfoxide. Mar. Biol. 12:184-188.

Smith, R.G., Wheeler, W.N. and Srivastava, L.M., 1983. Seasonal photosynthetic performance of Macrocystis integrifolia (Phaeophyceae). J. Phycol. (in press).

Stahl, E., 1969. Thin-Layer Chromatography, A Laboratory Handbook. Springer-Verlag, 1041 pp.

Steeman Nielsen, E.; 1975. Marine Photosynthesis with Special Empasis on the Ecological Aspects. Elsevier North Holland Inc., Amsterdam, 141pp.

Strickland, J.D.H., and Parsons, T.R., 1972. A practical handbook of seawater analysis. Bull. Fish. Res. Bd. Can. 167:1-310.

Stryer, L., 1981. Biochemistry. W.H. Freeman and Company, San Francisco, pg 299.

Talling, J.F., 1957. Photosynthetic characteristics of some freshwater plankton diatoms in relation to underwater radiation. New Phytol. 56:29-50.

Tuominen, T.M., 1981. Seasonal changes in the diel productivity of Macrocystis integrifolia Bory under near in situ conditions. M.Sc. thesis, Simon Fraser Univ., Burnaby, B.C., Canada. 88 pp..

Weidner, M. and Küppers, U., 1982. Metabolic conversion of ^{14}C-aspartate, ^{14}C-malate and ^{14}C-mannitol by tissue disks of Laminaria hyperborea: role of phosphoenolpyruvate carboxykinase. Z. Pflanzenphysiol. Bd. 108:353-364.

Welschmeyer, N.A. and Lorenzen, C.J., 1981. Chlorophyll-specific photosynthesis and quantum efficiency at subsaturating light intensities. J. Phycol. 17:283-293.

Wheeler, W.N., 1980. Pigment content and photosynthetic rate of the fronds

of Macrocystis pyrifera. Mar. Biol. 56:97-102.

Wheeler, W.N. and Srivastava, L.M., 1983. Seasonal nitrate physiology of Macrocystis integrifolia Bory. submitted manuscript.

Wheeler, W.N. and Weidner, W., 1983. The effects of external inorganic nitrogen concentration on the metabolism of growth and activities of key carbon and nitrogen assimilatory enzymes of Laminaria saccharina (Phaeophyceae) in culture. J. Phycol. 19:91-96.

Willenbrink, J., Kremer, B.P., Schmitz, K., and Srivastava, L.M., 1979. Photosynthetic and light independent carbon fixation in Macrocystis, Nereocystis, and some selected Pacific Laminariales. Can. J. Bot. 57:890-897.

Yamaguchi, T., Ikawa, T. and Nisizawa, K., 1966. Incorporation of radioactive carbon from $H\,^{14}CO_3$ into sugar constituents by a brown alga, Eisenia bicyclis, during photosynthesis and its fate in the dark. Plant Cell Physiol. 7:217-229.

Yentsch, C.S. and Lee, R.W., 1966. A study of photosynthetic light reactions, and a new interpretation of sun and shade phytoplankton. J. Mar. Res. 24:319-337.

Yokohama, Y., 1973. A comparitive study on photosynthesis-temperature relationships and their seasonal changes in marine benthic algae. Int. Revue ges. Hydrobiol. 58:463-472.

Zavodnik, N., 1973. Seasonal variations in rate of photosynthetic activity and chemical composition of the littoral seaweeds common to North Adriatic. Part I. Fucus virsoides (Bon) J. Ag.. Bot. Mar. 16:155-165.