CULTURE AND GROWTH KINETICS OF SELECTED NANOPLANKTERS

THESIS SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
OF THE
UNIVERSITY OF COCHIN

BY
AMMINI JOSEPH, M. Sc.

CENTRE OF ADVANCED STUDIES IN MARICULTURE
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
COCHIN-682 018

DECEMBER 1983

Declaration

I hereby declare that this thesis entitled "CULTURE AND GROWTH KINETICS OF SELECTED NANOPLANKTERS" has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

Cochin-682 018,
December, 1983.

(AMMINI JOSEPH)

Certificate

This is to certify that the thesis entitled "CULTURE AND GROWTH KINETICS OF SELECTED NANOPLANKTERS" is the bonafide record of the work carried out by Miss AMMINI JOSEPH under my guidance and supervision and that no part thereof has been presented for any other Degree.

Dr. P. V. Ramachandran Nair, M. Sc., Ph. D.
Scientist S 3,
Central Marine Fisheries Research Institute,
Cochin-682 018.

Cochin-682 018,
December, 1983.

P R E F A C E

The candidate after passing M.Sc. in Genetics and Plant Breeding from the University of Kerala joined the Centre of Advanced Studies in Mariculture, Central Marine Fisheries Research Institute as a Senior Research Scholar in the first batch of the Ph.D. programme in July, 1980. During the first semester, the candidate underwent an intensive course work in mariculture comprising the following aspects: Biology and culture of fishes, crustaceans, molluscs, seaweeds and live-food organisms, biochemistry, statistics, research methodologies, farm engineering and aquaculture management. The optional subject i.e. 'Structure and Reproduction of Algae' was studied in detail during the second semester. During the same period the candidate also passed the qualifying examination conducted by the Cochin University in December, 1981.

The research work for the thesis was started during the second semester. Several field trips were undertaken in the backwaters and inshore waters of Cochin on board the research vessels of the Institute. During this period she also got familiarised with various analytical techniques connected with the marine biology

and oceanography work programmes. For certain specialised work the candidate made trips to the Environmental Survey Laboratory and Reactor Research Centre, Kalpakkam. The Liquid Scintillation Counter available at the ESL was made use of for certain studies with metabolites of nanoplankters. The SEM facility at RRC helped in understanding the nature of flagella of the flagellates isolated by the candidate. In connection with field trials, the candidate made trips to Mandapam to familiarise with the higher algae and its cultivation as a part of the course work and to do feed trials with oysters at the newly developed hatchery at Tuticorin. Most of the other facilities were developed by the candidate on he own initiative and with the guidance of the scientists, technical staff and fellow scholars. The culture tube for continuous culture was developed locally and modified for the particular requirements based on a photograph of the tube used by Prof. Stemann Nielsen in the Danish Pharmaceutical Institute, Copenhagen. The modified version has got much more advantages in the fact that inoculation and sampling could be done without contamination.

Similarly the water bath for carrying out physiological study under different temperature was designed and fabricated at the Institute. It could maintain

temperature within ±0.5°C. Thus many of the facilities for the experimental work had to be developed or improvised locally and in certain cases make use of the facilities available with sister institutions elsewhere. This involved the co-operation from colleagues and scientists whose willing help enabled to complete this work in the minimum possible time. They have been individually acknowledged in the following pages.

ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude to Dr.P.V.Ramachandran Nair, Scientist S-3 and Head, Fishery Environment and Management Division, Central Marine Fisheries Research Institute, whose consistent encouragement and able guidance enabled this programme of work to materialise. The suggestions and directions given by Dr.E.G.Silas, Director, Central Marine Fisheries Research Institute, at various stages of the experimental work were equally beneficial. I extend my wholehearted thanks to him for the same and for providing the facilities of the CMFRI, Cochin and the regional stations at Mandapam and Tuticorin.

I offer my sincere thanks to Mr.B.S.Ajitha Kumar, Senior Research Fellow, CAS in Mariculture, Central Marine Fisheries Research Institute for all the help he has done right from setting up the laboratory, design of the culture tube, TLC study, biochemical analysis and microbiology. The help rendered by Mr.V.Kunjukrishna Pillai, Scientist S-1, Central Marine Fisheries Research Institute, regarding the instrumentation and arranging the trip to Kalpakkam is gratefully acknowledged. Thanks are due to Dr.K.V.K.Nair, Senior Scientist, ESL, Kalpakkam and Dr.P.Rodriguez, Head,

Metallurgy Programme, RMC, Kalpakkam, who helped me to carry out the scintillation counting work and SEM study. I wish to express my gratitude to Mr.S.Natarajan, Field Officer, Central Marine Fisheries Research Institute, who fabricated the temperature control unit, to Dr.C.P.Gopinathan, Scientist S-1, Central Marine Fisheries Research Institute and Mr.V.K.Balachandran, Senior Technical Assistant for their various suggestions and help with the nanoplankton culture and to Dr.K.Alagaraja, Scientist S-2, Central Marine Fisheries Research Institute, for helping with the statistical analysis. The directions given by Dr.P.V.Rao, Scientist S-3, Central Marine Fisheries Research Institute during the course work and in later stages is gratefully acknowledged. I sincerely thank Dr.C.V.Kurian, Emeritus Scientist, Central Marine Fisheries Research Institute, for going through the manuscript critically and offering many helpful suggestions. The suggestions given by Dr.J.C.Green, Plymouth Laboratory, U.K., regarding the taxonomy of nanoplankton is also gratefully acknowledged.

I express my gratitude to ICAR/UNDP for offering the Senior Research Fellowship to carryout this work at the Centre of Advanced Studies in Mariculture, Central Marine Fisheries Research Institute, Cochin.

Cochin-18,
November, 1983.

(AMALI JOSEPH)

CULTURE AND GROWTH KINETICS OF
SELECTED NANOPLANKTERS

C O N T E N T S

LIST OF TABLES

Table No.	Title
8.	Cell concentration and chlorophyll *a* content of *Chromulina freiburgensis* grown in culture media of salinities 14‰, 24‰ and 34‰ for a period of 24 days.
9.	Cell concentration and chlorophyll *a* content of *Chromulina freiburgensis* during phases of growth in culture media of different pH levels.
10.	Carbon-14 uptake by *C. freiburgensis* as function of cell concentration.
11.	Effect of photoperiod on growth of *C. freiburgensis* in batch culture.
12.	Rate of growth and ^{14}C uptake (as counts per minute per sample incubated) at different temperatures in batch culture.
13.	Specific growth rate (μ/day) during exponential phase of *C. freiburgensis*, *I. galbana* (C.s.) and *D. salina* as function of NO_3^- concentration in the medium.
14.	Specific growth rate (μ/day) during exponential phase of *C. freiburgensis*, *I. galbana* (C.s.) and *D. salina* as function of $PO_4^{\equiv}$ concentration.
15.	Variation in the amount of pigments expressed as $\mu g/10^6$ cells of *I. galbana* (C.s.) for a growth period of 30 days in batch culture.
16.	Biochemical composition of *Isochrysis galbana* (C.s.) raised in batch culture.

Table No.	Title
17.	Percentage of extracellular products (ECP) released by *I. galbana* (C.s.) during phases of growth in culture. (The % soluble represents the ECP as fraction of the total carbon fixed).
18.	Cell concentration and chlorophyll *a* content of *I. galbana* (C.s.) grown in culture media of salinities 14‰, 24‰ and 34‰ for a period of 24 days.
19.	Cell concentration and chlorophyll *a* content of *I. galbana* (C.s.) during phases of growth in culture media of different pH.
20.	Carbon-14 uptake by *I. galbana* (C.s.) as function of cell concentration.
21.	Effect of photoperiod on growth of *I. galbana* (C.s.) in batch culture.
22.	Variation in the amount of pigments expressed as $\mu g/l \times 10^6$ cells of *Synechocystis salina* for a growth period of 30 days in batch culture.
23.	Biochemical composition of *S. salina* raised in batch culture.
24.	Percentage of extracellular products (ECP) released by *S. salina* during phases of growth in culture (The % soluble represents the ECP as fraction of the total carbon fixed).

LIST OF FIGURES

Fig.No.	Title
Fig.1A.	Increase in cell number of *Chromulina freiburgensis* during growth in batch culture.
B.	Variation in the amount of photosynthetic pigments of the above species during growth in batch culture.
Fig.2A.	Rate of production of organic carbon by *C. freiburgensis* for a growth period of 30 days in batch culture (measured by the oxygen technique).
B.	Rate of production of organic carbon by the above species for a growth period of 30 days in batch cultures (measured by ^{14}C technique).
Fig.3.	Two-dimensional chromatograms of pigments in nanoplankters separated on silica-gel thin layer plates. A. *C. salina*, B. *C. freiburgensis*, C. *I. galbana* (C.s.), D. *I. gracilis*.
Fig.4.	Rate of carbon fixation (expressed as ^{14}C uptake in cpm) as function of different culture densities of *C. freiburgensis*.
Fig.5.	The rate of photosynthesis (expressed as ^{14}C uptake in cpm) as function of light intensity for *C. freiburgensis*.
Fig.6.	Relation between the concentration of nitrate in the culture medium and the specific growth rate of *C. freiburgensis*.

Fig.No.	Title
Fig.7.	Relation between the concentration of phosphate in the culture medium and the specific growth rate of C. freiburgensis.
Fig.8A.	Increase in cell number of Isochrysis galbana (C.s.) during growth in batch culture.
B.	Variation in the amount of photosynthetic pigments of the above species during growth in batch culture.
Fig.9A.	Rate of production of organic carbon by I. galbana (C.s.) for a growth period of 30 days in batch culture (measured by the oxygen technique).
B.	Rate of production of organic carbon by the above species for a growth period of 30 days in batch culture (measured by the ^{14}C technique).
Fig.10.	Rate of carbon fixation (expressed as ^{14}C uptake in cpm) as function of different culture densities of I. galbana (C.s.).
Fig.11.	The rate of photosynthesis (expressed as ^{14}C uptake in cpm) as function of light intensity for I. galbana (C.s.).
Fig.12.	Relation between the concentration of nitrate in the culture medium and the specific growth rate of I. galbana (C.s.).
Fig.13.	Relation between the concentration of phosphate in the culture medium and the specific growth rate of I. galbana (C.s.).

Fig. No.	Title
Fig.14A.	Increase in cell number of *Synechocystis salina* during growth in batch culture.
B.	Variation in the amount of photosynthetic pigments of the above species during growth in batch culture
Fig.15A.	Rate of production of organic carbon by *S. salina* for a growth period of 30 days in batch culture (measured by the oxygen technique).
B.	Rate of production of organic carbon by *S. salina* for a growth period of 30 days in batch culture (measured by the ^{14}C technique).
Fig.16.	Rate of carbon fixation (expressed as ^{14}C uptake in cpm) as function of different culture densities of *S. salina*.
Fig.17.	The rate of photosynthesis (expressed as ^{14}C uptake in cpm) as function of light intensity for *S. salina*.
Fig.18.	The relation between the concentration of nitrate in the culture medium and the specific growth rate of *S. salina*.
Fig.19.	The relation between the concentration of phosphate in the culture medium and the specific growth rate of *S. salina*.

LIST OF PLATES

Plate No.	Title
I.	Young motile cells and reproductive phases of *Isochrysis galbana* (C.s.)
II.	Young motile cells and reproductive phases of *Chromulina freiburgensis*.
III.	Figs.1-5. *I. galbana* (C.s.) Figs.6-10. *C. freiburgensis*
IV.	Cultures of *C. freiburgensis*, *I. galbana* (C.s.) and *S. salina*.
V.	Modified culture tubes for growing axenic cultures of nanoplankton in the laboratory.
VI.	Experimental set up for controlling ambient temperature for nanoplankton culture.
VII.	Liquid Scintillation Counting System and the quench correction curve for determining the counting efficiency of the system.
VIII.	The experimental set up of the cultures of *C. freiburgensis* and *S. salina* to test their tolerance to three different salinity levels.
IX.	Mass culture of *C. freiburgensis* and *I. galbana* (C.s.)

1. INTRODUCTION

The biomass of phytoplankton and primary productivity in the sea have always been the basis in the estimation of the relative fertility and potential fishery resources. Net sampling was one of the accepted and widely used methods for assessment of population composition and biomass. Lohmann (1903) drew attention to the fact that an unknown proportion of biomass was lost by net sampling since the cells of many organisms were too small to be retained. He applied the term 'nannoplankton' (more often referred to as the 'nanoplankton') to the autotrophic plankters that passed through the meshes of fine silk net. Holligan and Harbour (1977) restricted the term to photosynthetic organisms and used 'Nanophytoplankton' instead of nanoplankton.

Several schemes have been proposed for the size classification of the nanoplankton. The maximum size as visualized by Lohmann (1903) is 50–60μ. But later, when plankton nets of smaller mesh size became available, further fractionation was possible. 'Some authors suggest separating the very smallest cells (less than 5 microns) as 'ultra plankton' and to limit the nanoplankton to algae not exceeding 30μ' (Raymont, 1980). Dussart (1965) and

Hannah and Boney (1983) consider the maximum dimensions to be less than 20μ.

Irrespective of size the nanoplankton includes algae from different classes: Chrysophyceae, Haptophyceae, Prasinophyceae, Chlorophyceae, Dinophyceae, Bacillariophyceae and Cyanophyceae. In structural organisation they may be naked flagellates, thick walled diatoms, dinoflagellates, silicoflagellates or coccolithophorids and coccoid forms. The life history and structure of many of these have been studied by investigators such as Parke, Manton, Green, Paasche, Dodge, Boney and Bernard.

The contribution of nanoplankton to the primary productivity of the seas has been much investigated (cf. Raymont, 1980). Most of these works show that the contribution of the nanoplankton fraction in terms of cell numbers, chlorophyll a content and carbon fixation, whilst variable, represent sizeable proportions (50-100% of the total) irrespective of whether these data refer to polar, temperate or tropical seas. Some investigators point out that nanoplankton dominate the oceanic waters and in low latitudes while net plankton are abundant in the neritic waters and high latitudes.

For temperate waters it has been suggested that in many areas, nanoplankton becomes important after the spring

diatom outburst (Holligan and Harbour, 1977). Flagellates of less than 10μ have been reported to form a stable component of the Scotland Sea. Coccolithophorids abound in some seas, though minute diatoms are also not excluded (Collier and Murphy, 1962; Beers et al., 1975; Throndsen, 1976).

Nanoplankton population of warmer seas have been found to be significantly high. Hulburt, Ryther and Guillard (1960) estimated that in the Bermuda Region the nanoplankton population was at least hundred times denser than the larger phytoplankton. Further studies on the nanoplankton of the tropical waters have been done by Wood (1963), Tundisi (1971) and Malone (1971a,b,c) to mention a few. Saijo (1964) did the size fractionation of the Indian Ocean phytoplankton. He observed that the size group between 0.8 and 90 microns contributed 50% or more to the productivity compared to the larger net plankton. Devassy and Bhattathiri (1981) reported that the nanoplankton chlorophyll a averaged 0.034 mg/m^3 as against 0.043 mg/m^3 for the net plankton in the Andaman sea during the year 1979.

Subrahmanyan (1959) and Subramanyan and Sarma (1965) did the pioneering studies on the nanoplankton of the Indian coast. These studies showed that in the south west coast

near Calicut, about 30-50% of the standing crop was due to the nanoplankton fraction. They did not observe any seasonal trends in their occurrence.

Qasim et al. (1974) reported that in the Cochin backwaters the relative contribution of photosynthesis by nanoplankton varied from 45-96% while that of net plankton was only 4-55% of the total production. The amount of nanoplankton chlorophyll registered higher values throughout the year. However, during the monsoon and premonsoon months, the net plankton occurred in higher numbers. In a similar study in the coastal waters of Cochin, Vijayaraghavan et al. (1974) estimated that nanoplankton contributed 66.4% of the total productivity.

The dynamics of phytoplankton growth in nature depends on the behaviour of individual species. The latter can be understood only by laboratory studies on unialgal cultures. Miquel (1890) was among the pioneers to initiate the culture of microalgae. Adequate amounts of nitrate and phosphate added to filtered sea water was used as the basic growth medium for phytoplankton.

Pringsheim (1946) has summarised the history and procedure of phytoplankton culture. In the 1950's, Provasoli, Droop and others modified the culture media

by adding trace elements, chelating compounds and vitamins (Provasoli et al., 1957; Provasoli, 1958; Droop, 1954,1958a,1961a,b). The mineral requirements of algae were further reviewed by Lewin (1962), O'Kelly (1968), Stein (1973) and Stewart (1974).

Many axenic cultures of phytoplankton were developed using such enriched media. About 50% of these were diatoms and dinoflagellates and the rest composed of small green flagellates (Chlorophyceae, Prasinophyceae, Euglenophyceae), golden brown flagellates and coccolithophoric (Chrysophyceae, Haptophyceae, Chloromonadophyceae), cryptomonad flagellates (Cryptophyceae), red algae (Rhodophyceae) and blue-green algae (Cyanophyceae). Some well known species in culture are: Skeletonema costatum, Asterionella japonica, Phaeodactylum tricornutum, Chaetoceros calcitrans, Ceratium tripos, Amphidinium carteri, Anabaena cylindrica, Isochrysis galbana, Monochrysis lutheri, Chlorella pyrenoidosa and Dunaliella tertiolecta.

The cultures are raised in the laboratory in a limited volume of the medium inoculated with a small number of cells and exposed to suitable conditions of light, temperature and aeration. These batch cultures are maintained by periodic reculturing.

For experimental purposes many investigators make use of continuous cultures. These are of two types: Chemostat culture (Monod, 1950; Novick and Szilard, 1950) and 'Turbidostat' culture (Myers and Clark, 1944). Synchronous cultures are also used in experimental studies (Tamiya et al., 1953).

The culture collections of phytoplankton have been used to study many basic processes relating to photosynthesis, growth, algal phylogeny and biomass estimates. Experiments with algal cultures are providing clues to important ecological processes such as succession patterns, mechanism of phytoplankton blooms and many other phenomenon of basic importance to plant productivity in the sea.

In the 1960's the idea of single cell protein (SCP) was mooted. The International Biological Programme (IBP) clearly stated that 'Investigation should be carried out on those unused and little used plant and animal products which might be converted into nutritionally exploitable materials by bio-engineering techniques'. Microalgae was identified as a rich source of SCP and consequently large scale production of different species was undertaken to be incorporated into human food as well as cattle and poultry feed.

In nature nanoplankton is the source of food for the minute zooplankters and the larvae of many aquatic animals (Atkins, 1945; Thorson, 1950; Beers and Stewart, 1969; Parsons and Le Brasseur, 1970). Hence these microalgae are used as live-food in rearing the larvae of fish and shell fish in aquaculture practices. Though in natural environment they feed on a variety of organisms, in culture systems only a few species have been found successful. Diatoms such as Skeletonema, Chaetoceros, Thalassiosira and Nitzschia are known to be good food for Crustacea (Kinne, 1977). May (1970) and Lasker et al. (1970) have reported success in growing marine fish larvae on algal diet alone. The larvae of the bivalve molluscs feed and grow best on nanoplankters especially the naked golden brown flagellates (Bruce et al., 1940; Guillard, 1958; Waine, 1963,1970,1974; Ukeles, 1975).

Recently micro-particulate diets have gained some progress in the rearing of bivalve larvae. But many authors opine that live-food is superior to non-living diets and the former is still being widely used in hatcheries (Jirasek et al., 1977; Kinne, 1977). As such, the culture of nanoplankton remains a necessary component of molluscan hatcheries.

The micro-flagellate Isochrysis galbana Parke is used to rear the larvae of molluscs in the laboratory of CMFRI. The nutritional value of this flagellate is well recognised (cf. Loosanoff and Davis, 1963). But, I. galbana is a temperate strain the growth of which is retarded above 22°C (Ukeles, 1961). So mass cultures of I. galbana is quite unstable in the tropical climate. Hence the need to isolate tropical strains of nanoplankters that could be of use in mariculture.

There has been a few attempts in the past to isolate nanoplankters from the Indian waters. Samuel (1973) collected nine species of nanoplankton from the sea off Cochin. Studies on the culture and growth kinetics of a few species have been attempted by Nair (1974), Vijayaraghavan et al. (1975) and Gopinathan (1981). But there has been no comprehensive study on the culture of nanoplankton in relation to its food value.

So these investigations were initiated with a view to isolate strains of nanoplankton that could be of food value to bivalve larvae and to study the growth requirements of these algae in culture. This has been presented in detail in the six chapters to follow. The introductory chapter explains the relevance of the work together with the literature review. Chapter 2 gives a taxonomic account

of some nanoplankters of the Indian coast including the present isolates. Chapter 3 presents an exhaustive description of the material and methods adopted for the study. The results of the various experiments on growth and other physiological activities are presented in Chapter 4. Chapter 5 deals with the mass culture and food value of the two microflagellates isolated during the period of investigation. The conclusions arrived at by the study are discussed in Chapter 6.

Growth kinetics: Literature

Literature on the growth kinetics of unicellular algae in culture is voluminous (cf. Strickland, 1960; Fogg, 1975). In batch cultures under suitable conditions, the microalgae grow and multiply rapidly in a sequential manner. Thus it is possible to observe a lag phase, exponential phase, stationary phase and death phase in such cultures. Lag phase has been detailed by Fogg (1944), Gerloff *et al.* (1950), Spencer (1954) and Eberly (1967). The exponential or the logarithmic phase produces the peak growth after which the growth rate decreases and stationary phase begins. The stationary phase may be maintained for several weeks before decline sets in. The duration of these phases, however, varies with the species and the prevailing environmental conditions.

There is no single parameter to measure growth of unicellular algae. When cell number is known, the rate of doublings per day is often used as a useful index of growth (Eppley and Strickland, 1968). Specific growth rate (μ) or the relative growth constant (k) and generation time (t_g) are also useful parameters for measuring growth (Fogg, 1975). The growth rate is usually calculated from cell number, cell carbon, or the amount of chlorophyll a. Values of relative growth rates for different species in culture have been summarised by Hoogenhout and Amesz (1965). Thus a study of growth kinetics envisages different aspects such as population density, the pigments and cellular composition.

In regular oceanographic analysis the phytoplankton biomass is often determined as photosynthetic pigments. Analysis of the same by spectrophotometry has been popularised by Richards and Thompson (1952) and Parsons and Strickland (1963). In the 1960's chromatography became widely used for more detailed analysis of the algal pigments (Jeffrey, 1961, 1965,1968). These studies have shown that algae contain three basic groups of pigments namely chlorophylls, carotenoids and biliproteins. These function as photoreceptors in photosynthesis. The distribution of these in the various algal taxa has been ascertained by separating the component pigments on chromatograms. At first, paper chromatography

was used to separate the chlorophylls and carotenoids of marine algae in culture (Jeffrey, 1961,1965) and sea water samples (Jensen and Liaaen Jensen, 1959; Jeffrey, 1974). Further, micro-chromatographic techniques were developed which needed only small amounts of the material and gave rapid and good resolution of the components. Investigators like Riley and Wilson (1965), Madgwick (1965) and Jeffrey (1968,1974,1981) employed the thin layer technique to study the algal pigments. Different adsorbents and solvent systems were used by these authors so as to get maximum resolution.

The solar energy trapped by the pigments is used to produce organic matter which further is partitioned between proteins, carbohydrates and lipids and within each of these are numerous organic substances. Some data are given by Spoehr and Milner (1949), Myers (1949), Parsons et al. (1961), Lewin and Guillard (1963) and Myklestad (1974). All these workers point out that the microalgae are mainly protein synthesizers. According to Goldman (1980) a balanced nutritional state produces about 50-60% protein, 30-40% carbohydrate and 10-20% lipids. However, prolonged growth under nitrogen deficiency cause synthesis of lipids or carbohydrates at the expense of protein. The changing protein-carbohydrate ratio during batch growth has been

studied by Fogg (1959) and Healey (1975). Reviewing the development of the work in the field Morris (1981) concludes that besides the general similarity between species in the relative amounts of crude protein, lipid and carbohydrate there exist important differences as function of species and ecological conditions.

Another integral component of algal photosynthesis is the release of organic metabolites into the surrounding medium (Fogg, 1975; Mague et al., 1980). However, there is little agreement on the relative amounts of the material released (Hellebust, 1965; Watt, 1966; Fogg, 1966; Thomas, 1971). The exponentially growing cells may release from 1% to 50% of their photoassimilated carbon while cells in lag or stationary phase release more (cf. Hellebust, 1974). The variation in the rate of excretion has been attributed to different factors - physical, chemical and biological (Guillard and Wangersky, 1958; Marker, 1965; Fogg, 1966; Samuel, Shah and Fogg, 1971; Williams and Yentsch, 1976).

The method of estimation of the extracellular products (ECP) employed by these investigators makes use of the carbon-14 incubation method. The carbon-14 incubated samples are filtered and the organic fraction in the filtrate (ECP) is determined with the aid of Geiger Counter or Scintillation Counter (cf. Vollenweider, 1974).

Apart from these central features of growth and photosynthesis the physiological ecology of microalgae have been much discussed. The algae in general show high metabolic diversity. This allows extensive variability to be superimposed on the basic processes. The nature of this variability is regulated by the environmental factors and the physiological state of the organism.

In nature phytoplankton populations are subject to a variety of factors like light, temperature and nutrient fluctuations acting simultaneously. Nature has normally established an equilibrium between all the factors governing phytoplankton production. The incidence of any one factor becoming limiting in such systems is only occasional. However, in culture systems single factors like light intensity, nitrate or phosphate concentration can become limiting resulting in growth arrest. This has been illustrated in experimental cultures (cf. Fogg, 1975; Stewart, 1974; Platt, 1981).

Solar radiation was early understood to be a prime factor in photosynthesis (cf. Rabinowitch, 1951). As such, in productivity studies the euphotic zone and light - photosynthesis relationship gained importance. Talling (1957) introduced the term I_K to describe the light intensity that marks the onset of saturation of photosynthesis. This saturation level has been defined for a number of

species (Steemann Nielsen and Jensen, 1957; Steemann Nielsen and Hansen, 1959,1961; Steemann Nielsen and Jorgensen, 1962, 1968a; Jorgensen, 1964,1969; Steemann Nielsen and Willemoes, 1971).

The algal growth and species competition in culture systems and natural ecosystems is also a function of temperature. Eppley (1972) has discussed in detail the effect of temperature on phytoplankton growth rates. He found that there is a gradual and exponential increase in the specific growth rates with increasing temperature upto 40°C. He quantified the temperature - growth rate relationship in terms of Q_{10}. However, this response to temperature need not be direct. In fact many authors have tried to find out the combined effect of temperature, light, salinity and nutrient status in axenic cultures (cf. Eppley and Strickland, 1968; Stewart, 1974). Goldman and Carpenter (1974) correlates the effect of temperature to nutrient concentration in many fresh water and marine algae. Nedalje and Lewe (1983) has illustrated in Thalassiosira alenii Takano that the effect of temperature and nutrients interact to govern how a particular parameter like protein content vary with temperature while light affected the range of these values.

The kinetics of uptake of nutrients and the relation between nutrient uptake and growth of phytoplankton has

received much attention. The results of Ketchum (1939) and Harvey (1957) indicated a hyperbolic relationship between the rate of uptake of nutrients and the concentration of the same in the medium. Dugdale (1967) and Caperon (1968) established that the rate of uptake of a limiting nutrient and the specific growth rate are related to nutrient concentrations in the medium in a way similar to that of the Michaelis-Menten relation of enzyme kinetics. Further work on this aspect has been done by Eppley and Coatsworth (1968); Eppley and Thomas (1968); Eppley, Rogers and McCarthy (1969); Droop (1973); Qasim et al. (1973); Goldman (1977); Goldman and McCarthy (1978) and McCarthy (1981).

The nutrient requirement of a species is expressed in terms of half-saturation constant(K_S). It is defined as the concentration supporting half the maximum growth (K_μ) or half the maximum uptake (K_S) and is calculated using the Monod expression (Monod, 1942). Most of the research in this line has centred around the kinetics of uptake of nitrate, phosphate, ammonium, silicate and vitamin B_{12}.

Other than light, temperature and nutrients, phytoplankton of coastal waters is subject to high fluctuations of salinity, pH and many other factors due to land drainage and other human activities. The salinity and pH requirements of marine algae have received attention of

certain investigators (Droop, 1958a; McLachlan, 1961; Soeder et al., 1966; Qasim et al., 1972; Majumder et al., 1980). Cells of Dunaliella salina are found to respond drastically to the changes in salinity by changing the pigment content (Loeblich, 1982). Thus investigations on microalgae have envisaged all aspects of growth and assimilatory products in connection with their eco-physiology.

2. SYSTEMATICS

A taxonomic account of some nanoplankters of the coastal and estuarine waters of India suitable for development as axenic cultures for feed.

The diatoms and dinoflagellates of the Indian waters are well documented (Menon, 1945; Subrahmanyan, 1946,1958a, 1959,1968,1971; Subrahmanyan and Sarma, 1960,1965; Nair, 1959,1960; Gopinathan, 1975,1981). These works deal mostly with the net plankton. Except for a few reports information on nanoplankton is scanty. Subrahmanyan and Sarma (1965) identified 58 species of nanoplankters of which 27 are diatoms and 26 dinoflagellates. The remaining are species of Carteria, Chlamydomonas, Coccolithus pelagicus, Euglena and Meriamopedia. Sarma and Khan (1980) have listed some nanoplankters reported from the Indian seas which again include mostly diatoms and dinoflagellates. Nine species of the smaller nanoplankters (less than 20 microns) have been described by Samuel (1970). A few species have been isolated from the coastal region of Cochin and is available in the culture collections of CMFRI. In order to present a complete picture of this study, the taxonomy and salient features of the smaller nanoplankters occurring in Indian waters are included in this section, so as to provide information on the species suitable for culture of live-food

in hatchery development. Only those species below 20 microns in size are listed here; all diatoms, dinoflagellates, chain forming and colonial types have been omitted.

Classification

Algal systematics has been subjected to much change since the time of publication of the exhaustive work on algae by Fritsch (1935,1945). Many taxa had been reshuffled and a few new groups created based on advanced studies. Recent revisions of algal systematics are provided by Christensen (1962), Parke and Dixon (1976), Bold and Wynne (1978) and many others.

The classification followed here is that of Parke and Dixon (1976).

CYANOPHYTA

CYANOPHYCEAE

CHROOCOCCALES

Chroococcaceae

SYNECHOCYSTIS Sauvageau, 1892

Sauvageau, C. 1892. Algues recolt. en Algerie. Bull. Soc. Bot. France, 39: 115.

Fritsch, F.E. 1935. Structure and Reproduction of the Algae. Vol.II, p.814.

Desikachary, T.V. 1959. Cyanophyta, p.144.

Spherical cells usually single; sometimes occur in two's or rarely in colonies of a few cells. No distinguishable mucilage envelope.

Distribution: The genus occurs as plankton in the fresh and marine waters. The genus is also reported to be epizoic on ascidians and within mangrove roots.

Key to the species

1) Spherical cells, solitary or in two's and no characteristic motility. ... S. pevalekii Geitler

2) Solitary, spherical cells with jerky movements ... S. salina Wislouch

Synechocystis pevalekii Geitler, 1932

Geitler, L. 1932. Kryptogamenflora, p. 269.
Desikachary, T.V. 1959. Cyanophyta, p. 145.

Spherical cells; sometimes two together and appear hemispherical after division. The cells measure 2.5–3.5μ in size. The cell contents is bluish green and homogeneous.

Distribution: The species is reported from the fresh waters of Madras, Deira Lake and Ceylon by Desikachary. The strain available in CMFRI culture collection was isolated from the coastal region of Tuticorin.

Synechocystis salina Wislouch, 1924

Wislouch, S.M. 1924. Acta. Soc. Bot. Pol., 2: 99.
Geitler, L. 1942. Schizophyceae in Engler-Prantl Die Naturlichen pflanzenfamilien, II Ed. 1a.
Lund, J.W.G. 1950. Naturalist, London, 835: 143-148.

Small spherical cells of diameter 3μ with bluish green colour. Sometimes occur in two's. The species is characterised by the jerky movements of the cells.

Distribution: The species was isolated from the coastal region of Cochin.

CRYPTOPHYTA

CRYPTOPHYCEAE

CRYPTOMONADALES

Cryptomonadaceae

CRYPTOCHRYSIS Pascher, 1911

Pascher, A. 1911. Ber. Deutch. Ges. Bod., 29: 139-203.
Fritsch, F.E. 1935. Structure and Reproduction of the Algae. Vol.I, p.652.
Pierre P. Grassе. 1953. Traite De Zoologie, 285-308.
Butcher, R.W. 1952. J. mar. biol. Ass. U.K., 31: 185-187.
Samuel, S. 1970. J. mar. biol. Ass. India, 12: 35-42.

Motile naked cells with slight dorso-ventral constriction. A narrow furrow extents from the slightly

constricted anterior end to the posterior part. The flagellae are of unequal length. Chromatophore single, two or numerous with diverse shades of brown.

Distribution: Distributed in the coastal seas of both temperate and tropical waters.

Key to the species

1) Ovoid cells with a vertical furrow running down to the basal pyrenoid ... C. fulva Butcher
2) Ovoid cells with a shallow furrow extending upto the centre of the cell. Pyrenoid sub-median in position ... C. virescens Butcher

Cryptochrysis fulva Butcher, 1952

Butcher, R.W. 1952. J. mar. biol. Ass. U.K., 31: 185-187.
Samuel, S. 1970. J. mar. biol. Ass. India, 12: 35-42.

Motile naked flagellates measuring 6-8x3-4μ. The cells appear ovoid in dorsal view. The apex is truncate and posteriorly the cell tapers. A vertical furrow extents from the apex to the pyrenoid which is posterior in position.

The motile cell or the resting cell divides longitudinally to form daughter cells. Palmelloid stages also occur.

Distribution: Found in the shell fish tanks of Conway, Northwales. In India it has been reported from the west coast.

Cryptochrysis viresceng Butcher, 1952

Butcher, R.W. 1952. J. mar. biol. Ass. U.K., 31: 187.
Samuel, S. 1970. J. mar. biol. Ass. India, 12: 35-42.

Cells measure 5-7x3-4μ in size; ovoid with a convex dorsal side and flattened ventral side. A shallow furrow runs down laterally from the anterior end to the centre. There are two flagellae that are unequal in length. Chromatophore single, large, bluish green, smooth and almost fills the cell. There is a single large and sub-median pyrenoid.

Reproduction occure by the longitudinal division of the motile cell into two. Palmelloid stages are also reported.

Distribution: The species is reported from the river Crouch, Essex. The Indian report is from the Cochin coast.

HAPTOPHYTA

HAPTOPHYCEAE

ISOCHRYSIDALES

Gephyrocapsaceae

DICRATERIA Parke, 1949

Parke, M. 1949. J. mar. biol. Ass. U.K., 28: 255-286.
Bourrelly, P. 1958. Review Algologique Memoir. Hors. Serie. 1. 229.
Samuel, S. 1970. J. mar. biol. Ass. India, 12: 35-42.
Green, J.C. and R.M. Pienaar. 1977. J. mar. biol. Ass. U.K., 57: 7-17.

Cells solitary, spherical and golden brown in colour. Two flagellae of equal length. Asexual reproduction by the division of the motile cells. Cysts are also reported.

Distribution: Reported from the coastal waters of Britain and Europe. In India it has been collected from the Laccadive sea.

Dicrateria gilva Parke, 1949

Parke, M. 1949. J. mar. biol. Ass. U.K., 28: 255-286.
Samuel, S. 1970. J. mar. biol. Ass. India, 12: 35-42.

Biflagellate spherical cells measuring about 3.5-7μ in diameter. Two flagella of equal length. There are two golden brown chromatophores that are parietal in position. The rest of the cell is occupied by the nucleus, leucosin, oil bodies and contractile vacuole.

The species is characterised by the spherical shape, slow rotatory movements and the absence of stigma. As the

cells grow old, the number of chromatophores and leucosin increase.

Reproduction takes place by the binary fission of the cells. Cysts and palmelloid phases also occur.

Distribution: The species was first reported from the plankton samples taken from the Plymouth Sound, Britain. The Indian strain was found in the Laccadive sea.

Isochrysidaceae

ISOCHRYSIS Parke, 1949

Parke, M. 1949. J. mar. biol. Ass. U.K., 28: 255-286.
Billard, C. and P. Gayral. 1972. Br. phycol. J., 7: 289-297.
Green, J.C. and R.N. Pienaar. 1977. J. mar. biol. Ass. U.K., 57: 7-17.

Slightly elongated motile cells with a capacity to change shape. Two smooth apical flagella, once to twice the length of the cell. The motile cell has haptophycean scales on the cell surface. Benthic phases occur usually.

Distribution: The genus has been reported from the fish ponds of Britain, from below the chalk-cliffs of the French and British coasts and from the shrimp culture ponds of Tahiti.

Isochrysis galbana Parke, forma nova

CMFRI Culture No.12: Isolated from the inshore waters of Cochin on the west coast of India in November, 1981.

Motile cell

Length = 12–14μ

Breadth = 5–6μ

Length of the flagellum = 14μ

Golden-yellow flagellate moving actively. The cell is slightly elongated and compressed. There is marked capacity to change shape. The cells are surrounded by a thin mucilage layer.

Two flagella of equal length arise from the anterior end. They are visible feebly in the light microscope as two stiff hair like structures directed away from the body. The two are equal in diameter throughout their length. During movement the flagella are directed forwards with the body rotating and gyrating in the course. The dry specimens observed under the electron microscope showed a knob like haptonema in between the flagellae (Plate I).

There is a single chromatophore that is golden-brown in colour and parietal in position. Embedded in the base of

EXPLANATION OF PLATE I

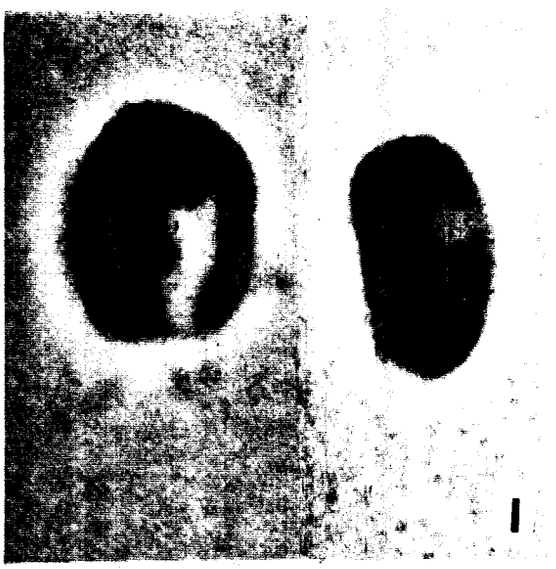
Figs.1–4. *Isochrysis galbana forma nova*

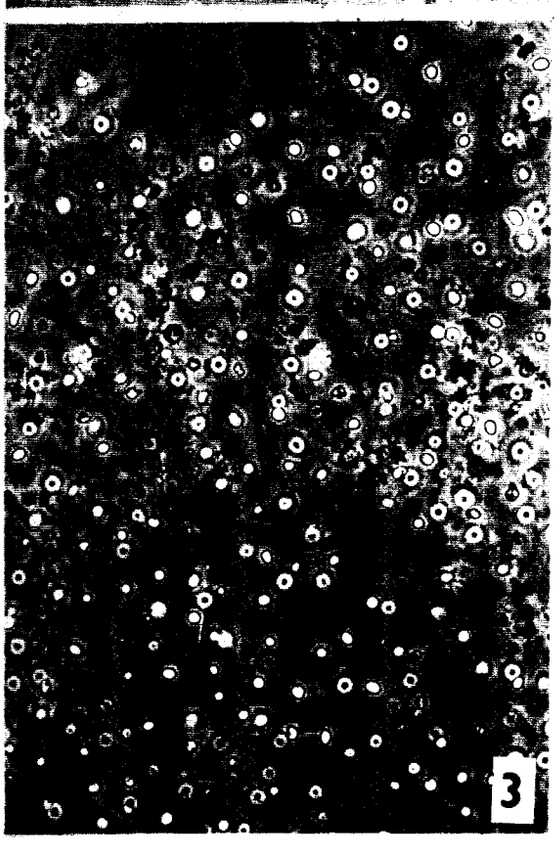
Fig.1. Young motile cell viewed under light microscope (x 3000).

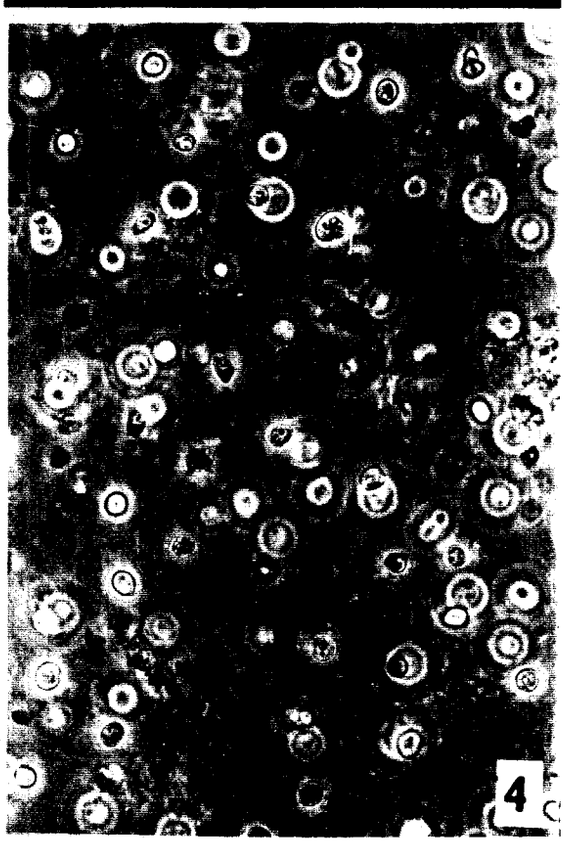
Fig.2. Scanning electron micrograph of young motile cell (x 6000); h, haptonema; f, (broken) flagella.

Figs.3–4. Reproduction in the palmelloid phase (fig.3, x 320; fig.4, x 640).

PLATE I







the chromatophore is the pyrenoid that appears as a part of the chromatophore.

The young motile cells show a median red body. In older cells three or four of such round bodies are observed. The nucleus is median in position.

The species show phototactic response, though a clear stigma has not been found. At the posterior end of the cell leucosin body is prominent. Small oil drops are distributed throughout the cell (Plate III).

Asexual reproduction occurs in the young cultures by the longitudinal division of the motile cells. When cultures are two to three months old, the cells begin to settle at the bottom of the culture vessel forming a layer at the bottom. The cultures on examination was observed to contain non-motile cells embedded in a thin layer of mucilage (Plate I). It is inferred that this is a form of reproduction in palmelloid phase. These spheroidal individuals have large masses of leucosin and two to four chromatophores. Most of these cells are observed to be in the division phase with or without completing the separation of the daughter cells (Plate III). On inoculating into fresh medium, all these non-motile cells completed division, developed flagella and became active. Division in

the non-motile phase have been reported by Parke (1949) for *I. galbana*. However, cysts as observed by Parke (1949) was not obtained even in 6 month old cultures.

The particulars of the present strain agrees with that of the genus *Isochrysis*. Three species of *Isochrysis* are reported of which *I. maritima* and *I. littoralis* are distinguished by the absence of haptonema and the dominant benthic phase (Billard and Gayral, 1972). As revealed by the SEM study, the present strain has got the much reduced haptonema similar to *I. galbana*, Parke. The new isolate has also been compared with the live cultures of *I. galbana*, Parke which is available in CMFRI culture collection. The characteristic capacity to change shape, the dominance of motile phase, the single chromatophore and the absence of well defined stigma supports the assigning of the present strain to *I. galbana*.

However, the present strain differs from *I. galbana*, Parke in the cell dimensions. The latter measures 3-6μ in length, 2-4μ in breadth and the flagella 7μ in length whereas the present strain is almost double the size. Moreover, *I. galbana* is purely a temperate water species while the present strain is tropical. A similar flagellate is reported from the tropical waters of Tahiti designated T-ISO (J.L. Martin, Centre Oceanologique Du Pacifique,

personal communication). The ultra-structural aspects of these tropical strains have not been carried out. So the present strain is given the designation *I. galbana forma nova** pending additional studies.

Distribution: The species is reported from the fish ponds of Port Erin, U.K. The clone T-ISO was isolated from the lagoon water of Tahiti. The present strain in the culture collection of CMFRI was isolated from the inshore region of Cochin.

CHRYSOPHYTA

CHRYSOPHYCEAE

CHROMULINALES

Chromulinaceae

CHROMULINA Cienkowski, 1870

Cienkowski, L. 1870. Arch. mikroskop. Anat., 6: 421-438.

Conrad, W. 1931. Mem. Mus. Roy. Hist. Nat. Belgique., 8: 65.

Bourrelly, P. 1968a. Les algues d'eau douce. Initiation a'la systematique. II. Les algues jaunes et brunes, Chrysophycees, Xanthophycees.

Bold, H.C. and M.J. Wynne. 1978. Introduction to the Algae, Structure and Reproduction, P.358.

Cells solitary, free swimming. Cells are naked with no well defined form. There is a single pleuronematic flagellum. One or two chloroplast present.

---

*Hereafter referred to as I. galbana (C.s) meaning Isochrysis galbana (Cochin strain)

About 120 species are known. The species are distinguished by the presence or absence of stigma and pyrenoid, structure and formation of cysts or number and form of chloroplast.

Distribution: The genus occurs both in freshwater and marine habitats.

Chromulina freiburgensis Dofl. 1923

Doflein, F. 1923. Arch. Protistenk., 46: 267.
Fritsch, F.E. 1935. Structure and Reproduction of the Algae. Vol.I, p. 512.

C. freiburgensis (CMFRI culture No.11). Isolated from the inshore waters of Cochin in August, 1981.

Motile cell

Diameter = 10μ
Length of the flagellum = 25μ

Cells spheroidal and motile with a smooth periplast that permits only slight change of shape. The cell has a thin layer of mucilage forming a halo around it. A single flagellum arises apically. As shown by the electron microscopy, the flagellum ends in a bulb like expansion (Plate II). No evidence of cilia was obtained in dry specimens. The live cells move slowly in a rotating motion with active forward movement occasionally.

EXPLANATION OF PLATE II

Figs.1–6. *Chromulina freiburgensis*

Fig.1. Young motile cell viewed under light microscope (x 1500).

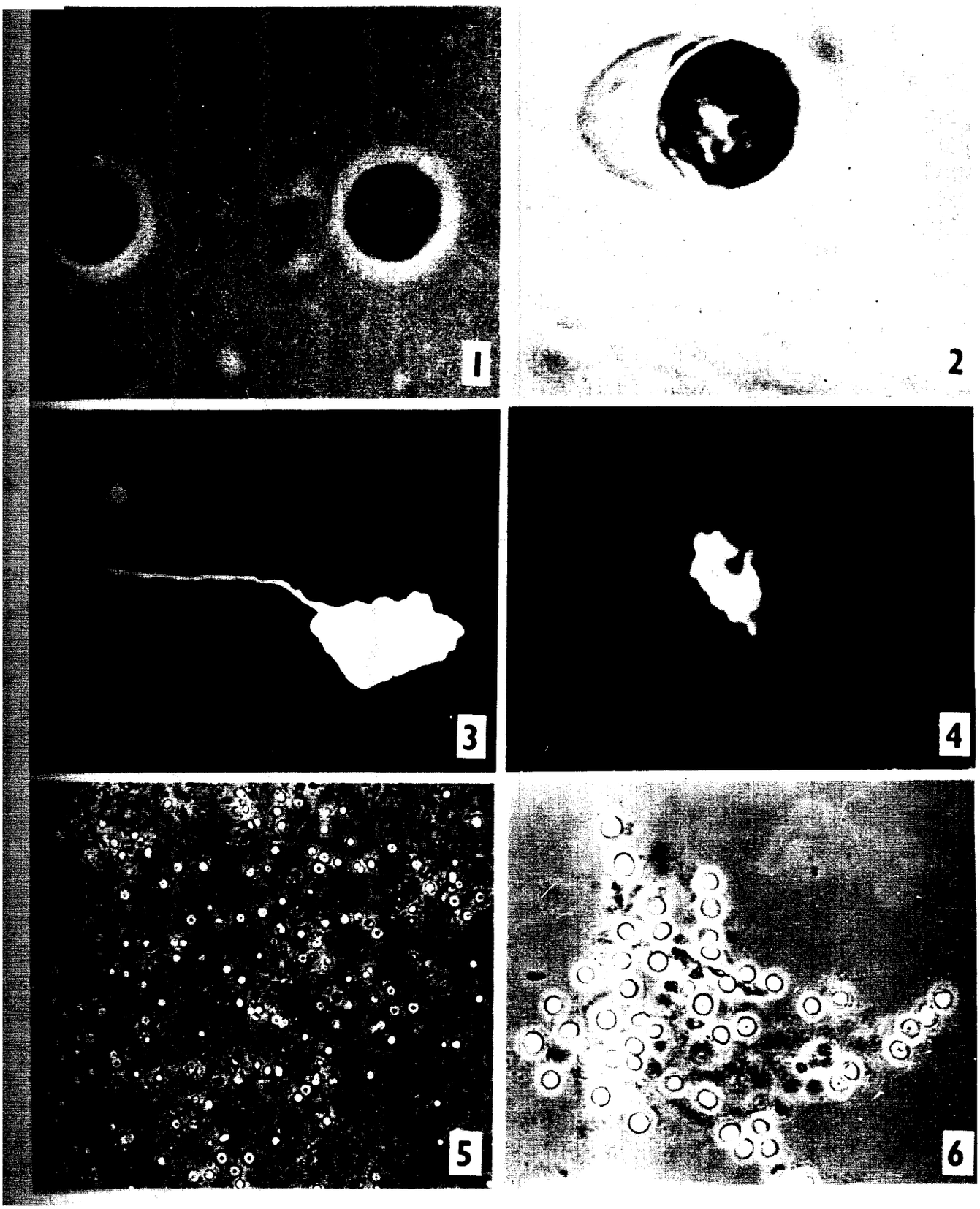
Fig.2. Older motile stage showing the mucilage envelope (x 1500).

Fig.3. Scanning electron micrograph of young motile cell with stretched flagella (x 3500).

Fig.4. Scanning electron micrograph of young motile cell with the flagella bent upon itself (x 3500).

Figs.5–6. Reproductive phase in six month old culture showing dividing cells and cysts (Fig.5, x 320; Fig.6, x 640).

PLATE II



The single cup-like chromatophore is golden-brown in colour. This imparts the characteristic colour to the culture. At the posterior end of the cell, in the cup of the chromatophore lies a lump of leucosin. Pyrenoid is found as a rounded protrusion within the chromatophore. The single nucleus is displaced to the anterior end (Plate III).

The species shows phototactic response. A red coloured stigma is visible along the anterior edge of the chromatophore. There is a spherical body at the base of the flagellum that appears granular. A few membrane bound bodies occur randomly in the cytoplasm.

Asexual reproduction occurs in the young cultures by the division of the motile cells. In cultures that are over a month old, non-motile cells are found to settle down forming a mat-like layer at the bottom of the culture vessel (Plate II). These non-motile cells have been observed to divide into two or four cells that remain together before being released. These non-motile cells are sometimes surrounded by mucilage. Cultures that are still older, showed cyst formation. The large non-motile cell divides into two within the cyst. The cyst is almost ovoid in shape with smooth outer wall and the plug region raised above the surface (Plate III).

EXPLANATION OF PLATE III

(Figs.1-10, x 1500)

Figs.1-5. *Isochrysis galbana* (S.s.)

Figs.1-2. Young motile stage illustrating the variation in shape and position of the chromatophore. c, chromatophore; l, leucosin; n, nucleus; s, stigma; v, vesicles.

Figs.3-5. Reproduction in palmelloid phase.

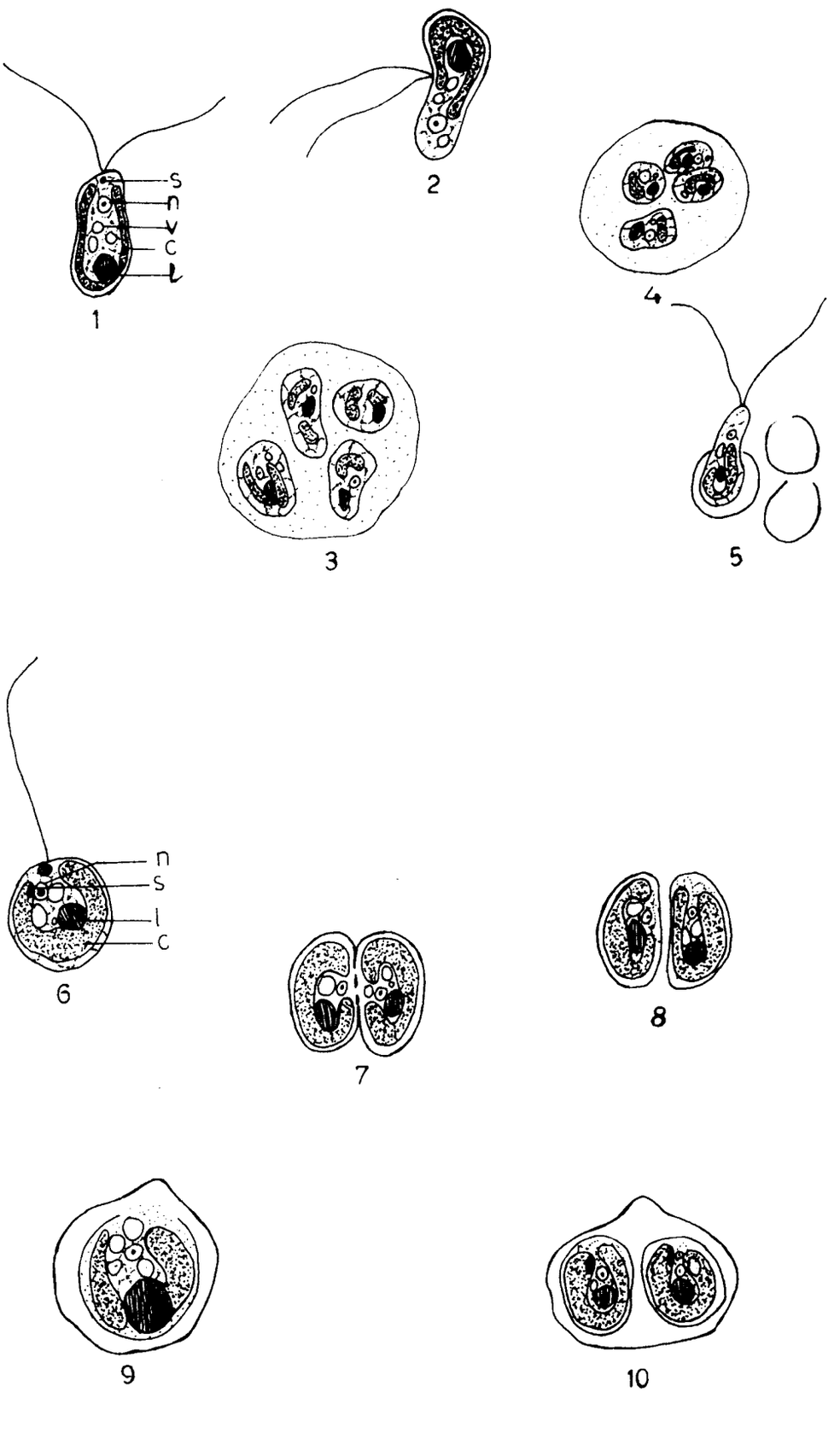
Figs.6-10. *Chromulina freiburgensis*.

Fig.6. Young motile cell illustrating the internal structure. c, chromatophore; l, leucosin; n, nucleus, s, stigma.

Figs.7-8. Cell division in the non-motile phase.

Figs.9-10. Cyst formation in older cultures.

PLATE III



Based on the observation that the present strain has a single cup-like chromatophore that lodges the pyrenoid, a large posterior leucosin body and a single flagellum, it has been assigned to the species Ca. freiburgensis.

Distribution: The present strain was obtained from the inshore waters of Cochin.

CHLOROPHYTA

PRASINOPHYCEAE

PRASINOCLADALES

Prasinocladaceae

TETRASELMIS Stein, 1878

Stein, F. 1878. Der Organismus der Infusions thiere. 3: 1.
West, G.S. 1916. J. Bot. London. 54: 3 (Platymonas).
Kylin, H. 1935. Kungl. Fysiogr. Sallsk. Lund. Forhandl., 5: 22.
Butcher, R.W. 1959. Fish. Invest. Ser.IV. 67-71.
Manton, I. and Parke, 1965. J. mar. biol. Ass. U.K., 45: 753-754.
Samuel, S. 1970. J. mar. biol. Ass. India. 12: 35-42.
Pett, S. 1971. Preselia. 43: 289-308.
Parke, M. and P.S. Dixon.1976. J. mar. biol. Ass. U.K., 56: 527-594.

Actively swimming green flagellates; ellipsoid or ovoid in shape. Flagella four in number arising from an

apical furrow. Distinct eye-spot and pyrenoid present. Reproduction is by longitudinal division of the protoplast. Cyst formation also occur.

The genus resembles Carteria, but with slight differences in the flattening of the cells, position of the eye-spot and in the nature of the cyst.

The genus has been used synonymous with Platymonas West. However, the evidence is inconclusive.

Distribution: Found in the inshore water of both temperate and tropical seas.

Key to the species

1) Ovoid body with reniform starch sheath and sub-median stigma ... I. carteriiformis Butcher

2) Body tapering posteriorly with 'U' shaped starch sheath and median stigma ... I. gracilis Kylin

I. carteriiformis Butcher, 1959

Butcher, R.W. 1959. Fish. Invest. Ser. IV, 67-71.
Samuel, S. 1970. J. mar. biol. Ass. India, 12: 35-42.
Parke, M. and P.S. Dixon. 1976. J. mar. biol. Ass. U.K., 56: 527-594.

Cells measure 12–16 x 8–11 x 7μ. It is broadly ovoid at the anterior end and tapers posteriorly. Four flagella arise from the shallow furrow in front. The length of the flagellae is almost equal to that of the cell.

There is a single, smooth, cup-shaped chromatophore that is pale green in colour. It becomes coarse and granular as the cell grows old. A large basal pyrenoid with reniform starch sheath is present.

Reproduction is effected by the longitudinal division of the resting cell. Thick walled cysts are observed in older cultures.

Distribution: The species is reported from the rock pools on the Isle of Cumbrae, Scotland. In India it was collected from the coastal waters of Cochin.

I. gracilis Kylin, 1935

Kylin, H. 1935. Kungl. Fysiogr. Sälsk. Lund. Förhandl. Bdv. Nr. 22. (P. gracilis)
Butcher, R. W. 1959. Fish. Invest. Ser. IV. 67–71.
Samuel, S. 1970. J. mar. biol. Ass. India, 12: 35–42.
Parke, M. and P.S.Dixon. 1976. J. mar. biol. Ass. U.K., 56: 527–594.

Cells cylindrical with rounded ends measuring 8–12 x 6.5–8 x 4.5μ. From the shallow apical furrow arise

four flagella of equal length. A long narrow sinus extents from the furrow to the base of the cell.

Single pale green chromatophore. Pyrenoid with a 'U' shaped starch sheath and median stigma.

Reproduction by division of the cells or by cyst formation.

Distribution: Reported from the ditches, salt marshes and pools in Kent-Isle of Wight and in the west coast of Sweden and India.

CHLOROPHYCEAE

CHLOROCOCCALES

Coccystaceae

* CHLORELLA Beijerinck, 1890

Beijerinck, W. 1904. Rec. Trav. Bot. Neerl., 1: 14-27.
Fritsch, F.E. 1935. Structure and Reproduction of the Algae. Vol. I, p.147.
Butcher, R.W. 1952. J. mar. biol. Ass. U.K., 31: 179-181.
Samuel, S. 1970. J. mar. biol. Ass. India, 12: 38-42.
Parke, M. and P.S. Dixon. 1976. J. mar. biol. Ass. U.K., 56: 527-594.

Coccoid green algae with spherical or ovoidal shape. Cells single or in groups. Reproduction is by the division of the cells into two to eight non-motile spores.

Distribution: The genus is predominantly fresh water in habitat. A few species are marine.

Key to the species

1) Cells oval, chromatophore bright green, granular, pyrenoid absent ... *C. marina* Butcher

2) Cells oval, chromatophore green, smooth, pyrenoid absent ... *C. ovalis* Butcher

3) Cells spherical, chromatophore green, granular, pyrenoid present ... *C. salina* Butcher

Chlorella marina Butcher, 1952

Butcher, R.W. 1952. J. mar. biol. Ass. U.K., 31: 179-181.
Samuel, S. 1970. J. mar. biol. Ass. India, 12: 35-42.
Parke, M. and P.S.Dixon. 1976. J. mar. biol. Ass. U.K., 56: 527-594.

The cells are oval measuring 8-13 x 5-8μ in size and is limited by a thin cell wall. The single chromatophore is bright green in colour, plate-like parietal in position and almost fills the cell.

During reproduction the mother cell divides to form 8-16 daughter cells.

Distribution: Reported from the offshore region of Quilon, west coast of India. It has been isolated from Port Erin, U.K.

Chlorella ovalis Butcher, 1952

Butcher, R.W. 1952. J. mar. biol. Ass. U.K., 31: 175–191.
Samuel, S. 1970. J. mar. biol. Ass. India, 12: 35–42.
Parke, M. and P.S. Dixon, 1976. J. mar. biol. Ass. U.K., 56: 527–594.

The oval or ellipsoid cells measure 3–5 x 5–10μ. There is a single chloroplast that is green, parietal and smooth filling the entire cell.

The mother cells divide asexually to produce 4–8 daughter cells.

Distribution: The species was first isolated from river Crouch, Essex. In India it is reported from the water samples collected from the sea off Cochin.

Chlorella salina Butcher, 1952

Butcher, R.W. 1952. J. mar. biol. Ass. U.K., 31: 174–180.
Samuel, S. 1970. J. mar. biol. Ass. India, 12: 35–42.
Parke, M. and P.S. Dixon. 1976. J. mar. biol. Ass. U.K., 56: 527–594.

Cells 5–7μ in size; bright green and spherical in shape. Chloroplast single, granular and saucer-shaped.

Pyrenoid is large and centrally placed. It is surrounded by starch sheath.

Reproduction is by the successive division of the cell contents into eight cells.

Distribution: The species was first isolated from the Conway tanks. The Indian report is from the Laccadive sea.

Chlorococcaceae

**Tetracystis Brown and Bold, 1964

Brown, R.M. and H.C. Bold. 1964. The University of Texas Publication No.6417.

Bold, H.C. and M.J. Wynne, 1978. Introduction to the Algae. Structure and Reproduction, p. 129.

The cells are spherical occurring singly or in groups. Chloroplast is parietal and cup-shaped. Pyrenoid is conspicuous.

Asexual reproduction occurs by the division of the cell into 2-8 daughter cells. Zoospore formation also does occur. Sexual reproduction occurs by biflagellate gametes.

Tetracystis sp.

Samuel, S. 1970. J. mar. biol. Ass. India. 12: 35-42.

**The genus is included in the family due to its resemblance to Chlorococcum. Parke and Dixon (1976) have not dealt with the genus. Bold and Wynne (1978) have placed it under Chlorosarcinales.

The ovoid or spherical cells measure about 13-16μ in size. The cell wall is thin while young and later becomes thick and striated. The chloroplast is parietal and almost fills the cell. The median pyrenoid is surrounded by starch grains.

The species reproduces asexually by cell division. Zoospores and aplanospores are also reported. Sexual fusion of isogametes have also been noticed.

The organism resembles Chlorococcum in appearance and life cycle.

Distribution: The species is reported from the inshore marine regions of the west coast of India.

3. MATERIAL AND METHODS

3.1. Isolation of nanoplankton

Methods of isolation and maintenance of microalgae in axenic culture are based on the classical methods used in bacteriology. Despite many attempts to refine and simplify the conventional techniques, the process of isolation of unicellular algae remains lengthy and tedious. The standard methods used in the literature with necessary modifications were used to isolate nanoplankters during the present study.

The water samples for the isolation of the nanoplankton were collected from the coastal region of Cochin ($9^{\circ}58'N$, $76^{\circ}15'E$) near barmouth. As this group of algae is not retained by the standard phytoplankton net (No.25 bolting silk net of mesh size 64μ) no net samples were taken. Instead, surface water samples were collected. Collections were made from April to August, 1981. The water samples were stored in polythene bottles and transported to the laboratory within one to two hours of collection.

In the laboratory, the samples were filtered through Whatman No.542 filter paper to remove the larger size fraction of phytoplankters. The filtrate was

transferred to culture flasks, enriched with nutrients and incubated for a few days under fluorescent lamps. The enrichment promoted rich growth of diatoms and dinoflagellates. The mixed cultures were then filtered again through filter papers. Filtration was quite effective in removing the diatoms and similar large sized plankton. The process was repeated several times so that only small flagellates and small coccoid algae were left in the mixture.

Further isolation was done by agar plating and dilution technique. 1.5% agar was prepared in Miquel's medium and inoculated with the algal mixture. These were incubated for a week. Certain green and brown growths of algae were observed on the agar plates following incubation. These were transferred to liquid medium. However, agar plating method was not found to be effective for the isolation of flagellates.

Dilution technique was found to be quite successful for the isolation of flagellates. Culture medium (Miquel's medium) was taken in a series of test tubes and each inoculated with a drop of algal mixture. These were examined under the microscope every fourth day and the successful cultures were diluted. The process of successive

dilution was done repeatedly. By this method two unialgal cultures of micro-flagellates which were not available in the regular stock cultures of the Institute were obtained.

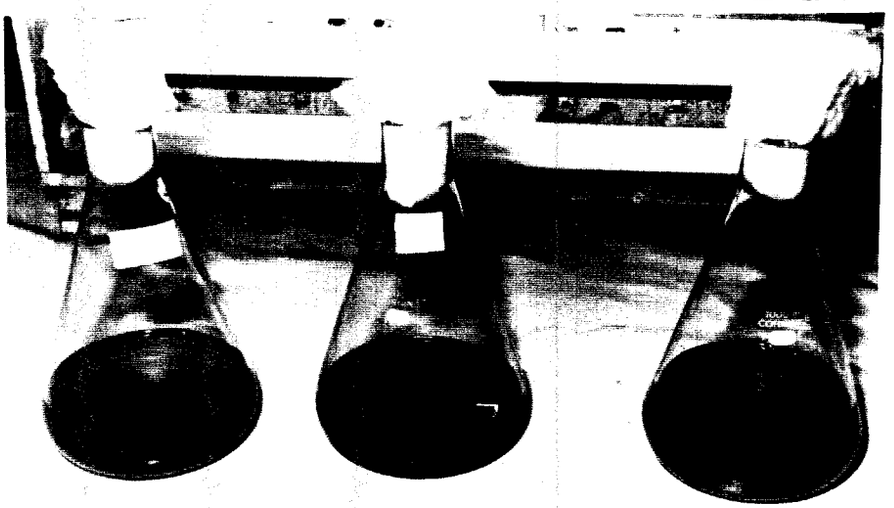
The new isolates were identified to be Chromulina freiburgensis Doflein and Isochrysis galbana Parke forma nova. The morphological features and the physiological characteristics were used for the identification of these flagellates. Live specimens were examined under the microscope to study the pattern of movement and the internal organisation. Size measurements were taken from the live specimens. The nature and number of the flagellae could not be ascertained from the light microscopic observation. So the specimens were scanned in Scanning Electron Microscope (PSEM 501). For SEM study the cultures were fixed in 2% osmium tetroxide in Millonig's buffer for one hour at low temperature. The fixed cells were washed with de-ionized water. A drop of the concentrated culture was transferred to a glass substratum and dried in dust free atmosphere. The dry samples were coated with gold and examined in the SEM.

The two new isolates were maintained in the laboratory to study the physiological characteristics. From the stock cultures maintained in the CMFRI culture

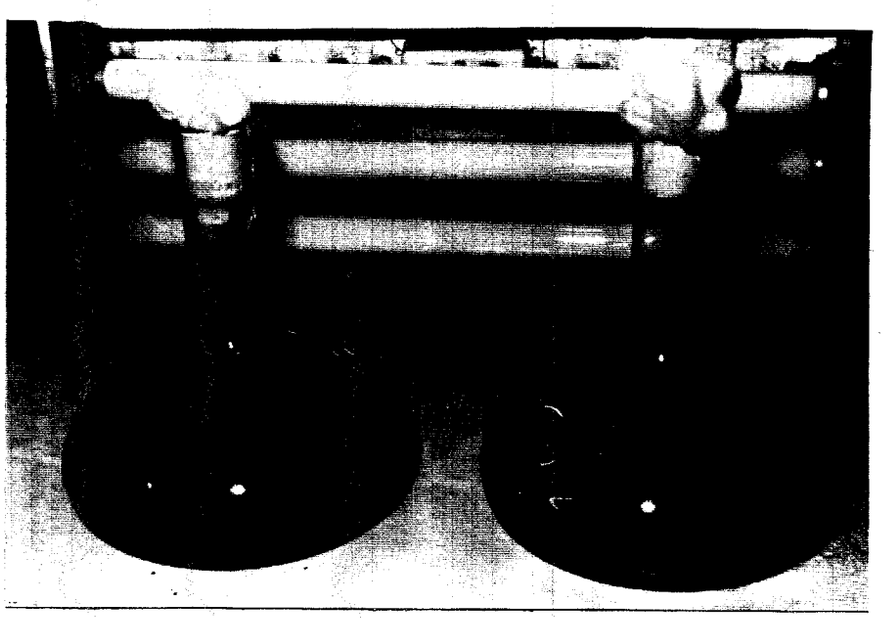
EXPLANATION OF PLATE IV

1. Stock cultures of *C. freiburgensis*, *S. salina* and *I. galbana* (C.s.)
2. Cultures of *S. salina* grown in Haffkine flasks.

PLATE IV



1



2

collection, two species: Synechocystis salina Wislouch and Tetraselmis gracilis Kylin were also used for making comparative studies (Plate IV).

3.2. Culture conditions

The nanoplankters were grown in enriched sea water as batch cultures. Sea water collected from offshore was allowed to age in carbuoys before preparing the culture medium. The sea water was filtered through Whatman GF/C filter paper; enriched with nutrients and autoclaved. The cool sterilised medium was equilibrated with filtered air for 24 hours and transferred to sterile culture flasks. Care was taken to keep the bacterial contamination low. However, the cultures were not totally bacteria free.

Different nutrient solutions like Miquel's medium (Miquel, 189), Erd Schreiber solution (Schreiber, 1925) and Walne's medium (Walne, 1974) were used to culture the plankton. However, after a few experiments the standard media were modified based on the observations. The composition of this modified Miquel's medium is given below:

Solution A

Potassium nitrate (KNO_3)	...	20 gms
Distilled water	...	100 ml

Solution B

Sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$)	...	2.5 gms
Distilled water	...	100 ml

Solution C

Calcium chloride ($CaCl_2 \cdot 6H_2O$)	...	4 gms
Ferric chloride ($FeCl_3$)	...	2 gms
Con. Hydrochloric acid	...	2 ml
Distilled water	...	98 ml

Solution D

Disodium ethylene diamine tetra acetic acid (EDTA)	...	300 mgs
Manganese chloride ($MnCl_2 \cdot 4H_2O$)	...	12 mgs
Zinc chloride ($ZnCl_2$)	...	1.5 mgs
Cobaltous chloride ($CoCl_2 \cdot 6H_2O$)	...	3 mgs
Copper sulphate ($CuSO_4 \cdot 5H_2O$)	...	0.12 mgs
Ortho Boric acid (H_3BO_3)	...	60 mgs
Distilled water	...	100 ml

Culture medium

Solution A	...	.9 ml
Solution B	...	0.5 ml
Solution C	...	0.5 ml

Solution 3	...	0.25 ml
Sea water	...	1000 ml
Tris buffer	...	50 mgs

The salinity of the sea water used to prepare the medium was 15–20‰ for the culture of *C. salina* and *I. gracilis* while *C. freiburgensis* and *I. galbana* (C.s.) were grown at 30–35‰ salinity.

The stock cultures and experimental cultures were grown in one litre conical flask or three litre Haffkine flask made of corning glass. The flasks were plugged with non-absorbent cotton. The cultures were placed on shelves illuminated with fluorescent tubes giving an illuminance of 20,000 lux on a 10:14 light-dark regime. This light condition was used for all the experiments unless specified. The ambient temperature was 24°C at night hours to 35°C at noon in summer months.

The cultures were not aerated. Once a day, the flasks were swirled manually to give three to four rotations of the suspension. The planktors grew well without settling for about a month; but the older cultures showed the tendancy to settle down. This was avoided by using culture tubes that could be aerated from below.

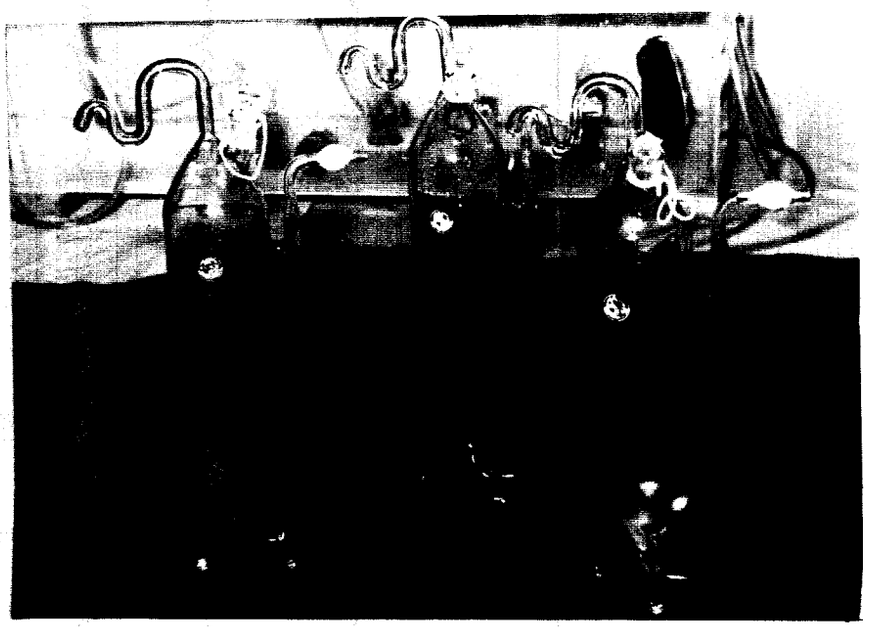
The culture tube used for the purpose was a modified version of the one used by Prof. Steemann Nielsen in his laboratory (Plate V). It consists of a tube of diameter 7.5 cm and 500 ml capacity, ending in a conical bottom which is drawn out into a narrow tube of diameter 1.5 cm. The narrow tube recurves and runs parallel to the wide tube and ends in a bulb that opens out. The wide tube is provided with an outlet fitted with glass stopper. This facilitates inoculation and sampling. The upper end of the tube narrows into a curved tube. The culture tube is also provided with three supports at the base. Sterile air is let in through the narrow tube. The curved tube favours the exit of the air as well as prevents the bacterial contamination.

More controlled conditions of growth were obtained by immersing the culture tubes in a temperature bath (Plate VI). The temperature bath consisted of a Perspex tank of 100 litre capacity filled with water and heated by an immersion heater. The heater was connected to a Jumo thermometer through a thermostat unit. The water in the tank was kept in circulation so that heating was uniform. This set up could be used to attain as well as maintain temperatures at 30°, 35°, 40° and 45°C. The heating system could not be used for lower temperatures. This was accomplished by adding ice to the tank. The entire

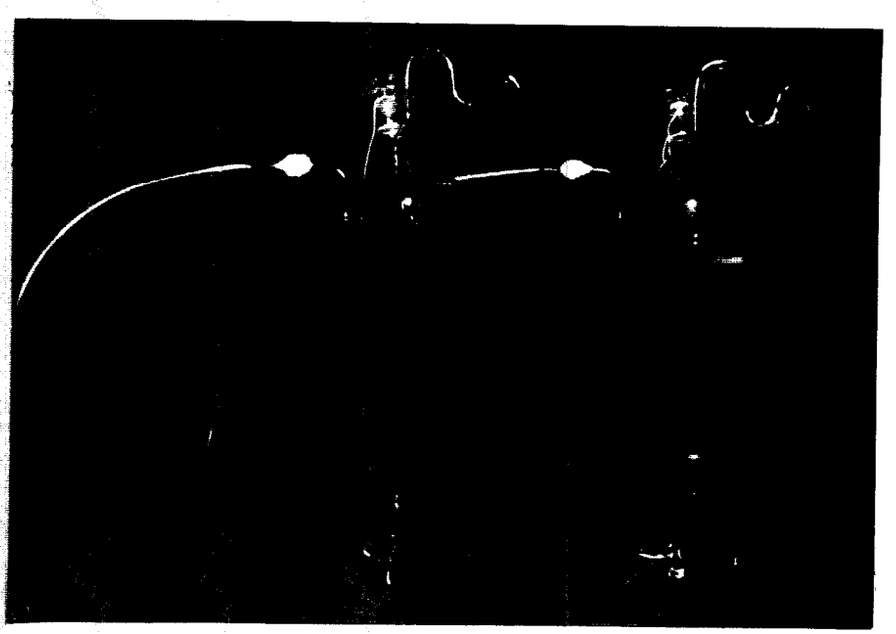
EXPLANATION OF PLATE V

odified culture tubes for growing axenic cultures of nanoplankton in the laboratory.

PLATE V



1

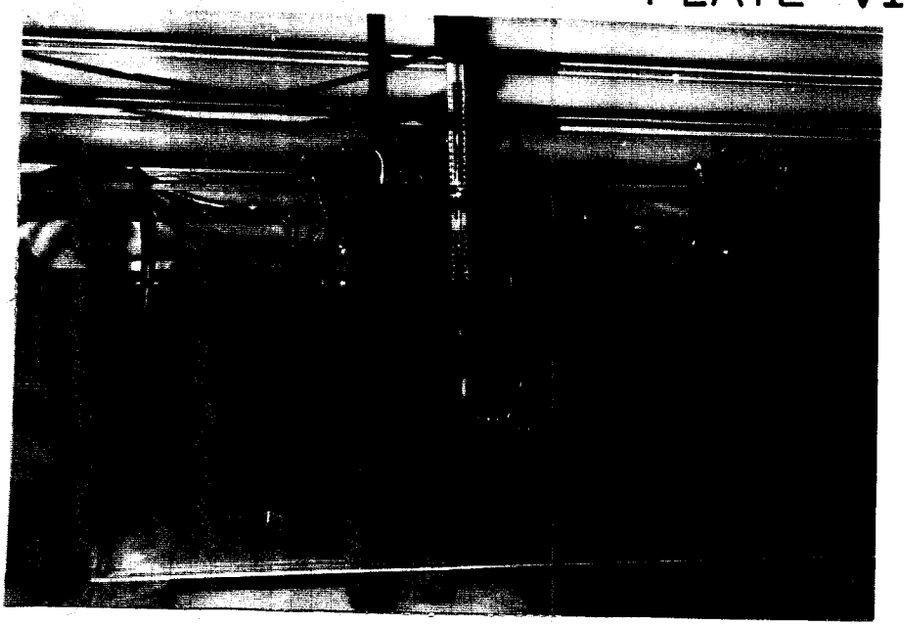


2

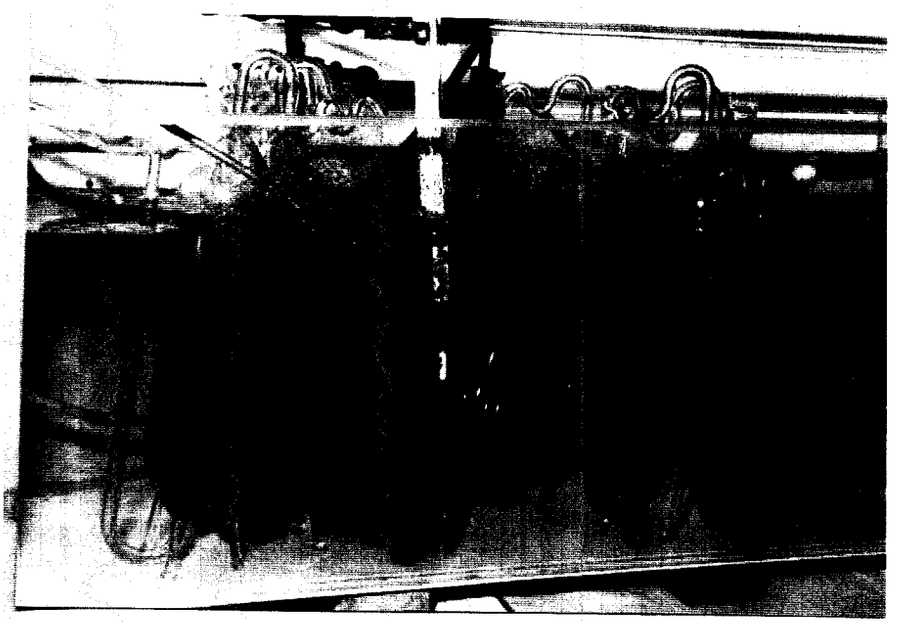
EXPLANATION OF PLATE VI

Experimental set up for controlling ambient temperature for nanoplankton culture.

PLATE VI



1



2

set up was illuminated from above as in the other experiments.

Whenever necessary the incident light also was controlled by using appropriate filters to cut off the light.

3.3. Growth measurements

3.3.1. Determination of cell number

Aliquots of the culture (5–10 ml) were taken after thorough mixing and fixed in Lugol's iodine. The cells were counted with a previously calibrated Haemocytometer.

The efficiency of growth was calculated as growth rate or doublings per day (k) given by the equation (1) (Epoley and Strickland, 1968).

$$k(\text{division/day}) = \frac{3.32}{t-t_o} (\log_{10} n_t - \log_{10} n_{to}) \quad \ldots \text{ eq.1}$$

where time is in 24 hour day and $3.32 = \log_2 10$.

The instantaneous rate of growth or the specific growth rate (μ/day) was computed from 'k' following the equation given by Goldman, 1979 (eq. 2).

$$\text{doublings per day} \times 0.693 = \mu \quad \ldots \text{ eq.2}$$

The mean generation time (t_g) was calculated from eq.3 (Strickland, 1960).

$t_g = \frac{0.7}{k}$ where k = relative growth constant/hr =

$$\frac{\ln n_t - \ln n_0}{t}$$

3.3.2. Determination of the physiological activity

The rate of photosynthesis and the amount of the pigments were used as indices of physiological activity.

The rate of photosynthesis was determined by the carbon-14 technique (Steemann Nielsen, 1952; Steemann Nielsen and Jensen, 1957). The cultures were diluted about ten times and taken in 60 ml bottle. Dilution was necessary to prevent any shading effect and also the self absorption of the beta rays on the filters. The ^{14}C was added in the form of Sodium bicarbonate at pH 9.0. The isotope was supplied by BARC, Bombay. 1 ml of the hot solution having an activity of 5μCi was added to each sample. The operations were done in subdued light. Dark bottle controls were also kept simultaneously. The hot samples were incubated for two to four hours. The incubation was done in a rotary incubator illuminated by a set of fluorescent lamps or they were left alongside the cultures for incubation. All samples had replications.

The incubated samples were filtered immediately through Millipore HA filters (0.45μ). The filter papers

were rinsed with filtered sea water to remove any adhering inorganic ^{14}C and dried in desiccator over silica gel.

The activity of the hot samples was determined with the aid of Geiger Counter (GCS 16, EC) of efficiency 3.2%. The rate of production was expressed as mg C/litre/hr.

$$\text{Rate of production} = \frac{\text{net activity x carbon dioxide}}{\text{added activity x hrs of incubation}}$$

where net activity was determined and added activity was computed by the biological method of Steemann Nielsen, 1965 and compared with the Liquid Scintillation technique of Watts and Scott, 1961 (Nair, 1966).

The amount of carbon dioxide was determined by a double titration method using phenolphthalein followed by methyl orange titration (APHA, 1980).

The light and dark bottle technique of Gaarder and Gran (1927) was also used to determine the physiological state of the cultures. Diluted cultures were incubated for 6-8 hours in bottles of 125 ml capacity. The oxygen content of the initial, dark and light bottles were determined by the Winkler titration. The oxygen values were converted to their carbon equivalents.

$$\text{Production (mg C)} = \frac{O_2 \text{ ml} \times .536}{P.Q.}$$

where P.Q. = Photosynthetic quotient = 1.25

The amount of chlorophylls and carotenoids were determined by spectrophotometric analysis of the acetone extracts applying the equations of Parsons and Strickland, 1963 and Lorenzen, 1967.

50–100 ml of the cultures were filtered through Millipore HA filters. 1–2 drops of 1% Magnesium carbonate was added to the samples while filtering. The pigments were extracted by adding 10 ml of 90% acetone for each filter. The extraction was carried out at low temperature for about 20 hours. The extracts were centrifuged and the extinction of the clear solution was measured by spectrophotometer - Unicam (SP 500) and (SP 8650).

3.4. Organisation of individual experiments

3.4.1. Growth and activity of Chromulina freiburgensis, Isochrysis galbana (C.s.) and Synechocystis salina in batch culture.

Healthy cultures of C. freiburgensis, I. galbana (C.s.) and S. salina were inoculated into modified Miquel's medium taken in Haffkine flasks. The culture volume was restricted to two litres. Duplicates were kept for each species. The cultures were illuminated artificially at ambient temperature.

The cultures were maintained for 30 days. Aliquots were withdrawn on alternate days to determine the cell number, pigment content and physiological activity (described in 3.3).

3.4.2. Pigment composition of C. freiburgensis, I. galbana (C.s.), S. salina and I. gracilis.

Unialgal cultures of the species were grown as in the above experiment. The cultures were harvested during the exponential phase.

Preparation of the pigment extract

The algae from 1 to 2 l ml of cultures were harvested by filtering through Millipore HA filters and the pigments extracted with 1 ml of 90% acetone. The pigment extract was immediately transferred to an equal volume of diethyl ether. The mixture was taken in a separating flask and shaken with about ten times its volume of 1% sodium chloride. The pigments migrated to the ether layer. The ether phase was repeatedly washed with sodium chloride solution to remove any excess acetone and water soluble impurities. The ether layer was concentrated under a stream of nitrogen. Condensed water was removed from the samples by centrifugation.

Preparation of silica gel thin-layer plates

Silica gel was made into a slurry with distilled water (1:2 w/v) and coated on to chromatographic plates (20 x 20 x 0.2 cm) at a thickness of 0.25 mm using an applicator. The coated plates were air-dried. The plates were activated at 100°C for half an hour and stored in desiccator. The plates were used within a week of preparation.

The concentrated pigment extracts were applied to the TLC plates using a capillary spotting aid. Along with the samples standard chlorophyll a (supplied by Sandoz Ltd., Switzerland) was also developed. The extracts were dried over the TLC plates in a gentle stream of nitrogen. The entire process was carried out in diffused light.

Chromatographic tanks were lined with black paper and equilibrated with the appropriate solvent mixture before the plates were developed. The solvent system used was n-propanol: light petroleum (60°-80° fraction) (2.5:97.5; v/v) for the first dimension and chloroform: light petroleum: acetone (25:75:0.5; v/v/v) for the second dimension. After the first run, the plates were dried in dark and then developed in the second dimension. The first run took 60 minutes to complete and the second run, 45 minutes.

The dry plates were observed under UV light to locate the pigments. The solvent front and the pigment spots were marked. The colour of the pigments also was noted. The R_f value of the pigments were computed. Chlorophyll a was identified based on the colour of the spot and R_f of the standard. The other pigments were identified with the help of the guidelines given by Jeffrey, 1963 and 1981.

3.4.3. Estimation of biochemical composition

The cultures of C. freiburgensis, I. galbana (C.s.), I. salina and I. gracilis were grown in modified Miquel's medium under artificial illumination with light-dark cycle at ambient temperature. Samples were taken after five days, ten days and twenty days of growth for analysis. The cells were collected by either centrifugation or filtration. The adhering NaCl crystals were removed by washing the cells with an isotonic solution of Ammonium formate. As the samples could not be analysed immediately they were deep-frozen. Six replicates of each sample were analysed. The cultures were not totally bacteria free. So the bacterial counts also were taken during sampling.

Cell number, dry weight and moisture content

The cell number was counted by Haemocytometer. The dry weight of the algae was determined by the standard

method of evaporating off the water content from filtered samples.

Millipore HA filters were washed with distilled water, dried and weighed. 50-100 ml of cultures and corresponding volume of media were filtered through filter papers of identical weight. After filtration the samples were rinsed with an isotonic solution of ammonium formate to remove any sodium chloride crystals retained. The papers were sucked dry, folded, blotted and weighed immediately.

The samples were dried to constant weight at 90±5°C; cooled and weighed. The weight of the filter papers were deducted to get the weight of the algae.

$$\text{Dry weight (\%)} = \frac{\text{Wt. of the dry sample}}{\text{Wt. of the wet sample}} \times 100$$

$$\text{Moisture content (\%)} = \frac{\text{Wet wt. - dry wt.}}{\text{Wet wt.}} \times 100$$

Carbohydrate

The carbohydrate content in algae is composed of cell wall polysaccharides and reserve carbohydrate in addition to intermediate sugars of the photosynthetic pathway. A good part of these algal polysaccharides break up into simple sugars on acid hydrolysis. These simple sugars can be estimated by Nelson-Somogyi method (Nelson, 1944; Somogyi, 1945).

The nanoplankton derived by the centrifugation of 25 ml of the culture was hydrolysed with 30% sulphuric acid in a waterbath. Hydrolysis was done for six hours to ensure complete breakdown of the polysaccharides. The samples were cooled and centrifuged. 1 ml of this clear solution was pipetted into a clean test tube and the arseno-molybdate reagent added. The absorbance of the coloured solution was measured at 530 nm using a spectrophotometer (Spectronic 20). Glucose was used as the standard.

Protein

The protein content of the nanoplankton cells was determined by Lowry's method (Lowry et al. 1951) using Folin-Ciocalteu reagent. The algal pellet obtained by centrifuging 250 ml of cultures was extracted with 80% ethanol. The protein precipitated was separated by centrifugation. The precipitate was dissolved in 1 N sodium hydroxide. It was again centrifuged to remove any remaining cell particles. The protein content in the extract was estimated by the Lowry's method. The optical density of the blue colour developed was measured at 530 nm using spectrophotometer (Spectronic 20). The standard graph was prepared using Bovine serum albumin.

Lipid

The lipids of algae comprise photosynthetic pigments - chlorophylls and carotenoids and other compounds like phospholipids and glycolipids. These are extractable by organic solvents. The method employed was that of Bligh and Dyer (1959).

Adequate amounts (25 ml) of the cultures were centrifuged and to the algal pellet added a mixture of chloroform and methanol (2:1 v/v). The extraction was done repeatedly with small volumes of the solvent for about 3-4 hrs. The mixture was transferred to a separating flask and shaken with distilled water. The lipid layer was stripped off, centrifuged to remove any cell particles and the solvent evaporated off in a waterbath. The weight of the lipid fraction was determined.

The level of carbohydrate, protein and lipid, in the nanoplankters was calculated as percent dry weight.

Isolation of bacteria

The standard dilution plate technique (Fraser and Schmidt, 1966) was followed for the enumeration of bacteria. Nutrient agar (Allen, 1953) prepared in filtered sea water was used as the medium for growing bacteria. The composition of the medium is given below:

Nutrient glucose agar

Glucose	... 5 gms
Bactopeptone	... 5 gms
Beef extract	... 3 gms
Agar	... 15 gms
Filtered sea water	... 1000 ml
pH	... 7.5–8.0

The bacterial plates were incubated for 96 hours and the counts were taken.

3.4.4. Estimation of the extracellular products of the nanoplankters.

The sterile media (500 ml modified Miquel's medium) were inoculated with approximately 10^4 cells of the unialgal cultures. The species under study were *C. freiburgensis*, *I. galbana* (C.s.), *D. salina* and *I. gracilis*. Growth conditions were the same as in the previous experiment. Sampling was done on the 4th, 8th and 16th day of growth.

The samples were diluted and incubated in triplicate with carbon-14 for two hours in the same conditions as the culture phase. After incubation the samples were filtered through Millipore HA filters. The filtrate was retained. The filters were washed with filtered sea water sucked dry and stored in desiccator. 10 ml of the culture filtrate

was acidified with 1 ml of N/10 HCl and the radioactive bicarbonate was removed by bubbling with air. The solution was then neutralized with 1 ml of the N/10 sodium hydroxide. The radioactivity of the samples was measured using Scintillation Counter (LSS 2B, EC).

Liquid Scintillation counting

The liquid scintillation system works on the photon emission and its subsequent conversion to electronic pulse, the former being released by certain compounds when bombarded by nuclear radiation.

Procedure

1. Determination of counting efficiency

The activity of the quenched standards of ^{14}C was determined by two-channel counting and the channels ratio (AB/AC) was computed. From this the counting efficiency was worked out as presented in table 1 given below.

The counting efficiency was plotted against channels ratio to obtain the quench correction curve (Plate VII).

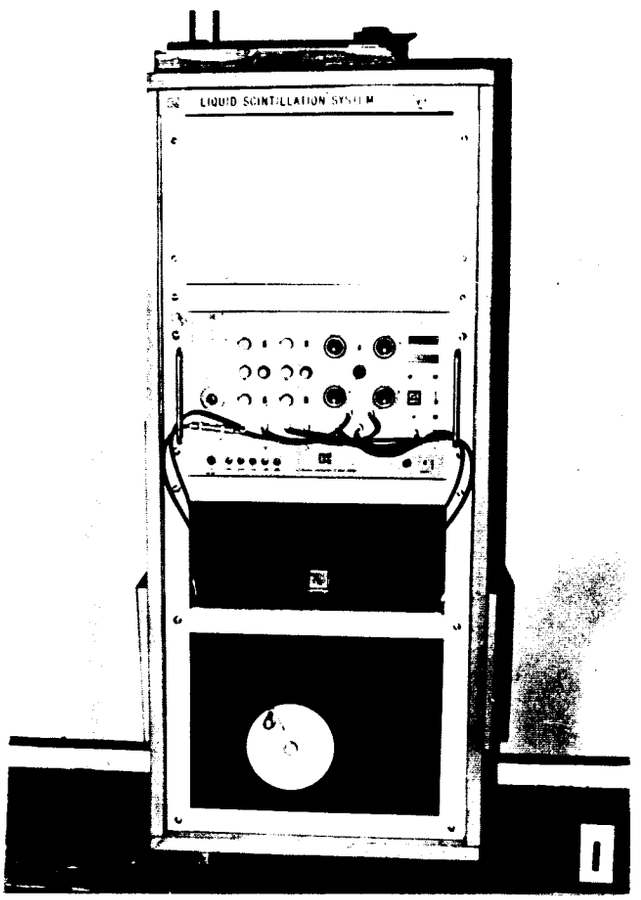
2. Standardization of ^{14}C ampoules

0.1 ml each of the radiocarbon was withdrawn from ampoules of 1 ml capacity and the cpm determined. The

EXPLANATION OF PLATE VII

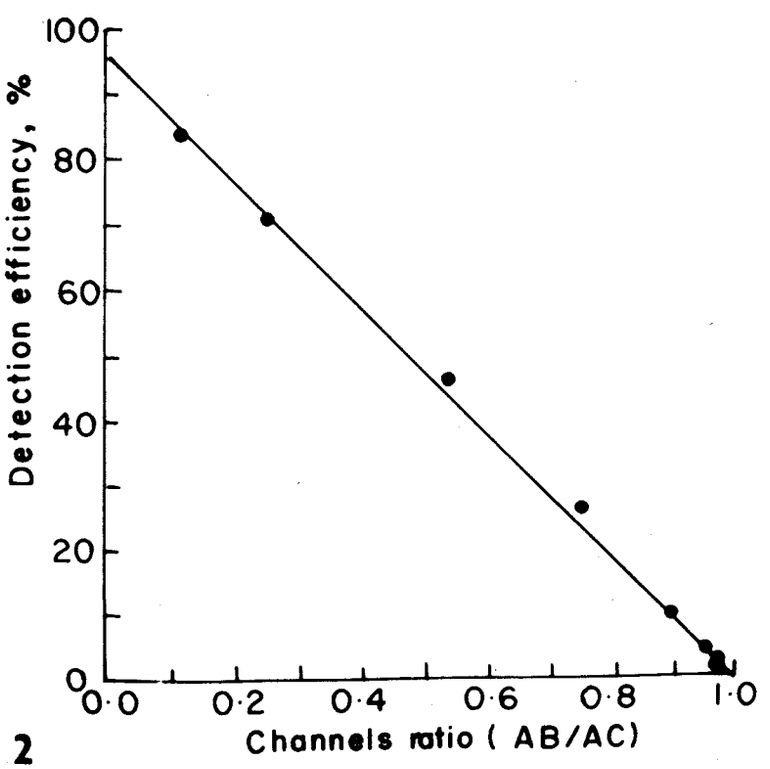
1. Liquid Scintillation Counting System used for the measurement of excretory products of nanoplankton.
2. Channels ratio versus Detection efficiency of the counting system.

PLATE VII



1

Channels ratio quench correction curve for the liquid scintillation counting of ^{14}C



2

Table. The count rate and channels ratio for the quenched standards of ^{14}C.

Quenched standard	C_1 or AC (cpm)	C_2 or AB (cpm)	C_2/C_1	Efficiency (%) $= \frac{C_1}{20,3\ldots} \times 1\ldots$
1	1,70,973	22075	0.12	84
2	1,45,99…	37559	0.25	71
3	96,367	514…4	.53	47
4	55,683	41536	0.74	27
5	21,671	19479	0.89	1…
6	9,39…	8968	0.95	4
7	4,398	42…	.97	2
8	2,5…3	2439	0.97	1

counting efficiency of these samples were determined from the quench correction curve and the absolute activity was calculated as μC/ampoule.

3. The activity of the filters was determined by liquid scintillation suspension counting (Krishnamurthy and Viswanathan, 1968). The filter paper was dissolved in 1 ml of acetone in a test tube, rinsed twice with dioxane and transferred to the scintillation vial containing polystyrine gel. The gel was prepared by dissolving 4.0 gms of polystyrine in 10 ml of dioxane scintillator. The composition of the dioxane fluor is given below:

PPO	:	4 gms
POPOP	:	50 mgs
Naphthalene	:	120 gms
Dioxane	:	1000 ml

The sample remained on the surface of the gel. This was fed into the counter and the counts for hundred seconds were taken.

Liquid scintillation solution counting was employed to measure the activity of the filtrate. 0.5 ml of the filtrate was taken in scintillation vial and 15 ml of scintillator was added to it. The vial was shaken to get

a homogeneous solution. The activity of this was counted for 100 seconds.

Unlabelled samples served as blank in each determination and these blank values were deducted from all sample readings. The absolute activities of the samples were determined from the quench correction curve.

3.4.5. Influence of salinity on growth

The nanoplankters under study were isolated from the coastal region of Cochin near the barmouth where the salinity fluctuates from brackish to strictly marine (Barbyshire, 1967). So the response of the nanoplankters to different salinity levels was studied in culture. The organisms used for the study were Chromulina freiburgensis, Isochrysis galbana (C.s.) and Synechocystis salina. The mother cultures were grown at salinity 34‰ which is often obtained in the sea water off Cochin. 1 ml of these cultures were inoculated into 50 ml of Modified Miquel's medium. Each alga was grown in three salinity levels 34‰, 24‰ and 14‰. Three replicates were kept for each treatment. The nine experimental cultures of each species were randomized completely to a uniform illumination of 2000 lux from white fluorescent tubes at ambient

temperature (Plate VIII). The design of the experiment is given below:

I	II	II
II	I	III
III	I	III

Treatment I : 14‰.

Treatment II : 24‰.

Treatment III : 34‰.

The lower salinities of 14‰ and 24‰ were obtained by diluting the sea water with distilled water. These were autoclaved and aerated for 24 hours before adding the nutrients. The pH was adjusted to 8.2 using Tris buffer.

The growth of the culture was followed for three weeks. The cell density and the amount of chlorophyll a was measured regularly. After three weeks the experiment was discontinued as it was found that the cell multiplication and the amount of chlorophyll had decreased considerably and the culture showed senescence.

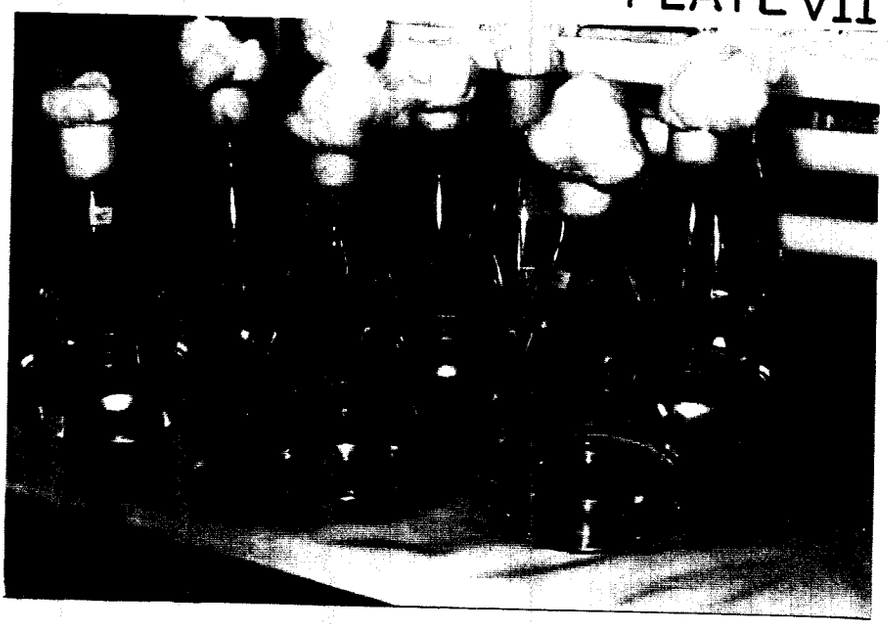
3.4.6. Effect of pH on growth

The pH of the sea water under normal conditions vary from 7.5 to 8.3. But factors like dilution, bacterial action and human activities are found to change this normal

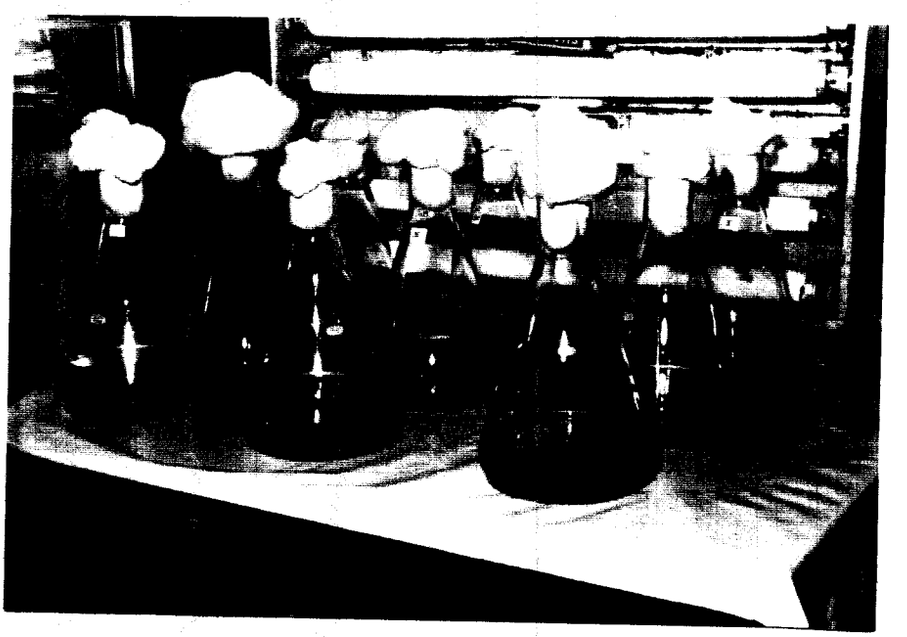
EXPLANATION OF PLATE VIII

The experimental set up of the cultures of *C. freiburgensis* (1) and *S. salina* (2) to test their tolerance to three different salinity levels.

PLATE VII



1



2

state. The response of the phytoplankton also may vary to the changed pH. This was tested in cultures of nanoplankton using four different pH levels. The experiment was done on similar lines as the previous one (3.4.5) using the same organisms.

Modified Miquel's medium was prepared in sea water of salinity 34‰ and pH 8. This medium was acidified to obtain the different pH levels: 6.5, 7.0 and 7.5. 500 ml of these media taken in one litre corning flasks were inoculated with the cultures that were growing at a pH 8. Each treatment was done in triplicate. The treatments were completely randomized as shown below.

IV	III	I	III
IV	II	II	IV
I	II	III	I

Treatment I : 6.5
Treatment II : 7.0
Treatment III : 7.5
Treatment IV : 8.0

The cultures were observed till they reached the stationary phase. The cell density at the peak growth

phase (4 days) and the final yield (on 16th) were compared. Simultaneously the amount of chlorophyll *a* was also measured.

3.4.7. Effect of cell concentration on the rate of carbon fixation.

While estimating the rate of photosynthesis many factors are reported to interfere with the accuracy of the measurements. The density of the phytoplankton is one such factor. This was tested in cultures of the nanoplankters - *C. freiburgensis*, *I. galbana* (C.s.) and *D. salina* so that the optimum cell density that must be used for the photosynthetic rate measurements could be fixed.

The cultures of the three organisms were grown in modified Miquel's medium. The flagellates were provided with medium of salinity about 34‰ and the blue-green algae were grown at a salinity of 15‰. The culture conditions were similar to those described in 3.2. The cultures were allowed to grow and multiply for a week so that sufficient cell population was built up. *C. freiburgensis* grew upto a density of 14.4×10^5/ml; *I. galbana* (C.s.) to a concentration of 6.3×10^5/ml and *D. salina* to 33×10^5/ml.

The samples of these cultures were withdrawn and diluted with the medium to get different densities. These were incubated in triplicate with carbon-14 for two hours in the same culture conditions as the culture. After incubation the samples were filtered through Millipore HA filters and the activity was measured using the Geiger counter.

3.4.8. Influence of light on growth and activity of nanoplankters.

Batch cultures of the three nanoplankters were raised in modified Allquel's medium under ambient temperature and 2[illegible],000 lux light. Two sets of duplicate cultures were maintained. One set was illuminated continuously from cool-white fluorescent lamps, while the other set was subjected to light-dark cycle of 10:14 hours. The increase in the cell population was studied for a month. The final yield and growth rate were compared for both the light conditions.

During the logarithmic phase of growth of the constantly illuminated cultures aliquots were withdrawn to study their photosynthetic activity at different light intensities. The samples were diluted suitably and incubated with ^{14}C in 60 ml bottles placed on a rotary incubator.

The rotary incubator used for the purpose was similar to that described by Steemann Nielsen and Hansen (1961) and Nair (1974). The sample bottles were fixed on the rotating wheel of the incubator and covered with appropriate light filters simulating the different light conditions. All samples had duplicates. The rotating wheel was lowered into the tank filled with water. The temperature of the water was 30°C. It was illuminated by a bank of ten white fluorescent tubes (20 watts each) and a battery of three incandescent bulbs (100 watts each). These together gave an illuminance of 68 x 10^{15} quanta x cm^{-2}_{x} sec^{-1} i.e. 28.80 m watt x cm^{-2} or 4,000 lux.

The samples were pre-exposed to the new light regime for one hour before the addition of ^{14}C. The experiment proper took two hours of incubation after which the samples were filtered and the activity measured in Geiger counter.

3.4.9. <u>Influence of temperature on growth and viability of nanoplankters</u>.

The nanoplankters were exposed to a range of temperatures from 5°C to 45°C to study the effect on motility and viability in cultures. Cultures growing at room temperature were exposed to the low temperature of a refrigerator (5°C). Samples from this were recultured into

fresh medium at room temperature every week. This was done for about six months. The cultures were similarly exposed to higher temperatures (upto 45°C) and their motility and viability were observed.

The growth rate of these nanoplankters was determined for the temperatures 20,25,30,35 and 40°C. The cells were inoculated into about 500 ml of the culture medium taken in the culture tubes (described in 3.2). The culture tubes were immersed into the temperature bath maintained at the required temperature. Each culture had its duplicate. The cultures were aerated constantly. The whole set up was illuminated from above with five fluorescent tubes of 40 watts each (Plate VI). The heating system with the immersion heater and the Jumo thermometer functioned to acquire and maintain temperatures above 30°C. The lower temperatures (20 and 25°C) were obtained by adding ice to the water in the tank. The temperature in the water bath was kept uniform by keeping the water in circulation by means of aerators.

The growth rate of the plankters was studied for six days taking the cell counts daily at each temperature. The results of the duplicate experiments were averaged.

During the exponential phase of these cultures, aliquots of 10 ml were withdrawn from each, diluted and the rate of photosynthesis determined by the ^{14}C method.

3.4.1.6. Kinetics of utilization of macronutrients (nitrate and phosphate).

Nitrate and phosphate are the major nutrients that contribute to the culture media. So the influence of different concentrations of these nutrients on growth rate of the nanoplankters was studied in batch cultures of limited volume.

To examine growth at different nitrate levels the cultures were first depleted of nitrate. This was done by growing cells of *C. freiburgensis*, *I. galbana* (C.s.) and *D. salina* in modified Miquel's medium devoid of added nitrogen source for a period of 10 days. The sea water used for preparing the medium contained only 1.5 µg at NO_3-N/litre.

Cells from the N-depleted cultures were then inoculated into flasks of sterile culture medium and various amounts of A.R. Potassium nitrate was added to each flask to yield concentrations from 2.6 to 3 µg-at.N/litre. Duplicates were maintained for each treatment. The cultures were grown for six days under artificial illumination and

ambient temperature. The cell counts were taken daily. The amount of NO^3-N in the medium was measured at the beginning of the experiment. The cultures were filtered and the filtrate was used for the determination of nitrate. The KO_3 in the medium was first reduced to KO_2 and determined colorimetrically employing the method described by Strickland and Parsons (1968).

A similar experiment was conducted to find out the amount of the phosphorus required by these organisms in culture. The source of phosphorus was $NaH_2PO.2H_2O$. Phosphorus starved cultures were inoculated into medium containing different concentrations of phosphate. The initial levels of dissolved phosphate was determined spectrophotometrically for each treatment following the methodology of Murphy and Riley (1962). As in the case of nitrate the cell counts were taken for six days. The mean of the duplicate experiments were taken and the half saturation constants for growth was computed using the relation $S = \mu max\ (S/\mu) - K\mu$.

R E S U L T S

4.1. Chromulina freiburgensis

Growth and activity in batch culture

Cultures of Chromulina freiburgensis were grown in modified Miquel's medium to study its growth kinetics (3.4.1). Actively growing cells were inoculated into fresh medium to give a concentration of 350 cells/ml. The cell number was counted after 48 hours to find whether there was any noticeable increase. It was found that the cell numbers increased gradually in the first 48 hours; between the 2nd and 4th day, cell divisions were rapid and the cell population increased four times. In the following days of observation, the cell concentration increased gradually, reaching a density of 1.4 million cells/ml in 30 days. The highest growth rate was found to occur between the 2nd and 4th day. The phase of declining growth rate was found to be between 4th and 12th day. After 12 days, the stationary phase began. The cell numbers remained stationary till the 30th day when the experiment was discontinued (Fig.1A). The highest growth rate recorded for the species during this study was 2.66 divisions per day corresponding to a mean generation time of 9 hours. The relative growth constant per hour was 0.079 (Table 2).

Table 2. Cell concentration and growth rate of three nanoplankters grown at a light intensity of 2[illegible],000 lux with a light-dark cycle of 1[illegible]:14 hours at ambient temperature.

Culture	Initial inoculum cells/ml	Highest density attained cells/ml	Maximum doublings 'k'	Minimum generation time 't_g'	Relative growth constant per/hr
Chromulina freiburgensis	33[illegible]	14,25[illegible]	2.66	9	0.079
Isochrysis galbana (C.s.)	35[illegible]	6,900[illegible]	1.43	16	[illegible].043
Synechocystis salina	1440	4,75,[illegible]	2.74	9	0.079

The live cultures were observed under the microscope to study their motility. The motility of the cells in the aged cultures was comparatively less. Many cells were non-motile and sank to the bottom of the culture flask forming a thin layer at the bottom. The colour of the young culture was golden-yellow. As the culture grew old, the yellow colour gave way to an orange-red tint.

The pigment content of the culture remained low immediately after inoculation. Between the 2nd and 4th day the amount of chlorophyll *a*, *c* and carotenoids rose up steeply. After the 4th day the increase in the amount of the pigments was gradual. It reached stationary phase by the 12th day

and this level was maintained till the 30th day (Fig.1B). However the amount of pigments per unit number of cells was highest during the first week of culture (Table 3). The maximum values for chlorophyll a, c and carotenoids was 0.245, 0.189 and 0.526 µg/10^6 cells. Chlorophyll a was always greater than chlorophyll c while carotenoids dominated over the chlorophylls. The amount of the carotenoid pigments was about double that of chlorophyll a till the 24th day of culture; but later the proportion of carotenoids became ten times that of chlorophylla a (Table 3).

The metabolic activity of the flagellate was measured in terms of the oxygen exchange. The rate of production was observed to increase during the period upto 10 days (Fig.2A); the rate of production decreased on 12th and 14th and remained more or less stationary for the rest of the period. The highest production was 12.5 µgC/10^6 cells/hour (Gross production) and 4.0 µgC/10^6 cells/hour (net production). After the 10th day, the rate of net production decreased to 1.5 µgC/10^6 cells/hour.

As per the ^{14}C uptake the photosynthetic activity increased upto 16 days and further declined (Fig. 2B). The highest production was observed to be 6.0 µgC/10^6 cells/hour.

Fig.1A. Increase in cell number of *Chromulina freiburgensis* during growth in batch cultures

Fig.1B. Variation in the amount of photosynthetic pigments of the above species during growth in batch culture.

Fig. 1.

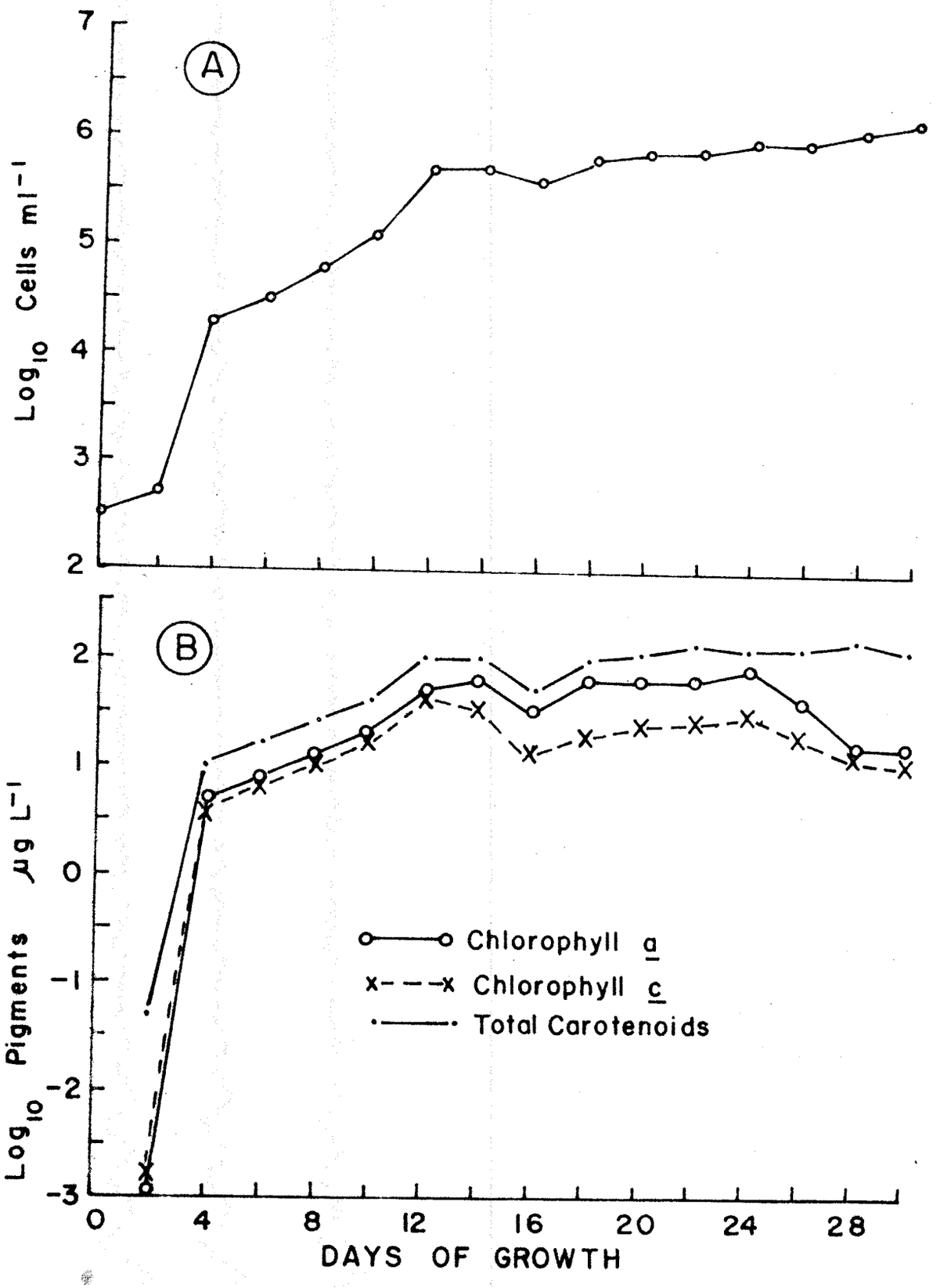

A
B
7
6
5
4
3
2
Log10 Cells ml-1
2
1
0
-1
-2
-3
Log10 Pigments µg L-1
Chlorophyll a
Chlorophyll c
Total Carotenoids
0
4
8
12
16
20
24
28
DAYS OF GROWTH

Fig.2A. Rate of production of organic carbon by *C. freiburgensis* for a growth period of 30 days in batch culture (measured by the oxygen technique).

Fig.2B. Rate of production of organic carbon by the above species for a growth period of 30 days in batch culture (measured by the ^{14}C technique).

Fig. 2.

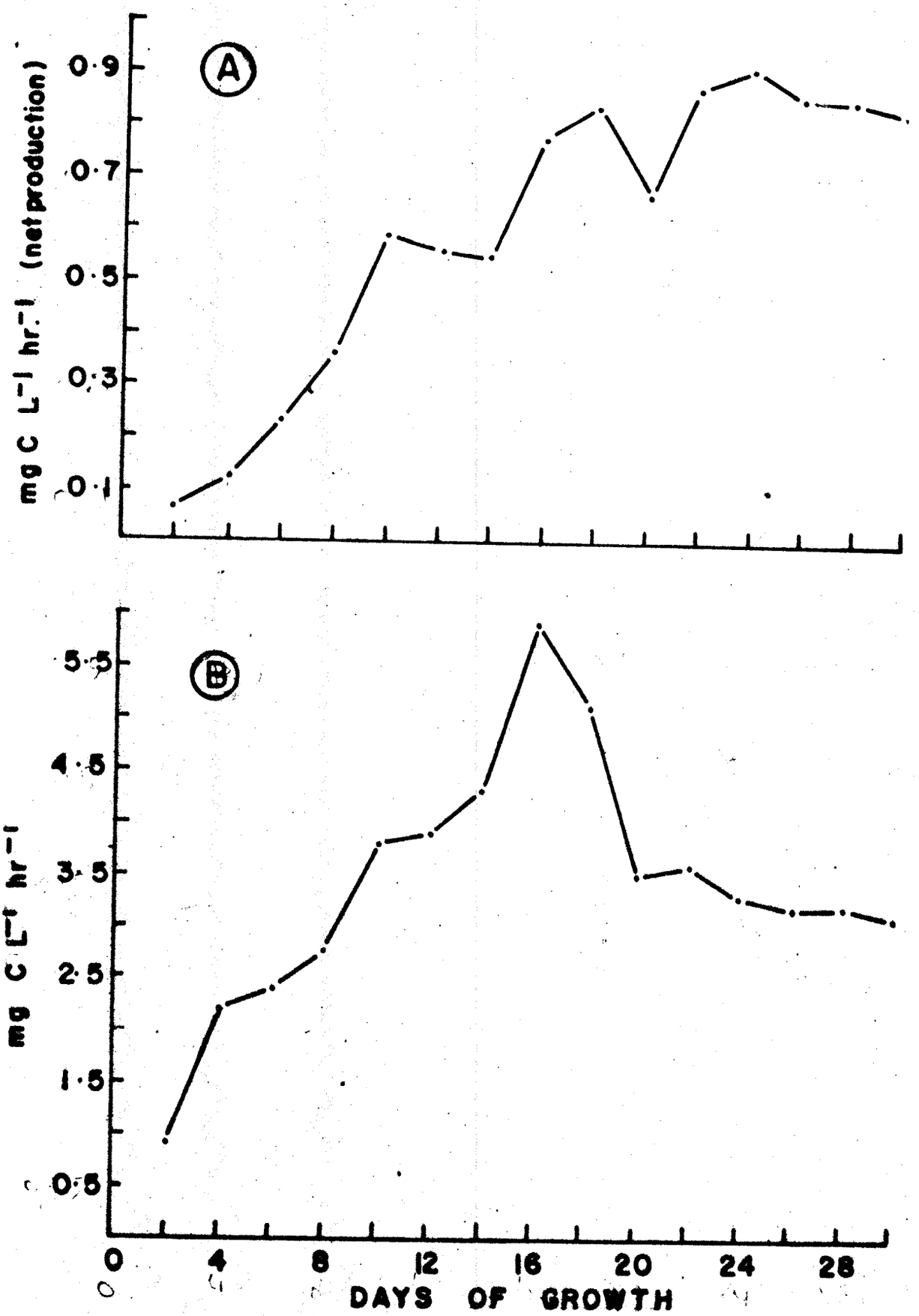

A
0·9
0·7
0·5
0·3
0·1
mg C L⁻¹ hr⁻¹ (net production)
B
5·5
4·5
3·5
2·5
1·5
0·5
mg C L⁻¹ hr⁻¹
0
4
8
12
16
20
24
28
DAYS OF GROWTH

Table 3. Variation in the amount of pigments expressed as $\mu g/10^6$ cells of *Chromulina freiburgensis* for a growth period of 30 days in batch culture

Age of culture (days)	Chlorophyll *a* $\mu g/10^6$ cells	Chlorophyll *c* $\mu g/10^6$ cells	Total Carotenoids $\mu g/10^6$ cells	Carotenoids/ Chlorophyll *a*
2	0.198	0.150	0.405	2.05
4	0.245	0.189	0.526	2.15
6	0.236	0.178	0.453	1.92
8	0.214	0.159	0.364	1.70
10	0.182	0.134	0.312	1.71
12	0.112	0.074	0.200	1.79
14	0.110	0.058	0.182	1.65
16	0.083	0.034	0.138	1.66
18	0.085	0.030	0.143	1.68
20	0.080	0.036	0.152	1.90
22	0.078	0.030	0.148	1.90
24	0.069	0.028	0.143	2.07
26	0.040	0.018	0.125	3.13
28	0.012	0.010	0.128	10.31
30	0.010	0.008	0.105	10.49

on the 4th day. The rate of production remained at about 10 $\mu gC/10^6$ cells/hour till the 12th day and then decreased further.

Pigment composition

The photosynthetic pigments of C. freiburgensis was analysed by TLC. The chromatogram resolved five fractions (Fig.38). The R_F value of these fractions are given in Table 4. The orange pigment, with the highest R_F value was identified as carotene. Chlorophyll a was blue-green in colour in UV light with R_F 0.34 and 0.37. The only other green pigment located was light green in colour and this was identified as chlorophyll c. The remaining two fractions were yellow in colour. These could probably be xanthophylls.

Biochemical composition

The biochemical components of the flagellate was estimated in terms of carbohydrate, protein and lipid. The water content of the flagellate varied from 91.56% in 5 day old cultures to 59.20% in 20 day old cultures. Simultaneously, the dry weight of the cells increased from 120 $\mu g/10^6$ cells to 253 $\mu g/10^6$ cells during the 20 days growth (Table 5). Protein was the major photosynthetic product during the exponential phase of culture. As the culture grew old the amount of protein decreased and that of carbohydrate increased. The ratio of protein to carbohydrate reduced by half within ten days of growth in culture. The change in the lipid content was less pronounce

Fig.3. Two-dimensional chromatograms of pigments in nanoplankters separated on silica-gel thin layer plates. A. *S. salina*, B. *C. freiburgensis*, C. *I. galbana* (C.s.), D. *I. gracilis*. Solvent system: first dimension, 0.8% n-propanol in light petroleum (60–80°C); second dimension, chloroform: light petroleum:acetone (25:75:0.5); 1. carotene (orange), 2. chlorophyll *a* (blue-green), 3. chlorophyll *b* (olive green), 4. chlorophyll *c* (light green), 5. fucoxanthin (orange), 6,7,8,9 unidentified pigments.

Fig.3.

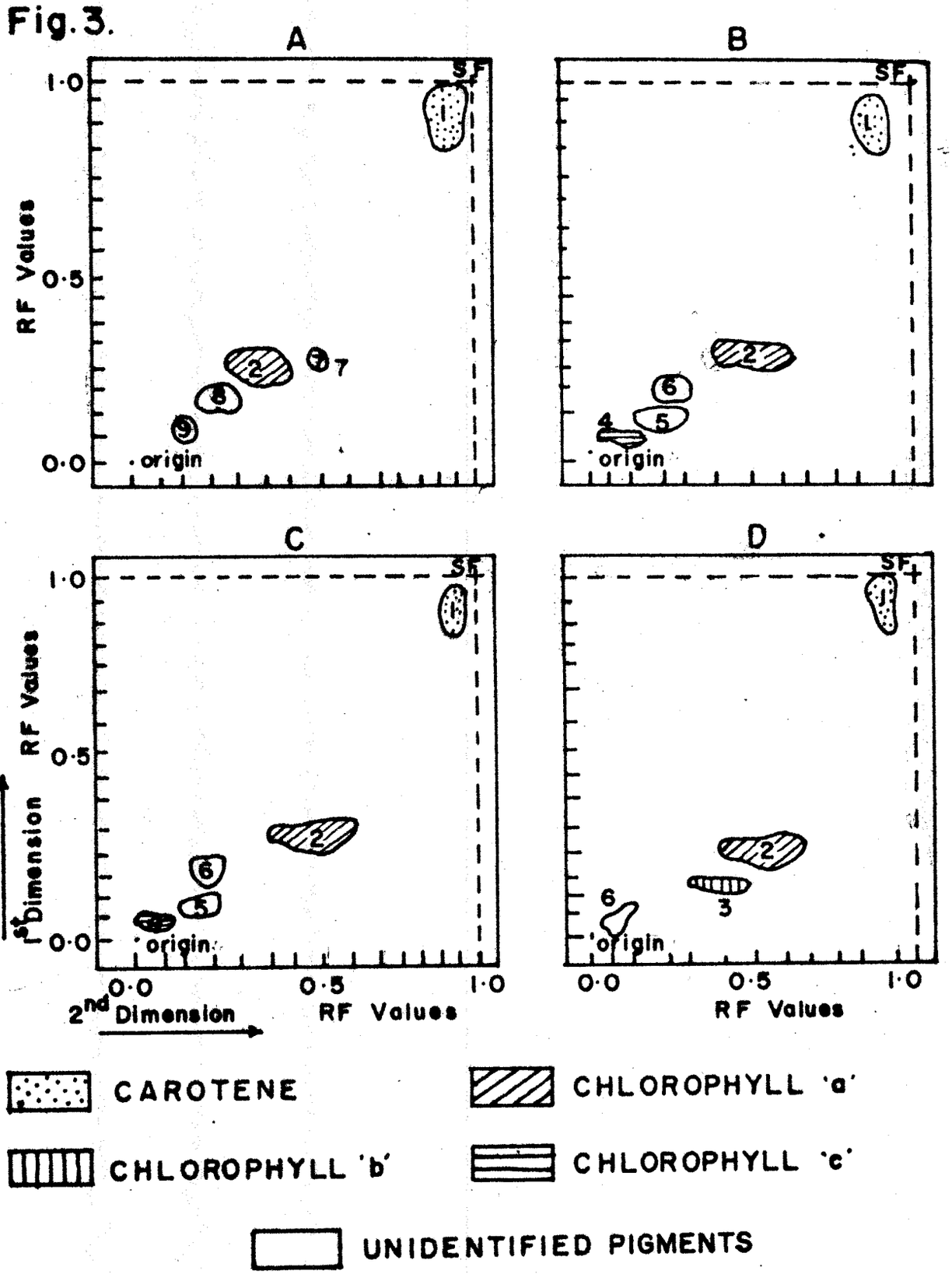

A
B
C
D
SF
origin
RF Values
1·0
0·5
0·0
1st Dimension RF Values
2nd Dimension RF Values
CAROTENE
CHLOROPHYLL 'a'
CHLOROPHYLL 'b'
CHLOROPHYLL 'c'
UNIDENTIFIED PIGMENTS

Table 4. R_F value of photosynthetic pigments of *Chromulina freiburgensis*, *Isochrysis galbana* (C.s.), *Synechocystis salina* and *Tetraselmis gracilis* separated on thin-layers of silica gel

	Spot number	1st Dimension: 0.8% n. Propanol in light petroleum (60 - 80°C)	2nd Dimension: Chloroform: light petroleum: acetone 25: 75: 0.5
Carotenes (Orange)	1	0.93	0.93
Chlorophyll *a* (Blue green)	2	0.34	0.37
Chlorophyl *b* (Olive green)	3	0.23	0.3[illegible]
Chlorophyl *c* (Pale green)	4	0.075	0.02
Fucoxanthin (Orange)	5	0.175	0.1[illegible]
Unidentified	6	0.18	0.09
"	7	0.35	0.46
"	8	0.29	0.18
"	9	0.3[illegible]	0.[illegible]1

Table 5. Biochemical composition of *Chromulina freiburgensis* raised in batch culture.

Fraction	Sampling interval		
	5 days	10 days	20 days
Percent moisture	91.56 ±3.68	86.0 ±4.71	59.20 ±3.16
Dry weight ($\mu g/10^6$ cells)	120.0	182.0	253.0
Protein (% dry wt.)	58.4 ±6.12	46.85 ±4.30	40.16 +11.02
Carbohydrate (% dry wt.)	26.6 ±6.48	38.5 ±7.64	43.83 ±4.98
Lipid (% dry wt.)	10.16 ±1.7	11.60 ±2.85	17.20 ±3.13
Protein: Carbohydrate	2.20	1.22	0.92
Bacterial count/ml	180	106	390

Estimation of extracellular production

The amount of extracellular products of the species was estimated by liquid scintillation technique. The rate of excretion was low during the phase of active growth. During the stationary phase the amounts excreted increased about five fold. The amounts varied from 3% to 12.57% of the total carbon fixed (Table 6). In terms of absolute activity, about 0.02–0.033 μC were retained in the medium for an added activity of 5μC (Table 7).

Table 6. Percentage of extracellular products (ECP) released by C. freiburgensis during phases of growth in culture. (The % soluble represents the ECP as fraction of the total carbon fixed)

Days of growth	Cells ml^{-1}	Particulate (mgC/l/hr)	soluble	% soluble
4 days	9 x 10^4	19.53	0.60	3.0
8 days	16 x 10^4	7.03	0.55	7.8
16 days	33 x 10^4	9.71	1.22	12.57

Table 7. The absolute activity in µC retained by filtrates of ^{14}C labelled nanoplankton cultures (Added activity = 5 µC).

Culture	Sampling interval		
	4 days	8 days	16 days
Chromulina freiburgensis	0.020	0.027	0.033
Isochrysis galbana (C.s.)	0.036	0.043	0.150
Synechocystis salina	0.129	0.141	0.273
Tetraselmis gracilis	0.01	0.018	0.028

Eco-physiology of Chromulina freiburgensis

Salinity: The salinity tolerance of the species was tested by growing the flagellate in culture media of salinities 14‰, 24‰ and 34‰. The rate of cell division was affected by the variation in salinity. As seen from Table 7, the cell counts showed consistently higher values for the treatment level III i.e. 34‰. Analysis of variance showed that the difference between the treatments was highly significant except on the 2nd day. The density of the culture was very low at 14‰ while at 24‰ it was higher; but the highest density was built up at the salinity level of 34‰.

The difference in the salinity of the culture medium was sharply reflected in the amount of chlorophyll *a*. Analysis of variance showed the treatments to be highly significant from the 4th to 24th. At the lowest salinity level the chlorophyll content was very low compared to the other two treatments. At 24‰, it was further high and the medium at 34‰ gave highest chlorophyll *a* value throughout the experiment. The variation in the amount of the pigment is given Table 8.

Table 3. Cell concentration and chlorophyll a content of Chromulina freiburgensis grown in culture media of salinities 14‰, 24‰ and 34‰ for a period of 24 days.

Age of culture (days)	Cell concentration per ml			Chlorophyll a (µg/10^6 cells)		
	14‰	24‰	34‰	14‰	24‰	34‰
0	350	350	350	-	-	-
2	530	470	530	0.096	0.116	0.132
4	841[illegible]	1,852[illegible]	2,170[illegible]	0.236	0.209	0.31[illegible]
6	1,639[illegible]	3,468[illegible]	3,644[illegible]	0.185	0.236	0.285
8	2,936[illegible]	5,687[illegible]	6,45[illegible]	0.194	0.216	0.286
10	4,668[illegible]	16,932[illegible]	24,413[illegible]	0.163	0.171	0.242
12	5,42[illegible]	42,138[illegible]	5[illegible],07[illegible]	0.079	0.083	0.101
14	6,61[illegible]	48,66[illegible]	54,497[illegible]	0.082	0.091	0.159
16	6,098[illegible]	65,[illegible]300	72,77[illegible]0	0.073	0.085	0.108
18	5,925[illegible]	7[illegible],[illegible]830	76,783[illegible]	0.077	0.081	0.102
20	[illegible],747[illegible]	72,895[illegible]	86,75[illegible]0	0.081	0.084	0.109
22	4,896[illegible]	83,510[illegible]	102,45[illegible]0	0.076	0.081	0.104
24	4,563[illegible]	90,740[illegible]	112,6[illegible]0	0.059	0.07[illegible]	0.1[illegible]

Effect of pH

The response of the species to the change in pH was found to be pronounced. However, the cell count was not

much affected in the pH range of 6.5 to 8. But the amount of chlorophyll *a* varied significantly (Table 9). At pH 8, the amount of the pigment was about double that at pH 6.5 both at the exponential phase and the stationary phase. The analysis of variance showed that the difference is significant at 1% level.

Table 9. Cell concentration and chlorophyll *a* content of *Chromulina freiburgensis* during phases of growth in culture media of different pH levels.

Treatment (pH level)	Logarithmic phase		Stationary phase	
	Cell density per ml	Chlorophyll *a* $\mu g/10^6$ cells	Cell density per ml	Chlorophyll *a* $\mu g/10^6$ cells
6.5	19,73[illegible]	[illegible].171	1[illegible],78[illegible]	[illegible].088
7.[illegible]	2[illegible],83[illegible]	[illegible].204	99,47[illegible]	[illegible].82
7.5	2[illegible],2[illegible]	[illegible].222	1[illegible]4,17[illegible]	[illegible].[illegible]8[illegible]
8.[illegible]	19,5[illegible]	[illegible].329	96,2[illegible]	[illegible].161

Effect of cell concentration on the rate of carbon fixation

While measuring the rate of carbon fixation the concentration of the culture used for the experiment was optimised so that self-shading was avoided. The cell density and the corresponding incorporation of carbon-14 is given in

Table 1. The rate of production increased upto a cell density of 10.8 x 10^5 per ml. At the next density level tested (14.4 x 10^5 per ml) there was drastic reduction in the production. Fig.4 represents this graphically. From the available data it may be assumed that cell concentrations above 1 x 10^5 per ml was inhibitory to the rate of ^{14}C fixation.

Table 1. Carbon-14 uptake by C. freiburgensis as function of cell concentration.

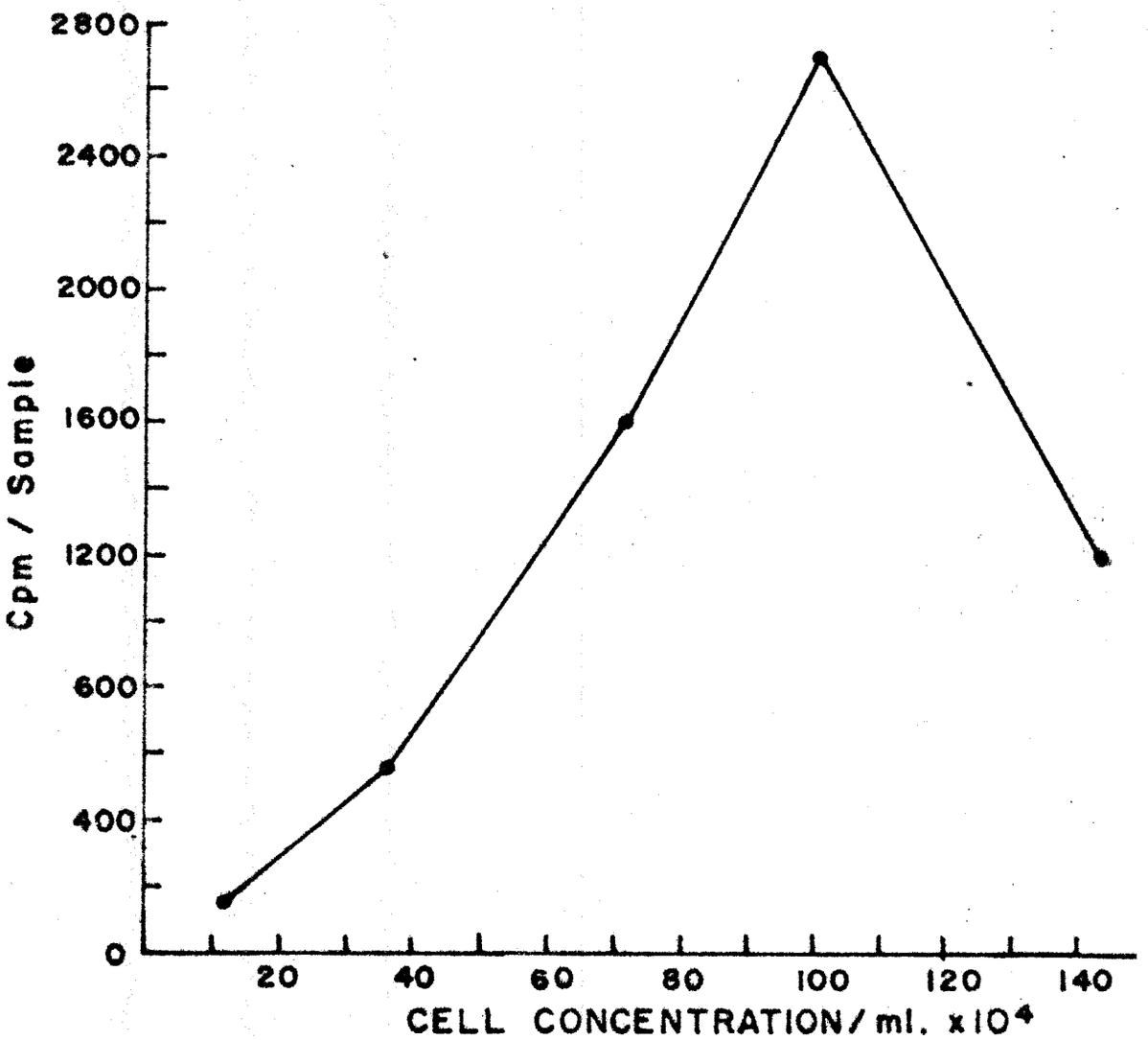
Cell density per ml	Counts per minute
12,000	148 ±15.1
36,000	575 ±91.0
72,000	1619 ±156.0
108,000	2763 ±63.2
144,000	1415 ±101.0

Effect of light

The light conditions to which the species was exposed affected growth profoundly. Cultures were exposed to alternating light-dark period and also constant illumination for a month. Those exposed to constant illumination developed rapidly compared to the others. From an initial

Fig.4. Rate of carbon fixation (expressed as ^{14}C uptake in cpm) as function of different culture densities of *C. freiburgensis*.

Fig. 4.


2800
2400
2000
1600
1200
600
400
0
Cpm / Sample
20
40
60
80
100
120
140
CELL CONCENTRATION / ml. x10⁴

density of 4[illegible] cells/ml, the population grew upto 12[illegible] x 10^4/ml within [illegible]6 days with light-dark cycle while constant illumination produced a higher population density [illegible]203 x 10^4 cells/ml (Table 11). As is observed, the continuous illumination induces higher growth rate of the flagellate.

Table 11. Effect of photoperiod on growth of C. freiburgensis in batch culture.

Age of culture (days)	Cell density per ml	
	10:14 Light-dark cycle	Constant illumination
[illegible]	4[illegible]	4[illegible]
2	1652[illegible]	2[illegible]5[illegible]
4	9,4[illegible]	12,5[illegible]
6	2[illegible],[illegible]	32,[illegible]
8	3[illegible],[illegible]	5[illegible],35[illegible]
1[illegible]	37,[illegible]	6[illegible],6[illegible]
12	46,[illegible]	7[illegible],96[illegible]
14	52,13[illegible]	89,87[illegible]
16	57,[illegible]	125,[illegible]
18	57,[illegible]	163,1[illegible]
2[illegible]	63,1[illegible]	1[illegible],3[illegible]
22	7[illegible],79[illegible]	158,5[illegible]
24	89,13[illegible]	178,[illegible]
26	12[illegible],[illegible]	2[illegible]3,[illegible]
28	63,1[illegible]	178,3[illegible]
3[illegible]	57,[illegible]	2[illegible],6[illegible]

The metabolic activity of the flagellate was affected significantly by the intensity of illumination. The light-photosynthesis curve followed the hyperbolic relation as per the equation $Y = a+bX+cX^2$ with an I_K of 24 x 10^{15} x cm^{-2} x sec^{-1} (or 14.1 klux) (Fig.5). The highest light intensity that supported the maximum rate of carbon fixation was 34.61 x 10^{15} quanta x cm^{-2} x sec^{-1}. At the lowest light intensity tested (1 x 10^{15} x quanta x cm^{-2} x sec^{-1}) the rate of production was very low, being 15.48% of that at light saturation. A similar reduction was noticed at the highest light intensity tested i.e. 16.55% of production at 68 x 10^{15} quanta x cm^{-2} x sec^{-1}.

Effect of temperature

Temperature was found to be one of the important factors controlling growth and proliferation of _. freiburgensis. When exposed to low temperature (5°C) the flagellate lost motility within five minutes. After a week when they were recultured at room temperature, the culture were viable. The viability of the cultures stored in the refrigerator was retained for three months. After three months the cultures did not regain viability on transfer to fresh medium at room temperature.

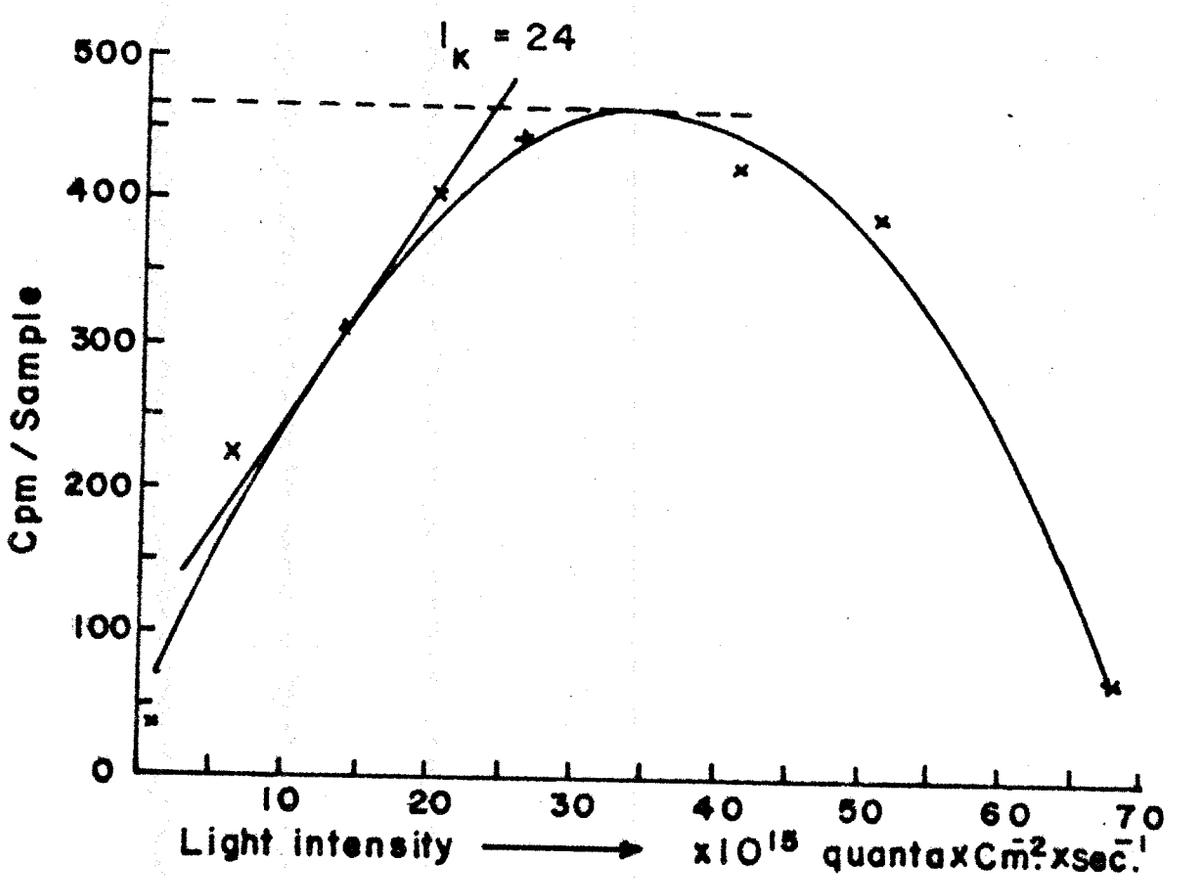
Fig.3. The rate of photosynthesis (expressed as ^{14}C uptake in cpm) as function of light intensity for C. freiburgensis.

Fig. 5.

$Y = 48{\cdot}343 + 24{\cdot}157\,X - 0{\cdot}349\,X^2$

X_{max} = 100 % light saturation = $34{\cdot}61 \times 10^{15}$ quanta.Cm^{-2} Sec^{-1}

I_K = 24·0 quanta. Cm^{-2} Sec^{-1}



The growth of the culture was studied within the temperature range 2[illegible]–4[illegible]°C. The motility of the cells also varied within this temperature range. At 2[illegible]°C, 30[illegible] of the cells were non-motile. At temperatures of 25, 3[illegible] and 35°C the cells behaved normally. When exposed to 4[illegible]°C all cells lost motility within 2–3 hours of exposure.

The growth of the culture within the temperature range was quantified by measuring the rate of growth. The rate of doubling did not show significant difference within the temperature range 2[illegible]–35°C. At 4[illegible]°C the rate of growth was considerably low and the culture did not survive more than five days. The mean doublings per day for the different temperatures is given in Table 12.

Table 12. Rate of growth and ^{14}C uptake (as counts per minute per sample incubated) at different temperatures in batch culture.

Temperature °C	Chromulina freiburgensis		Isochrysis galbana (C.s.)		Synechocystis salina	
	k	cpm	k	cpm	k	cpm
2[illegible]	2.66	498	2.56	123	1.99	1347
25	2.76	468	2.64	342	2.26	7479
3[illegible]	3.05	4392	2.76	2559	2.13	2829
35	2.84	2334	2.66	1399	2.13	2385
4[illegible]	[illegible].13	222	[illegible].86	42	1.16	1[illegible]99

The rate of fixation of carbon was measured simultaneously. The ^{14}C incorporation by the flagellate was observed to be highly sensitive to temperature. There was significantly high production at 30°C which was reduced to half at 35°C. At all other temperatures the rate of production was very low (Table 12).

Utilization of macro-nutrients

The nitrate and phosphate requirement of the species was also determined. The rate of cell division in the culture varied with the concentration of nitrate in the medium. Applying the linear transformation of the Monods expression, $S = \mu_{max} (S/\mu) - K_s$, the half-saturation constant for growth was obtained as 1.7 µg at 1 L^{-1} with a maximum specific growth rate of 0.961 per day (Fig.6). It was also observed that at a NO_3 level of more than 2[illegible] mg/litr the growth of the flagellate was highly inhibited.

Similarly, the PO_4 requirement of the species could be defined by the relation $S = 1.97 (S/\mu) - 1.31$ where 1.31 is the half-saturation constant for growth, and .97 being the maximum (unlimited) growth rate (Fig.7). It was observed that PO_4 concentrations at and above 25 mg/litre, the growth of the flagellate is inhibited.

Fig.6. Relation between the concentration of nitrate in the culture medium and the specific growth rate of C. freiburgensis.

Fig. 6.

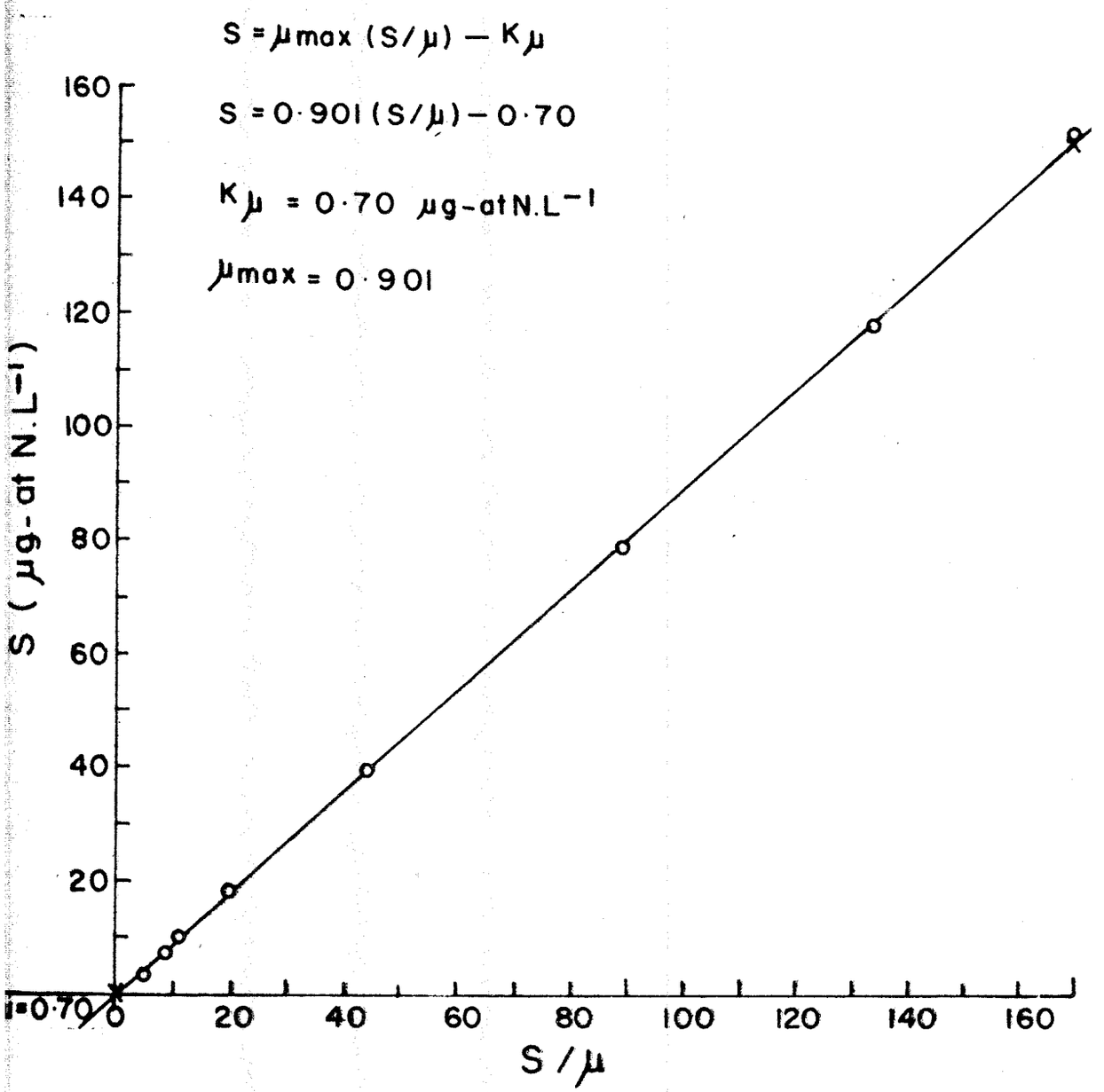
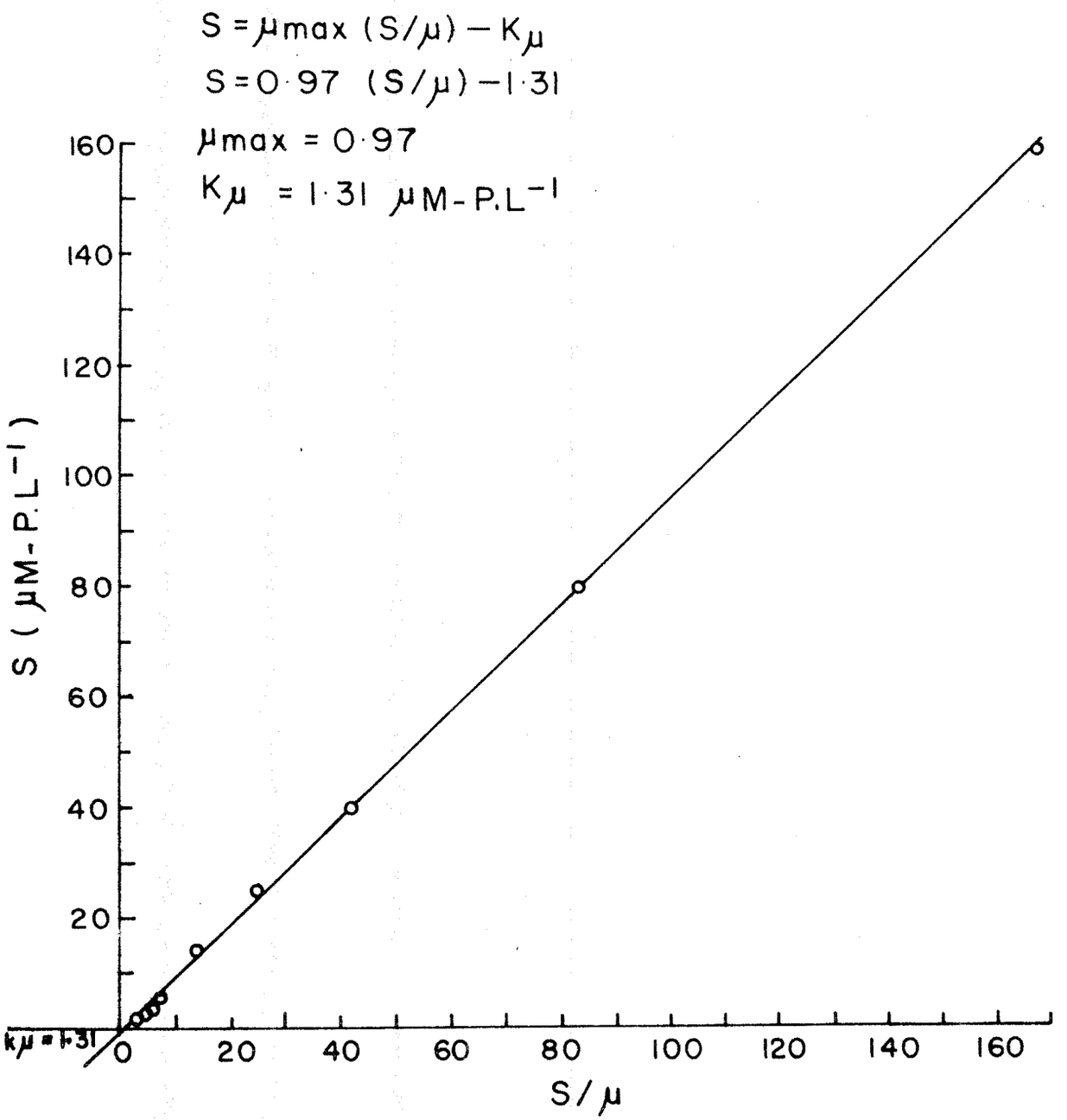

S = μmax (S/μ) — Kμ
S = 0·901 (S/μ) — 0·70
Kμ = 0·70 μg-at N.L⁻¹
μmax = 0·901
S (μg-at N.L⁻¹)
S / μ
160
140
120
100
80
60
40
20
0
20
40
60
80
100
120
140
160
I=0·70

Fig.7. Relation between the concentration of phosphate in the culture medium and the specific growth rate of C. freiburgensis.

Fig.7.


S = μmax (S/μ) − Kμ
S = 0·97 (S/μ) − 1·31
μmax = 0·97
Kμ = 1·31 μM-P.L⁻¹
S (μM-P.L⁻¹)
S/μ
kμ = 1·31
0
20
40
60
80
100
120
140
160

An obvious fact here was that the substrate concentration supporting, the μ_{max} (unlimited) was far lower than actually observed. Thus the observed growth rate neare the μ_{max} only at about 2[illegible] µg-at. $N.L^{-1}$ (Table 13) for nitrate and around 7.8 µg-P L^{-1} for phosphate (Table 14)

Table 13. Specific growth rate (µ/day) during exponential phase of S. freiburgensis, I. galbana (C.s.) and S. salina as function of NO_3^- concentration in the medium.

S. freiburgensis		I. galbana (C.s.)		S. salina	
NO_3^- concentration µg-at. NL^{-1}	µ/day	NO_3^- concentration µg-at. NL^{-1}	µ/day	NO_3^- concentration µg-at. NL^{-1}	µ/d
2.6	[illegible].96	2.6	0.46	2.6	0.52
7.[illegible]	[illegible].81	7.[illegible]	[illegible].60	5.[illegible]	0.54
18.[illegible]	0.90	1[illegible].[illegible]	0.73	1[illegible].[illegible]	0.66
4[illegible].[illegible]	0.9[illegible]	13.[illegible]	0.83	28.[illegible]	0.82
79.[illegible]	0.89	49.[illegible]	[illegible].94	49.[illegible]	0.88
118.[illegible]	[illegible].89	98.[illegible]	[illegible].938	98.[illegible]	0.91
152.[illegible]	[illegible].9[illegible]	12[illegible].[illegible]	[illegible].944	152.[illegible]	1.18
-	-	152.[illegible]	[illegible].92[illegible]	3[illegible].[illegible]	[illegible].95

Table 14. Specific growth rate (μ/day) during exponential phase of C. freiburgensis, I. galbana (C.s.) and S. salina as function of SO_4 concentration.

Substrate concentration μM-SO_4^{-1}	C. freiburgensis	I. galbana (C.s.)	S. salina
.75	0.28	0.26	.26
1.5	0.32	0.33	0.37
2.3	0.490	0.52	0.48
4.3	0.650	.64	.7
7.80	.980	.92	0.93
15.	.976	.94	0.96
24.5	0.979	.96	.97
4 .0	.950	0.96	0.96
8 .0	0.961	.96	.96
160.	.938	0.96	0.95

4.2. Isochrysis galbana (C.s.)

Growth and activity in batch culture

Cultures of Isochrysis galbana (C.s.) were grown in enriched seawater to study its growth kinetics. Under the defined conditions (3.4.1) the flagellate was found to grow rapidly. From an initial concentration of 35 cells/ml the

cell count increased to 1.95 x 10^5/ml on the 4th day. After the 4th day the increase in cell counts was slow (Fig.8A). The stationary stage persisted till 20 days after which the cell counts began to decline. The highest growth rate was during the logarithmic growth phase with a mean doubling rate of 1.43 divisions per day for a generation time of 16 hours. The corresponding relative growth constant was 0.043 per hour. The maximum density attained by the culture was 6.9 x 10^5 per ml (Table 2).

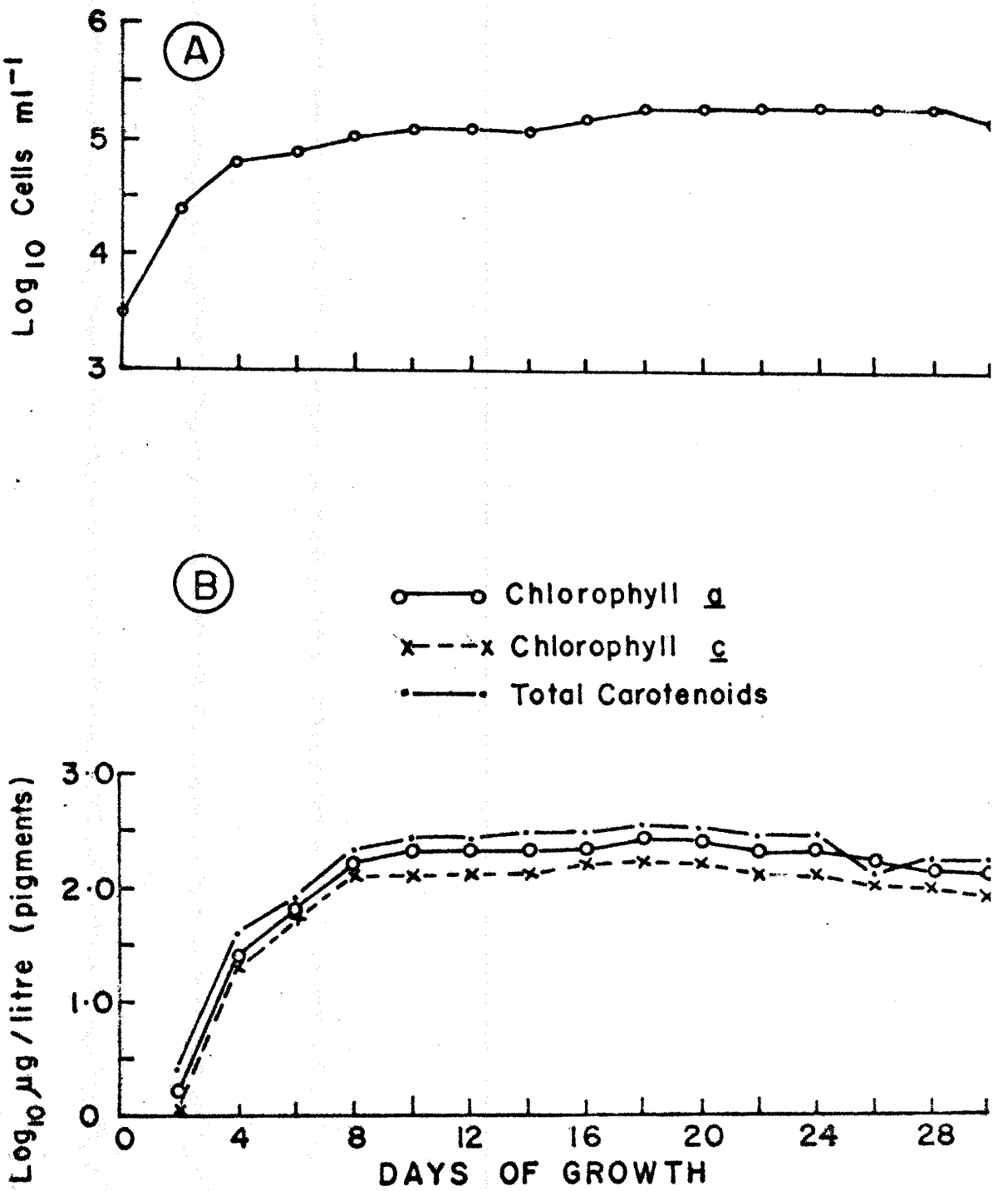
Corresponding to the decline in cell numbers it was also observed that the proportion of motile cells in the culture decreased. The non-motile cells settled to the bottom of the culture flask forming a thin layer. The golden-brown colour of the culture changed to a reddish brown by the 4th week.

The amount of chlorophyll *a*, *c* and carotenoids was very low on the 2nd day; but within the next two days there was a sudden increase of about twenty times. After the fourth day the pigments increased gradually till the 20th. The amount of all the pigments decreased on further aging of the culture (Fig.8B). The amount of chlorophyll *a*, *c* and carotenoids per unit number of cells was comparatively higher during 8-12 days (Table 15) with the maximum amounts of 0.584, 0.418 and 0.662 respectively. The quantity of

Fig.8A. Increase in cell number of *Isochrysis galbana* (C.s.) during growth in batch culture.

Fig.8B. Variation in the amount of photosynthetic pigments of the above species during growth in batch culture.

Fig. 8.


A
Log10 Cells ml-1
6
5
4
3
B
Chlorophyll a
Chlorophyll c
Total Carotenoids
Log10 µg / litre (pigments)
3·0
2·0
1·0
0
0
4
8
12
16
20
24
28
DAYS OF GROWTH

chlorophyll a was less than that of carotenoids while the latter was less in quantity compared to the total chlorophylls. The ratio of carotenoid to chlorophyll a did not show much fluctuation except for the last day of observation.

Table 15. Variation in the amount of pigments expressed as $\mu g/10^6$ cells of *I. galbana* (C.s.) for a growth period of 30 days in batch culture.

Age of culture (days)	Chlorophyll a $\mu g/10^6$ cells	Chlorophyll c $\mu g/10^6$ cells	Total carotenoids $\mu g/10^6$ cells	Carotenoids/ Chlorophyll a
2	0.068	0.042	0.100	1.47
4	0.142	0.105	0.195	1.38
6	0.265	0.202	0.364	1.37
8	0.584	0.418	0.662	1.13
10	0.502	0.380	0.612	1.22
12	0.468	0.330	0.594	1.27
14	0.430	0.316	0.570	1.33
16	0.424	0.304	0.553	1.30
18	0.420	0.300	0.550	1.31
20	0.405	0.253	0.468	1.16
22	0.365	0.230	0.460	1.26
24	0.318	0.192	0.390	1.23
26	0.226	0.165	0.280	1.24
28	0.228	0.152	0.264	1.16
30	0.108	0.101	0.256	2.30

The flagellate exhibited high metabolic activity on the 2nd and 14th day with a gross production of 4. µgC/1.^{6} cells/hour and a net production of 12 µgC/1.^{6} cells/hour as measured by the oxygen technique. From the 6th to 22nd the production remained uniform (Fig.9A) and later began to decrease.

As per the Carbon-14 experiment, the flagellate exhibited high metabolic activity during the period from -6 days with a maximum observed rate of 16/µgC/1.^{6} cells/hour. After the 6th day the rate of production decreased (Fig.9B).

Pigment composition

On chromatographic separation, the pigments of the strain resolved into five fractions (Fig.3C). The orange coloured carotene, the blue green chlorophyll a and the light green chlorophyll c were easily noticeable. The two other spots were yellow in colour and it is assumed that these may be xanthophylls. Table 4 gives the R_f values of the different pigments.

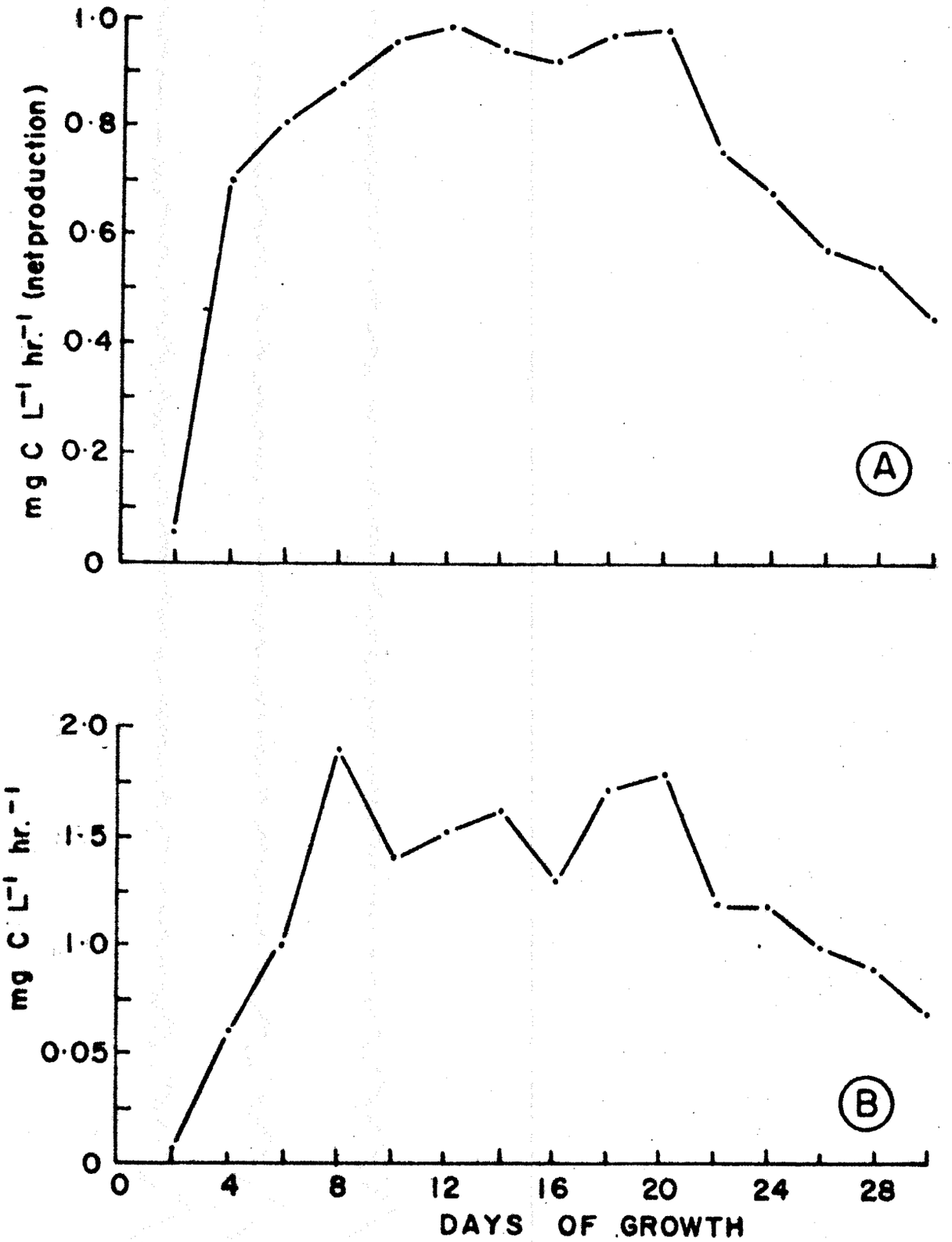
Bio-chemical composition

The water content of the cells varied from 71.4. to 91.74. from the 5th day to 2 th. There was consistent

Fig.9A. Rate of production of organic carbon by *I. galbana* (C.s.) for a growth period of 30 days in batch culture (measured by the oxygen technique).

Fig.9B. Rate of production of organic carbon by the above species for a growth period of 30 days in batch culture (measured by the ^{14}C technique).

Fig. 9.


1·0
0·8
0·6
0·4
0·2
0
mg C L⁻¹ hr.⁻¹ (net production)
A
2·0
1·5
1·0
0·05
0
mg C L⁻¹ hr.⁻¹
B
0
4
8
12
16
20
24
28
DAYS OF GROWTH

increase in the dry weight of the cells as culture aged. The dry weight per million cells was 130 µg on the 5th day whereas the weight increased to 373 µg and 465 µg during the 10th and 20th day respectively. The major product of synthesis was protein during the exponential phase. As the culture became old the amount of protein decreased while that of carbohydrate increased. As a result the protein: carbohydrate ratio decreased from 1.83 to 0.93 within 20 days (Table 16). The lipid content was comparatively low throughout.

Table 16. Biochemical composition of Isochrysis galbana (T.s.) raised in batch culture.

Fraction	Sampling interval		
	5 days	10 days	20 days
Percent moisture	71.4 ±6.17	66.6 ±5.62	61.74 ±4.81
Dry weight (µg/10^6 cells)	130.0	373.0	465.0
Protein (% dry wt.)	51.3 ±7.00	44.85 ±6.14	37.8 ±8.09
Carbohydrate (% dry wt.)	28.0 ±7.16	34.34 ±4.23	40.58 ±6.12
Lipid (% dry wt)	6.78 ±2.76	6.58 ±1.24	11.67 ±3.62
Protein: Carbohydrate	1.83	1.43	0.93
Bacterial count/ml	2[illegible].0	2[illegible]	11[illegible].0

Extracellular release

Table 17 gives the relative amount of excretion of I. galbana (C.s.). During the exponential phase of growth only 3.9% of the total photo-assimilate was released into the medium. In one week old cultures this increased to 13.57% and on the 16th day to 45.2%.

Table 17. Percentage of extracellular products (ECP) released by I. galbana (C.s.) during phases of growth in culture. (The % soluble represents the ECP as fraction of the total carbon fixed)

Days of growth	Cells ml^{-1}	Particulate	soluble mgC/l/hr	% soluble
4	7×10^4	31.32	1.22	3.9
8	25×10^4	12.01	1.63	13.57
16	$2[illegible] \times 10^4$	4.42	2.[illegible]	45.2

The amount of absolute activity retained as ECP in the medium was also determined. Out of the 5 µC added to each sample, 0.15 µC was retained as ECP in the 16 days old culture. On the 4th and 8th day this fraction was less being 0.036 and 0.043 respectively (Table 7).

Salinity:

The salinity of the culture medium affected the

growth of the flagellate. The culture density and the pigment content varied with the salinity levels 14, 24 and 34‰. (Table 18). Analysis of variance showed the

Table 18. The cell concentration and chlorophyll a content of I. galbana (C.s.) grown in culture media of salinities 14‰, 24‰ and 34‰ for a period of 24 days.

Age of culture (days)	Cell concentration/ml			Chlorophyll a ($\mu g/10^5$ cells)		
	14‰	24‰	34‰	14‰	24‰	34‰
0	10000	10000	10000	-	-	-
2	19300	56200	58300	0.070	0.115	0.128
4	65800	2,91500	3,65500	0.234	0.280	0.341
6	1,70200	4,62500	5,38300	0.167	0.210	0.234
8	1,82600	5,37700	6,13000	0.174	0.188	0.195
10	3,85800	6,80800	7,10800	0.114	0.152	0.154
12	4,82500	6,28300	6,85000	0.102	0.143	0.157
14	5,10000	6,92800	7,92000	0.103	0.143	0.157
16	5,69100	8,91300	8,91300	0.106	0.132	0.144
18	5,93200	12,59000	12,59000	0.080	0.100	0.109
20	5,70200	18,54100	20,56300	0.040	0.087	0.088
22	5,43000	18,23100	17,85600	0.025	0.084	0.089
24	5,38000	18,10000	17,53400	0.020	0.078	0.079

treatment effect to be highly significant. The cell density and chlorophyll a were consistently low for the lowest salinity (14‰) tested, but the culture densities at 24 and 34‰ did not differ much throughout the experiment. The

maximum cell density built up during the treatments was 5.952 lakhs/ml (T_1), 18.541 lakhs/ml (T_2) and 2[illegible].59 lakhs/ml (T_3).

The amount of chlorophyll a was very low in cultures grown at 14‰. At 24‰ the quantity was still higher; but the highest salinity level produced the greater quantity of pigments consistently.

Effect of pH

The pH requirement of the species was also found to be quite specific. The range of pH from 6.5 to 8 affected the cell multiplication and the content of chlorophyll a. Analysis of variance showed that both at logarithmic phase and stationary stage the treatments were highly significant. Both the cell counts and chlorophyll a registered higher values for the pH 8 (Table 19).

Table 19. Cell concentration and chlorophyll a content of *I. galbana* (C.s.) during phases of growth in culture media of different pH.

Treatment pH level	Logarithmic phase		stationary phase	
	Cell density per ml	Chlorophyll a $\mu g/10^6$ cells	Cell density per ml	Chlorophyll a $\mu g/10^6$ cells
6.5	1[illegible]000	[illegible].290	67[illegible]300	[illegible].168
7.[illegible]	1255[illegible]0	[illegible].303	7417[illegible]0	[illegible].212
7.5	121[illegible]00	[illegible].307	7453[illegible]0	[illegible].217
8.[illegible]	155[illegible]00	[illegible].315	787[illegible]00	[illegible].268

Cell concentration/rate of production

The amount of carbon-14 incorporation by the flagellate when incubated in short term experiments was observed to increase with the concentration of the cells in the culture (Table 2[illegible]). The increase in the rate of production was not quite linear (Fig.1[illegible]). At about the cell density of 5 x 10^5/ml, the rate of production was found to saturate. The production decreased around a density of 6 x 10^5 cells/ml.

Table 2[illegible]. Carbon-14 uptake by I. galbana (C.s.) as a function of cell concentration.

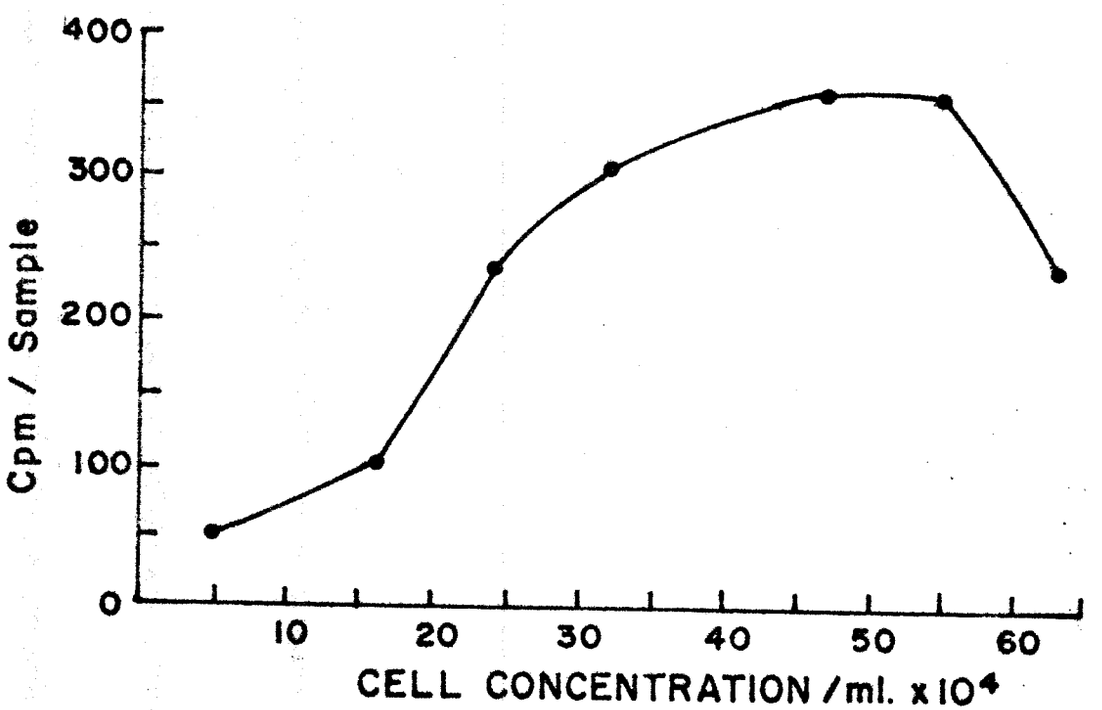
Cell density per ml	Counts per minute
5,[illegible]	53 ± 9.[illegible]
16,[illegible]	1[illegible]3 ±19.[illegible]
24,[illegible]	236 ± 8.3
32,[illegible]	309 ±13.1
47,[illegible]	363 ± 7.6
55,[illegible]	29[illegible] ±19.8

Influence of light

I.galbana (C.s.) was grown in constant illumination and also subject to light-dark cycle. The cell counts

Fig.1. Rate of carbon fixation (expressed as ^{14}C uptake in cpm) as function of different culture densities of I. galbana (c.s.)

Fig. 10.



showed that the photoperiod affected the cell division rate considerably. Starting from an initial concentration of 350 cells/ml, the culture developed a density of 23 x 10^5/ml in 30 days on constant illumination while it remained at 8.2 x 10^5/ml in the cultures exposed to light-dark regime (Table 21).

Table 21. Effect of photoperiod on growth of I. galbana (C.s.) in batch culture.

Age of culture (days)	Cell density per ml	
	10:14 Light-dark cycle	Constant illumination
0	3500	3500
2	2,5000	3,3300
4	19,5000	20,3000
6	19,9500	25,1200
8	17,0000	36,0000
10	24,0000	40,3000
12	28,1800	39,8100
14	40,5000	45,5000
16	50,1200	63,1000
18	56,2300	79,4300
20	69,0000	124,0000
22	50,1200	125,9000
24	62,0000	150,0000
26	60,0000	125,9000
28	70,0000	158,5000
30	82,0000	230,0000

The flagellate showed different rates of activity at various light intensities. The rate of photosynthesis was plotted against the respective light intensities to get the hyperbolic relation between these (Fig.11). As the figure shows the rate of production saturated at 34.71 x 10^{15} quanta x cm^{-2} x sec^{-1} with an I_K of 24 x 10^{15} x quanta x cm^{-2} sec^{-1}. The rate of photosynthesis was considerably low at the lowest (1 x 10^{15} quanta x cm^{-2} x sec^{-1}) and highest 68 x 10^{15} quanta x cm^{-2} x sec^{-1}) light intensities tested.

Effect of temperature

The motility, viability and growth rate of the species was related to the temperature conditions. This was illustrated by the temperature controlled experiments.

The cultures were exposed to 5°C to 40°C. At 5°C the immediate response was to lose motility and the effect was complete. At 20°C, 50% of the cells were motile. At 25-35°C all cells were active. When the cultures were exposed to 40°C, the cells gradually lost their active movements and within two hours 100% of the cells became non-motile.

The cultures remained viable for three months in the refrigerator (5°C). At higher temperature (40°C), the cells were viable for four days and did not regain viability on return to the room temperature.

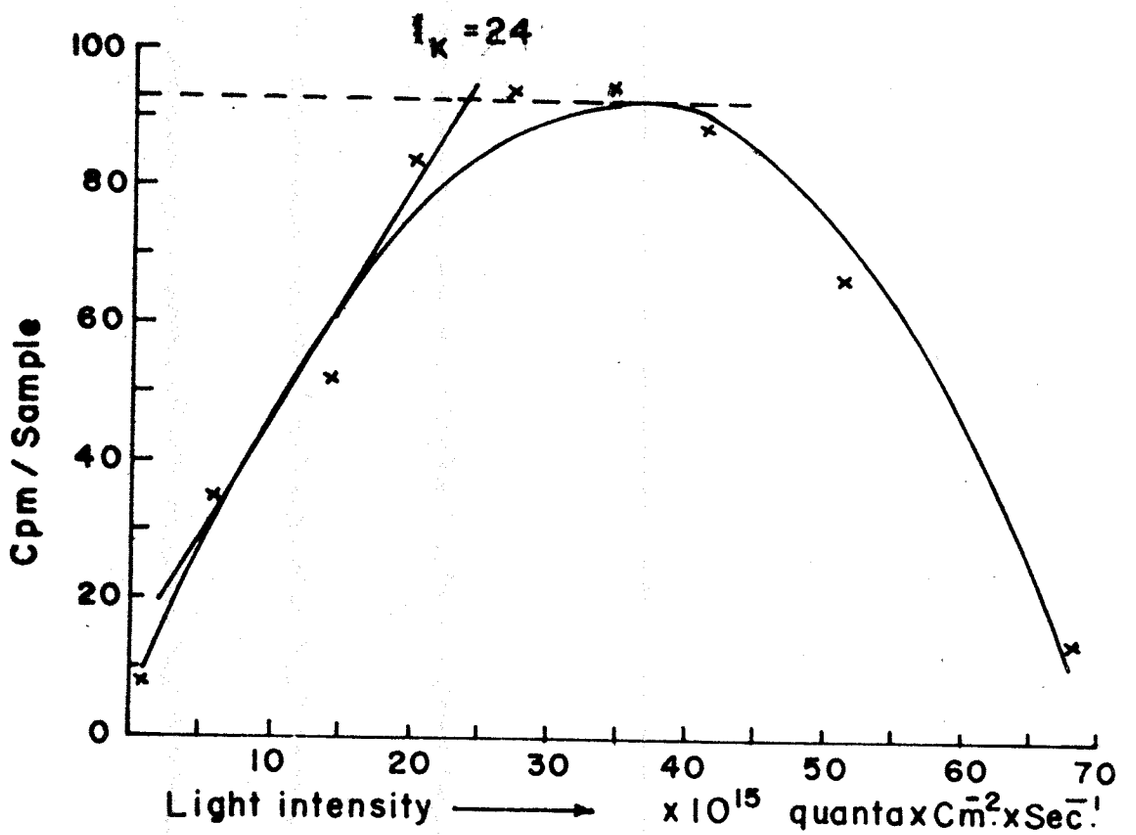
Fig.11. The rate of photosynthesis (expressed as ^{14}C uptake in cpm) as function of light intensity for *I. galbana* (C.s.).

Fig. 11.

$Y = 3{\cdot}988 + 5{\cdot}152\ X - 0{\cdot}0742\ X^2$

X_{max} = 100% Photosynthesis = $34{\cdot}71 \times 10^{15}$ quanta.Cm^{-2} Sec^{-1}

I_K = 24×10^{15} quanta. Cm^{-2} Sec^{-1}



The rate of growth of the flagellate for the temperature range 20-40°C was determined. There was no significant difference in the growth rate at temperatures from 20 to 35°C, but at 40°C the growth rate was considerably low (Table 11).

Temperature influenced the physiological activity significantly. The rate of production of the flagellate was highest at 30°C. At 35°C it was reduced to half of that at 30°C. The rate of production was very low at 20, 25 and 40°C (Table 12) compared to that at 30 and 35°C.

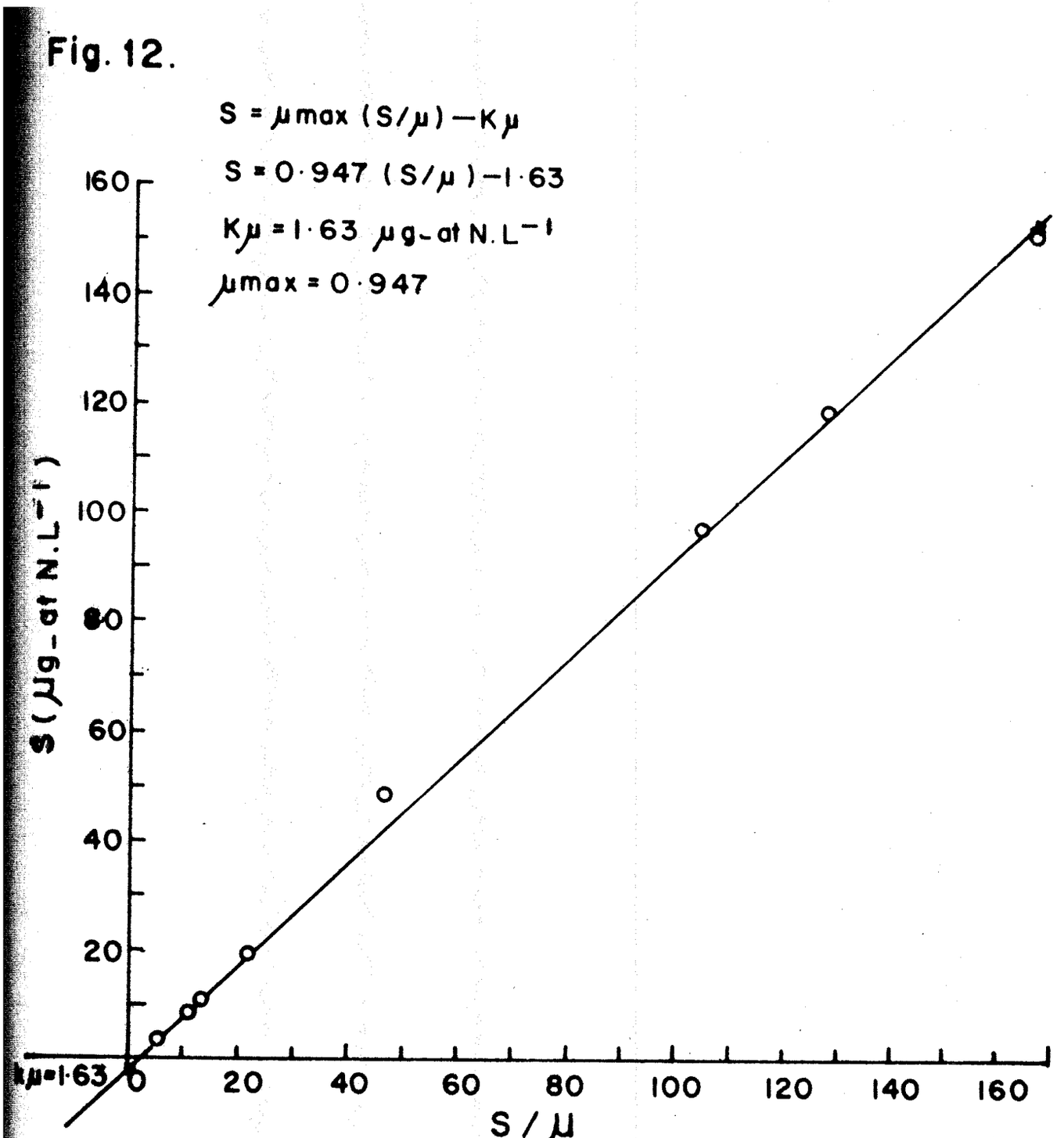
Requirement of nitrate and phosphate

The rate of growth of *I. galbana* (C.s.) was dependent on the concentration of nitrate and phosphate in the medium. Applying the Monod expression, it was found that the half-saturation constant of growth for nitrate was 1.63 µg at-N L^{-1} for a μ_{max} of 0.947 (Fig.12). However, it was observed that this maximum growth rate was attained only at a relatively higher substrate concentration i.e. at about 46.23 µg at-N L^{-1} (Table 13). Clear evidence of growth inhibition was found to occur at concentrations of 400 mg/litre of Potassium nitrate.

Similarly, the half-saturation constant for PO_4 was obtained from the relation, $S = 0.974 (S/\mu) - 1.63$

Fig.12. Relation between the concentration of nitrate in the culture medium and the specific growth rate of *I. galbana* (C.s.).

Fig. 12.


S = μmax (S/μ) − Kμ
S = 0·947 (S/μ) − 1·63
Kμ = 1·63 μg-at N.L⁻¹
μmax = 0·947
S (μg-at N.L⁻¹)
S / μ
Kμ=1·63
0
20
40
60
80
100
120
140
160

where K_μ = 1.63 $\mu M.P\ L^{-1}$. This was equivalent to a maximum growth rate of 0.974 (Fig.13). Table 14 gives the observed growth rate for each substrate concentration. Accordingly, the saturation level was reached only at a substrate concentration of about 25.42 $\mu M\text{-}P\ L^{-1}$. Phosphate levels above 35 mg/litre inhibited the growth of the organism.

4.3. Synechocystis salina

Growth rate and activity in batch culture

The culture of Synechocystis salina when grown in batch culture clearly showed a logarithmic phase and stationary phase which after a few days began to show senescence. No lag phase was observed in culture. The cell counts increased from an initial concentration of 1440 cell/ml to 9 lakhs/ml within four days. The cell doubling continued though at a decreased pace till 18th when the culture density was 47.5 million cells per ml. After 18 days the cell numbers began to fall (Fig.14A). By the 30th, the cell density came down to 30 million/ml. During the peak growth period the rate of doubling (k) was 2.74 for a generation time of 9 hours. The growth constant per hour was found to be 0.079 (Table 2). Though the growth rate decreased after four days it was observed

Fig.13. Relation between the concentration of phosphate in the culture medium and the specific growth rate of *I. galbana* (C.s.).

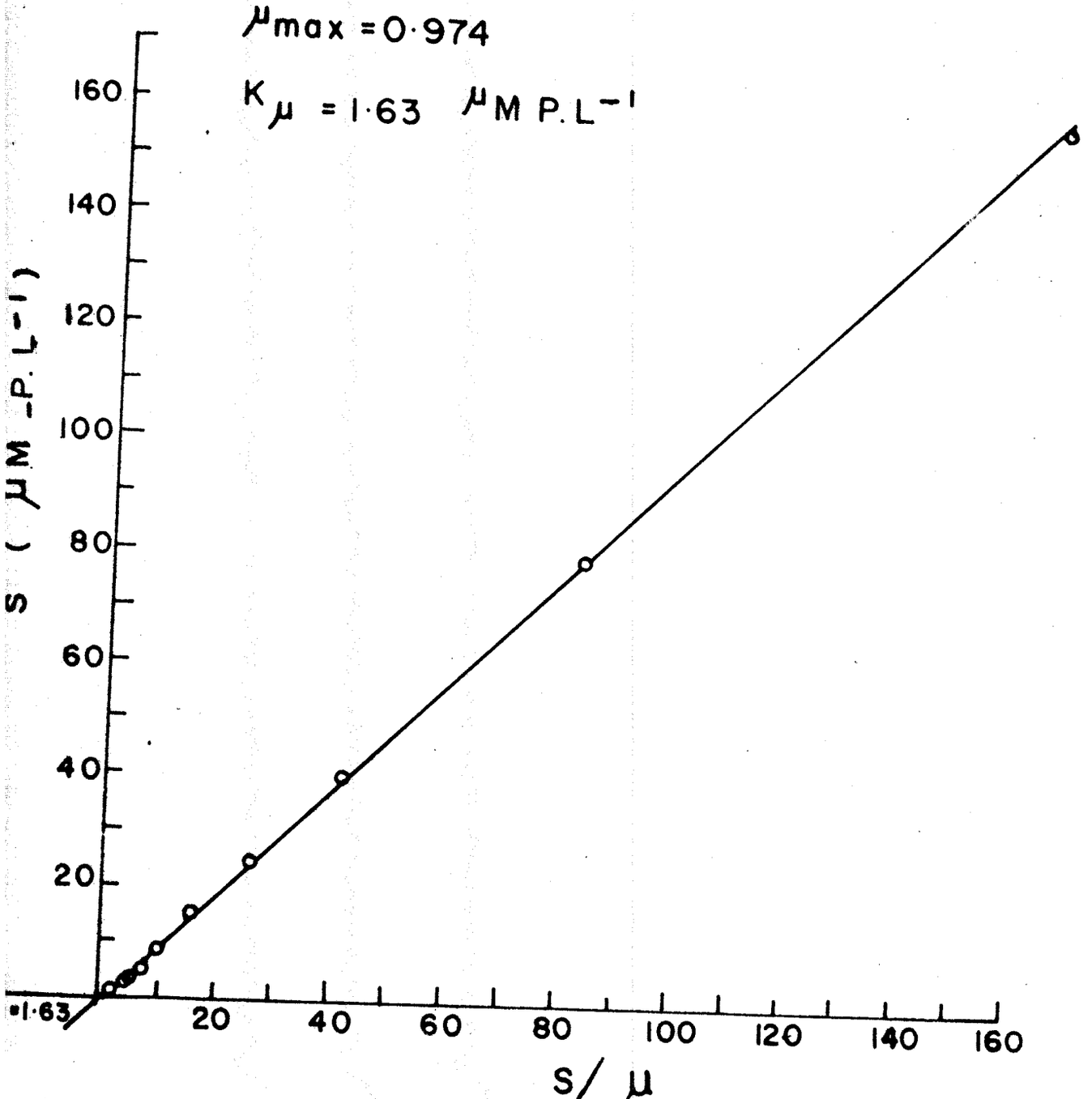
Fig. 13.

$S = \mu max\ (S/\mu) - K_\mu$

$S = 0.974\ (S/\mu) - 1.63$

$\mu max = 0.974$

$K_\mu = 1.63\ \mu M\ P.L^{-1}$



that upto 12th the population doubled itself every 48 hour thus building up a very dense culture.

The young cultures had a bluish-green colour. The blue tint reduced as the culture aged. The declining cultures were dull green in colour.

The amount of chlorophyll a and non-chlorophyllous pigments also followed the same trend as the cell numbers (Fig.14B). During the first four days the increase in the amount of the pigments was exponential; this was followed by a gradual rise till the 12th and later the culture was in the stationary state. Decline in the amount of the pigments began by 20th day. The amount of chlorophyll a per unit number of cells was high during 8-12 days; the maximum amount being 0.019 $\mu g/10^6$ cells. Similarly the non-chlorophyllous pigments also were found to be maximum from 8-14 days with a concentration of 0.012 $\mu g/10^6$ cells. On all days except the 2nd, chlorophyll a dominated over the non-chlorophyllous pigments. But in the declining cultures i.e. from the 20th, the amount of non-chlorophyllous pigments was higher (Table 22).

Fig.14A. Increase in cell number of *Synechocystis salina* during growth in batch culture.

Fig.14B. Variation in the amount of photosynthetic pigments of the above species during growth in batch culture.

Fig. 14.

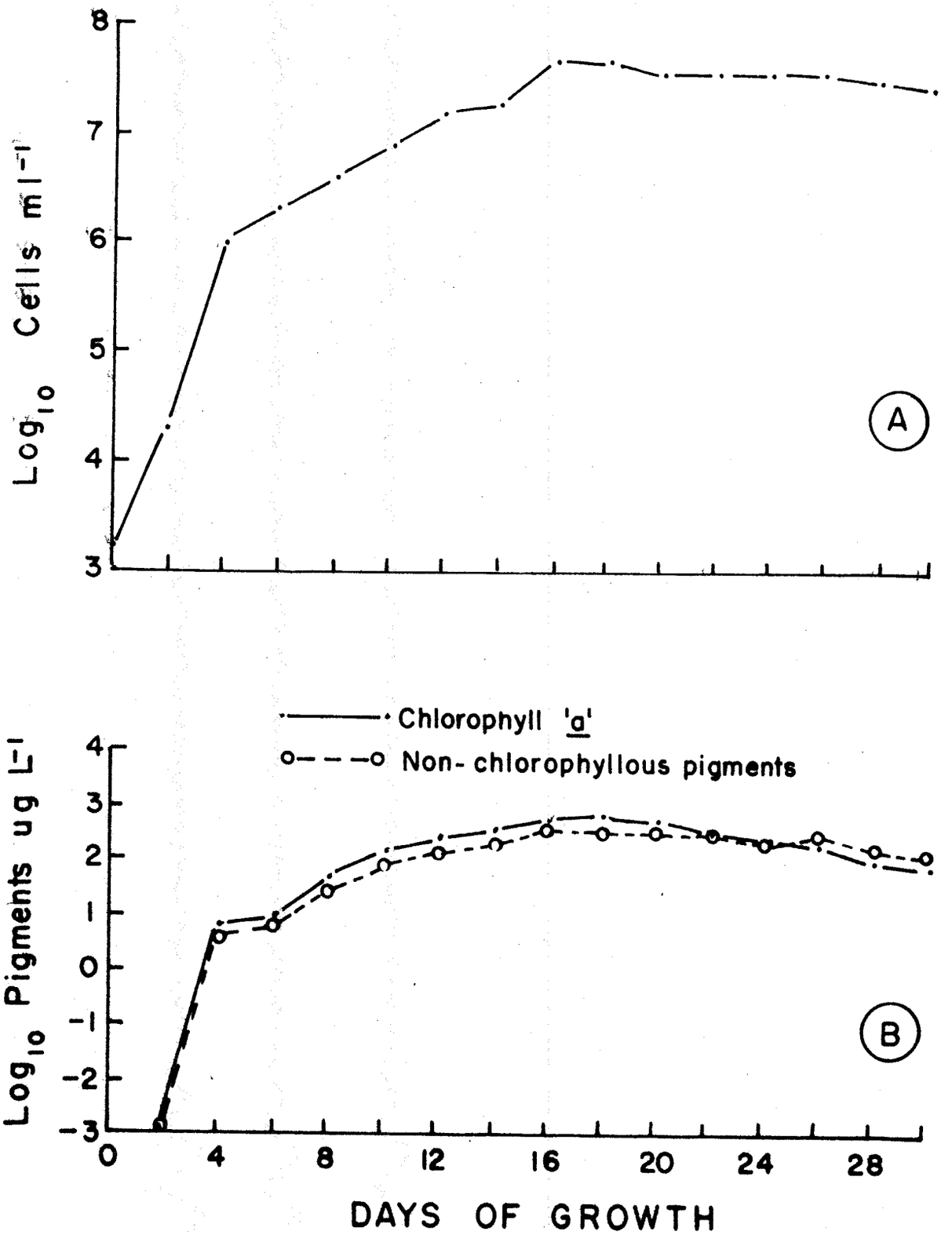

8
7
6
5
4
3
Log_{10} Cells ml^{-1}
A
Chlorophyll 'a'
Non-chlorophyllous pigments
4
3
2
1
0
-1
-2
-3
Log_{10} Pigments ug L^{-1}
B
0
4
8
12
16
20
24
28
DAYS OF GROWTH

Table 22. Variation in the amount of pigments expressed as $\mu g/10^6$ cells of *Synechocystis salina* for a growth period of 30 days in batch culture.

Age of culture (days)	Chlorophyll *a* $\mu g/10^6$ cells	Non Chlorophyllous pigments $\mu g/10^6$ cells	Non Chlorophyllous/ chlorophyll *a*
2	0.003	0.004	1.33
4	0.008	0.005	0.63
6	0.009	0.006	0.66
8	0.018	0.007	0.39
10	0.018	0.010	0.56
12	0.019	0.012	0.63
14	0.018	0.011	0.61
16	0.019	0.012	0.63
18	0.016	0.009	0.51
20	0.013	0.010	0.77
22	0.009	0.009	1.00
24	0.007	0.007	1.00
26	0.006	0.008	1.33
28	0.004	0.006	1.67
30	0.003	0.005	1.67

The rate of production as shown by the oxygen and ^{14}C methods gave the highest production rate on 6 to 8 days.

After this period the rate of production reduced considerably (Fig.15A). After ten days the activity remained stationary with slight fluctuations.

When the experiment was discontinued after 30 days the physiological activity was at the declining stage. In the carbon-14 experiments the outcome was more variable after 10 days of growth (Fig.15B). The rate of production increased on the 12th day followed by decrease on 14th and 16th. This fluctuation remained till the 30th day. The highest rate of production as measured by the oxygen experiment was 0.40 $\mu gC/10^6$ cells (gross production) and 0.25 $\mu gC/10^6$ cells (net production). As per the ^{14}C technique the highest rate of production was 0.30 $\mu gC/10^6$ cells.

Pigment composition

The pigment system of the blue-green algae was found to be composed of five fractions (Fig.3A) of which carotene and chlorophyll *a* were dominant. The other three fractions could not be identified. The R_f of these are given in Table 4.

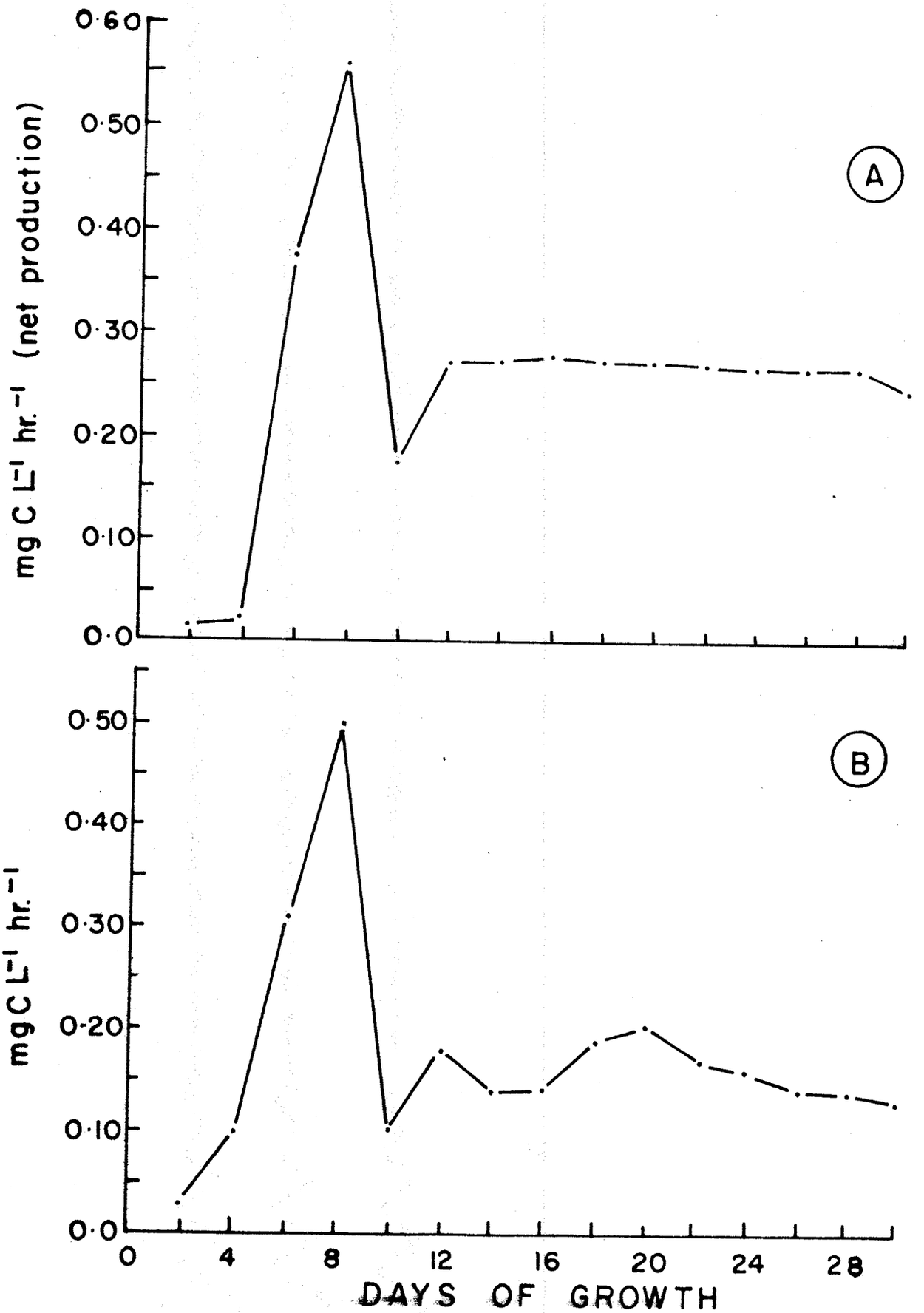
Biochemical composition

The biochemical composition of the species showed definite trends in the new and aged cultures. The water

Fig.15A. Rate of production of organic carbon by S. salina for a growth period of 30 days in batch culture (measured by the oxygen technique).

Fig.15B. Rate of production of organic carbon by the above species for a growth period of 30 days.

Fig. 15


0·60
0·50
0·40
0·30
0·20
0·10
0·0
mg C L⁻¹ hr.⁻¹ (net production)
A
0·50
0·40
0·30
0·20
0·10
0·0
mg C L⁻¹ hr.⁻¹
B
0
4
8
12
16
20
24
28
DAYS OF GROWTH

content of the cells was considerably reduced in old cultures. There was a difference of about 35% between the five day and twenty day old culture. Similarly increment in the dry weight was noticed. The young culture weighed 7 µg/10^6 cells and it doubled as the culture became twenty days old. The protein content was comparatively low with an average of 33–41% . The ratio of protein to carbohydrate decreased from 1.29 to 0.74 as the culture became old. The variation in lipid content was relatively low. The amount of various components are given in Table 23.

Table 23. Biochemical composition of *D. salina* raised in batch culture.

Fraction	Sampling interval		
	5 days	10 days	20 days
Percent moisture	88.57 ±5.14	80.62 ±3.88	50.29 ±4.14
Dry weight (µg/10^6 cells)	7	7.9	15.0
Protein (% dry wt.)	41.0 ±9.16	34.2 ±11.32	33.12 ±6.84
Carbohydrate (% dry wt.)	31.75 ±7.55	36.22 ±8.42	44.56 ±11.19
Lipid (% dry wt.)	12.02 ±2.38	10.16 ±3.12	9.24 ±2.16
Protein: Carbohydrate	1.29	0.94	0.74
Bacteria count/ml	10	1	4

Extracellular release

The species was observed to release a part of the photoassimilates to the culture medium. The four day old cultures excreted 38.91% of the total carbon fixed while the proportion increased to 64.5% in the cultures after 16 days of growth (Table 24). In terms of the absolute activity amounts ranging from 0.129–0.273 μC were retained in the medium as ECP out of the 5 μC added to each sample (Table 7).

Table 24. Percentage of extracellular products (ECP) released by *S. salina* during pheses of growth in culture. (The % soluble represents the ECP as fraction of the total carbon fixed).

Days of growth	Cells ml^{-1}	Particulate mgC/l/hr	soluble mgC/l/hr	% Soluble
4	16 x 10^4	24.62	9.58	38.91
8	360 x 10^4	18.95	7.00	36.94
16	1100 x 10^4	14.24	9.19	64.5

Eco-physiology of *S. salina*

Salinity:

The growth of *S. salina* varied with the salinity of the medium. As shown by the analysis of variance the cell

counts were significantly different till the 14th day following inoculation with exception on the 2nd. After 14 days there was virtually very little difference between the treatments as far as the cell counts were concerned (Table 24). But the culture at low salinity levels were found to be more healthy with a bluish-green tint while that at higher salinity (34‰) was dull green in colour.

The amount of chlorophyll a was high at the lower salinity levels (14‰ and 24‰). Analysis of variance revealed the treatment effect to be highly significant. Between 14‰ and 24‰ there was however, not much difference in the amount of the pigments (Table 25). At salinity 34‰ the amount of chlorophyll a was too low throughout the experiment.

Effect of pH

The difference in the pH of the culture medium was reflected gradually in the growing cultures. In the four day old cultures the cell density did not differ significantly in the pH range 6.5 - 8. But when the culture became still older, there was significant difference between the treatments (Table 26). The highest cell density was built up at pH 7.5 to be closely followed by that at 7 and 6.5. At the stationary stage, the cell density at pH 8

Table 25. Cell concentration and chlorophyll a content of S. salina grown in culture media of salinities 14‰, 24‰ and 34‰ for a period of 24 days.

Age of culture (days)	Cell concentration/ml			Chlorophyll a ($\mu g/10^5$ cells)		
	14‰	24‰	34‰	14‰	24‰	34‰
0	2400	2400	2400	-	-	-
2	20,0000	19,0000	28,0000	0.227	0.229	0.189
4	440,0000	300,0000	218,0000	0.158	0.159	0.100
6	1155,0000	925,0000	900,0000	0.083	0.083	0.016
8	2270,0000	2006,0000	1600,0000	0.032	0.036	0.002
10	3480,0000	3320,0000	2668,0000	0.016	0.016	0.002
12	4320,0000	3936,0000	3700,0000	0.007	0.008	0.001
14	5620,0000	5625,0000	5654,0000	0.006	0.006	0.001
16	8500,0000	8000,0000	8000,0000	0.006	0.007	0.002
18	8520,0000	8515,0000	8510,0000	0.005	0.005	0.001
20	8500,0000	8510,0000	8510,0000	0.005	0.005	0.001
22	7600,0000	7600,0000	7050,0000	0.005	0.005	0.001
24	7534,0000	7500,0000	6129,0000	0.003	0.004	0.001

was too low compared to the other three treatments. The amount of chlorophyll *a* showed highly significant difference between the treatments with the pH levels 6.5 and 7 giving higher values. The Table 26 gives the mean values for the cell counts and chlorophyll *a*.

Table 26. Cell concentration and chlorophyll *a* content of *Synechocystis salina* during phases of growth in culture media of different pH levels.

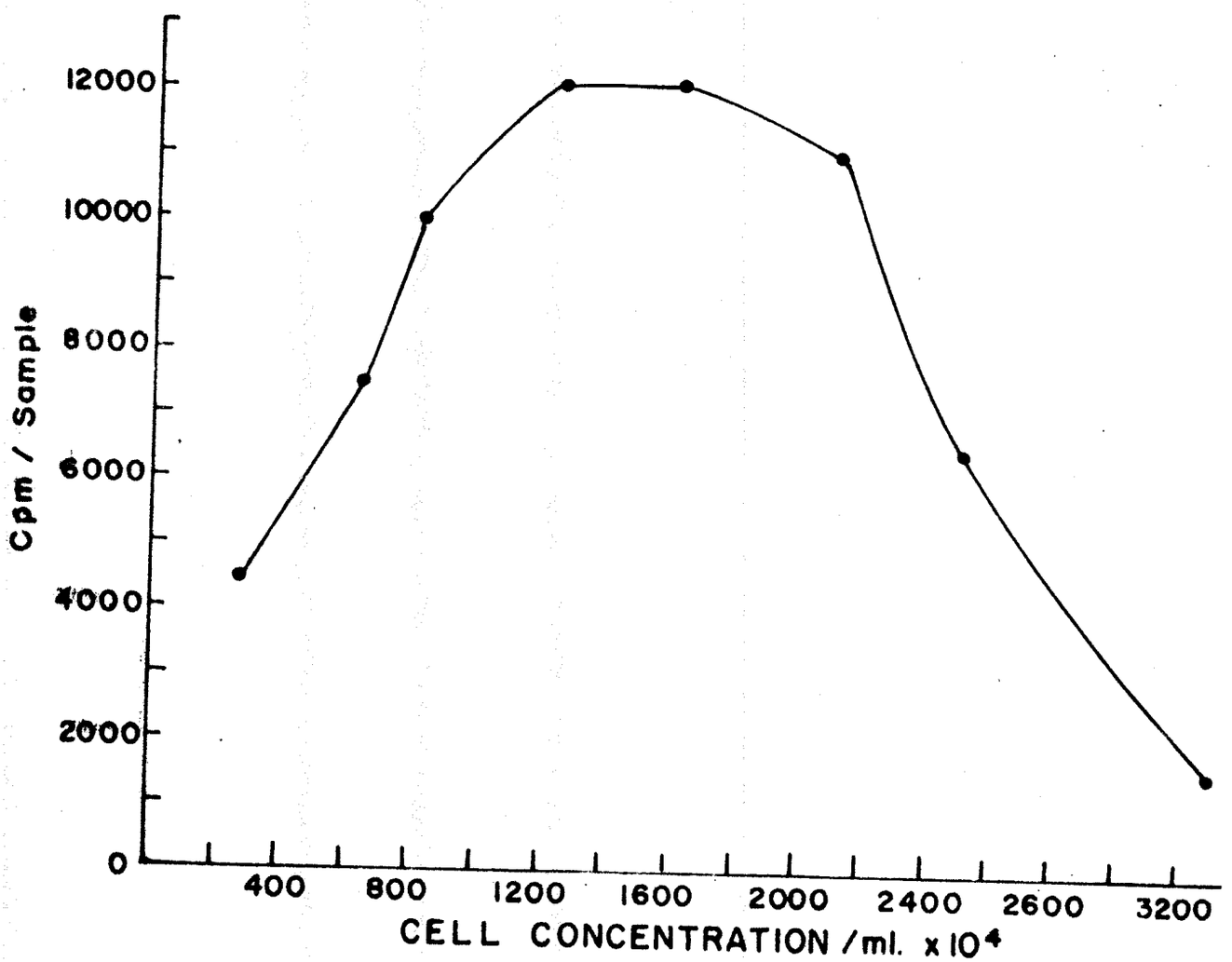
Treatments (pH level)	Logarithmic phase		Stationary phase	
	Cell density per ml	Chlorophyll *a* µg/10^6 cells	Cell density per ml	Chlorophyll *a* µg/10^6 cell
6.5	390,6700	0.162	4506,6000	0.018
7.0	401,0000	0.147	4507,6700	0.017
7.5	411,0000	0.103	4524,3000	0.009
8.0	346,0000	0.099	4048,3000	0.006

Effect of cell concentration on the rate of carbon fixation

The amount of ^{14}C incorporation in the short term experiment increased with the cell concentration. But the trend did not continue indefinitely as is seen from Fig.16. The increasing trend in the production persisted till a concentration of 125 x 10^5 cells/ml. At higher densities,

Fig.16. Rate of carbon fixation (expressed as ^{14}C uptake in cpm) as function of different culture densities of *D. salina*.

Fig. 16.


12000
10000
8000
6000
4000
2000
0
Cpm / Sample
400
800
1200
1600
2000
2400
2600
3200
CELL CONCENTRATION /ml. x 10⁴

upto 175 x 10^5 cells/ml, the production remained unaltered. Above this density, the rate of production decreased. The cell densities and the corresponding ^{14}C activity is given in Table 27.

Table 27. Carbon-14 uptake by Synechocystis salina as function of cell concentration.

Cell density	Counts per minute
275,0000	4450 ± 134.0
640,0000	7506 ± 98.0
825,0000	10089 ± 767.0
1250,0000	12120 ± 582.0
1650,0000	12156 ± 74.2
2100,0000	11910 ± 103.8
2500,0000	6451 ± 77.0
3300,0000	1568 ± 53.8

Influence of light

The response of the organism varied significantly with the photoperiod to which the cultures were exposed. The rate of cell division was higher in those subject to constant illumination. From an initial density of 1440 cells/ml a density of 45 x 10^6 cells/ml was reached in thirty days, by continuous illumination whereas the

density of the culture subject to light-dark cycle remained at 8 x 10^6 cells/ml (Table 28).

Table 28. Effect of photoperiod on growth of *S. salina* in batch culture.

Age of culture (days)	Cell density per ml	
	10:14 Light-dark cycle	Constant illumination
0	1440	1440
2	2,0000	7,0000
4	00,0000	350,0000
6	165,0000	1100,0000
8	290,0000	2000,0000
10	400,0000	2980,0000
12	500,0000	3000,0000
14	520,0000	3110,0000
16	560,0000	3650,0000
18	750,0000	3920,0000
20	780,0000	4200,0000
22	800,0000	4500,0000

The intensity of light to which the cultures were exposed affected the rate of production. From the hyperbolic relation between the light and photosynthesis it was observed that the rate of carbon fixation was

saturated at 34.71 x 10^{15} quanta x cm^{-2} x sec^{-1} with an I_K of 24 x 10^{15} quanta x cm^{-2} x sec^{-1} (Fig.17). The metabolic activity was very low below 14 and above 51 x 10^{15} quanta x cm^{-2} x sec^{-1}.

Effect of temperature

The viability, growth rate and rate of production of the blue-green alga was determined at different temperatures. The cultures were kept in refrigerator (5°C) and recultured weekly to fresh medium at room temperature. It was observed that the cultures were viable upto six months of storage under the low temperature. The cultures behaved normally from 20°C - 40°C. The thermal death point was observed to be 45°C.

The growth rate of the cultures differed slightly at the temperature range 20-40°C. The mean doubling rate per day is given in Table 12. The highest growth rate occurred at 25°C with slight reduction at 30 and 35°C. At 20°C, the rate of growth was less than that at 25° and it was still lower at 40°C. At this higher temperature the cells were viable though with a highly reduced growth rate.

Requirement of nitrate and phosphate

The concentration of nitrate in the culture medium and growth of *S. salina* in culture was related as per the

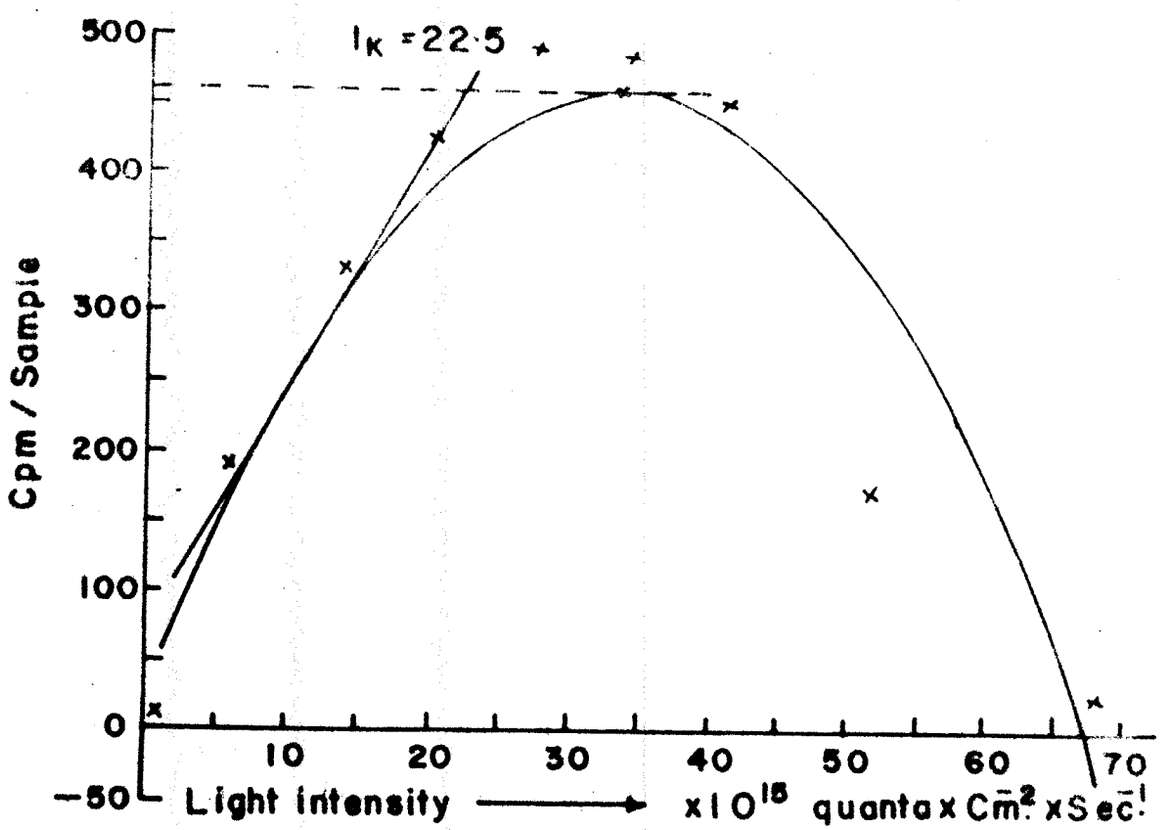
Fig.17. The rate of photosynthesis (expressed as ^{14}C uptake in cpm) as function of light intensity for *D. salina*.

Fig. 17

$Y = 33 \cdot 172 + 26 \cdot 18\ X - 0 \cdot 4\ X^2$

X_{max} = 100 % Photosynthesis = $32 \cdot 73 \times 10^{15}$ quanta $Cm^{-2}\ Sec^{-1}$

$I_K = 22 \cdot 5 \times 10^{15}$ quanta. $Cm^{-2}\ Sec^{-1}$



relation S = 0.983 (S/μ) - 0.81 where 0.81 = half-saturation constant for growth and 0.983 = maximum (unlimited) growth rate (Fig.18).

Similarly, the phosphate requirement of the species could be defined by the relation S = 0.963 (S/μ) - 1.34 where 1.34 is the half-saturation constant for growth and 0.963 is the maximum unlimited growth rate (Fig.19).

As in the case of flagellates, here also the trend was that the observed rate of growth neared μ_{max} only at very high substrate concentrations (Table 13 and 14). It was also observed that a nitrate concentration above 60 mg/litre and a phosphate concentration of about 30 mg/litre were inhibitory to the growth of the species.

4.4. Tetraselmis gracilis

The growth kinetics of the species has been described by Nair, 1974. T. gracilis isolated from the inshore region of Cochin was cultured in Miquel's medium and its growth in culture was studied for 35 days. The highest growth rate (k) as computed from the cell counts was 0.05 with a generation time of 14 hours. From an initial inoculum of 200 cells/ml, the culture density increased to 1 million by the end of the observation period. Chlorophyll-a and b were also measured

Fig.18. The relation between the concentration of nitrate in the culture medium and the specific growth rate of *S. salina*.

Fig. 18.

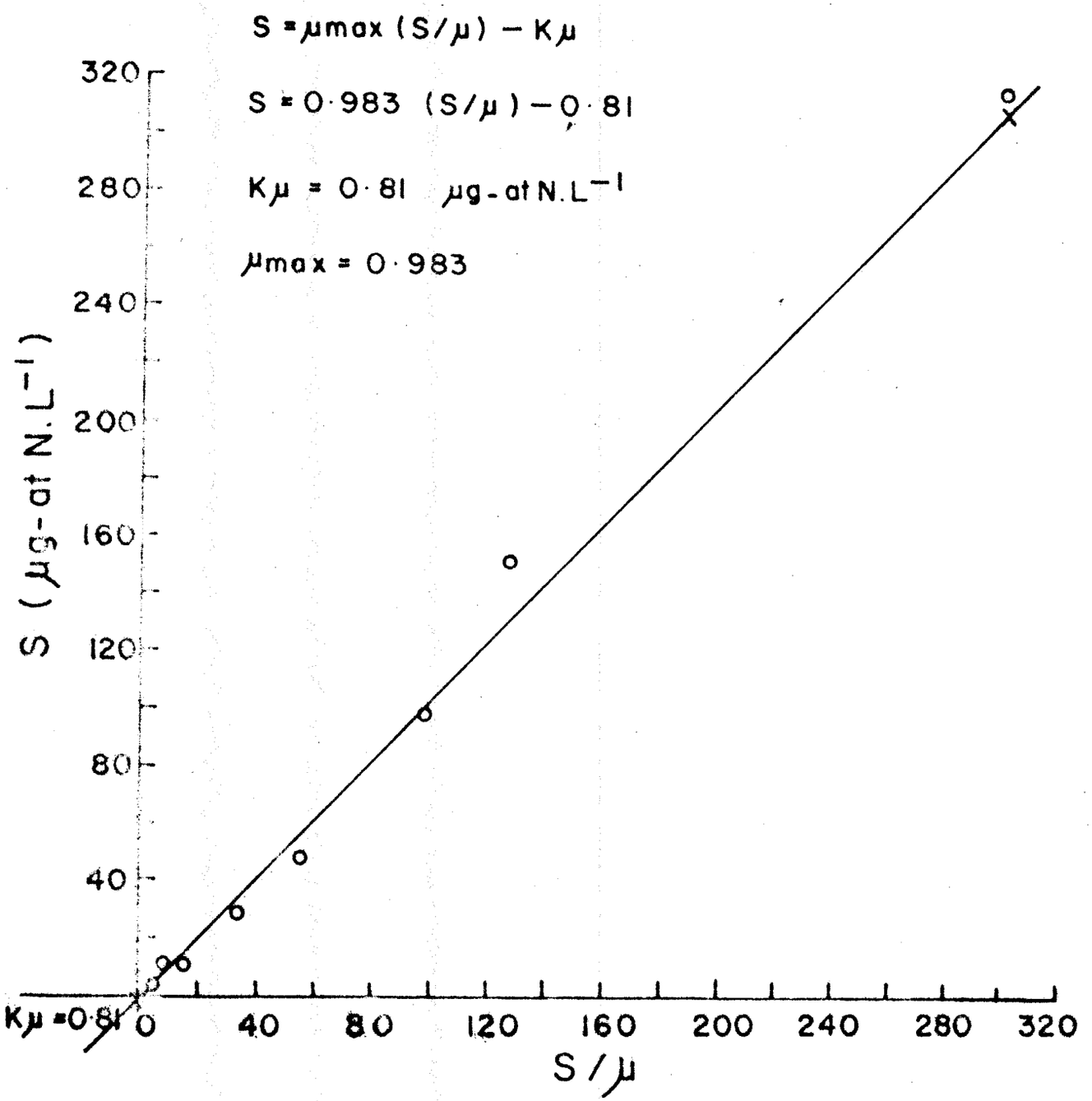

S = μmax (S/μ) − Kμ
S = 0·983 (S/μ) − 0·81
Kμ = 0·81 μg-at N.L⁻¹
μmax = 0·983
S (μg-at N.L⁻¹)
S/μ
Kμ = 0·81
0
40
80
120
160
200
240
280
320

Fig.19. The relation between the concentration of phosphate in the culture medium and the specific growth rate of *S. saline*.

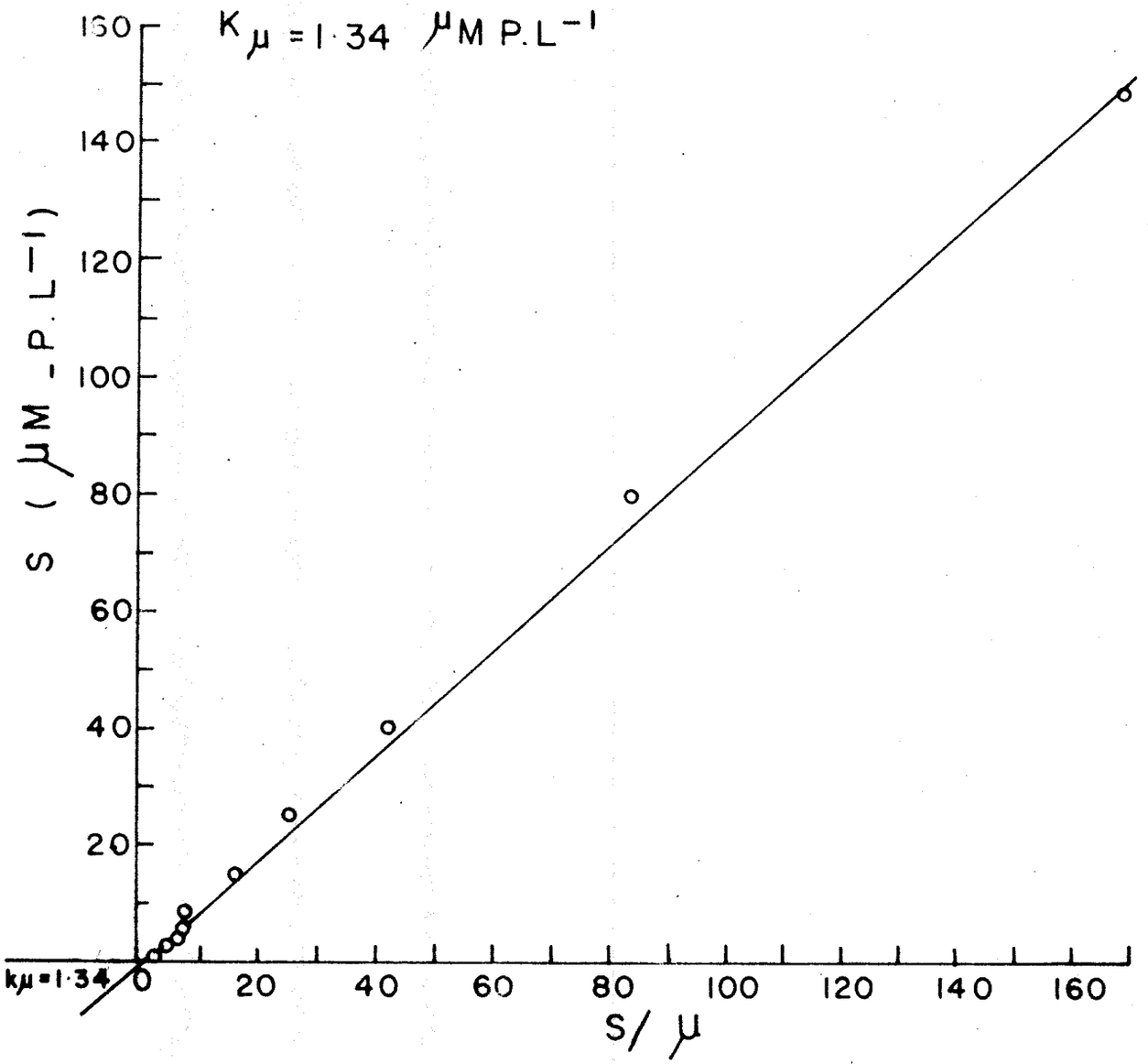
Fig. 19.

$S = \mu max\,(S/\mu) - K_\mu$

$S = 0{\cdot}963\,(S/\mu) - 1{\cdot}34$

$\mu max = 0{\cdot}963$

$K_\mu = 1{\cdot}34\ \mu M\ P.L^{-1}$



simultaneously. The gross carbon values per litre per hour ranged from 0.07 to 0.79 mgC measured by the oxygen technique, and from 0.03 to 0.34 mgC per hour by the ^{14}C technique.

Certain other aspects of growth of this strain was studied during the present investigation.

Pigment composition

The photosynthetic pigments of I. gracilis was analysed by TLC. The chromatogram resolved 4 fractions (Fig.3D). Chlorophyll a, Chlorophyll b and Carotene was quite prominent. The R_F values of these are given in Table 4. The remaining fraction was assumed to be xanthophyll.

Biochemical composition

The biochemical components proteins, carbohydrates and lipids of the flagellate varied in their relative amounts. The lipid level was comparatively lower than that of Carbohydrate and Protein. Protein and carbohydrate contributed the major share with predominant protein synthesis (Table 29). The ratio of protein to carbohydrate decreased by half from five days to twenty days of growth. Similarly there was high fluctuation in the water content and dry weight of the cells.

Table 29. Biochemical composition of Tetraselmis gracilis raised in batch culture.

Fraction	Sampling interval		
	5 days	10 days	20 days
Percent moisture	86.00 ±5.14	82.00 ±3.84	52.76 ±4.81
Dry weight (µg/10^6 cells)	579	720	1249
Protein (% dry wt.)	54.00 ±12.15	46.00 ±7.68	42.00 ±8.34
Carbohydrate (% dry wt.)	23.75 ±7.35	29.69 ±8.24	42.29 ±12.13
Lipid (% dry wt.)	2.20 ±0.09	2.36 ±0.76	1.86 ±0.81
Protein: Carbohydrate	2.13	1.55	0.99
Bacterial count/ml	80	5	12

Excretion of organic matter

The extent of organic excretion also changed with the age of the culture. 2.5% of the total metabolite was released into the medium on the 4th day whereas on the 16th day this fraction increased to 22.36% (Table 30). In terms of absolute activity 0.01–0.28 µC were obtained as ECP in each sample added with a total activity of 5 µC (Table 7).

Table 30. Percentage of extracellular products (ECP) released by *Tetraselmis gracilis* during phases of growth in culture. (The % soluble represents the ECP as fraction of the total carbon fixed)

Days of growth	Cells ml^{-1}	Particulate mgC/l/hr.	soluble mgC/l/hr.	% Soluble
4	2.4×10^4	2.35	0.06	2.5
8	7×10^4	2.18	0.30	13.76
15	15×10^4	3.22	0.72	22.36

5. MASS CULTURE OF CHAETOCEROS AFFINIS AND ISOCHRYSIS GALBANA (C.s.) AND EVALUATION OF THEIR FOOD VALUE IN MARICULTURE.

5.1. Mass culture

Mass production of nanoplankton has been taken up in mariculture operations in connection with the rearing of the filter-feeding larval stages of marine fish and other invertebrates.

The simplest method for the mass production of phytoplankton is by adding commercial fertilizers to sea water collected and kept in out-door tanks. In this system filtered sea water is let into open tanks of 2000 to 10,000 gallons capacity, fertilized with commercial fertilizers to promote a good growth of mixed phytoplankton. Such systems could be installed out-door or in green houses that are artificially lit to provide an year round supply of mixed phytoplankton culture.

Large scale culture of Chaetoceros affinis is taken up in the prawn culture laboratory of CMFRI, Cochin on similar lines. Fibre glass tanks of capacity 1000 litres are filled with filtered sea water and fertilized with commercial fertilizers. These are inoculated with stock

cultures of C. affinis. At temperatures above 28°C good diatom growth dominated by C. affinis is obtained.

Unialgal cultures of nanoplankton of proven food quality are raised on a large scale in controlled conditions. Many laboratories make use of pyrex carbuoys as growth chambers for axenic cultures. The carbuoys are maintained at constant temperature and illuminated artificially. The cultures are aerated by bubbling a mixture of air and CO_2. A fixed volume of the culture is harvested daily and is replaced by an equal volume of the medium. This is a semi-continuous system. Similar cultures are maintained in the molluscan hatchery of CMFRI at Tuticorin. The culture containers used are 2 litre carbuoys or perpex tanks that are artificially lit indoor.

Continuous culture systems have been built up in many laboratories for mariculture operations Palmer et al. (1975) and Trotta (1981). These laboratories use different culture containers like spherical or cylindrical glass bottles, perpex cylinders or plastic bags. These continuous culture systems may function as 'turbidostat' or 'chemostat' cultures (cf. Fogg, 1975). The continuous culture production system usually is comprised of rows of pyrex carbuoys or plastic bags with provision for addition of

nutrients, withdrawal of the culture and aeration. It has also the added advantage that the culture can be maintained in steady state so that a more uniform product can be fed to the larvae continuously (Palmer et al., 1975). The present investigation pertains to mass culture of Chromulina freiburgensis and Isochrysis galbana forma nova. These were grown as discontinuous culture under controlled conditions.

The flagellates were inoculated into Walne's medium taken in 20 litre glass carbuoys. The cultures were exposed to a light-dark cycle of 12:12 hours with illumination of about 20,000 lux from fluorescent lamps at a temperature of 30±2°C (Plate IX) with constant aeration.

The growth of the culture was followed by taking the cell counts for twenty days.

Observation

Chromulina freiburgensis:

The culture was inoculated at a density of 9400 cells/ml. From this initial concentration it increased to 1.015 million cells/ml in six days. After

the 12th day the cell numbers began to decrease. However, the culture declined completely only by the 24th day. The cell counts for the various days are given in Table 31. The growth rate of the flagellate was calculated from the cell counts. The maximum growth rate was observed to be on the 2nd day being 2.1 doublings per day.

Isochrysis galbana (C.s.):

The culture had a density of 326[illegible] cells/ml initially. During the course of growth it increased to [illegible].9 lakhs per ml in ten days. Later the cell numbers were found to decrease (Table 31). It was found that the growth

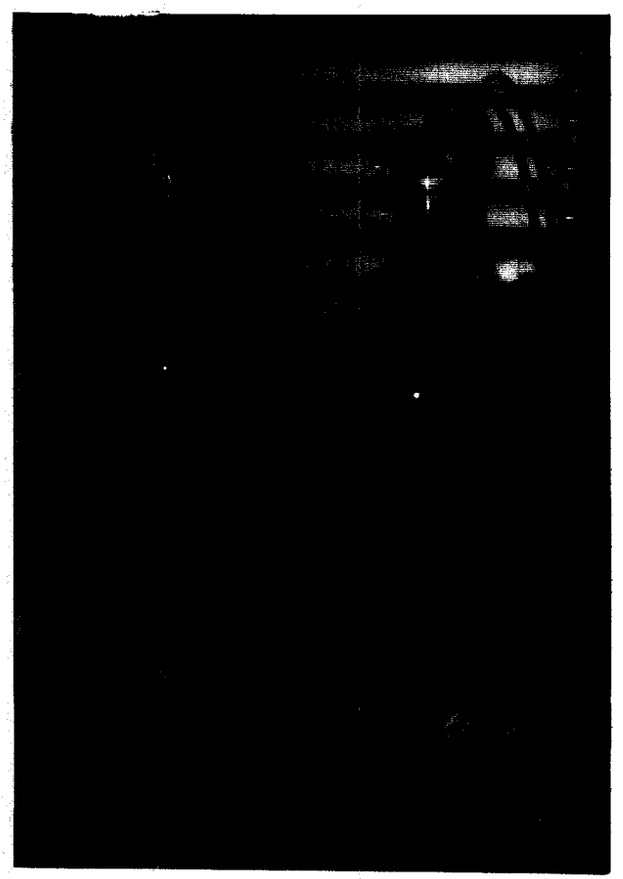
Table 31. Cell concentration per ml, in mass cultures of Chromulina freiburgensis and Isochrysis galbana (C.s

Age of culture (days)	Chromulina cells/ml	Isochrysis cells/m
0	94[illegible]	326[illegible]
1	1,8[illegible]	71[illegible]
2	7,75[illegible]	2,75[illegible]
3	22,5[illegible]	13,[illegible]
4	67,[illegible]	3[illegible]
5	1[illegible]1,5[illegible]	65,[illegible]
6	1[illegible]5,[illegible]	79,[illegible]
8	1[illegible]3,[illegible]	87,[illegible]
1[illegible]	93,[illegible]	99,[illegible]
12	48,[illegible]	42,[illegible]
14	46,[illegible]	46,[illegible]
16	38,[illegible]	36,[illegible]
18	53,[illegible]	35,[illegible]

EXPLANATION OF PLATE IX

Mass culture of *L. freiburgensis* and *L. galbana* (U.S.)

PLATE IX



rate was maximum on the 2nd to 3rd day with a maximum value of 2.24 doublings per day. The cultures declined completely and the cells settled down by the 20th day after the initiation of the experiment.

5.2. Evaluation of the acceptability of *C. freiburgensis* and *I. galbana* (C.s.) as food for the larvae of edible oyster, *Crassostrea madrasensis*.

Bivalve hatcheries around the world utilise different feeds to rear the larvae from the beginning of the planktonic stage to metamorphosis. The relative efficiency of the feed is verified in terms of the larvae that survive. Loosanoff and his collaborators in the Milford laboratory and Walne, Davis, Guillard, Imai and others in Europe, America and Japan have successfully reared the larvae of several genera of bivalves (cf. Loosanoff and Davis, 1963).

It has been now well established that bivalve larvae can be reared in the laboratory on a diet of small naked flagellates. It is also observed that the phytoplankters are not equal in nutritive value. Cole (1936) suggested that the algae like *Chlorella* and *Coccomyxa* were unsuitable as food for oyster larvae probably because of the thick cellulose cell wall which the larvae may find

difficult to digest. However, Newell (1953) demonstrated that the style enzymes of adult oyster can degrade cellulose. Loosanoff and Karak (1951) raised the larvae of certain bivalves on mixed Chlorella culture. Davis (1953) observed that the larvae of Crassostrea virginica utilised thick walled nanoplankton: Chlorella, Platymonas sp., Chlorococcum sp. and Phaeodactylum tricornutum. This led to the suggestion that certain bivalves can utilise only a few species of microalgae while certain others have a wider option. As Ukeles (1975) sums up, the three basic requirements of the feed are:-

1. The feed must be non-toxic.
2. It must be easily accessible by the animals.
3. It must provide the nutrients for normal growth and development.

It has also been observed that a mixture of algal species is superior for feeding compared to single species. A mixture of Isochrysis galbana, Monochrysis lutheri, Platymonas sp. and Dunaliella euchlora induced rapid growth of both clam and oyster larvae compared to equal quantities of any of these fed separately (cf. Loosanoff and Davis, 1963).

Production of toxic products at a concentration lethal to the larvae is considered to be a drawback of

ertain flagellates as Prymnesium parvum (Davis and uillard, 1953). A few studies have also been directed o the chemical composition of the nanoplankters.

The size of the feed particle is an obvious limitation in the development of live-food in bivalve hatcheries. Diatoms are not usually used to rear the oyster larvae as these are larger and difficult to digest due to the silica walls. However, Japanese workers have successfully reared molluscan larvae mainly that of scallop on a diatom diet comprising Cyclotella nana, Skeletonema costatum, Isochrysis galbana, Chaetoceros gracilis, Chaetoceros simplex and Chaetoceros calcitrans (Ukeles, 1975).

Colourless flagellates have been tried by many workers. But they have not proved to be quite successful food for rearing oyster larvae (Waine, 1956). The Milford laboratory team also has arrived at a similar conclusion.

The motile green algae like Dunaliella and Pyramimonas are also considered to be satisfactory food for rearing the larvae. However, the yellow-brown chrysomonads in single and in combinations have proved to be the best food for bivalve larvae. Flagellates like

Isochrysis galbana and Monochrysis lutheri were found to be of equal food value and among the best to feed the bivalve larvae (Loosanoff and Davis, 1963). Walne (1956) also observed rapid growth of the larvae of Ostrea edulis on a diet of I. galbana and another Chrysomonad Chromulina pleiades. The significance of Isochrysis galbana in bivalve hatcheries has further been proved by Walne (1970) and Epifanio (1979). I. galbana is used in molluscan hatchery of CMFRI to rear the larval stages of pearl oyster, Pinctada fucata and edible oyster, Crassostrea madrasensis. Recently success was achieved in rearing the larvae of Indian mussels, Perna indica and Perna viridis from fertilization to spat stage. A tropical strain of I. galbana has been used with great success by Ewart and Epifanio (1981) to rear the larvae of the American oyster, Crassostrea virginica.

The present experiment was conducted to find out the suitability of the tropical flagellates Chromulina freiburgensis and Isochrysis galbana (C.s.) as food for oyster larvae. Newly hatched larvae of edible oyster, Crassostrea madrasensis was used for this purpose. In the laboratory of CMFRI these larvae are usually raised on a diet of I. galbana. The nutritional quality of the present isolates were compared against this control feed.

Organization of the experiment

Naturally spawned larvae of C. madrasensis were collected after 24 hours of fertilization. The size of the larvae was 55µ. These larvae were suspended in sea water (filtered through 1µ net and UV irradiated) of salinity 34‰ at a temperature of 28±2°C.

Filtered and sterilized sea water was taken in corning glass beakers of capacity 2.5 litres and equal number of larvae were suspended in them at a density of five larvae per ml. These were placed as three sets and fed on one of the three diets given below.

Diet A - Chromulina freiburgensis
Diet B - Isochrysis galbana (C.s.)
Control - Isochrysis galbana

The feeding schedule was once in a day at a rate of 5000 cells per larva.

The larval density and feeding level were fixed arbitrarily. Every alternate day the sea water in which the larvae were suspended was changed. The nanoplankters used to feed the larvae were cultured in Walne's medium as described in section 5.1. These were fed to the larvae on every 5th day of inoculation.

Samples of larvae were collected from each group at 2–4 days interval and the size of the larvae was measured. The number of days taken by the larvae to settle as spat and the percent survival of the larvae were also noted.

Results

The mean size of the larvae on the various days of observation is given in Table 32. All the three diets supported the growth of the larvae to metamorphosis. But as presented in Table 32, the diets influenced the growth rate (reflected by the size of the larva) differently.

Table 32. Mean size (in microns) of the larvae of Crassostrea madrasensis reared on three nanoplankton diets (Initial size of the larvae = 55µ).

Diet	Sampling interval				
	5 days	7 days	11 days	14 days	19 days
Chromulina freiburgensis	93.25 ±1.08	120.0 ±4.30	160.75 ±7.24	298.0 ±8.15	573.3 ±7.91
Isochrysis galbana (Exp.)	90.00 ±2.36	91.25 ±5.80	150.25 ±3.16	261.25 ±6.74	533.20 ±5.30
Isochrysis galbana (Control)	87.25 ±1.76	106.50 ±6.81	137.25 ±3.95	201.75 ±7.18	492.50 ±8.22

The mean size of the larvae was larger in those fed with C. freiburgensis and I. galbana (C.s.) compared to the control. Results of the student t-test indicated highly significant difference between the size of the spat as measured on the 19th day of observation. The results of t-test with the three diets is given below.

Diet A/control - $P \leq 0.01$, d.f. = 18, t = 13.32**
Diet B/control - $P \leq 0.01$, d.f. = 18, t = 5.91**
Diet A/diet B - $P \leq 0.01$, d.f. = 18, t = 5.98**

These data show that the present isolates induced higher growth rates of the oyster larvae. It was also observed that the larvae fed on C. freiburgensis settled on the 17th day while in the other two the larval settlement started only on the 19th day.

**Significant at 1% level.

The percent survival of the larvae was significantly higher in those fed with the present isolates: C. freiburgensis and I. galbana (C.s.) (Table 33).

Table 33. Percent survival of the larvae of C. madrasensis fed with three nanoplankton diets (Initial number of larvae = 10,000)

Diet	No. of spat obtained	Percent survival of larvae
Chromulina freiburgensis	279	2.79
Isochrysis galbana (C.s.)	101	1.01
Isochrysis galbana (Control)	24	0.24

Conclusion

This experiment clearly indicates that the present isolates, Chromulina freiburgensis and Isochrysis galbana (C. are quite successful feeds in oyster rearing. Among these C. freiburgensis offers the greatest potential to be developed as live-food.

In the bivalve hatcheries in India and abroad greater importance has been placed on Monochrysis lutheri and Isochrysis galbana as these have been found to promote good growth of the larvae. There are some random reports of Chromulina pleiades being used successfully for rearing

the larvae of Mercenaria mercenaria (Davis and Loosanoff, 1953), Ostrea edulis (Walne, 1956) and Crassostrea virginica (Loosanoff and team, Milford laboratory). However, these authors consider I. galbana and M. lutheri superior to C. pleiades in promoting the growth of these bivalve larvae.

Ewart and Epifanio (1981) have presented the tropical strain of Isochrysis (T-ISO) as a suitable substitute for Isochrysis galbana for both the larval and juvenile American oysters, Crassostrea virginica. T-ISO is reported to be suitable for development in mass cultures in green house environment of 15-30°C under which conditions this gives 0.8 to 2 doublings per day.

The present investigation has projected to tropical flagellates with 2.5 to 3.5 doublings per day in the temperature range 20-35°C. It has been shown experimentally that both these species thrive well in indoor mass culture system. In the case of stability C. freiburgensis shows greater potential to be developed in hatcheries. The feeding experiment also projects Chromulina freiburgensis superior in food value than Isochrysis galbana (T.s.). These findings indicate the greater potential for the use of Chromuline freiburgensis in the tropical oyster hatcheries.

DISCUSSION

The nanoplankters, Chromulina freiburgensis, Isochrysis galbana (C.s.) and Synechocystis salina raised in culture to study their growth rate and activity grew asymptotically building up dense populations. As seen from the results the logarithmic phase, the phase of declining growth rate and stationary phase were well marked in the course of development of the culture. In most of the cultures the lag phase was not noticed. Probably the absence of the lag phase is because the inocula were taken from exponentially growing cultures. In fact Spencer (1954) and Fogg (1944) have correlated the length of the lag phase to the age of inoculum. They report that in Phaeodactylum tricornutum and in Anabaena cylindrica the lag phase is totally absent if the inoculum had been growing exponentially.

The maximum growth rates observed (Table 1) was 2.66, 1.48 and 2.74 for C. freiburgensis, I. galbana (C.s.) and S. salina respectively corresponding to a relative growth constant of 0.079, 0.043 and 0.079. Previous literature shows that the relative growth constant (k) for various phytoplankters varies between 0.01 and 0.1 per hour at optimum conditions (Oppley and Strickland, 1968).

The present results are also in confirmity with this. The growth rates observed during this study were higher compared to that of similar strains of the temperate waters (cf. Fogg, 1975). The temperate strain of I. galbana has a generation time of 30.2 hours (Kain and Fogg, 1960) whereas the present strain took only 16 hours. In mass culture experiments, the strain was found to divide in 9 hours. The tropical strain of the green flagellate Tetraselmis gracilis divided once in 14 hours as reported by Nair (1974). The increased growth rate could have been due to the tropical conditions and the nutritional aspects of the culture medium.

For a given species the growth rate is a function of temperature, light intensity and nutrients. As in this study, the culture conditions to which all the three species were exposed were identical, any difference in the growth rate must be species specific. The nature of the inoculum may also have a role to play. Comparing the flagellates with Synechocystis salina, an obvious feature is that the latter maintained increased doublings of cell for a longer period than the flagellates so that it could attain very high densities (40-50 million cells per ml). S. salina is a minute coccoid blue-green alga of volume $14\mu^3$. The higher growth rate may be in accordance with the fact that smaller species grow faster (cf. Raymont, 1980).

Blue-green algae in general are observed to have low generation time, the lowest t_g recorded being two hours for Anacystis nidulans (Kratz and Myers, 1955a). I. galbana (C.s.) (396 μ^3) took more time to divide than C. freiburgensis (524 μ^3). In fact in other experiments in the same series I. galbana (C.s.) showed similar growth rate as C. freiburgensis. But the higher generation time (t_g) of I. galbana (C.s.) shown in the experiment 3.4.1. may be due to some factor concerned with the inoculum. These results show that apart from culture conditions and cell volume, factors like health of the inoculum and other features characteristic of a species or taxon can affect the growth rate in culture.

The amounts of Chlorophylls a, c and total carotenoids were high during the exponential phase for the flagellates. This may be because the cells have access to abundant nutrients. The amount of chlorophyll a decreased considerably in a week's time. Similar results have been obtained in batch growth of cultures by previous workers (Morris and Glover, 1974; Vijayaraghavan et al. 1975). The relative proportion of carotenoids increased in the old cultures resulting in the change of colour of the culture from golden yellow to orange-red. Accumulation of carotenoids in ageing cultures has been frequently

encountered (Fogg, 1975). The possible reason for this as suggested by Droop (1954) is the depletion of nitrogen or phosphorus.

The culture of Synechocystis salina behaved in a slightly different way. In this the pigment content kept on increasing for eight days, remained at the maximum till 14th and then began to decrease. The decrease in chlorophyll a was reflected in change of colour of the culture from bluish green to dull green.

The rate of production of Chromulina freiburgensis and Isochrysis galbana (C.s.) was at the highest level during the first week of culture coinciding with the maximum pigment concentration. This confirms the fact that production is proportional to the pigment content. But in Synechocystis salina the period of peak metabolic activity preceded that of maximum chlorophyll a content. Probably, other factors such as high culture concentration and its further consequences interfered with the measurements. The decreased rate of production in the ageing cultures may be attributed to the decreased content of chlorophyll a and nutrient depletion which is probable to occur in batch cultures. Moreover many cells in the old cultures were in the non-motile resting stage at which time metabolic activity is normally reduced.

Chromatographic analysis of the photosynthetic pigments of the species revealed the major fractions and hence their taxonomic affinities. Chlorophyll *a* and carotene was observed in all the four species. These components are characteristic of all algal classes (Strain, 1951). Chlorophyll *b* is unique to the green algal line and the results showed it to be present only in *Tetraselmis gracilis*. Chlorophyll c was located in *C. freiburgensis* and *I. galbana* (C.s.) indicating them to belong to the brown algal line. No such indicative pigments could be spotted for *Synechocystis salina*. The suggestion that the yellow pigments of the Chrysomonads could be xanthophyll is substantiated by the work of Jeffrey (1968) on *I. galbana*. She fractionated the pigments of *I. galbana* into seven fractions including chlorophyll *a*, *c*, carotene, fucoxanthin and three other yellow xanthophylls.

The nanoplankters were observed to have predominant protein synthesis in the exponential growth phase while the relative proportion of protein decreased in the aged cultures. Algae have been established as mainly protein synthesizers (Spoehr and Milner, 1949; Lewin and Guillard, 1963). Parsons *et al.* (1961) observed in algae harvested at logarithmic phase of growth, lower contents of lipids and carbohydrate. Ricketts (1966) using stationary phase

cultures obtained relatively higher lipid and carbohydrate content. Platt and Irwin (1973) also suggest that lipids are minor components of phytoplankton. The present observation of changing ratios of protein to carbohydrate is on par with that of the results obtained by Handa (1969), Myklestad (1974) and Goldman (1980). Fogg (1956) working with the diatom, Navicula pelliculosa observed that the predominant protein synthesis is replaced by lipid in nitrogen deficient algae. Nutrient depletion leading to carbohydrate accumulation in natural populations has been reported by Myklestad and Haug (1972) and Haug, et al. (1973).

The bacterial counts in the cultures used for the present analysis was negligible. As such the role of bacteria in the changing metabolic pattern of the algae seems to be insignificant. The variation in the chemical composition of the cell, though apparently related to the age of the culture as observed in the present study, could be due to the influence of physical and chemical factors of the environment to which the species were exposed. It was also observed that the amounts of protein, carbohydrate and lipid in the four species were not identical though they were grown under similar conditions. The difference is therefore solely a species characteristic.

The amount of organic metabolites released by the nanoplankters C. freiburgensis, I. galbana (C.s.), D. salina and I. gracilis varied from 2.5% to 64.5%. Liberation of organic metabolites is shown by all phytoplankters. However, there is little agreement on the absolute amounts released. The consensus of opinion is that exponentially growing cells release comparatively less amounts of organic metabolites while the rate of excretion increases during the stationary phase (cf. Hellebust, 1974). The same was observed in the present study. C. freiburgensis excreted 3% during the exponential phase and it increased to 12.57% by the 16th day. Similarly the other algae showed increased rates of excretion with ageing.

Guillard and Wangersky (1958) have shown that marine Chrysophytes release organic metabolites at increased rates during the post exponential phase of growth. I. galbana produced upto 25 mg/l and Prymnesium parvum, 125 mg/l of excreted material. In the present study the Cochin strain of I. galbana (C.s.) produced upto 2.3 mg/l, C. freiburgensis, 1.22 mg/l and I. gracilis, 0.72 mg/l. In D. salina it was 9.58 mg/l for the given culture densities (see Chapter 4).

The present data agree with the work of Samuel et al., 1971 on a few tropical flagellates. According to their observation seven day old cultures of Tetraselmis gracilis, Tetraselmis certeriiformis, Dicrateria sp. and Chlorella sp. excreted 3.1%, 1.43%, 4.35% and 1.65% of organic matter respectively. Hellebust (1965) studying the rate of excretion of 22 species of phytoplankters concluded that at logarithmic phase under similar conditions the majority of the species excreted only 3.6% of the carbon assimilated. A few algae excreted upto 25%. In the present experiment the cultures of Synechocystis salina raised under the same conditions as the flagellates exhibited a higher percentage of excretion. During the exponential phase, the flagellates excreted less than 4% of the ^{14}C incorporated while S. salina showed an excretion of 38.91%. Probably this is explained by the work of Malewajko (1966). In dilute suspensions of microalgal cultures excretion was less than 2% of the total carbon fixed. Excessive population densities reduced photosynthesis as well as increased excretion. He relates it to the decreased carbon dioxide concentration and light intensity that take place due to cell crowding, resulting in higher rates of exretion. The higher excretion rate

by S. salina observed in the present study may be thus attributed to the increased population density.

The percentage of dissolved organic matter may tend to rise with decreasing physiological activity. The higher content of organic metabolites in the culture medium in ageing cultures could also be due to the passive release of organic matter from the dead and moribund cells (Fogg, 1975).

A study of the kinetics of growth of phytoplankters is never complete without an appraisal of its immediate environmental factors that control growth. An exclusive factor in the cultivation of marine microalgae is salinity of the culture medium. The response of the flagellates under study was similar to the variation in salinity of the medium while Synechocystis salina had different salinity preference. The chrysomonads grew well at higher salinities (greater than 24‰) while S. salina multiplied rapidly and had a higher pigment content at the lowest salinity level (14‰) tested. In the case of flagellates, both cell number and chlorophyll a was low at salinity of 14‰. In S. salina higher salinities affected the pigment content profoundly as shown by the chlorophyll a measurements and it could be discerned from the colour of the culture that they were not healthy. But cell counts

were nearly uniform after the culture entered stationary phase. It has been reported that sub-optimum salinities can affect the chlorophyll a content of the algae while the growth may be unaffected (McLachlan, 1961). Sharp changes in the colour of the culture is reported to occur in cultures of Dunaliella salina grown at different salinity levels (Loeblich, 1982). The colour of this flagellate vary from green at lower salinity to red at very high salinities. Obviously, at higher salinities there is a reduction in chlorophyll synthesis and increase in the carotenoid pigments. This particular behaviour of D. salina is also shown to be brought about by changes in pH, light intensity, and deficiency of nitrogen and phosphorus. In the present study, the pigment content was affected significantly during the exponential phase also when nutrients could not have been limiting. So the reseponse of the algae is not due to nutrient deficiency. The chance of high light intensity affecting the pigment content of any particular treatment was also eliminated as all the cultures were exposed to similar conditions. As the pH was adjusted using buffer, the behaviour of the species is attributable to salinity change alone. It may be noticed that the rate of cell division and synthesis of chlorophyll a was promoted by higher salinities in the flagellates while the same salinity levels inhibited

the chlorophyll synthesis in S. salina. It may be that salinity is interacting with intracellular features that ultimately define the growth and activity of the organism. Though the optimum salinity level for the species differ, all show adaptability to the range of salinity tested and the experiment is a good example to show how the algae can control its metabolic activity to the requirement of the environment.

Phytoplankton has marked capacity to adjust osmotically. Most neritic marine flagellates can grow in 12‰ to 40‰ S with an optimum at 20‰ to 24‰ S (Provasoli, 1963a). Among the Chrysomonads, I. galbana tolerates salinities from 15‰ to 40‰; Monochrysis lutheri also does not show a distinct salinity optimum (Droop, 1958a). Further, the salinity tolerance may vary significantly with extremes of temperature, light and nutrients (Bonin et al., 1981.

Qasim et al. (1972) have attempted to define the optimum salinity for maximum photosynthesis in some of the tropical diatoms and dinoflagellates isolated from the inshore region of Cochin. All the species tested showed wide adaptability to changes in salinity and in all, the maximum photosynthesis occurred at low salinities (less than 20‰). They relate this to the ecological feature

of the region from which the species were isolated. In the inshore region of Cochin, the salinity varies from brackish (14‰) to marine (Darbyshire, 1967). The reduction in salinity is coupled with enrichment of nutrients enabling dense phytoplankton growth (Subrahmanyan, 1954; Prakash and Sarma, 1964). The same, they reason, may be responsible for accelerating photosynthesis in the cultures at low salinities. But as the present investigations reveal, the flagellates, S. freiburgensis and I. gelbana (C.s.) that were also isolated from the inshore region of Cochin exhibited better growth at higher salinities (>24‰) while S. salina grew better at lower salinity. S. salina is a blue-green alga, the group being mostly represented in fresh water (Desikachary, 1959). May be it is this basic affinity to fresh water environment, that is responsible for the better growth of the species at the lowest salinity (14‰) tested.

The results of the experiment with the three species reveal that salinity tolerance is predominantly a species characteristic though the same may by induced by an ecological need for survival. These results lend support to the statement of Qasim et al. (1972) that 'tropical phytoplankton species show a wide adaptability to changes in salinity'; but is at variance with the generelization

that 'their maximum photosynthetic rates occur at low salinities' assuming that photosynthetic rate reflects growth.

The hydrogen ion concentration of the medium affected the cell counts as well as the chlorophyll *a* synthesis though the effect on the latter was more pronounced. The flagellates grew best at pH 8. The acidic pH was found to be unsuitable for them. But at pH levels less than 8, *S. salina* exhibited good growth. The marine species in culture are reported to tolerate 6.8 to 9.6 pH levels with optimum at pH 8 (Kain and Fogg, 1958b). Pigment changes are also reported to occur in relation to pH change. It is believed that pH affects the organism through the transport mechanisms of the membrane systems or it may interfere directly with the metabolic activities or indirectly by controlling the dissociation rates of the inorganic and organic compounds in the medium (*cf.* Soeder and Stengel, 1974).

It may be noticed that *S. salina* has low salinity and low pH optima while the flagellates prefer higher salinity and higher pH. It may be remembered that in aquatic bodies fresh water influx that bring about reductio in salinity may lower pH. In the laboratory studies it

has been shown that the species concerned can adapt to both these factors simultaneously.

The rate of carbon-14 uptake by the nanoplankters was found to increase with the cell concentration. However the trend did not continue indefinitely. Beyond a culture density of 6-10 x 10^5 cells/ml, the rate of photosynthesis of the flagellates was reduced. However in S. salina, cultures at densities exceeding 175 x 10^5 cells/ml showed a decreasing trend. The probable reason for this inhibitic may be that the amount of light received by individual cel was reduced due to crowding or the amount of carbondioxide would have been limiting in high density cultures.

Light influences photosynthesis by its quality, quantity and photoperiod. The algae used in the present investigations were maintained in L:D cycle of 10:14 from fluorescent lamps. However, all the three grew better at constant illumination. It may be reasoned that longer lig period enabled light processes of photosynthesis to be carried on further resulting in more synthesis of organic carbon. It is generally held that blue-green algae do not need a light-dark cycle for good growth. Reference may b made to Fogg et al. (1973) who state that 'intermittent illumination (16:8 L:D) does not give better yields than

continuous illumination and blue-green algae do not appear to require a diurnal alternation of light and dark periods'.

Response to photoperiod has been found to occur in the green alga Dunaliella tertiolecta (Eppley and Coatsworth, 1966) and in the marine diatoms (Curbin, 1974; Holt and Smayda, 1974). Foy et al. (1976) observed that the smaller species of Oscillatoria namely O. redekii grew faster in constant illumination though the larger species O. agardhii exhibited better growth on the L:D cycle. They reason that, in the smaller species where the surface area/volume ratio is large the rate of excretion tend to be high in the dark. So on constant illumination the rate of loss is minimised. Probably the same reason may explain the better growth of the present species studied when subject to constant illumination.

The rate of photosynthesis in all the three cultures increased with increasing light intensity. The increased rate of photosynthesis with increasing light intensities may be brought about either by an increased pigment content or by enhancement of the photosynthetic rate itself (Steemann Nielsen and Jorgensen, 1968a; Jorgensen, 1969). However, all species invariably showed 100% photosynthesis around 34 x 10^{15} quanta/cm^2/sec. with I_k ranging from

22-24 x 10^{15} quanta/cm^2/sec. Light - photosynthesis relationship has been described early by Talling (1957), Steemann Nielsen (1962), Jorgensen (1964), McAllister et al. (1964) and Steemann Nielsen and Willemoes (1971). They have recorded, differences in the light adaptation capacity (indicated by I_K) and light saturated photosynthesis, depending on the species under study and the pre-incubation light intensity. It is noticed that, in the present investigation, there is not much difference in the response of the three species concerned. It may be that all these organisms were grown under the same light condition. Jorgensen (1964) reports that Chlorella vulgaris and Cyclotella meneghiniana adapted to 3 klux and 21°C had nearly same I_K i.e. 12 klux for Cyclotella and 13 klux for Chlorella, but when grown at 3 klux and 21°C, this similarity in I_K was not obtained. This shows that both species factor and pre-incubation factor can affect the I_K not to exclude the influence of temperature. Maddux and Jones (1964) have also related the light adaptation capacity of microalgae to temperature and the chemical composition of the medium. With the available evidence it can be said that the I_K of the species tested has been influenced by the pre-incubation light intensity. Generally, the temperate species have low light saturation levels compared to tropical forms. Photosynthesis in

Phaeodactylum tricornutum is saturated at 12,000 lux (Hayward, 1968c) while for Monochrysis lutheri it saturates at 2000 lux (Droop, 1961c). In the present investigation the saturating light intensities were quite high; may be because these are tropical strains and acclimatized to light of 25,000 lux. Qasim et al. (1972) have also reported similar I_K values for certain tropical diatoms and flagellates.

Within the temperature range 2 -35°C the growth rate of the three species did not differ significantly. The growth was seriously affected at 4°C; but Synechocystis salina continued to survive upto 45°C. However, the temperature range for photosynthesis was found to be very narrow. Maximum photosynthesis occurred at 30°C in the flagellates while it was at 25°C for S. salina. The rate of production was considerably reduced at all other temperatures. The temperature range for survival was however large for all species. Flagellates could survive the low temperature storage for 3 months and S. salina for 6 months. Discrepancies in the temperature optima for growth and photosynthesis have been reported early. It may be that the enzymatic processes controlling the cell division and photosynthesis are different (Innis and Ingraham, 1974; Rhee and Gotham, 1981). Another aspect is that the optimum temperature for growth is higher

compared to the temperate water strains, that usually prefer 15–22°C. According to Kain and Fogg (1958b) and Ukeles (1961) *I. galbana* does not survive above 27–30°C. But the present isolate of *I. galbana* grows well at 25–35°C with maximum photosynthetic activity at 30°C. In fact tropical strains are unable to grow at lower temperature. A tropical strain of *Gymnodinium* did not grow at 15°C, but at higher temperatures upto 37°C growth was normal. The same has been shown by certain tropical diatoms (Guillard and Ryther, 1962; Thomas, 1966). Thus the present investigation lends support to the view that different species and strains of the same species isolated from different geographical locations, may differ in their temperature responses. The temperature of acclimation has a significant role in this adaptation process. Adaptation may be brought about by many changes within the cell, like changing the pigment content or enzymes.

The thermal death point is also high for the tropical plankters. The concerned flagellates were alive only for a week when exposed to 40°C; *S. salina* survived at 40° though with highly reduced growth rate. However 45°C seemed to be lethal to the species. These observations are in keeping with the remark of Hall (1953) that 'active stages of 'protists' are usually killed as the temperature

approaches 45°C'. The thermal death may be caused by an increase in the viscosity of the protoplasm, denaturation of proteins or nutritional starvation (Heilbrunn, 1952; Hutner et al., 1957).

The amount of nitrate and phosphate as indicated by K_μ supporting the maximum growth rate of the flagellates and Synechocystis salina were different. The K_μ indicates the capacity of the species to adapt to low substrate levels. A species with low K_μ and high growth rate can dominate those with higher K_μ and low growth rate in mixed cultures and natural populations. Comparing the two flagellates, it is found that Chromulina freiburgensis has a K_μ of 0.7 μg-at. l^{-1} which is almost half of that for Isochrysis galbana (C.s.). The μ_{max} of I. galbana (C.s.) was not proportionately higher. It may be assumed that in mixed cultures of these species C. freiburgensis will dominate at low nitrate levels at least during the initial phase of culture. Probably the ratio may change as the culture ages due to the relatively higher growth rate of I. galbana (C.s.).

The phosphate utilization capacity of the flagellates does not differ markedly. However, there can be marginal difference in the popula ion density in

the mixed culture of these species at the initial stages with a dominance of C. freiburgensis.

S. salina had a K_μ of 0.81 µg-at. N L^{-1} for a μ_{max} of 0.983. This shows that this blue-green alga is better equipped to outcomplete the chrysomonads. The K_μ and μ_{max} for phosphorus utilization of S. salina was nearer to that of C. freiburgensis being 1.34 (K_μ) and 0.963 (μ_{max}) and 1.31 (K_μ) and 0.97 (μ_{max}) respectively. This would give an almost equal chance for proliferation to the species.

It is also seen from the data that the substrate concentration supporting maximum growth rate as per the observation is quite high than that computed. In fact the K_μ measures the ability of phytoplankters to utilize low substrate concentrations. This justifies the low K_μ values that has also been obtained for many other phytoplankters (Thomas et al. 1968; Eppley et al. 1969). It may be remembered that the Monod expression assumes that 'there is only a single substrate or in the case of multisubstrate reactions the concentration of all other substrates are held constant'.(McCarthy, 1981). In these experiments the nitrate and phosphate concentrations were made to vary; but the extent of interaction of these substrates at different concentrations to other substrates and the algae itself was not known. Similarly the physical

factors also have a decisive role in controlling the growth rate. These factors may explain the difference in the observed and expected saturation concentrations.

At this instance it is worthwhile to note the statement of Pringsheim (1946) that 'most algae are not affected by minute changes in the composition of the medium, otherwise they could not live under natural conditions. Changes effected by algae themselves are often more decisive than the differences between various media'. To conclude it has to be remembered that metabolic activities of phytoplankters are controlled primarily by nutrients, light and temperature. The optimum amounts of these vary with the species. The nutrient sufficiency, optimum light, optimum temperature and other such factors have been, however, studied on the basis of one-species and one-factor experiments. The final result i.e. growth, is actually the combined effect of all these parameters: physical, chemical and biological. But the present investigation has revealed that factors independently can be manipulated in culture systems to modify the yield.

The growth rate obtained in the small volume laboratory cultures of the flagellates, Chromulina freiburgensis and Isochrysis galbana (C.s.) have been retained in mass cultures also. C. freiburgensis developed

into a more stable culture reaching a density of 6.7×10^3 cells/ml in five days time starting from an inoculum of 9.4×10^3 cells/ml. I. galbana (C.s.) also showed similar growth rate though the cultures were not that stable as the former species. These results as well as the field trials for acceptability indicate that both these flagellates could be developed in mass culture systems in tropical hatcheries and used successfully as live-food in rearing the oyster larvae.

SUMMARY

The development of mass cultures of phytoplankton as live-food, forms an integral part of hatchery systems in mariculture. Among such photosynthetic microalgae nanoplankters with cell dimensions of 50–60µ and less are essential for providing the right type of food for early larval stages of bivalve molluscs. Hence the isolation and development of nanoplankters in axenic cultures *in vitro* conditions have been undertaken for further mass production.

The present study on nanoplankton is based on the isolation and development of unialgal cultures from the inshore waters of Cochin, characterization of their growth, assimilation products, eco-physiology and evaluation of nutritional quality. The work was carried out during the period 1980–1983. The nanoplankters were isolated and grown in the laboratory as batch cultures to study the increase in cell population, the photosynthetic pigments and physiological activity. The chemical composition of these organisms and their rate of excretion were also determined. The environmental factors – physical and chemical – that influence the growth of these cultures

were defined by conducting independent experiments. Mass cultures of the isolated nanoplankters were raised indoor and fed to the larvae of edible oyster to test their suitability as live-food.

Taxonomic description of thirteen species of nanoplankters in the Cochin estuarine and coastal regions have been given. Of these two were isolated during the period of this investigation. They are Chromulina freiburgensis Doflein and Isochrysis galbana Parke forma nova (referred as I. galbana (C.s.) and these are new records for the Indian waters. The latter is considered as a new tropical form differing from the widely known temperate species.

The identification of C. freiburgensis and I. galbana (C.s.) was made from their morphological and anatomical features as obtained with light microscope and confirmed by Scanning Electron Microscope and analysis of photosynthetic pigments using thin-layer chromatography.

The various techniques and media employed in isolation and development of axenic cultures of nanoplankton and analysis of chlorophylls, chemical composition, rate of excretion as well as the methods employed to determine the role of various environmental

factors on growth kinetics of these nanoplankters have been discussed under methodology.

The kinetics of growth of the present isolates _. freiburgensis and I. galbana (C.s.) were studied in detail along with two other nanoplankters Synechocystis salina and Tetraselmis gracilis. The growth of these species was defined by measuring the growth rate (as evidenced by the cell counts), the amount of chlorophyll and carotenoid pigments and photosynthetic production (given by the measurement of oxygen exchange and carbon-14 uptake) for a period of thirty days.

The various growth measurements of the above species showed that they exhibited peak growth and activity from two to six days of inoculation and then the growth rate declined gradually. Within 12 to 16 days all cultures attained stationary phase. As the cultures became one month old, their growth and activity reduced drastically with I. galbana (C.s.) and S. salina showing senescence while C. freiburgensis was more stable.

The amount of protein, carbohydrate and lipid of C. freiburgensis, I. galbana (C.s.), S. salina and I. gracilis was estimated at different phases of growth in culture. The relative proportion of the protein was

high in all the species during the exponential phase of growth-being 59.4% of the dry weight in S. freiburgensis and 51.3% in I. galbana (C.s.). In S. salina and I. gracilis it was 41% and 54% respectively. The relative amount of protein decreased in the ageing cultures while that of carbohydrates increased. Lipids were comparatively less.

The amount of extracellular products released by the above nanoplankters during their phases of growth in culture was estimated by the carbon-14 method using a Liquid Scintillation Counter. It was observed that the rate of excretion increased with the age of cultures. During exponential phase, cultures of S. freiburgensis released 3% of the total carbon fixed to the medium while this fraction increased to 12.57% in stationary phase cultures. In the cultures of I. galbana (C.s.) there was an increase from 3.9% to 45.2% and in S. salina the range was from 38.91% to 64.5% . In I. gracilis the amount of excretion varied from 2.5% to 22.36% from the 5th to 16th day.

Salinity tolerance studies conducted with media of 14‰, 24‰ and 34‰ salinity showed that the flagellat S. freiburgensis and I. galbana (C.s.) grew best at 34‰ while S. salina showed better growth at 14‰.

The optimum pH for the species varied with S. freiburgensis and I. galbana (C.s.) growing best at a pH of 8 while S. salina proliferated better at a pH of 7 to 7.5.

The rate of carbon fixation by the nanoplankters was found to be affected by the density of culture. The flagellates had a lower optimal saturation density i.e. 6-10 x 10^5 cells/ml compared to cultures of Synechocystis salina for which the rate of ^{14}C uptake increased with cell concentration upto 175 x 10^5 cells/ml after which there was decline. This difference could be related to the relative volume of the two species.

Cultures maintained on a light-dark cycle of 10:14 hours exhibited higher production rate when exposed to constant illumination. Similarly the light intensity supporting the maximum production of these cultures also did not differ significantly. The light adaptation of the species as defined by the constant I_K was found to be 22-24 x 10^{13} quanta x cm^{-2} x sec^{-1} for S. freiburgensis, I. galbana (C.s.) and S. salina.

The influence of temperature on the growth and activity of the nanoplankters was studied by growing them in thermostatically controlled water baths. The

flagellates, *C. freiburgensis* and *I. galbana* (C.s.) exhibited maximum productivity at 30°C while *S. salina* was more active at 25°C. But the optimum range of temperature for growth was wider (20–35°C) for all the three species.

The thermal death point was 40°C for *C. freiburgensis* and *I. galbana* (C.s.) and 45°C for *S. salina*. *S. salina* survived longer periods (six months) of exposure to low temperature (*ca.* 5°C) than the flagellates (3 months).

The rate of growth of *C. freiburgensis*, *I. galbana* (C.s.) and *S. salina* was studied with respect to varying concentrations of nitrate and phosphate in the culture medium and the nutrient requirement of these species were defined in terms of half-saturation constants for growth. Among the chrysomonads, *C. freiburgensis* was found to have a lower half-saturation constant and higher growth compared to *I. galbana* (C.s.) giving the former better chances for nitrate utilization. *S. salina* was better equipped to compete with the flagellates at low nitrate levels.

The phosphate utilization capacity of *C. freiburgensis* was higher than that of *I. galbana* (C.s.) while *S. salina* was more or less at par with *C. freiburgensis*.

From the nutrient kinetic studies of the flagellates it was evident that C. freiburgensis has better chances of survival than I. galbana (C.s.) in culture.

Mass culturesof the present isolates C. freiburgensis and I. galbana (C.s.) were raised in 20 litre carbuoys. The flagellates grew and multiplied rapidly. In these mass cultures also C. freiburgensis showed more stability.

The acceptability of the two species C. freiburgensis Doflein and I. galbana (C.s.) to the molluscan larvae was tested. Newly hatched larvae of Crassostrea madrasensis fed separately with C. freiburgensis and I. galbana (C.s.) settled as spat in 17 and 19 days respectively. The results of the experiment showed that these flagellates were not only acceptable to the larvae but also induced higher growth rate and larval survival compared to control feed i.e. I. galbana Parke, the temperate water strain that is widely in use in hatcheries. C. freiburgensis seems to be the more potent species for development as live-food in oyster hatcheries.

ALLEN, C.M. 1953. Experiments in Soil Microbiology. Burgess Publ. Co., Minneapolis, Minn., 127 pp.

APHA, 1980. Standard methods. For the Examination of Water and Wastewater. 15th Ed. APHA - AWWA - WPCF., Washington, 1193 pp.

ATKINS, W.R.G. 1945. Autotrophic flagellates as the major constituent of the oceanic phytoplankton. Nature, Lond., 156: 446-447.

BEERS, J.R. AND G.L. STEWART. 1969. The vertical distribution of microplankton and some ecological observations. J. Cons. Int. Explor. Mer., 33: 30-44.

BEERS, J.R., F.M.H. REID AND G.L. STEWART. 1975. Microplankton of the North Pacific Central Gyre. Population structure and abundance June 1973. Int. Rev. Gesamten Hydrobiol., 60: 607-638.

BILLARD, C. AND P. GAYRAL. 1972. Two new species of Isochrysis with remarks on the genus Ruttnera. Br. Phycol. J., 7: 289-297.

BLIGH, E.G. AND W.J. DYER. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem Physiol., 37: 911-917.

BOLD, H.C. AND M.J. WYNNE. 1978. Introduction to the Algae. Structure and Reproduction. Prentice-Hall Inc., Englewood Cliffs, New Jersey, 706 pp.

BOWIN, D.J., S.Y. MAESTRINI AND J.W. LEFTLEY. 1981. Some processes and physical factors that affect the ability of individual species of algae to compete for nutrient partition. In: T. Platt (ed.) Physiological Bases of Phytoplankton Ecology. Can. Bull. Fish. Aquat. Sci., 210: 292-309.

BRUCE, J.R., M. KNIGHT AND M. PARKE. 1940. The rearing of oyster larvae on an algal diet. J. mar. biol. Ass. U.K., 24: 337-374.

CAPERON, J. 1968. Population growth response of Isochrysis galbana to nitrate variation at limiting concentrations. Ecology, 49: 866-872.

CHRISTENSEN, T. 1962. Alger. In: T.W. Bocher, M. Lange and T. Sorenson (ed.) Botanik. Kobenhavn: Munkegaard, p. 178.

COLE, H.A. 1936. Experiments on the breeding of oysters (Ostrea edulis) in tanks, with special reference to the food of the larva and the spat. Fish. Invest., Lond. Ser. II, 15: 1-28.

COLLIER, A. AND A. MURPHY. 1962. Very small diatoms: preliminary notes and description of Chaetoceros galvestonensis. Science, 136: 780-782.

DAMODARAN, A. 1967. The surface waters off the coast of Kerala, south-west India. Deep-Sea Res., 14: 295-320.

DAVIS, H.C. 1953. On food and feeding of larvae of the American oyster, C. virginica. Biol. Bull., Woods Hole, 104: 334-350.

DAVIS, H.C. AND V.L. LOOSANOFF. 1953. Utilization of different food organisms by clam larvae. Anat. Rec., 117: 646.

DAVIS, H.C. AND R.R.L. GUILLARD. 1958. Relative value of ten genera of micro-organisms as food for oysters and clam larvae. Fish. Bull. No.136, U.S. Fish. Wildlife. Serv. Bull., 58: 293-304.

DESIKACHARY, T.V. 1959. Cyanophyta. ICAR, New Delhi. 700 pp.

DEVASSY, V.P. AND P.M.A. BHATTATHIRI. 1981. Distribution of phytoplankton and chlorophyll a around Little Andaman Island. Indian J. Mar. Sci., 10: 248-252.

DROOP, M.R. 1954. Conditioning governing haematochrome formation and loss in the alga Haematococcus pluvialis Flotow. Arch. Mikrobiol., 20: 391-397.

DROOP, M.R. 1958a. Optimum relative and actual ionic concentrations for growth of some euryhaline algae. Verh. int. Verr in theor. angew. Limnol., 13: 722-730.

DROOP, M.R. 1961a. Recent developments in the culturing of algae. Chemical and ecological considerations in the design of the synthetic culture media for marine algae. In: D.L. Bailey (ed.) Recent Advances in Botany, 9th International Botanical Congress, Montreal, 1959. University of Toronto Press, Toronto: 243-247.

_____ 1961b. Some chemical considerations in the design of synthetic culture media for marine algae. Botanica mar., 2: 231-246.

_____ 1961c. Vitamin B_{12} and marine ecology: the response of Monochrysis lutheri. J. mar. biol. Ass. U.K., 41: 69-76.

_____ 1973. Some thoughts on nutrient limitation in algae. J. Phycol., 9: 264-272.

DUGDALE, R.C. 1967. Nutrient limitation in the sea: dynamics, identification and significance. Limnol. Oceanogr., 12: 685-695.

DURBIN, E.G. 1974. Studies on the autecology of the marine diatom Thalassiosira nordenskioldii Cleve. 1. The influence of day length, light intensity and temperature on growth. J. Phycol., 10: 220-225.

•OUSSART, B.M. 1965. Les diff'erentes cate'gories de plancton. Hydrobiologia, 26: 72-74.

EBERLY, W.R. 1967. Problems in the laboratory culture of planktonic blue-green algae. In: W.R. Eberly (ed.) Environmental Requirements of Blue-green Algae. Corvallis, Oreg.: 7-34.

EPIFANIO, C.E. 1979. Comparison of yeast and algal diets for bivalve molluscs. Aquaculture, 16: 187-192.

EPPLEY, R.W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. U.S., 70: 1063-1085.

EPPLEY, R.W. AND J.L. COATSWORTH. 1966. Culture of the marine phytoplankter Dunaliella tertiolecta with light-dark cycles. •Arch. Mikrobiol., 55: 66-80.

_____ 1968. Uptake of nitrate and nitrite by Ditylum brightwellii - kinetics and mechanisms. J. Phycol., 4: 151-156.

EPPLEY, R.W. AND J.D.H. STRICKLAND. 1968. Kinetics of marine phytoplankton growth. In: M.R. Droop and E.J. Ferguson Wood (ed.) Advances in Microbiology of the sea. Academic Press, London, 1: 23-62.

EPPLEY, R.W. AND W.H. THOMAS. 1968. Comparison of half-saturation constants for growth and nitrate uptake of a marine phytoplankton. J. Phycol., 5: 375-379.

EPPLEY, R.W., J.N. ROGERS AND J.J. McCARTHY. 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr., 14: 912-920.

EWART, J.W. AND C.E. EPIFANIO. 1981. A tropical flagellate food for larval and juvenile oysters, Crassostrea virginica Gmelin. Aquaculture, 22: 297-300.

FOGG, G.E. 1944. Growth and heterocyst production in Anabaena cylindrica Lemm. New Phytol., 43: 164-175.

_____ 1956. Photosynthesis and formation of fats in a diatom. Ann. Bot., Lond. N.S. 20: 265-285.

_____ 1959. Nitrogen nutrition and metabolic patterns in algae. Symp. Soc. Exp. Biol., 13: 106-125.

_____ 1966. The extracellular products of algae. Oceanogr. Mar. Biol. Ann. Rev., 4: 195-212.

_____ 1975. Algal Cultures and Phytoplankton Ecology. (2nd ed.). University of Wisconsin Press, London, 175 pp.

FOGG, G.E., W.D.P. STEWART, P. FAY AND A.E. WALSBY. 1973. The Blue-green Algae. London and New York, 459 pp.

FOY, R.H., C.E. GIBSON AND R.V. Smith. 1976. The influence of day length, light intensity and temperature on the growth rates of planktonic blue-green algae. Br. phycol. J., 11: 151-163.

FRITSCH, F.E. 1935. The Structure and Reproduction of the Algae. I. Cambridge University Press, 791 pp.

_____ 1945. The Structure and Reproduction of the Algae. II. Cambridge University Press, 939 pp.

GAARDER, T. AND H.H. GRAN. 1927. Investigations of the production of plankton in the Oslo Fjord. Rapp. Proc. - Verb. Cons. Expl. Mer., 42: 1-48.

GERLOFF, G.C., G.P. FITZGERALD AND F.SKOOG. 1950. The isolation, purification and nutrient solution requirements of blue-green algae. In: J. Brunel, G.W. Prescott and L.H.Tiffany (ed.) Symposium on the culture of algae. Dayton, Ohio: 27-44.

GOLDMAN, J.C. 1977. Biomass production in mass cultures of marine phytoplankton at varying temperatures. J. Exp. Mar. Biol. Ecol., 27: 161-169.

_____, _____. 1980. Physiological processes, nutrient availability and the concept of relative growth rate in marine phytoplankton ecology. In: Paul G. Falkowski (ed.) Primary Productivity in the Sea. Plenum Press: 179-208.

GOLDMAN, J.C. AND E.J. CARPENTER. 1974. A kinetic approach to the effect of temperature on algal growth. Limnol. Oceanogra. 19: 756-766.

GOLDMAN, J.C. AND J.J. McCARTHY. 1978. Steady state growth and ammonium uptake of a fast - growing marine diatom. Limnol. Oceanogr., 23: 695-730.

GOLDMAN, J.C., J.J. McCARTHY AND D.G. PEAVEY. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature, Lond., 279: 210-215.

GOPINATHAN, C.P. 1975. On new distributional records of plankton diatoms from the Indian Seas. J. mar. biol Ass. India. 17: 223-240.

_____ 1981. Studies on phytoplankton in estuarine and marine environments. Ph.D. Thesis Univ. Cochin.

HEALEY, F.P. 1975. Physiological indicators of nutrient deficiency in algae. Res. Dev. Dir. Freshwater Inst. Tech. Rep.: 382: 00-00.

HELLBRUNN, L.V. 1952. An outline of General Physiology. W.B. Saunders Co., Philadelphia, Paris.

HELLEBUST, J.A. 1965. Excretion of some organic compounds by marine phytoplankton. Limnol. Oceanogr., 10: 192-206.

_____ 1974. Extracellular Products. In: W.D.P. Stewart (ed.) Algal Physiology and Biochemistry. Blackwell Scientific Publ., Oxford: 838-863.

HOLLIGAN, P.M. AND D.S. HARBOUR. 1977. The vertical distribution and succession of phytoplankton in the western English channel in 1975 and 1976. J. mar. biol. Ass. U.K., 57: 1075-1093.

HOLT, M.G. AND T.J. MAYDA. 1974. The effect of day length and light intensity on the growth rate of the marine diatom Detonula confervacea (Cleve) Gran. J. Phycol., 10: 231-237.

HOOGENHOUT, H. AND J. AMESZ. 1965. Growth rates of photosynthetic micro-organisms in laboratory cultures. Arch. Mikrobiol., 50: 10-24.

HULBURT, E.M., J.H. RYTHER AND R.R.L. GUILLARD. 1960. J. Cons. Int. Explor. Mer., 25: 115-128.

HUTNER, S.H., H. BAKER, S. AARONSON, H.A. NATHAN, E. RODRIGUEZ, S. LOCKWOOD, M. SANDERS AND R.A. PETERSON. 1957. Growing Ochromonas malhamensis above 35°C. J. Protozool., 4: 259-269.

GUILLARD, R.R.L. 1958. Some factors in the use of nanoplankton cultures as food for larval and juvenile bivalves. Proc. Natl. Shell Fish Assoc., 48: 134–141.

GUILLARD, R.R.L. AND P.J. WANGERSKY. 1958. The production of extracellular carbohydrates by some marine flagellates. Limnol. Oceanogr., 3: 449–454.

GUILLARD, R.R.L. AND J.H. RYTHER. 1962. Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt, and Detonula confervacea (Cleve) Gran. Can. J. Microbiol. 8: 229–239.

HALL, R.P. 1953. Protozoology. Prentice-Hall Inc., N.Y.

HAMA, J. 1969. Carbohydrate metabolism in the marine diatom Skeletonema costatum. Mar. Biol., 4: 208–214.

HARNAH, F.J. AND A.D. BONEY. 1983. Nanophytoplankton in the Firth of Clyde, Scotland: Seasonal abundance, carbon fixation and species composition. J. Exp. Mar. Biol. Ecol., 67: 105–147.

HARVEY, H.W. 1957. The Chemistry and Fertility of Sea Waters (2nd ed.). Cambridge University Press, London, 224 pp.

HAUG, A., S. MYKLESTAD AND E. SAKSHAUG. 1973. Studies on the phytoplankton ecology of the Trondheimsfjord. I. The chemical composition of phytoplankton populations. J. Exp. Mar. Biol. Ecol., 11: 15–26.

HAYWARD, J. 1968c. Studies on the growth of Phaeodactylum tricornutum. VI. Comparison of different isolates. J. mar. biol. Ass. U.K., 48: 657–666.

INNIS, W.E. AND J.L. INGRAHAM. 1978. Microbial life at low temperature: Mechanisms and molecular aspects. In: D.J. Kushner (ed.) Microbial life in extreme environments. Academic Press: 73-104.

JEFFREY, S.W. 1961. Paper chromatographic separation of chlorophylls and carotenoids from marine algae. Biochem. J., 80: 336-342.

_____ 1965. Paper chromatographic separation of pigments in marine phytoplankton. Austr. J. Mar. Freshw. Res., 16: 307-313.

_____ 1968. Quantitative thin-layer chromatography of chlorophylls and carotenoids from marine algae. Biochim. Biophys. Acta., 162: 271-285.

_____ 1974. Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. Mar. Biol., 26: 101-110.

_____ 1981. An improved thin-layer chromatographic technique for marine phytoplankton pigments. Limnol. Oceanogr., 26: 191-197.

*JENSEN, A. AND S. LIAAEN-JENSEN. 1959. Quantitative paper chromatography of carotenoids. Acta. Chem. Scand., 13: 1863-1868.

*JIRASEK, J. AND Z. ADAMEK. 1977. The effect of a type of food on oxygen consumption by carp fry. Zivociana Vyroba, 22: 833-838.

JITTS, H.R. AND B.S. SCOTT. 1961. The determination of zero thickness activity in Geiger counting of ^{14}C solutions used in marine productivity studies. Limnol. Oceanogr., 6: 116-123.

JORGENSEN, E.G. 1964. Adaptation to different light intensities in the diatom Cyclotella meneghiniana Kutz. Physiol. Plant., 17: 136–145.

_____ 1969. The adaptation of plankton algae. IV. Light adaptation in different algal species. Physiol. Plant., 22: 1307–1315.

KAIN, J.M. AND G.E. Fogg. 1958b. Studies on the growth of marine phytoplankton. II. Isochrysis galbana Parke. J. mar. biol. Ass. U.K., 37: 781–788.

_____ 1960. Studies on the growth of marine phytoplankton. III. Prorocentrum Micans Ehrenberg. J. mar. biol. Ass. U.K., 39: 33–50.

KETCHUM, B.H. 1939. The absorption of phosphate and nitrate by illuminated cultures of Nitzchia closterium. Am. J. Botany, 26: 399–407.

KINNE, O. 1977. Marine Ecology. A Comprehensive Integrated Treatise on Life in Oceans and Coastal Waters. John Wiley and Sons, London, 3(2): 579–1293.

KRATZ, W.A. AND J. Myers. 1955a. Nutrition and growth of several blue-green algae. Am. J. Botany, 42: 282–287.

KUSUMAKUMARI, T.M. AND R. VISWANATHAN. 1968. Primary productivity studies in Bombay Harbour Bay using ^{14}C. Indian J. exp. Biol., 6: 115–116.

LASKER, R., H.M. FEDER, G.H. THEILACKER AND R.C. MAY. 1970. Feeding, growth and survival of Engraulis mordax larvae reared in the laboratory. Mar. Biol., 5: 345–353.

LEWIN, J.C. AND R.R.C. GUILLARD. 1963. Diatoms. Annu. Rev. Microbiol., 17: 373-414.

LEWIN, R.A. 1962. Physiology and Biochemistry of Algae. Academic Press, London. 929 pp.

LOEBLICH, L.A. 1982. Photosynthesis and pigments influenced by light intensity and salinity in the halophile Dunaliella salina (Chlorophyta). J. mar. biol. Ass. U.K., 62: 493-508.

*LOHMANN, H. 1903. Neue Untersuchungen uber den Reichtum des Meeres an Plankton und uber die Brauchbarkeit der verschiedenen Fangmethoden. Wiss. Meeresuntersuch. Kiel, 2: 47-67.

LOOSANOFF, V.L. AND R.R. MARAK. 1951. Culturing lamellibranch larvae. Anat. Rec., 111: 129-130.

LOOSANOFF, V.L. AND H.C. DAVIS. 1963. Rearing of bivalve mollusks. In: F.S.Russel (ed.) Advances in Marine Biology. Academic Press, 1: 1-136.

LORENZEN, C.J. 1967. Determination of chlorophyll and pheopigments: Spectrophotometric equations. Limnol. Oceanogr., 12: 343-346.

LOWRY, O.H., N.H. ROSEBROUGH, A.L. FARR AND R.J. RANDALL. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.

MADDUX, W.S. AND R.F. JONES. 1964. Some interactions of temperature light intensity and nutrient concentrations during the continuous culture of Nitzschia closterium and Tetraselmis sp. Limnol. Oceanogr., 9: 79-86.

MAGNICK, J.C. 1965. Quantitative chromatography of algal chlorophylls on thin-layers of glucose. Deep-Sea Res., 12: 233-236.

MAGUE, T.H., E. FRIBERG, D.J. HUGHES AND I. MORRIS. 1980. Extracellular release of carbon by marine phytoplankton: a physiological approach. Limnol. Oceanogr., 25: 262-279.

MAJUMDER, S.K., A.C. NANDY AND P.R. DAS. 1980. On the optimum salinity level for the growth of two brackishwater diatoms, Navicula lanceolata (Kuetzing) cleve and Skeletonema costatum (Greville) Cleve. Abstract No.202. NBA Symposium on Coastal Aquaculture, Cochin, India. 12-18 January 1980.

MALONE, T.C. 1971a. The relative importance of nanoplankton and netplankton as primary producers in tropical oceanic and neritic phytoplankton communities. Limnol. Oceanogr., 16: 633-639.

_____ 1971b. The relative importance of nanoplankton and netplankton as primary producers in the California Current System. Fish. Bull., U.S., 69: 799-820.

_____ 1971c. Diurnal rhythms in netplankton and nanoplankton assimilation ratios. Mar. Biol., 10: 285-289.

MARKER, A.F.H. 1965. Extracellular carbohydrate liberation in the flagellates Isochrysis galbana and Prymnesium parvum. J. mar. biol. Ass. U.K., 45: 755-772.

MAY, R.C. 1970. Feeding larval marine fishes in the laboratory: A review. Calif. Mar. Res. Comm. Calcofi. Rept., 14: 76-83.

McALLISTER, C.D., N. SHAH AND J.D.H. STRICKLAND. 1964. Marine phytoplankton photosynthesis as a function of light intensity: A Comparison of Methods. J. Fish. Res. Bd. Canada, 21: 159-181.

McCARTHY, J.J. 1981. The Kinetics of Nutrient Utilization. In: T. Platt (ed.) Physiological Bases of Phytoplankton Ecology. Can. Bull. Fish. Aquat. Sci., 210: 292-309.

McLACHLAN, J. 1961. The effect of salinity on growth and chlorophyll content in representative classes of unicellular marine algae. Can. J. Microbiol., 7: 399-406.

MENON, M.A.S. 1945. Observations on the seasonal distribution of the plankton of Trivandrum Coast. Proc. Indian Acad. Sci., 22: 31-62.

*MIQUEL, P. 1890-92. De la culture artificielle des Diatome'es. Le Diatomiste, 8.

*MONOD, J. 1942. Recherches sur la croissance des cultures bacte'riennes. Hermann and Cie, Paris, 210 pp.

* _____ 1950. La technique de culture continue; the'orie et applications. Annls. Inst. Pasteur, Paris, 72: 390-401.

MORRIS, I. 1981. Photosynthetic products, physiological state and phytoplankton growth. In: I. Platt (ed.) Physiological Bases of Phytoplankton Ecology. Can. Bull. Fish. Aquat. Sci., 210: 83-102.

MORRIS, I. AND H.E. GLOVER. 1974. Questions on the mechanisms of temperature adaptation in marine phytoplankton. Mar. Biol., 24: 147-154.

MURPHY, J. AND J.P. RILEY. 1962. A modified single solution method for the determination of phosphate and total phosphate in seawater. Anal. Chim. Acta, 27: 31-36.

MYERS, J. 1949. The pattern of photosynthesis in Chlorella. In: J. Franck and W.E. Loomis (Ed.) Photosynthesis in plants. Am. Soc. Plant. Physiol. Iowa state college Press: 349-364.

MYERS, J. AND L.B.CLARK. 1944. Culture conditions and the development of the photosynthetic mechanism II. An apparatus for the continuous culture of Chlorella. J. gen. Physiol., 28: 103-112.

MYKLESTAD, S. 1974. Production of carbohydrates by marine planktonic diatoms. I. Comparison of nine different species in culture. J. Exp. Mar. Biol. Ecol., 15: 261-274.

MYKLESTAD, S. AND A. HAUG. 1972. Production of carbohydrates by marine diatom. Chaetoceros affinis var. willei (gran) Mustedt. I. Effect of the concentration of the nutrients in the culture medium. J. Exp. Mar. Biol. Ecol., 9: 137-144.

NAIR, P.V.R. 1959. The marine planktonic diatoms of the Trivandrum Coast. Bull. Cent. Res. Inst. Univ. Kerala. Ser.C, 7: 1-63.

_____ 1960. On the diatoms from the inshore waters of Palk Bay. J. mar. biol. Ass. India, 2: 196-198.

_____ 1966. Standardization Carbon-14 stock solution using scintillation and biological methods in primary production studies. Proc. Atomic Energy Establishment India: 15-20 (Mimeo).

_____ 1974. Studies on the Primary Production in the Indian Seas. Ph.D. thesis Univ. Cochin.

NALEWAJKO, C. 1966. Photosynthesis and excretion in various planktonic algae. Limnol. Oceanogr., 11: 1-10.

NELSON, D.H. 1944. A photometric adaptation of the Somogyi's method for the determination of the glucose. J. Biol. Chem., 153: 373-380.

NEWELL, B.S. 1953. Cellulolytic activity in the lamellibranch crystalline style. J. mar. biol. Ass. U.K., 32: 491-495.

NOVICK, A. AND L. SZILARD. 1950. Description of the chemostat. Science, Washington, D.C., 112: 715-716.

O'KELLEY, J.C. 1968. Mineral nutrition of algae. A. Rev. Pl. Physiol., 19: 89-112.

PALMER, F.E., K.A. BALLARD AND F.B. TAUB. 1975. A continuous culture apparatus for the Mass Production of Algae. Aquaculture, 5: 319-331.

PARKE, M. 1949. Studies on marine flagellates. J. mar. biol. Ass. U.K., 28: 255-286.

PARKE, M. AND P.S. DIXON. 1976. Check-list of British marine algae - third revision. J. mar. biol. Ass. U.K., 26: 527-594.

PARSONS, T.R., K. STEPHENS AND J.D.H. STRICKLAND. 1961. On the chemical composition of eleven species of marine phytoplankton. J. Fish. Res. Bd. Canada, 18: 1001-1016.

PARSONS, T.R. AND J.D.H. STRICKLAND. 1963. Discussion of spectrophotometric determination of marine plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. J. Mar. Res., 21: 155-163.

PARSONS, T.R. AND R.J.LE BRASSEUR. 1970. The availability of food to different trophic levels in the marine food chain. In: J.H.Steele (ed.) Marine food chains. Oliver and Boyd, Edinburgh: 325-343.

PLATT, T. 1981. Physiological Bases of Phytoplankton Ecology. Can. Bull. Fish. Aquat. Sci., 210: 346 pp.

PLATT, T. AND B. IRWIN. 1973. Caloric content of phytoplankton. Limnol. Oceanogr., 18: 306-310.

PRAKASH, A. AND A.H.V. SARMA. 1964. On the occurrence of 'red water' phenomenon on the west coast of India. Curr. Sci., 33: 169-170.

PRAMER, D. AND E.L. SCHMIDT. 1966. Experimental Soil Microbiology. Burgess Publ. Co., Minneapolis, Minn., 107 pp.

PRINGSHEIM, E.G. 1946. Pure cultures of algae. Their preparation and maintenance. Cambridge Univ. Press. 119 pp.

PROVASOLI, L. 1958. Nutrition and ecology of protozoa and algae. Annu. Rev. Microbiol., 12: 279-308.

_____ 1963a. Organic regulation of phytoplankton fertility. In: M.N.Hill (ed.) The Sea. Wiley, New York, 2: 165-219.

PROVASOLI, L., J.J.A. McLAUGHLIN AND M.R. DROOP. 1957. The development of artificial media for marine algae. Arch. Mikrobiol., 25: 392-428.

QASIM, S.Z., P.M.A. BHATTATHIRI AND V.P. DEVASSY. 1972. The influence of salinity on the rate of photosynthesis and abundance of some tropical phytoplankton. Mar. Biol., 12: 200-206.

_____ 1972. The effect of intensity and quality of illumination on the photosynthesis of some tropical marine phytoplankton. Mar. Biol., 16: 22-27.

_____ 1973. Growth Kinetics and Nutrient Requirements of two Tropical Marine Phytoplankters. Mar. Biol., 21: 299-304.

QASIM, S.Z., S. VIJAYARAGHAVAN, K.J. JOSEPH AND V.K. BALACHANDRAN. 1974. Contribution of Microplankton and Nanoplankton in the Waters of a Tropical Estuary. Indian J. Mar. Sci., 3: 146-149.

RABINOWITCH, E.J. 1951. Photosynthesis and Related Processes. Wiley, New York, 605 pp.

RAYMONT, J.E.G. 1980. Plankton and productivity in the oceans. Vol.I. Phytoplankton (2nd ed.). Pergamon Press, Oxford, 489 pp.

REDALJE, D.G. AND E.A. LAWS. 1983. The effects of environmental factors on growth and the chemical and biochemical composition of marine diatoms. I. Light and temperature effects. J. Exp. Mar. Biol. Ecol., 68: 59-79.

RHEE, G.Y. AND I.J. GOTHAM. 1981. The effect of environmental factors on phytoplankton growth: Light and the interactions of light with nitrate limitation. Limnol. Oceanogr., 26: 649-659.

RICHARDS, F.A. AND T.G. THOMPSON. 1952. The estimation and characterization of plankton populations by pigment analysis. II. A spectrophotometric method for the estimation of plankton pigments. J. Mar. Res., 11: 156-172.

RICKETTS, R.T. 1966. On the chemical composition of some unicellular algae. Phytochem., 5: 67-76.

RILEY, J.P. AND T.R.S. WILSON. 1965. The use of thin-layer chromatography for the separation and identification of phytoplankton pigments. J. mar. biol. Ass. U.K., 49: 583-591.

SAIJO, Y. 1964. Size distribution of photosynthesizing photoplankton in the Indian Ocean. J. Oceanogr. Soc. Japan, 19: 19-21.

SAMUEL, S. 1970. An introductory account on some algal nanoplankters of the south-west coast of India. J. mar. biol. Ass. India, 12: 35-42.

SAMUEL, S., N.M. SHAH AND G.E. FOGG. 1971. Liberation of extracellular products of photosynthesis by tropical phytoplankton. J. mar. biol. Ass. U.K., 51: 793-798.

SARMA, Y.S.R.K. AND M. KHAN. 1980. Algal Taxonomy in India. Botanical Records and Monographs - 2. Today and Tomorrow's Printers and Publishers, New Delhi, 151 pp.

*SCHREIBER, E. 1925. Zur kenntnis der Physiologie und sexualitat hoperer Volvocales. Z. Bot., 17: 336.

SOEDER, C. AND E. STENGEL. 1974. Physico-chemical factors effecting metabolism and growth rate. In: W.D.P. Stewart (ed.) Algal Physiology and Biochemistry. Blackwell Scientific Publ., Oxford: 714-740.

SOEDER, C.J., H. STROTHMANN AND R.A. GALLOWAY. 1966. Carbon dioxide induced delay of cellular development in two Chlorella species. J. Physol., 2: 117-120.

SOMOGYI, M. 1945. Determination of blood sugar. J. Biol. Chem., 160: 69–73.

SPENCER, C.P. 1954. Studies on the culture of a marine diatom. J. mar. biol. Ass. U.K., 33: 265–290.

SPOEHR, H.A. AND H.W. MILNER. 1949. The chemical composition of chlorellas; effect of environmental conditions. Pl. Physiol., 24: 120–149.

STEEMANN NIELSEN, E. 1952. The use of radio-active carbon (^{14}C) for measuring organic production in the sea. J. Cons. int. Explor. Mer., 18: 117–140.

_____ 1962. Inactivation of the photochemical mechanism in photosynthesis as a means to protect cells against too high light intensities. Physiol. Plant., 15: 161–171.

_____ 1965. On the determination of the activity in ^{14}C ampoules for measuring primary production. Limnol. Oceanogr., 10 (Suppl.): R.247–R.252.

STEEMANN NIELSEN, E. AND E.A. JENSEN. 1957. Primary oceanic production. The autotrophic production of organic matter in the oceans. Galathea Repts., 1: 49–136.

STEEMANN NIELSEN, E. AND V.K. HANSEN. 1959. Light adaptation in marine phytoplankton populations and its interrelationship with temperature. Physiol. Plant., 12: 353–370.

_____ 1961. Influence of surface illumination on plankton photosynthesis in Danish waters (56°N) throughout the year. Physiol. Plant., 14: 595–613

STEEMANN NIELSEN, E. AND E.G. JORGENSEN. 1962. The adaptation to different light intensities in Chlorella vulgaris and the time dependence on transfer to a new light intensity. Physiol. Plant. 15: 505-517.

_____ 1968a. The adaptation of algae. I. General Part. Physiol. Plant. 21: 401-413.

STEEMANN NIELSEN, E. AND M. WILLEMOES. 1971. How to measure the illumination rate when investigating the rate of photosynthesis of unicellular algae under various light conditions. Int. Rev. Ges. Hydrobiol. 56: 541-556.

STEIN, J.R. 1973. Handbook of Physiological Methods. Culture Methods and Growth Measurements. New York. 448 pp.

STEWART, W.D.P. 1974. Algal Physiology and Biochemistry. Blackwell Scientific Publ. 999 pp.

STRAIN, H.H. 1951. The pigments of algae. In: G.M. Smith (ed.) Manual of Physology. Ronald Press Co. New York: 243-262.

STRICKLAND, J.D.H. 1960. Measuring the production of marine phytoplankton. Bull. Fish. Res. Bd. Canada. 122: 1-172.

STRICKLAND, J.D.H. AND T.R. PARSONS. 1968. A practical handbook of water analysis. Bull. Fish. Res. Bd. Canada. No.167, 311 pp.

SUBRAHMANYAN, R. 1946. Marine Plankton Diatoms of the Madras Coast. Proc. Indian Acad. Sci. 24: 85-197.

_____ 1954. A new member of the Euglenineae, Protoeuglena noctilucae Gen. et. sp. nov., occurring in Noctiluca miliaris sur., causing green discolouration of the sea off Calicut. Proc. Indian Sci., 24: 85-197.

_____ 1958a. Phytoplankton organisms of the Arabian Sea off the west coast of India. J. Indian Bot. Soc., 37: 435-441.

_____ 1959. Studies on the phytoplankton of the West Coast of India, Parts I and II. Proc. Indian Acad. Sci., 50B: 113-137; 189-252.

_____ 1968. The Dinophyceae of the Indian Seas. Part I. Genus Ceratium Schrank. Mem. II., J. mar. biol. Ass. India. 118 pp.

_____ 1971. The Dinophyceae of the Indian Seas. Part II. Family Peridiniaceae Schutt emend Lindemann. Mem. II., J. mar. biol. Ass. India, 334 pp.

SUBRAHMANYAN, R. AND A.H.V. SARMA. 1960. Studies on the phytoplankton of the west coast of India. Part III. Indian J. Fish., 7: 307-336.

_____ 1965. Studies on the phytoplankton of the west coast of India. Part IV. Magnitude of the standing crop for 1955-'62, with observations on nanoplankton and its significance to Fisheries. J. mar. biol. Ass. India, 7: 406-419.

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

TAMIYA, H., E. IWAMURA, K. SHIBATA, E. HASE AND T. NIHEI. 1953. Correlation between photosynthesis and light - independent metabolism in the growth of Chlorella. Biochim. biophys. Acta, 12: 23-40.

THOMAS, J.P. 1971. Release of dissolved organic matter from natural populations of marine phytoplankton. Mar. Biol., 11: 311-323.

THOMAS, W.H. 1966. Surface nitrogenous nutrients and phytoplankton in the north-eastern tropical Pacific Ocean. Limnol. Oceanogr., 11: 393-400.

THOMAS, W.H. AND A.N. DODSON. 1968. Effects of phosphate concentration on cell - division rates and yield of a tropical oceanic diatom. Biol. Bull. mar. biol. Lab., Woods Hole, 134: 199-208.

THORSON, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev., 25: 1-45.

THRONDSEN, J. 1976. Occurrence and productivity of small marine flagellates. Norw. J. Bot., 23: 269-293.

TROITA, P. 1981. A simple and inexpensive system for continuous monoxenic mass culture of marine microalgae. Aquaculture, 22: 383-388.

TUNDISI, J.G. 1971. In: J.D. Costlow (ed.) Fertility of the Sea. Gordon and Breach, New York, 603-612.

UKELES, R. 1961. The effect of temperature on the growth and survival of several marine algal species. Biol. Bull., 120: 255-264.

_____ 1975. Views on Bivalve Larvae Nutrition. In Proceedings of the First International Conference on Aquaculture Nutrition. October, 1975: 127-162.

VIJAYARAGHAVAN, S., K.J. JOSEPH AND V.K. BALACHANDRAN. 1974. Preliminary studies on nanoplankton productivity. Mahasagar, 7: 125-129.

VIJAYARAGHAVAN, S., K.J. JOSEPH AND V.K. BALACHANDRAN AND V. CHANDRIKA. 1975. Production of dissolved carbohydrate (DCHO) in three unialgal cultures. J. mar. biol. Ass. India, 17: 206-212.

WOLLENWEIDER, R.A. 1974. A manual on methods for measuring primary production in aquatic environments. IBP Handbook No.12. (2nd edition) Oxford: Blackwell Scientific Pub., 225 pp.

WALNE, P.R. 1956. Experimental rearing of larvae of Ostrea edulis L. in the laboratory. Fish. Invest. Lond., Ser.II, 20: 1-23.

_____ 1963. Observation on the food value of seven species of algae to the larvae of Ostrea edulis. I. Feeding experiments. J. mar. biol. Ass. U.K., 43: 767-784.

_____ 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria and Mytilus. Fish. Invest., Lond., Ser.II, 26: 1-62.

_____ 1974. Culture of bivalve molluscs. 50 years experience at Conwy. Fishing News (Books) Ltd., 173 pp.

WATT, W.D. 1966. Release of dissolved organic material from the cells of phytoplankton populations. Proc. R. Soc. Lond., Ser.B.164: 521-551.

WILLIAMS, P.J. LeB AND C.S. YENTSCH, 1976. An examination of photosynthetic production, excretion of photosynthetic products and heterotrophic utilization of dissolved organic compounds with reference to results from a Coastal Subtropical sea. Mar. Biol., 35: 31-42.

WOOD, E.J.F. 1963. In: C.H. Oppenheimer (ed.) Marine Microbiology. Thomas, Springfield, 3: 275-285.

---

*Not referred to in original