Primary production of phytoplankton in Loch Leven, Kinross, Scotland

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This thesis has been composed by myself and the data presented in it are my own, except that many of the chlorophyll a determinations were by Dr. A. E. Bailey-Watts; nitrogen, phosphorus and particulate carbon data were provided by the Freshwater Fisheries Laboratory, Pitlochry, and Merlewood Research Station, Grange-over-Sands, Lancashire, and the daily radiation values were obtained from Mr. I. R. Smith.

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SUMMARY

Photosynthetic productivity of phytoplankton in Loch Leven, a shallow, nutrient-rich temperate lake, was studied over a 4-year period (1968-71).

Gross photosynthesis was measured as oxygen evolution, using the light and dark bottle method. Respiratory oxygen uptake was also measured, but included an unavoidable contribution, of unknown magnitude, from bacteria and zooplankton.

Results are interpreted in relation to concurrent changes in crop density (estimated as chlorophyll a), water temperature, pH, dissolved oxygen and carbon dioxide, nitrogen and phosphorus supply, surface-incident solar radiation and underwater light penetration.

Seasonal changes in hourly and daily rates of gross photosynthetic productivity are described within the range 0.02 to 1.59 g O_2/m^2.h and 0.4 to 21.0 g O_2/m^2.day respectively. Annual values ranged from 1.6 to 2.6 kg O_2/m^2. In general, gross rates were high compared to values published for other temperate lakes.

The area of the photosynthesis-depth profile was closely approximated by a mathematical model devised by Talling. The components of this model formed the basis for the analysis of factors affecting gross productivity.

The light intensity denoting the onset of light-saturation of photosynthesis increased with temperature.

Hourly integral rates of gross photosynthesis were relatively insensitive to variations in surface irradiance, whereas daily rates were influenced to a considerable extent by daily surface irradiance.

Phytoplankton crop density was usually unstratified and often high (up to 240 mg chlorophyll a/m^3) and the chlorophyll a content per unit area in the euphotic zone often approached its estimated

theoretical upper limit (430 mg chlorophyll *a*/m^2).

The phytoplankton itself exerted a dominant influence on underwater light penetration, accounting for *ca* 75% of light extinction at highest crop densities.

Field and laboratory estimates of k_s (the increment in the minimum vertical extinction coefficient for unit increase in population density) were lower than other published values for small-celled algae. The implications of the self-regulation of the underwater light-climate by self-shading for gross and net productivity are discussed.

In general, increase in photosynthetic capacity (per unit content of chlorophyll *a*) accompanied increase in water temperature.

During certain periods an inverse relationship between photosynthetic capacity and population density was evident. Field and laboratory evidence indicated that reduction in photosynthetic capacity could be attributed, in part, to the high pH values (> 9.5) produced by photosynthetic CO_2 uptake by dense crops. By means of a pH titration procedure the extent of CO_2 depletion at high pH was shown to be greater than predicted from classical pH/alkalinity calculations.

The possible influence on photosynthetic capacity of other population density-dependent factors (including nitrogen and phosphorus supply, light history and dissolved oxygen concentration) are discussed; the evidence is considered to be inconclusive.

Estimates of net photosynthetic productivity were frequently zero or negative, even over periods when algal populations were increasing and dissolved oxygen and pH values were above their respective air-equilibrium values. Underestimation of gross photosynthesis due to photochemical oxidation, photorespiration or the use of stationary bottles could not account for these anomalies. The most probable sources of error in the estimates of net photosynthetic productivity are discussed.

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SYMBOLS AND ABBREVIATIONS

DCMU	3',(3,4-dichlorophenyl)-1',1'-dimethylurea
i	relative index of mean daily light intensity experienced by algal cells circulating in a mixed water column
I_o	mean light intensity at the water surface over exposure period, in kerg (Ph.A.R.)/cm^2.sec
I'_o	mean light intensity immediately below the water surface over exposure period, in kerg (Ph.A.R.)/cm^2.sec
$\bar{I}'_o$	mean light intensity immediately below the water surface over the daylength, in kerg (Ph.A.R.)/cm^2.sec
ΣI_o	total irradiance per day, in cal/cm^2.day
I_k	light intensity indicating onset of light-saturation of photosynthesis, in kerg (Ph.A.R.)/cm^2.sec (Talling, 1957a)
I.B.P.	International Biological Programme
k	vertical extinction coefficient, in ln units/m
k_{min}	minimum value (over the visible spectrum) for k
k_s	increment in k_{min} for unit increase in population density(n)
K'	gradient of the initial linear, light-limited region of the photosynthesis/light intensity curve; equal to P_{max}/I_k (Talling, 1957a,b)
n	population density, in mg chlorophyll a/m^3
Σn	population content per unit area, within the euphotic zone, in mg chlorophyll a/m^2
Σn_{max}	theoretical upper limit of Σn
nP	rate of gross photosynthesis per unit water volume (mg O_2/m^3.h)
nP_{max}	value of nP at light-saturation
ΣnP	hourly rate of gross photosynthesis per unit area (mg O_2/m^2.h)
$\Sigma\Sigma nP$	daily rate of gross photosynthesis per unit area (g O_2/m^2.d)

P_{max}	light-saturated rate of gross photosynthesis per unit population, in mg O_2/mg chlorophyll *a*.h (measure of photosynthetic capacity)
Ph.A.R.	photosynthetically-available radiation (spectral region 400-700 nm)
R	rate of community respiration expressed per unit of chlorophyll *a* (mg O_2/mg chlorophyll *a*.h)
r	relative respiration rate (ratio between respiratory rate and light-saturated gross photosynthetic rate)
Δ t	daylength, in hours
z	depth, in m
z_{eu}	depth of euphotic zone, in m
z_m	depth of mixed layer, in m
z'_m	effective depth of mixed layer, in m
$\bar{z}$	mean depth, in m

SECTION I: GENERAL INTRODUCTION

1. Background and scope

Energy capture in photosynthesis is a vital initial step in every food chain. In aquatic ecosystems planktonic algae are often dominant primary producers. Their photosynthetic and respiratory characteristics, and their response to environmental factors are, therefore, of fundamental importance for an understanding of the efficiency of energy transfer from solar radiation to higher trophic levels.

Photosynthetic productivity by phytoplankton has been extensively studied, under field conditions, during the past fifty years. Productivity has been estimated either directly, by measuring oxygen evolution (Gaarder & Gran, 1927) or ^{14}C incorporation (Steemann Nielsen, 1952a) in enclosed samples, or assessed indirectly from diurnal changes in dissolved oxygen (e.g. Talling, 1957c) or carbon dioxide (e.g. Verduin, 1956a, 1957) in the open water.

Most such investigations of freshwater algal populations have been concerned with relatively deep lakes which undergo thermal stratification (e.g. Windermere; Talling, 1957a, 1966). Many such lakes are relatively poor in plant nutrients and phytoplankton crops. In contrast, knowledge of events in shallow (mean depth < 10 m), unstratified water-bodies and in those supporting very dense phytoplankton crops is more restricted. Information on such systems comes from tropical and temperate mass algal cultures (Burlew, 1953; Oorschot, 1955; Tamiya, 1957; Myers & Graham, 1959), fishponds (Hepher, 1962; Fott, 1972; Imevbore *et al*, 1972; Prowse, 1972) and sewage ponds (Bartsch & Allum, 1957). Shallow, rich, tropical lakes have been studied (Talling, 1965a; Ganf, 1969, 1972; Talling *et al*, 1973) but until recently there have been few or no detailed studies of such lakes in temperate regions.

This thesis presents a study of primary productivity by phytoplankton in Loch Leven, a shallow, temperate lake rich in nutrients and phytoplankton.

Studies on the photosynthetic behaviour and growth yields of phytoplankton in other temperate, shallow freshwater bodies have been reported recently, e.g. Lake Norrviken, Sweden (Ahlgren, 1970), Lough Neagh, N. Ireland (Gibson et al, 1971), Tjeukemeer, the Netherlands (Beattie et al, 1972), Abbot's Pond, U.K. (Hickman, 1973), Neusiedlersee, Austria (Dokulil, 1973), Crose Mere, U.K. (Reynolds, 1971, 1973), Rybinsk reservoir, U.S.S.R. (Sorokin, 1972), Marion Lake, Canada (Dickman, 1969; Efford, 1972), Canadian Shield Lakes (Sakamoto, 1971; Schindler & Holmgren, 1971; Schindler, 1972), and the Rivers Thames and Kennet, U.K. (Kowalczewski & Lack, 1971). As will be discussed later, these waters differ from Loch Leven in various ways; many develop thermal stratification and none support phytoplankton crops as dense or as prolonged as those recorded at Loch Leven.

Knowledge of the factors controlling high levels of phytoplankton productivity is desirable for an understanding of many natural and artificially produced situations. In fishponds or mass algal cultures, where high phytoplankton yields are desired, it is of practical importance to define the factors preventing actual rates of production reaching theoretically calculated upper limits (Talling et al, 1973).

Nutrient-enrichment of freshwaters due to man's activities (cultural eutrophication) often results in excessive and undesirable growths of algae (Hasler, 1947; Lund, 1972a). Attempts to prevent or remedy the effects of this eutrophication are likely to be helped by an understanding of factors controlling rates of algal productivity, particularly in algal-rich systems.

The work reported here is part of a comprehensive study of productivity at all trophic levels in Loch Leven. The study was initiated in 1966 as a U.K. contribution to the International Biological Programme (Royal Society, 1967). The scope of the work at Loch Leven, together with some preliminary results, has been described by Morgan (1972). A collection of papers describing in detail all aspects of the work is published as Vol. 74 of the Proceedings of the Royal Society of Edinburgh (1974). The main food chains studied were those leading from algae via chironomid larvae to fish (Salmo trutta and Perca fluviatilis) and diving duck (Aytha fuligula).

The work on phytoplankton productivity had two main aims. Firstly, to quantify phytoplankton productivity and associated environmental factors. These data were required by the I.B.P. for a world-wide assessment of resources and for comparison of production in aquatic and terrestrial environments (I.B.P. synthesis volumes, in prep.). Secondly, the work aimed to define the factors controlling phytoplankton productivity and its seasonal changes at Loch Leven.

Planktonic algae were probably the dominant primary producers at the loch during the study period. Macrophytes were sparse but considerable quantities of benthic algae were recorded on the shallow, sandy sediments (Bailey-Watts, 1973, 1974). The productivity of the benthic algae was not measured.

Previous records have suggested that, particularly in recent times, Loch Leven has supported dense phytoplankton crops (Wesenberg-Lund, 1905; Murray & Pullar, 1910; Rosenberg, 1938; Brook, 1957, 1958, 1964, 1965). However, these reports were based on single samples or short-period investigations; prior to the I.B.P. no extended observations on the phytoplankton crops had been made and their productivity had not been measured.

I measured gross photosynthetic productivity of the phytoplankton at frequent intervals over a 4 year period (1968-71), using the oxygen light and dark bottle method. Respiratory rates were routinely measured but included an unavoidable contribution from bacteria and zooplankton. Related factors, including crop density (chlorophyll a), water temperature, pH, alkalinity, dissolved O_2 and CO_2, incident and underwater light intensity, were also monitored. Dissolved and particulate nitrogen and phosphorus concentrations were determined routinely by Mr. A. V. Holden, Mr. L. A. Caines and Mr. R. Harriman (Freshwater Fisheries Laboratory, Pitlochry). From consideration of these changes it was possible to evaluate the relative importance of various factors in controlling phytoplankton productivity. Some causal relationships, suggested by the seasonal observations, were experimentally investigated in the laboratory; these included the influences of pH, and of nitrogen and phosphorus supply, on photosynthetic activity.

A simultaneous study of species composition and other aspects of the phytoplankton crops was carried out by Bailey-Watts (1973, 1974).

2. General features of the site

Loch Leven (Fig. 1) is a lowland freshwater loch situated on the agriculturally fertile plain of Kinross, about 40 km north of Edinburgh. It lies at latitude 56°10'N, longitude 3°22'W, at an altitude of 107 m. A general description of the loch, its history, fauna and flora is given by Morgan (1970, 1974). Its physical and chemical characteristics have been described in detail by Smith (1974) and Holden & Caines (1974) respectively. The following description is based largely on the above four publications.

The loch covers an area of 13.3 km^2 and fills a depression left by melting ice from the last glacial period. The bedrock is Old Red

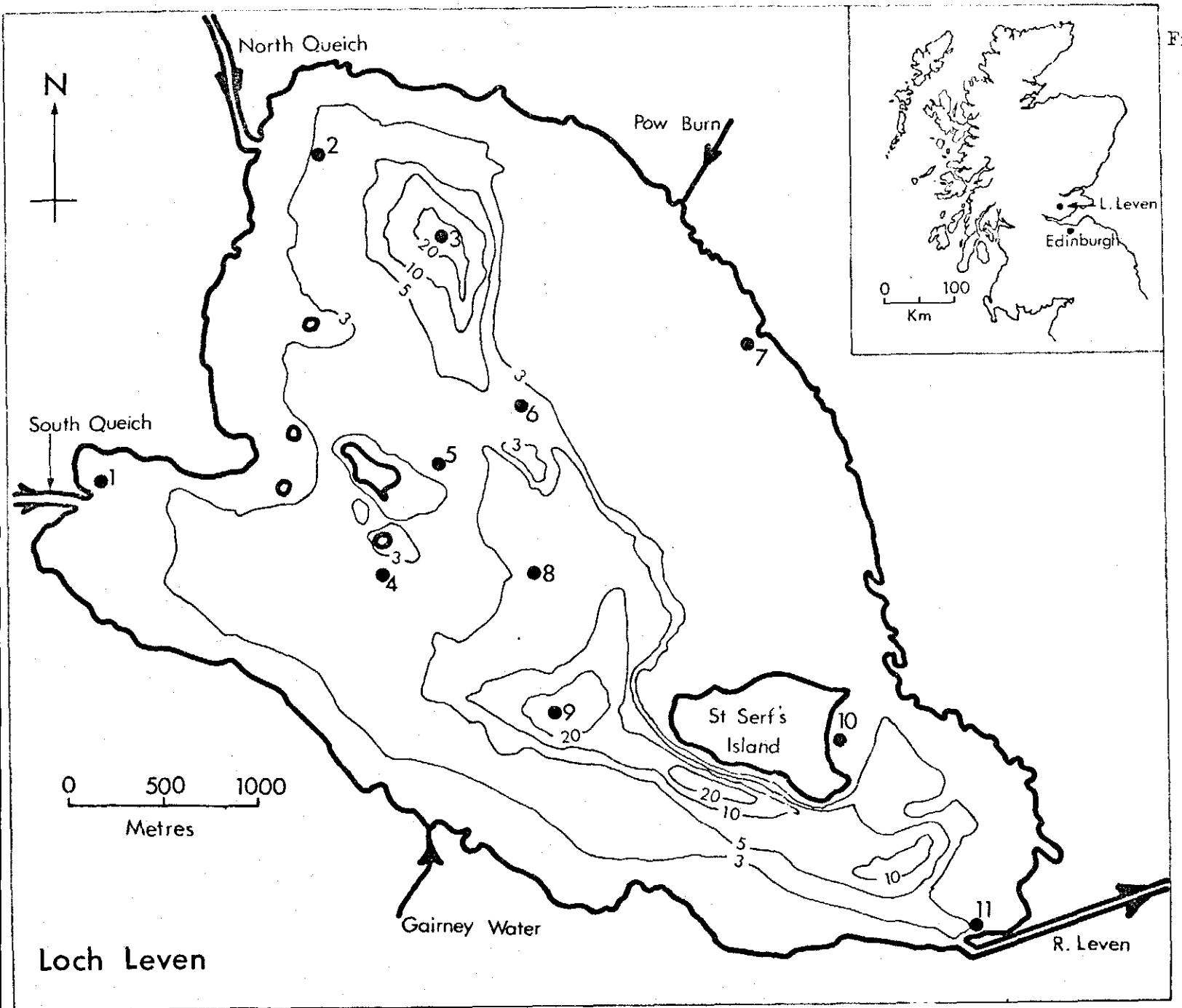


Fig. 1 Map of Loch Leven showing depth contours (metres) and sampling stations (●). Inset shows the location of the loch in Scotland. The depths of the numbered sampling stations are listed below:

station 1	1.0 m
2	3.5 m
3	22.0 m
4	4.5 m
5	4.5 m
6	4.3 m
7	0.5 m
8	5.8 m
9	23.0 m
10	0.5 m
11	1.0 m

Sandstone overlain by glacial drift. It is shallow, with a mean depth of 3.9 m and a maximum depth of 25.5 m. Only 10.3% of the total loch volume lies below 10 m. About 42% of the area of the loch is less than 3 m deep. The situation is exposed and the loch is subject to frequent wind action. This, coupled with the overall shallowness and relatively large surface area, accounts for the virtual absence of thermal stratification revealed during the course of the study. The volume of the loch is 52.4×10^6 m^3 and the mean retention time 5.2 months. It is fed by four major streams, the North and South Queich, the Gairney Water and the Pow Burn, and by several smaller streams. These drain a catchment area of 145 km^2 of which about 70% is rich agricultural land and about 30% upland grassland and moor. The loch receives the treated sewage of two small towns, Kinross and Milnathort (combined population about 3,500), and the effluent from a woollen mill. The discharge of dieldrin, an organo-chlorine insecticide, to the loch by the woollen mill was stopped in 1964 (Holden, 1966).

The level of the loch was lowered by 1.5 m in 1830 and is now artificially controlled by sluice-gates at the outlet to the River Leven. Maximum water level fluctuation is about 1 m.

In areas less than 3 m deep the sediment is mainly sand. In deeper areas the sediment is a soft organic mud.

The chemical status of the loch is summarised in Table 1.

About 96% of the total nitrogen input to the loch is as nitrate in the streams. This originates from cultivation and the extensive use of nitrogenous fertilisers in the catchment. A small amount of nitrogen is supplied in sewage and an insignificant amount from industry (the woollen mill). Seventy per cent of all phosphorus entering the loch originates from water softeners and detergents used by the woollen mill. The remaining phosphorus comes from sewage and land drainage via the streams.

Table 1. Chemical characteristics of Loch Leven - typical values for the whole water column (expressed, with the exception of pH and alkalinity, in mg/l). Vertical stratification was very rare.

Calcium	16-27	Alkalinity	1.0-1.6 m-equiv./l
Magnesium	6-10	Nitrate-N	0.1-1.9
Sodium	5.6-8.2	Ammonia-N	0.02-0.06
Potassium	1.0-2.9	Nitrite-N	None detected
Sulphate	ca 25	Organic N	0.5-1.8
Chloride	11-16	Phosphate P	0.002-0.040
pH	7.5-10.0	Total P	0.04-0.15
		Silica(SiO_2)	0.1-11.0

Over the past five years the specific supply loadings (inputs per unit area) of phosphorus and nitrogen ranged from 0.5-1.0 g P/m^2.year and 14-24 g N/m^2.year. These are considerably higher than the loadings of 2.0 g N and 0.13 g P/m^2.year judged by Vollenweider (1968) to be threshold values above which eutrophication problems are likely in lakes up to 5 m deep.

At 56°N the loch experiences wide seasonal fluctuations in day-length, daily incident radiation and temperature. These factors may be expected to influence seasonal patterns of primary productivity.

Studies of diatom stratigraphy (Haworth, 1972) suggest that the loch has long been eutrophic; there are indications that increasing enrichment of the already rich environment began prior to A.D. 1816; a change in the diatom composition of the sediments after A.D. 1816 is correlated with the lowering of the loch level in 1830.

It is likely that an increase in nutrient inflow has occurred in the last 100 years. Although the population of the catchment area (approx. Kinross county) has not changed appreciably since 1801 (General Registry Office, 1964, 1971), the amount of nutrient in domestic sewage has probably increased due, e.g., to the increased use of phosphorus-containing detergents since the war. The establishment of a woollen mill discharging its effluent to the loch has also increased the phosphorus loading. The increased use of nitrogenous fertilisers on the surrounding agricultural land during the last 25 years has increased the loch's nitrogen supply (Holden & Caines, 1974).

Changes in the fauna and flora of Loch Leven between 1891 and 1966 are reviewed by Morgan (1970). These include a decline in rooted submerged and emergent vegetation, a probable increase in phytoplankton density, and a change from a zooplankton dominated by Daphnia hyalina var. lacustris (a herbivore) to one dominated by the omnivorous or carnivorous Cyclops strenuus var. abyssorum.

Morgan considers that some of these, and other, changes may be related to nutrient enrichment of the loch, but points out that some are more difficult to explain (Morgan, 1972, 1974). During the I.B.P. study period, further changes in the loch ecosystem were recorded. These included the reappearance of Daphnia hyalina (Johnson & Walker, 1974) and an increase in the quantity of submerged macrophytes (Jupp, Spence & Britton, 1974).

The loch is famous as a brown trout fishery and as a wildfowl habitat. It was declared a National Nature Reserve in 1964.

SECTION II: METHODS

1. Sampling

Weekly or fortnightly sampling was carried out at station 4 or 5 (in 1968, 1970 and 1971) or station 3 (in 1969). Measurements of phytoplankton biomass were made frequently by Bailey-Watts (1973) at these and the other stations marked on the map (Fig. 1). No evidence of significant horizontal or vertical variability in phytoplankton distribution was found. A number of comparisons of the photosynthetic activity of samples from different stations were made, again revealing little horizontal variation. Further evidence of the general absence of station-to-station variation was provided by the chemical analyses of Holden (pers.comm.). Measurements at any open water station have, therefore, been considered representative of the loch as a whole.

Water samples were collected from discrete depths using a 2 l glass Ruttner sampler. Integrated samples were obtained with a weighted polyethylene tube (Lund, 1949).

2. Estimation of chlorophyll a

Chlorophyll a was used as an index of phytoplankton density in the water samples used for productivity measurements. The phytoplankton was filtered from a suitable volume of water (50 ml-1 l) onto a Whatman GF/C glass fibre filter (diameter 4.25 cm) using a Büchner funnel and reduced pressure. A small quantity of magnesium carbonate suspension was added to the filter to aid retention and to prevent the development of acidity - and hence pigment degradation - in the extract.

Pigments were extracted in 90% methanol or 90% acetone for 16 hours in the dark at 4-5°C. Usually two methanol and two acetone extracts were prepared from each water sample and mean values determined for each solvent.

Incomplete extraction of pigments after 16 hours occurred periodically, usually when green algae dominated the crop, and was most marked in acetone extracts. Extraction efficiency was improved on these occasions by grinding the filter plus algae in a small quantity of solvent, either by hand using a pestle and mortar and some silver sand, or electrically using a Potter-Elvehjem tissue grinder. Alternatively, with methanol, extraction could also be improved by boiling the solvent plus filter for 2 minutes. The chlorophyll *a* values reported in this study were calculated on each occasion from samples in which extraction was as complete as possible.

The extracts were clarified by centrifugation and their optical densities (O.D.) measured in 4 cm path length cuvettes at 665 nm and 750 nm using a Unicam SP600 spectrophotometer. Correction for any residual turbidity and for any optical differences between cuvettes was made by subtracting the optical density at 750 nm from that at 665 nm. The corrected O.D. at 665 nm was then used to calculate the optically equivalent concentration of chlorophyll *a*, using the following equations of Talling & Driver (1963):

For 90% methanol extracts,

$$\text{chlorophyll } a = 13.9\ (O.D._{665} - O.D._{750})$$

For 90% acetone extracts,

$$\text{chlorophyll } a = 11.9\ (O.D._{665} - O.D._{750}),$$

where the concentration of chlorophyll *a* is in mg/l of solvent, and optical densities are for an optical path length of 1 cm.

Degradation products of chlorophyll *a* were estimated from the acetone extracts using the methods of Lorenzen (1967) and Moss (1967a,b). The method of Lorenzen (1967) is based on the reduction in O.D. at 665 nm which accompanies the conversion of chlorophyll *a* to phaeophytin *a*.

This conversion is induced, in the cuvette, by the addition of dilute HCl. In this study 4 drops of 1 N HCl were added per 4 cm cuvette (14.5 ml extract). Optical densities were read at 665 nm and 750 nm before and after acidification. Results are expressed as an acidification ratio (665_o:665_a) equal to ($O.D._{665}$-$O.D._{750}$) before acidification divided by ($O.D._{665}$-$O.D._{750}$) after acidification. Concentrations of chlorophyll a and of phaeophytin a were calculated from equations given by Lorenzen (1967).

The method of Moss (1967a,b) is based on the difference in absorption maximum between chlorophyll a (430 nm) and phaeophytin a (410 nm). The ratio of the optical density of an extract at 430 nm to that at 410 nm (the 430:410 ratio) is determined. By reference to a calibration curve relating this ratio to a series of known mixtures of fresh and acid-degraded extract, the fraction of degraded pigment in an extract can be found (Moss, 1967a). For this study a calibration curve prepared from Loch Leven material by Dr. A. E. Bailey-Watts was used. Absolute concentrations of chlorophyll a and phaeophytin a were calculated using equations derived by Moss (1967b).

3. Measurement of photosynthetic productivity of the phytoplankton

The oxygen light and dark bottle technique was used. The general procedure was similar to that used by Talling (1957a) and discussed in Vollenweider (1969). Dissolved oxygen was measured by the Winkler method (described in Mackereth, 1963). 100 ml samples were titrated with N/200 sodium thiosulphate using starch as indicator. The thiosulphate solution was standardised against N/200 potassium dichromate solution. Variation between duplicate bottles, from which mean values were calculated, was normally less than 0.06 mg O_2/l. After analysis, bottles and stoppers were rinsed with ca N/100 thiosulphate to remove

all traces of iodine and then thoroughly rinsed with tap water followed by deionised water.

(a) in the loch

Water from 0.5 m depth was siphoned into 190 ml 'Monax' borosilicate glass bottles fitted with ground-glass stoppers. The bottles were fastened in pairs to a rope and suspended from a float attached to an anchored buoy. The float consisted of a 5 ft Dexion metal strip supported at each end by two large plastic containers. This arrangement ensured minimal shading of the experimental bottles by the float or the buoy. For a productivity measurement under ice (24.2.69), bottles were suspended 0.5 m away from the hole in the ice, from the ends of a Dexion cross-bar arrangement. The bottles, each covering 10 cm depth, were suspended vertically with their centres at 0.10, 0.35, 0.60, 1.10, 1.60, 2.10, 3.10 or 4.10 metres depth. A parallel series of bottles were sometimes also used with their centres at 0.25, 0.50 or 0.75 metres depth. Pairs of dark bottles (covered with black cloth) were suspended at 0.6 and 1.6 metres. Dark bottles at every depth were considered unnecessary in view of the general uniformity of temperature distribution within the euphotic zone. An additional pair of bottles, filled with water from the same sample, was reserved for the determination of the initial dissolved oxygen concentration of the sample. The bottles were exposed for about 3 hours near mid-day. Exposure began immediately after the bottles were filled. At the end of the exposure period the bottles were removed to a light-proof rubber box and Winkler reagents added immediately. Occasionally, in stormy weather, the Winkler reagents were added after returning to the field laboratory. This involved a delay of about 30 minutes and was allowed for in calculations of respiratory rates. Bottles were acidified and titrated the following day. Samples stored at the precipitate stage (i.e. before acidification)

for 12 days in the dark at 4°C showed no change in oxygen content. Gross photosynthesis was measured from the difference in dissolved oxygen concentration between clear and darkened bottles. Rates of gross photosynthesis per m^2 were estimated by planimetric integration of the depth-profiles of photosynthetic rate per m^3.

Respiratory oxygen uptake was determined from the difference between initial and final concentrations in darkened bottles.

Photosynthesis-depth profiles obtained using 0.5 m water differed very little from those obtained either using a more truly in situ exposure, with bottles filled with water from the depth of exposure, or using an integrated 0-3 m tube sample to fill all bottles. Samples taken from various depths (to 18 m) below the euphotic zone generally gave the same photosynthesis-depth profile as samples from within the euphotic zone. These results reflect the efficiency of turbulent mixing at Loch Leven which generally maintains a uniform depth-distribution of phytoplankton, temperature and nutrients and is rapid enough to prevent any physiological adaptation of the phytoplankton to a particular light intensity.

Calculations by Smith (pers. comm.), based on measurements of current velocity at different depths, have suggested that, on average, an algal cell would be transported from the surface to the bottom (4 m) and back to the surface again in about 2 hours.

(b) in the laboratory

Bottles were exposed horizontally in an illuminated, constant temperature water bath. A bank of seven closely spaced 'daylight' fluorescent tubes (65/80W) with internal reflectors, placed immediately below the glass base of the bath, provided a maximum light intensity of 23.7 klux (120 kerg/cm^2.sec).

This light intensity proved sufficient for light-saturation of photosynthesis to be realised at the temperatures studied. Lower light intensities were obtained by placing various thicknesses of black plastic gauze on the bottom of the bath in compartments optically isolated from each other by partitions of black plastic.

Even temperatures ($\pm$ 0.5°C) were maintained throughout the bath in the range 3° to 22°C using a cooling coil (model CC75, Grant Instruments, Cambridge) together with a Grant Instruments SU2P pump/heater/thermostat unit.

4. Limitations of the oxygen light and dark bottle technique and their evaluation under Loch Leven conditions

Measurements of phytoplankton productivity using unstirred algal suspension enclosed in sealed bottles at constant depth have been criticised for a variety of reasons (Soeder & Talling, 1969). These include:-

1. Reduced turbulence may lead to cell sedimentation and mutual shading and to reduced diffusion and uptake rates of nutrients.
2. Lack of equilibration with the atmosphere and of mixing with water from other depths may result in atypical chemical conditions developing in the bottles which may modify rates of photosynthesis and/or respiration.
3. Algal cells are maintained at a single point in the underwater light gradient whereas in nature their vertical motion is unrestricted. This could introduce errors if light history significantly affects photosynthetic behaviour.
4. The rate of respiratory oxygen uptake may vary with light intensity or with oxygen concentration.

Most bottle errors become more pronounced in longer exposures. Bottle errors are likely to vary, both seasonally in any one lake, and

between lakes, due to variation in phytoplankton density, chemical status, degree of turbulence etc. It is therefore important to establish the magnitude of possible errors for the particular conditions of any individual lake. The following aspects were investigated for Loch Leven.

a) Duration of exposure

Although 24 h exposure periods are commonly used in primary production studies many authors have found a decline in photosynthetic rate in exposures longer than 4-6 h (Verduin, 1956a; Ichimura & Saijo, 1958; Rodhe, 1958; Vollenweider & Nauwerck, 1961).

Two in situ experiments carried out at different times of year at Loch Leven, compared the sum of the photosynthetic yields in consecutive short-time (ca 3 h) exposures with yields in simultaneous long-time (ca 6 h) exposures. Despite considerable differences in the population density, pH and oxygen levels of the samples used (Table 2), results of the two experiments were very similar (Fig. 2). On both occasions the longer exposure gave only a slightly lower mean hourly rate (ca 10% lower at light-saturation) than the sum of consecutive short exposures. In view of reports that high oxygen tension may reduce photosynthesis (Turner & Brittain, 1962), it is noteworthy that no marked decline in photosynthetic rate occurred in the 6 h experiment on 21.5.69 when oxygen production at light-saturation gave rise to an oxygen level of 17.01 mg/l (equivalent to 156% saturation). The oxygen levels reached in the optimal depth bottles during routine 3 h exposures, whilst higher than those normally measured in the open water, were never as high as 156% saturated. Bubbles were very rarely seen in the experimental bottles after exposure. Thus, although atypical oxygen conditions develop in bottles at Loch Leven, they are probably not sufficient appreciably to modify photosynthetic rate, even in a 6 h exposure.

Table 2. Essential features of field experiments to compare long and short time exposures. n = phytoplankton population density, as mg chlorophyll a/m^3; I'_o = mean intensity of surface-incident radiation over the exposure period corrected for surface loss (10%), in kerg /Ph.A.R.)/cm^2.sec

Exposure period	Temperature (°C)	n (mg/m^3)	Initial pH	Initial O_2 (mg/l)	Initial O_2 (% saturation)	Final O_2 (in bottles at best depth) (mg/l)	Final O_2 (in bottles at best depth) (% saturation)	I'_o
Experiment 1 (21.5.69)								
10.40-13.50	11.6	196	9.60	12.92	118	15.33	140	348
13.50-16.55	11.6	185	9.70	12.65	116	14.89	136	281
10.40-16.55	11.6	196	9.60	12.92	118	17.01	156	315
Experiment 2 (20.8.69)								
11.25-14.35	17.9	45	8.40	8.14	85	9.74	102	139
14.35-17.35	17.9	61	8.65	8.70	91	10.09	106	118
11.25-17.35	17.9	45	8.40	8.14	85	10.94	115	129

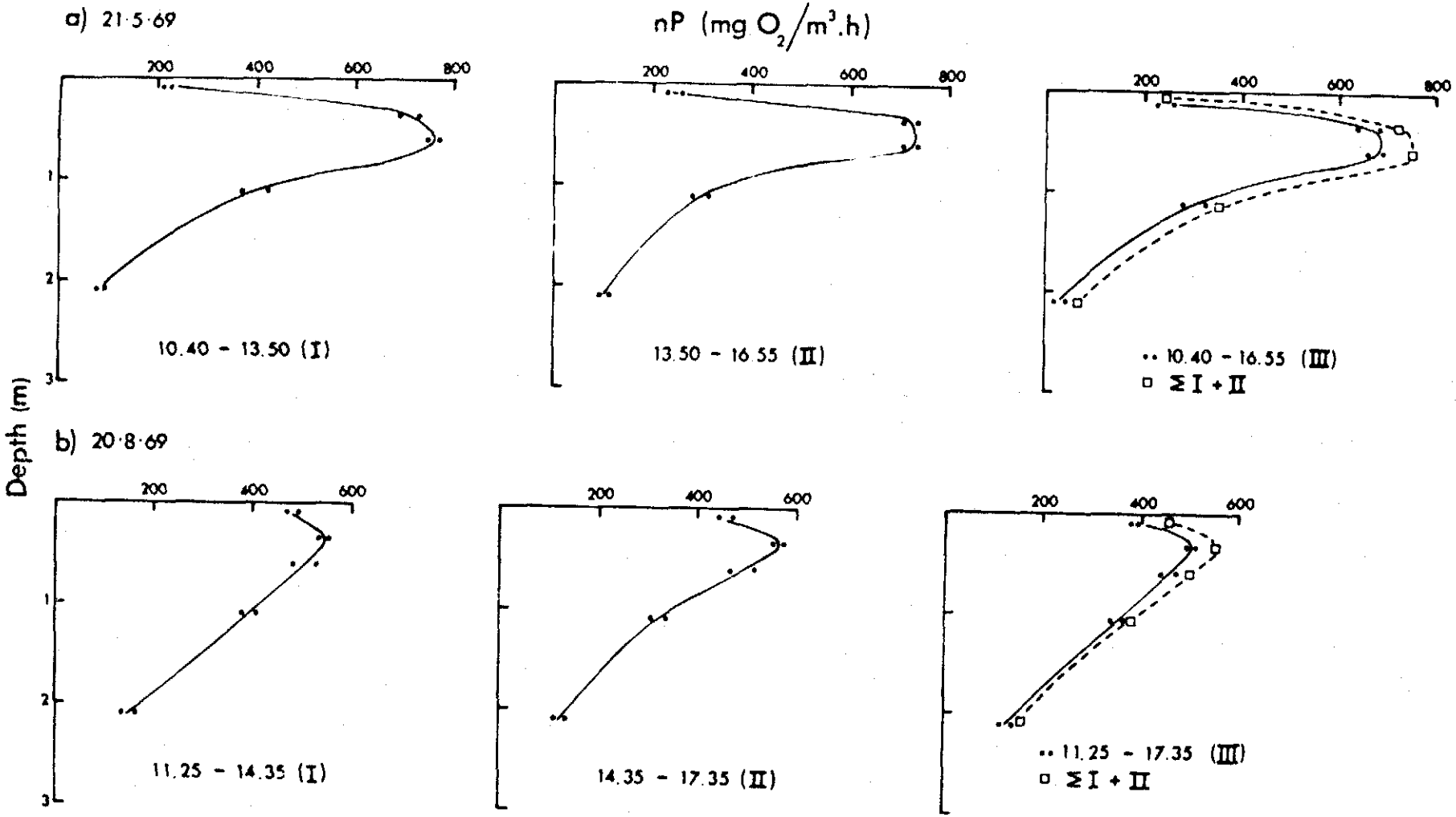


Fig. 2 Photosynthesis-depth profiles obtained from consecutive short-time (ca 3 h) exposures (I-II) and a simultaneous long-time (ca 6 h) exposure (III) in Loch Leven on two dates. The time of the start and end of each exposure period is shown. Rates of gross photosynthesis in individual light bottles are indicated (•) for exposures I-III. The mean rate obtained from the sum of the results of the consecutive exposures (Σ I and II) is also shown (□).

The initial oxygen concentration of the sample used to fill the light and dark bottles was usually closer to 100% oxygen saturation than was the *in situ* oxygen concentration, determined within 1 h of sampling, using the oxygen probe. A small amount of aeration occurred due to sampling. Deliberate aeration of samples has sometimes been used (Jenkin, 1937; Steemann Nielsen, 1955a; Ganf, 1969), when oxygen production is large, to avoid excessive bubbles and to reduce the discrepancy between oxygen conditions developing during exposure and those of the open water.

Variation in the light-saturated rate of photosynthesis with time was investigated on five occasions in the laboratory, using replicate bottles sampled in succession and exposure times varying between 1.5 and 9.5 h. No significant decline in photosynthetic rate was found with increase in duration of exposure up to 5-6 h. Exposures longer than 5 h generally gave reduced photosynthetic rates up to a maximum reduction of 30% in a 9.5 h exposure. This reduction may in part be explained by a 13% decline in chlorophyll *a* content of the sample recorded after a 9.5 h exposure to continuous light. Oxygen saturation levels reached in these laboratory exposures were on no occasion as high as that recorded in the 6 h field exposures on 21.5.69 and are therefore unlikely to have caused the reduced rates.

Decline in photosynthetic rate with prolonged illumination is discussed in more detail in section V , part 3c.

b) Agitation and density of phytoplankton suspensions

A concentrated phytoplankton suspension was prepared by sedimentation and the overlying water decanted. The concentrate was then rediluted with the overlying water to produce suspensions of differing algal density. The photosynthetic rate of each suspension was measured in the laboratory tank at 23.7 klux fluorescent illumination and 18°C.

Cell density, within the range 50-240 mg chlorophyll a/m^3, was found to have no effect on measured rates of photosynthesis per unit of chlorophyll a. This range corresponds to all but the lowest values of chlorophyll a concentration found in the loch. Higher cell densities were not investigated.

In a parallel experiment bottles were shaken by hand at 0.5 h intervals. No effect on photosynthetic rate at light-saturation was observed.

Limitation of photosynthetic rate by cell sedimentation is even less likely in the field since movement of bottles by wave action may partially eliminate sedimentation. Much of the criticism of the use of unstirred bottles originates in macrophyte work where surface area/volume relations are much less favourable than in suspensions of planktonic algae.

However, it may be noted that Kowalczewski & Lack (1971) found *in situ* gross photosynthetic rates of phytoplankton to be 1.38 ± 0.31 times higher in mechanically rotated bottles than in stationary bottles. However, these authors used 24 h exposure periods in which settling of algal cells is more likely than in the 3 h exposure periods used at Loch Leven.

c) Alternating light and dark periods

The rate of photosynthesis in a 3 h exposure at a saturating light intensity, supplied in 0.5 h light periods separated by 0.5 h dark periods, was the same as the rate recorded in a continuous 3 h exposure at the same light intensity.

d) Method of suspension

Horizontally suspended bottles (vertical depth range 5 cm) gave the same results as vertically suspended bottles (vertical depth range 10 cm).

Presumably, movement of bottles by wave action was sufficient to obliterate any effect of the difference in depth range covered in the two methods of suspension.

e) Oxygen uptake in the light and in the dark

Oxygen uptake rates in clear and dark bottles could differ if respiration is affected by light intensity or oxygen concentration, or if photochemical oxidation of dissolved organic matter occurs. Brown (1953) found that the respiration rate of Chlorella and other (unspecified) algal cultures was not affected by light intensity. This evidence, based on mass spectrometer studies with labelled oxygen, is often used to justify the assumption that oxygen uptake rates are the same in the light and dark bottles. Other published evidence, discussed below, has suggested that this assumption may not always be valid.

i) Photochemical oxidation

Using DCMU (3'-(3,4-dichlorophenyl)-1',1'-dimethylurea) to inhibit photosynthesis, Golterman (1971) found that the rate of oxygen uptake in Tjeukemeer lake water was greater in strong light than in the dark. In weak light no effect was found. The difference, attributed to photochemical oxidation of dissolved organic matter, leads to an underestimation of gross photosynthesis by the normal light and dark bottle method. Tests for the occurrence of photochemical oxidation in Loch Leven water were carried out in October 1972. DCMU (K. & K. Laboratories, Inc., California) was obtained from Kodak Ltd., Kirkby, Lancashire, England. Oxygen uptake was measured in dark bottles, with and without DCMU, and in light bottles with DCMU. Bottles were prepared in triplicate with a DCMU concentration of 5×10^{-6}M. They were incubated in

the laboratory bath for 4 h at 15°C at a light intensity of 23 or 121 kerg/cm^2.sec or in the dark. Oxygen uptake rates were found to be the same at both light intensities as in the dark. The presence of DCMU did not affect dark oxygen uptake rates. Photochemical oxidation does not, therefore, appear to be a source of error in the bottle method as used at Loch Leven. The possibility that the occurrence of photochemical oxidation might vary seasonally was not examined. The fact that levels of dissolved organic nitrogen and phosphorus (possible substrates for photochemical oxidation) did not show marked seasonal variation is some evidence against this possibility.

ii) Photorespiration

A light-dependent stimulation of respiration, which occurs only during photosynthesis, has been recorded in measurements of uptake of labelled oxygen ($^{18}O_2$) by algal cultures (Hock, Owens & Kok, 1963; Lex, Silvester & Stewart, 1972) or has been inferred from changes (interpreted as after-effects) in subsequent dark respiration rates (Brackett, Olson & Crickard, 1953; Verduin, 1957). These photorespiratory effects are sensitive to DCMU and would not, therefore, be revealed in the experiments described in (i) above. Photorespiration cannot be disccunted as a possible source of error in the estimates of gross photosynthesis reported here. The error is likely to be greatest under conditions of high pH (low CO_2) and high O_2 concentration which, according to Lex, Silvester & Stewart (1972), enhance photorespiration.

iii) Oxygen concentration

Gessner & Pannier (1958) found that the respiration rate of

natural populations of diatoms and of blue-green algae increased with increase in oxygen tension. At Loch Leven the difference in oxygen concentration between clear and darkened bottles after a 3 h exposure period was never greater than 3 mg O_2/l. According to the data of Gessner & Pannier (1958) such a difference would not be sufficient to cause significant differences in respiration rate between light and dark bottles.

f) Conclusions

Photorespiration, which was not investigated experimentally here, is a possible source of error in the oxygen light and dark bottle method, particularly when CO_2 concentration is low and O_2 concentration high. Possible errors due to photorespiration will be considered in the interpretation of results.

Otherwise, the available evidence suggests that, for Loch Leven, photosynthetic oxygen evolution rates measured over 3 h, in sealed unshaken bottles were close to the instantaneous rates occurring at any depth.

Exposures longer than 6 h were likely to underestimate production rates. Daily rates could not, therefore, be determined directly from 24 h exposures.

5. Extrapolation of hourly rates to longer time periods

Hourly rates were converted to daily rates using equation (5) of Talling (1965a). The equation, and its limitations, are described in the relevant section below.

6. Measurement of surface-incident solar radiation

Total solar radiation at the loch surface was measured using a Moll-Gorczynski thermopile (Kipp & Zonen, Delft, Holland), situated on

the roof of the field laboratory at the loch side. The thermopile output was recorded continuously on a millivolt chart recorder (Cambridge Instruments Ltd., Glasgow) and on a digital millivolt integrator (Lintronic Ltd., London) and converted to absolute units of radiant energy per unit area from the maker's calibration factors.

Mean surface intensities (I_o) for the exposure periods were calculated planimetrically from the chart records. The chart records were used for this purpose, in preference to the integrator readings, because the latter could not be recorded at exactly the start and end of the exposure period. I_o measurements were occasionally based on integrator readings when faults in the chart recorder occurred. The delay between reading the integrator and exposing the bottles was about 0.5 h and between removing the bottles and re-reading the integrator about 0.3 h. Ten-day mean daily radiation totals were calculated from the mV integrator readings. During periods when the recording equipment was defective daily radiation values were calculated by Mr. I. R. Smith using the equation given in Vollenweider (1969, p.162) and the hours of bright sunshine recorded on a Campbell-Stokes recorder maintained at the Kinross weather station, 1 km from the loch. Checks showed a close agreement between computed radiation values and those directly measured at the loch (Smith, 1974).

Photosynthetically available radiation (Ph.A.R.), in the spectral region 400-700 nm, was calculated as 0.46 of the total energy recorded (Talling, 1957a).

7. Measurement of underwater light intensities

Light penetration was measured with a selenium cell photometer, microammeter and red (RG 1), green (VG 9), blue (BG 12) and orange (OG 2 + BG 18) Schott filters. In order to define spectral attenuation

more precisely additional measurements were occasionally made with far red (RG 5) and near ultra violet (UG 2 + BG 12) filters. The optical properties of the filters are described by Sauberer (1962). From the transmission curves of the filters, the spectral sensitivity of the photocell, the colour filter action of the water column and the data of Taylor & Kerr (1941) on the spectral energy distribution in daylight, the optical midpoints of the filters in combination with the photocell in their working depth range (0.75 m) were estimated as approximately 630 nm (RG 1), 540 nm (VG 9), 460 nm (BG 12), 590 nm (OG 2 + BG 18), 680 nm (RG 5) and 380 nm (UG 2 + BG 12).

Corrections for changes in surface illumination during underwater measurements were made using a second photocell fitted with a duplicate filter. The underwater cell was suspended on the illuminated side of the boat from a boom ca 1 m long to prevent shading by the boat. The photocurrent was recorded at 0.5 m depth intervals and after correction for any changes in surface illumination used to calculate vertical extinction coefficients (k values). The latter are based on natural logarithms as defined by Sverdrup, Johnson & Fleming (1942) and were calculated from semi-log plots of μA readings against depth, taking the value at zero depth as 100% and dividing 3 by the depth interval required to reduce it to 5%.

Light intensities at various depths were calculated from the mean surface intensities, reduced by 10% to allow for 'surface loss' by direct reflection and upward scattering (Talling, 1957a), and the vertical extinction coefficients of blue, green and red light. The method of calculation was as described by Talling (1957a). The spectral region 400-700 nm was subdivided into three spectral blocks 400-490 nm, 490-585 nm and 585-700 nm and assigned the relative energy fractions 0.30, 0.35 and 0.35 respectively. The radiant energy in each block was

calculated for each depth from the appropriate vertical extinction coefficient and the total energy calculated by summation of the contributions from the three spectral blocks. The results obtained by this '3-block' method were very similar to those derived by a more elaborate procedure (Talling, 1960a) in which radiant energy at each depth is determined by planimetric integration of the areas under the spectral distribution curves constructed from values of percentage transmission per metre in the spectral regions covered by the six filters or filter combinations listed above.

Secchi disc readings were also taken, using a 20 cm black-and-white quartered metal disc, as an auxiliary measure of underwater light penetration.

8. Daylength was determined from the Smithsonian Meteorological Tables (1951).

9. Measurement of light intensity in the laboratory bath

Light intensities in the various compartments were measured with a selenium cell photometer (Lightmaster, Evans Electroselenium Ltd., Halstead, Essex) immersed within a glass beaker. Photometer readings in kilolux (klux) were converted into units of energy flux by a factor of 5.1 kerg/cm^2.sec per klux obtained from intercalibration with a Lintronic solarimeter and integrator (Lintronic Ltd., London).

10. Dissolved oxygen and temperature

The vertical distribution of dissolved oxygen in the loch was measured, at 0.5 m depth intervals, using a Mackereth oxygen probe (Mackereth, 1964) of sensitivity approx. 0.1 mg/l. Temperature was measured (to 0.02°C) using a thermistor thermometer (Mortimer & Moore, 1953) incorporated in the oxygen probe or with a mercury thermometer in a Ruttner water sampler.

Percentage oxygen saturation was calculated from the table of oxygen concentrations at different temperatures given by Welch (1948, p.352).

11. pH

pH was measured, with an accuracy of approx. 0.05 unit, using a model 30C portable pH meter (Electronic Instruments Ltd., Richmond, England) and appropriate standard buffers.

12. Alkalinity

Routine measurements of alkalinity were made by titration with N/10C hydrochloric acid to pH 4.5 (Mackereth, 1963) using B.D.H. '4.5' indicator (British Drug Houses Ltd., Poole, England).

Other measurements of alkalinity were made using the Gran titration method (Gran, 1952). The working procedure was essentially as described by Talling (1973). Details are given in the relevant section below.

13. Other chemical constituents

The following analyses were carried out on a sample of the water used for primary productivity measurements. Analyses were carried out at the Freshwater Fisheries Laboratory, Pitlochry, by Mr. A. V. Holden, Mr. L. A. Caines and Mr. R. Harriman. A surface water sample from near the outflow was also analysed at weekly intervals and samples from other stations and other depths at less frequent intervals.

Water samples, in polyethylene bottles, were transported to Pitlochry either on the day of sampling or on the following day after overnight storage in a refrigerator. Analyses began within three hours of arrival at Pitlochry.

Samples were passed through a zooplankton net (25 meshes/cm) before analysis.

Particulate and soluble fractions were separated by filtration through a 0.45 µm cellulose membrane filter.

Nitrogen

Total organic nitrogen was determined from unfiltered samples and soluble organic nitrogen from filtered samples. The particulate organic fraction was determined from the difference between total organic and soluble organic values. Inorganic forms (nitrate, nitrite and ammonia) were determined separately.

Organic nitrogen was determined by the Kjeldahl method (American Public Health Association (A.P.H.A.), 1967). Nitrate was determined by the phenol-disulphonic acid method (A.P.H.A., 1967). Nitrite and ammonia were determined by standard A.P.H.A. methods.

Phosphorus

Total (organic + inorganic) phosphorus was determined from unfiltered samples. Soluble organic and inorganic forms were determined separately from filtered samples. Particulate organic phosphorus was determined as the difference between total and soluble (organic + inorganic) forms.

Phosphate was determined by the stannous chloride method (A.P.H.A., 1967). Total phosphorus and soluble organic phosphorus were determined by perchloric/nitric acid digestion followed by phosphate determination.

Additional analyses of the chemical composition of the phytoplankton

Large volumes (e.g. 30 l) of lake water were filtered through a zooplankton net (25 meshes/cm) and through a 63 µm mesh Endecott steel sieve (Endecotts (Test Sieves) Ltd., London, England). This procedure removed most of the zooplankton (including rotifers) and other non-algal particulate material. However, some small zooplankters (e.g. Protozoa) and other small-sized non-algal particles were not removed. The remaining particulate matter in the filtrate was concentrated in an Alfa-Laval continuous centrifuge (Alfa-Laval Ltd., Middlesex, England),

and rinsed with 2 l of deionised water. The dry weight and chlorophyll a content of the concentrated algal suspension were determined. Dry weight was determined by drying to constant weight at 90°C. Dried samples were ground in an agate mortar and analysed for carbon, nitrogen, phosphorus and ash content by Mr. S. Allen et al (Merlewood Research Station, Grange-over-Sands, Lancashire) using the methods given below:

Carbon and nitrogen. A weighed sample was burnt in an inert gas in a carbon, nitrogen, hydrogen combustion apparatus (Hewlett-Packard, Slough, England). The oxides of carbon and nitrogen produced were converted to carbon dioxide and nitrogen gas respectively and estimated by gas-liquid chromatography.

Phosphorus. A weighed sample was digested with mixed sulphuric, nitric and perchloric acids. The phosphate content of the digest was determined by the molybdenum blue reaction using a Technicon Autoanalyser (Technicon Instruments Ltd., Basingstoke, England).

Ash. This was determined as the dry weight remaining after ignition in a furnace at 550°C for 30 minutes.

SECTION III: DISTRIBUTION OF GROSS PHOTOSYNTHETIC ACTIVITY WITH DEPTH

Depth-profiles of gross photosynthetic activity per unit volume of water (nP, mg $O_2/m^3.h$) are shown in Fig. 3. A key to the symbols used is given on p. 1. These profiles represent the photosynthetic behaviour of a 0.5 m depth sample taken from an approximately isothermal water column in which the phytoplankton was evenly distributed with depth. Each point corresponds to the centre of the integrated depths (10 cm) covered by the experimental bottles and is the mean of determinations from duplicate bottles.

The general profile shape is similar to that described for other unstratified waters (e.g. Jenkin, 1937; Talling, 1957b) and is primarily determined by the relation between photosynthesis and the vertical gradient of light intensity. Three main zones may be distinguished:-

1. A zone of inhibited photosynthesis near the surface (the light-inhibition zone).
2. A zone of maximum photosynthesis somewhat below the surface (the light-saturated zone).
3. A deeper zone in which photosynthesis declines more or less exponentially with depth in parallel with a similar gradient in light intensity (the light-limited zone).

Surface inhibition was recorded in most profiles and was most pronounced at higher surface light intensities. The maximum depth to which surface inhibition extended was 60 cm but it was usually confined to the uppermost 35 cm. The light intensity at the depth at which inhibition first began, calculated as the light intensity at the top of the light-saturated zone, varied between 20 and 195 kerg (Ph.A.R.)/cm^2.sec but was usually less than 100 kerg (Ph.A.R.)/cm^2.sec. The values are generally lower than those reported in other work on phytoplankton photosynthesis

Fig. 3 (following 4 pages) Depth-profiles of rates of gross photosynthesis per unit water volume (nP), for Loch Leven on the dates given. The mean surface-incident light intensity (I_o) during experiments and the value of I_K are given in kerg(Ph.A.R.)/cm^2. sec. Arrows indicate the depth of the euphotic zone (i.e.the depth corresponding to 1% of the photosynthetically-available radiation entering the water column). Each point corresponds to the centre of the integrated depths (10 cm) covered by the experimental bottles and is the mean of determinations from duplicate bottles. The profile for 24.2.69 was obtained from measurements under 5 cm of ice.

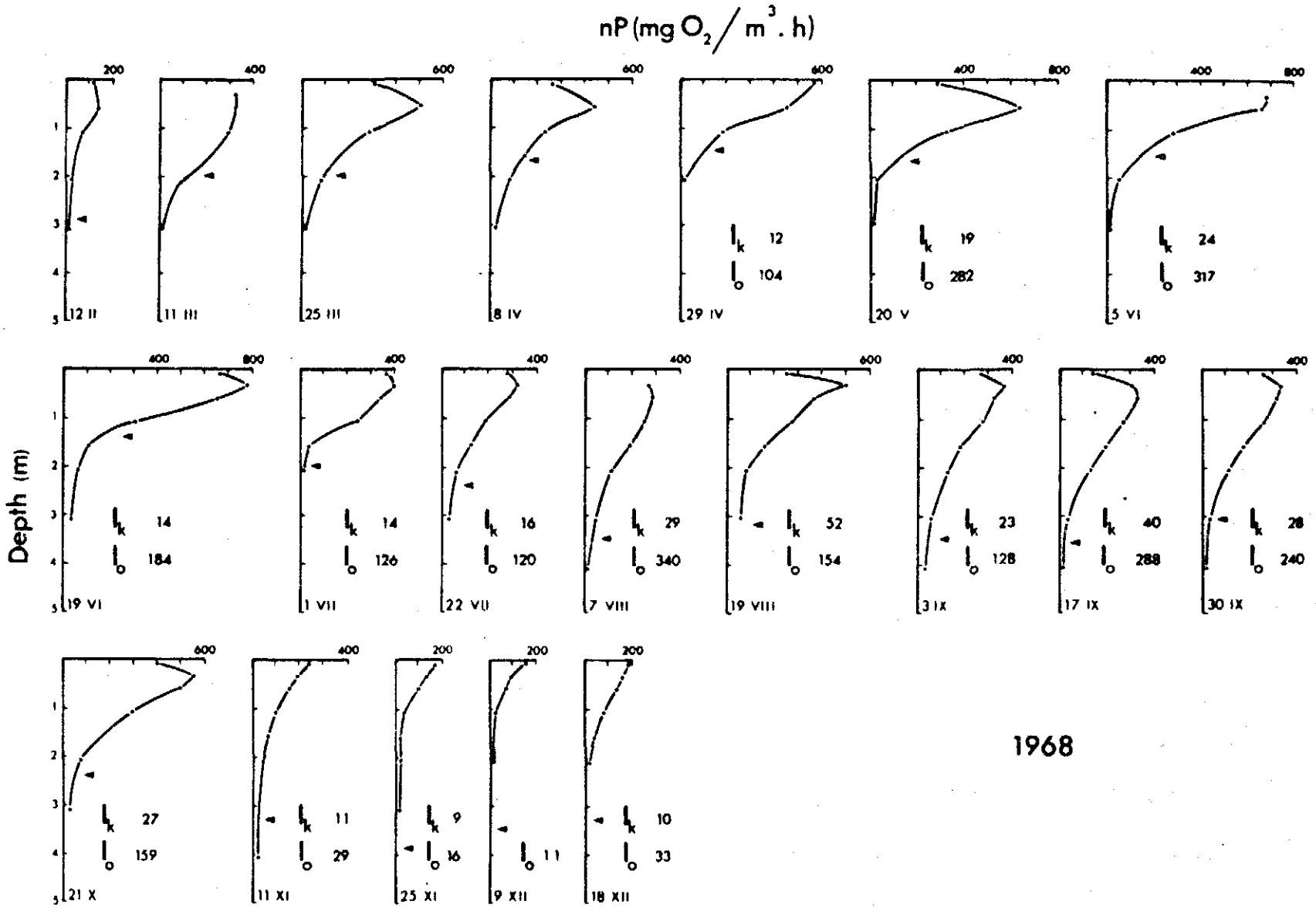


Fig. 3 Legend opposite.

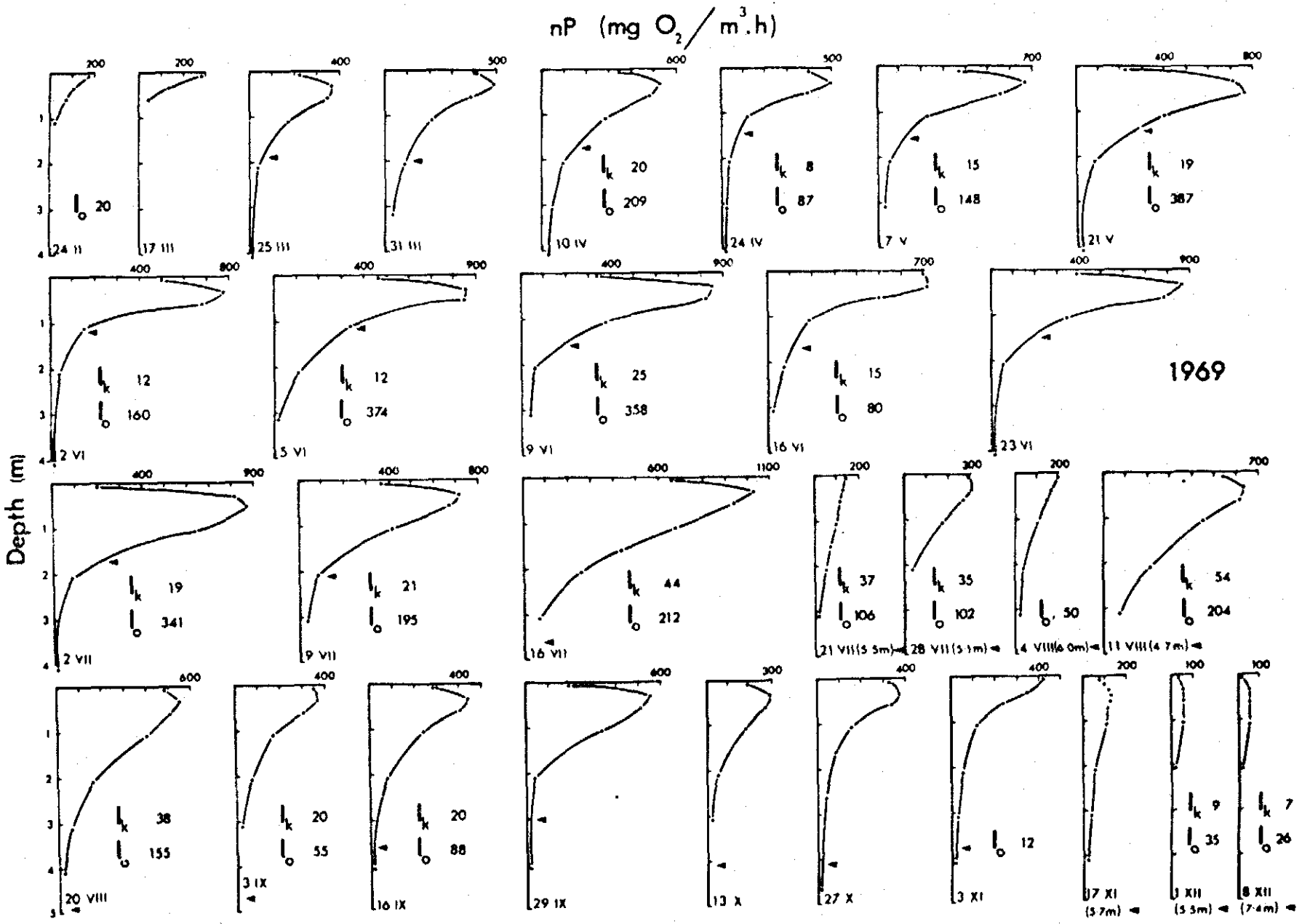

nP (mg O2 / m3.h)
Depth (m)
1969
24 II
17 III
25 III
31 III
10 IV
24 IV
7 V
21 V
2 VI
5 VI
9 VI
16 VI
23 VI
2 VII
9 VII
16 VII
21 VII(5.5m)
28 VII(5.1m)
4 VIII(6.0m)
11 VIII(4.7m)
20 VIII
3 IX
16 IX
29 IX
13 X
27 X
3 XI
7 XI (5.7m)
3 XII (5.5m)
8 XII (7.4m)

Fig. 3 (cont'd)

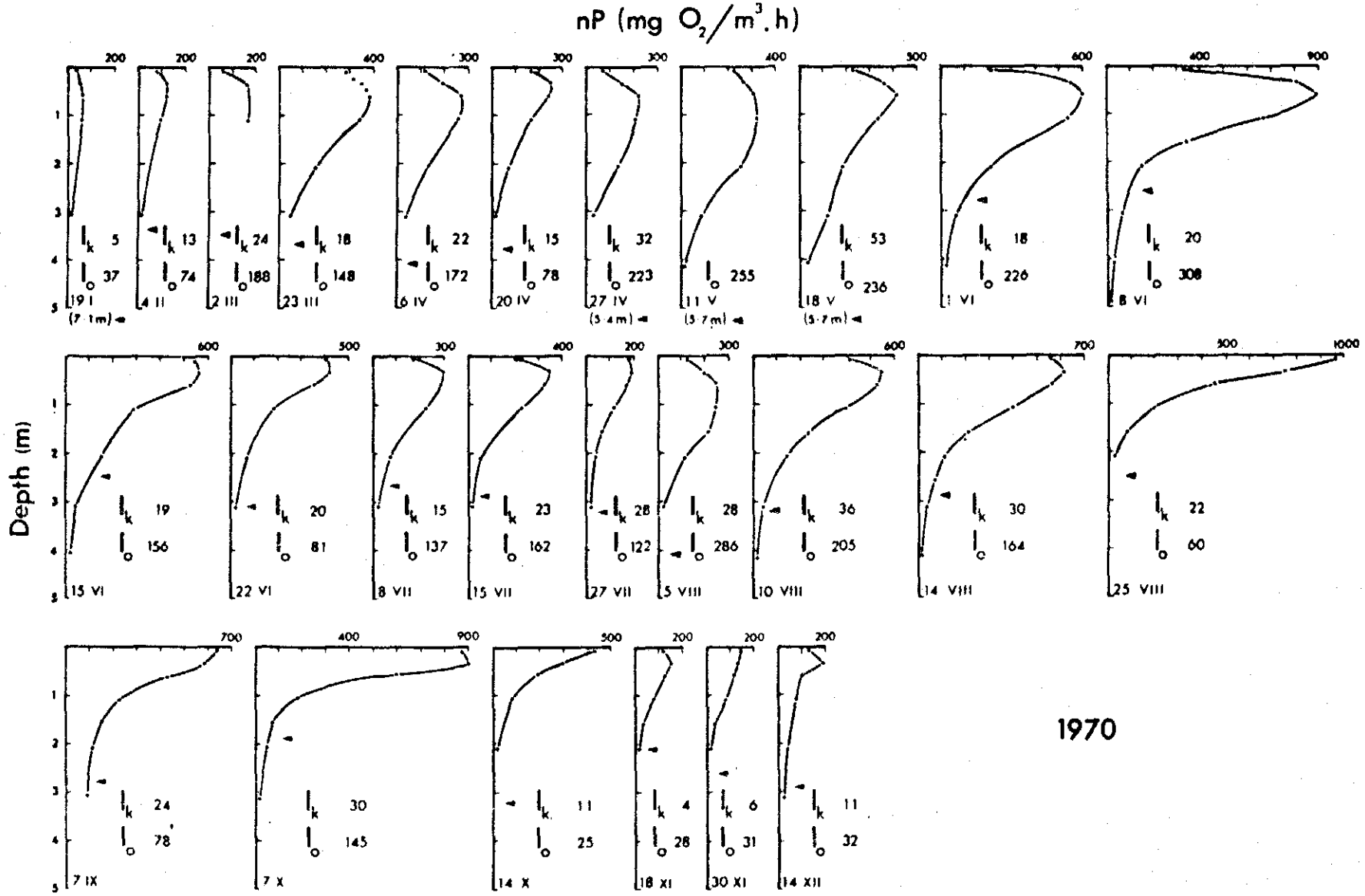

nP (mg $O_2/m^3.h$)
Depth (m)
1970
19 I
4 II
2 III
23 III
6 IV
20 IV
27 IV
11 V
18 V
1 VI
8 VI
15 VI
22 VI
8 VII
15 VII
27 VII
5 VIII
10 VIII
14 VIII
25 VIII
7 IX
7 X
14 X
18 XI
30 XI
14 XII

Fig. 3 (cont'd)

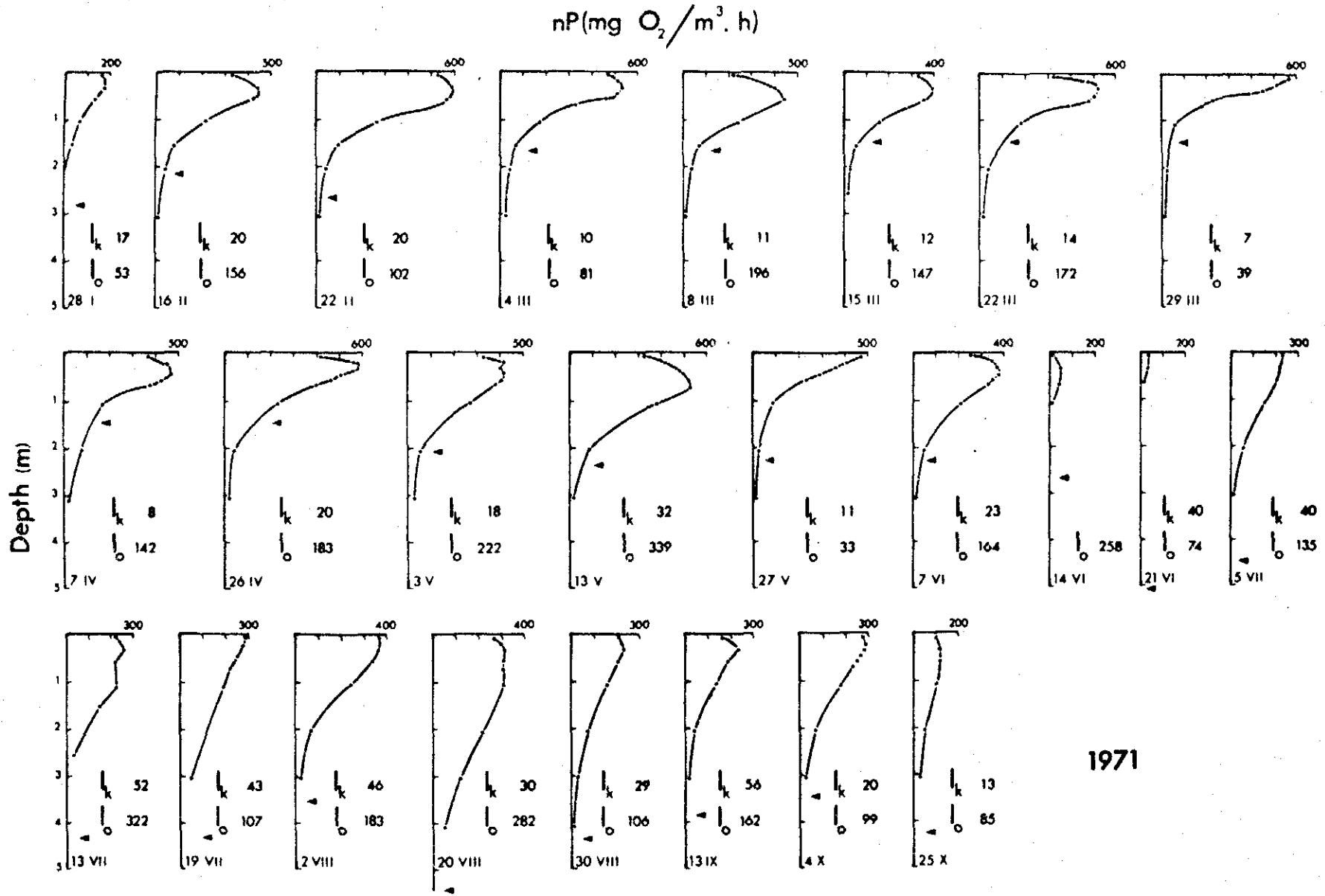

nP(mg O2/m3. h)
Depth (m)
28 I
16 II
22 II
4 III
8 III
15 III
22 III
29 III
7 IV
26 IV
3 V
13 V
27 V
7 VI
14 VI
21 VI
5 VII
13 VII
19 VII
2 VIII
20 VIII
30 VIII
13 IX
4 X
25 X
1971

Fig. 3 (cont'd)

where inhibition was found to begin at about 100-150 kerg/cm^2.sec (Jenkin, 1937; Talling, 1957a). Enhanced susceptibility to light inhibition has been reported for shade-adapted phytoplankton growing under the relatively poor light conditions of autumn (Verduin, 1956a) or of deep water (Ryther & Menzel, 1959).

Precise definition of the onset of light inhibition is difficult at Loch Leven because light extinction is so rapid with depth. However, if the generally lower values found here are true, they may reflect adaptation by the phytoplankton to the relatively low average light intensity likely to be experienced by a circulating algal cell in the turbid water of Loch Leven. Water temperature did not appear to be related to the onset of surface inhibition (cf. Aruga, 1965).

Light-saturation of photosynthesis usually began approximately 25-30 cm below the surface and occupied a very narrow depth zone. This was often no greater than the 10 cm column covered by the experimental bottles. In the absence of a well defined 'plateau' in the depth-profiles of photosynthesis, some uncertainty must be attached to the estimates of nP_{max} (the light-saturated rate of photosynthesis per unit volume of water), and of P_{max} (the light-saturated rate of photosynthesis per mg chlorophyll *a*) which is derived from it. True values of nP_{max} and P_{max} may be higher than those estimated over a 10 cm column.

Below the zone of light-saturation photosynthetic rates declined rapidly, reaching very low values below 3 m. Appreciable photosynthesis was often recorded (Fig. 3) below the depth, corresponding to 1% of available radiation (400-700 nm), usually used to define the lower limit of the euphotic zone.

The narrow zone of optimum light and the sharp decrease of photosynthetic rate with depth reflect the generally rapid extinction of light in this lake which in turn, as shown later, is determined largely

by the density of the phytoplankton crop it supports.

The overall shape of, and area enclosed by, the photosynthesis-depth profile shows marked seasonal variation, the causes of which will be discussed in subsequent sections.

SECTION IV: HOURLY GROSS PHOTOSYNTHESIS PER UNIT AREA AND THE COMPONENT VARIABLES OF THE PHOTOSYNTHESIS-DEPTH PROFILE

The area enclosed by each depth-profile is a measure of the integral rate of gross photosynthesis per unit area of lake surface (Σ nP, units mg $O_2/m^2.h$). Midday hourly rates of gross photosynthesis per unit area (Σ nP), determined by planimetric integration of the photosynthesis-depth profiles, are given in Fig. 4. Over the 4 year period Σ nP showed marked seasonal variation within the range 21-1586 mg $O_2/m^2.h$. Values of Σ nP determined planimetrically were in good agreement (Fig. 5) with those obtained using the following equation, devised by Talling (1957b), to describe integral phctosynthesis in an unstratified water column:

$$\Sigma nP = \frac{nP_{max}}{1.33\ k_{min}} \ln 2 \left(\frac{\log I_o' - \log 0.5\ I_k}{\log 2}\right) \qquad (1)$$

This expression can be reduced to:

$$\Sigma nP = \frac{nP_{max}}{1.33\ k_{min}} \ln \left(\frac{I_o'}{0.5\ I_k}\right) \qquad (2)$$

where,

Σ nP = hourly rate of gross photosynthesis per unit area (mg $O_2/m^2.h$)

n = population density per unit volume of water, as mg chlorophyll a/m^3

k_{min} = the minimum value (over the visible spectrum) of the vertical extinction coefficient (ln units/m)

P_{max} = light-saturated rate of gross photosynthesis per unit population, in mg O_2/mg chlorophyll a.h (photosynthetic capacity)

nP_{max} = light-saturated rate of gross photosynthesis per unit water volume (mg $O_2/m^3.h$)

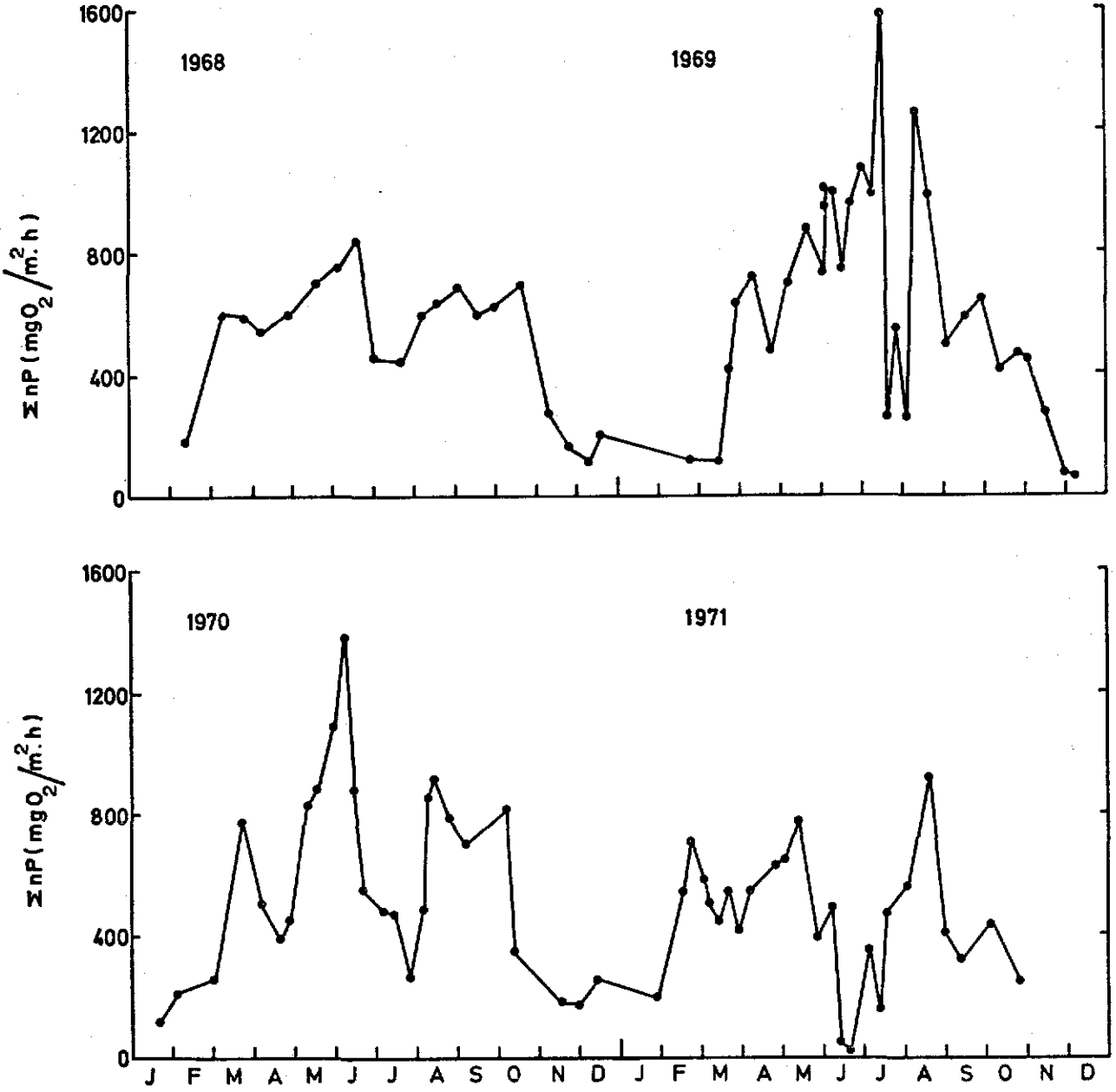


Fig. 4 Midday hourly rates of gross photosynthesis per unit area (Σ nP), 1968-71.

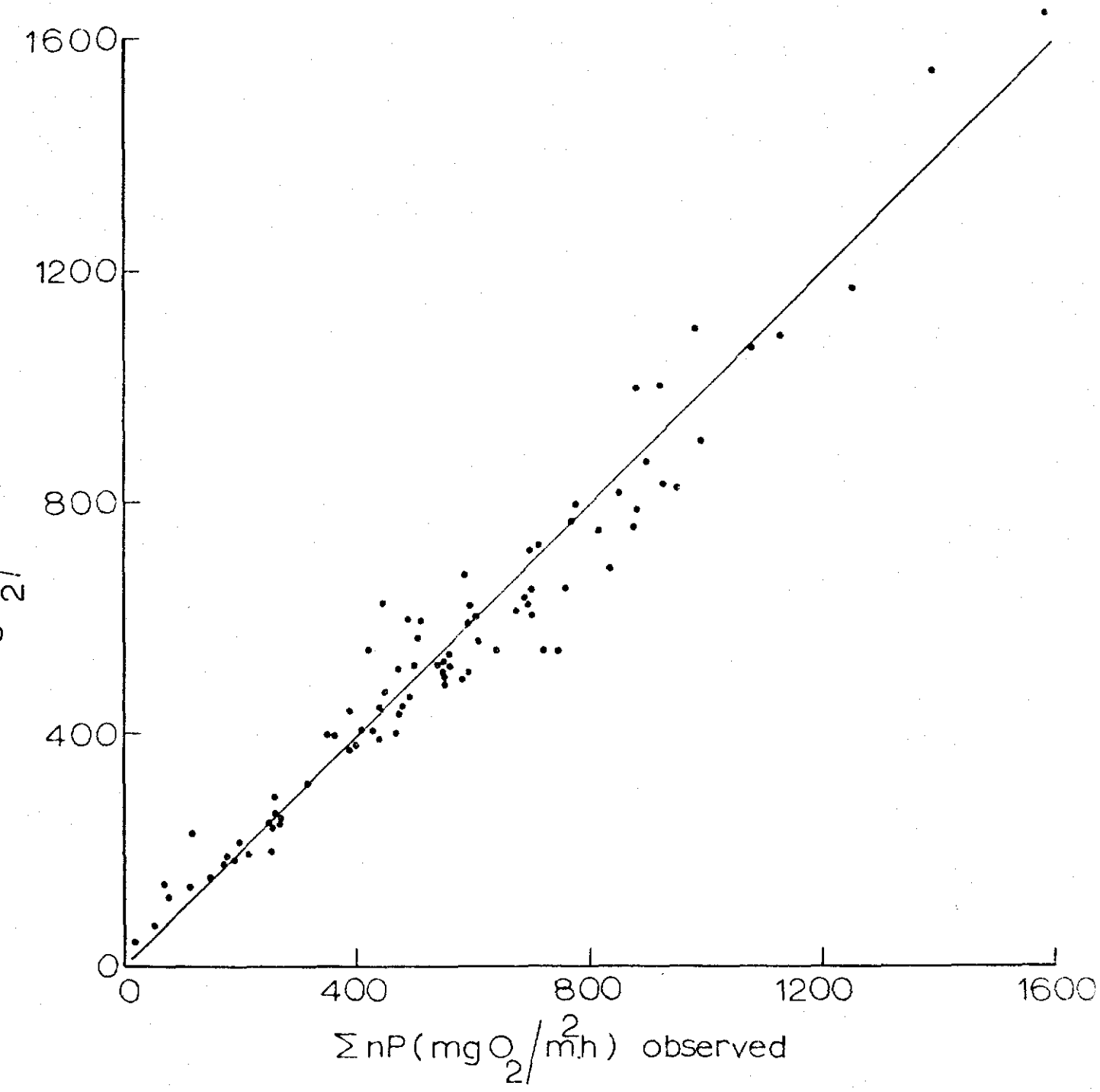


Fig. 5 Midday hourly rates of gross photosynthesis per unit area (Σ nP) observed in Loch Leven in relation to values calculated using equation 3 of Talling (1957b). The inserted line corresponds to a 1 : 1 relationship.

I_o' = light intensity immediately below the water surface (i.e. surface light intensity, I_o, corrected for surface reflection loss of 10%), in kerg (Ph.A.R.)/cm^2.sec

I_k = light intensity defining the onset of light-saturation of photosynthesis, in kerg (Ph.A.R.)/cm^2.sec. Characteristic introduced by Talling (1957a) and equivalent to the light intensity at which an extrapolation of the initial linear region of the rate-intensity curve would reach the light-saturated rate.

The indirect calculation of integral photosynthesis also works if the I_k value was obtained independently in the laboratory.

Talling's mathematical model usefully identifies the components of the photosynthesis-depth profile and illustrates their relative influence on the area-based integral rate (ΣnP). Essentially these components fall into two groups whose product is equivalent to the area of the profile (ΣnP). One group, which determines the maximum extent of the profile along the horizontal axis, contains the components n and P_{max}; the other group contains the components I_o', I_k and k_{min}, which determine the vertical displacement of the profile.

Most of the variation in the measured hourly rates of gross photosynthesis per unit area (ΣnP) at Loch Leven was accounted for by variation in the ratio nP_{max}/k_{min} (Fig. 6); the remaining variation in ΣnP was largely accounted for by variations in the ratio I_o'/I_k (Fig. 7). The following average relationship was observed between ΣnP and nP_{max}/k_{min} and is indicated as a broken line in Fig. 6:

$$\Sigma nP = \frac{nP_{max}}{k_{min}} \quad 1.9$$

Multiplication of the minimum extinction coefficient, k_{min}, by 1.33 yields a measure of the effective vertical extinction (k_e) of total

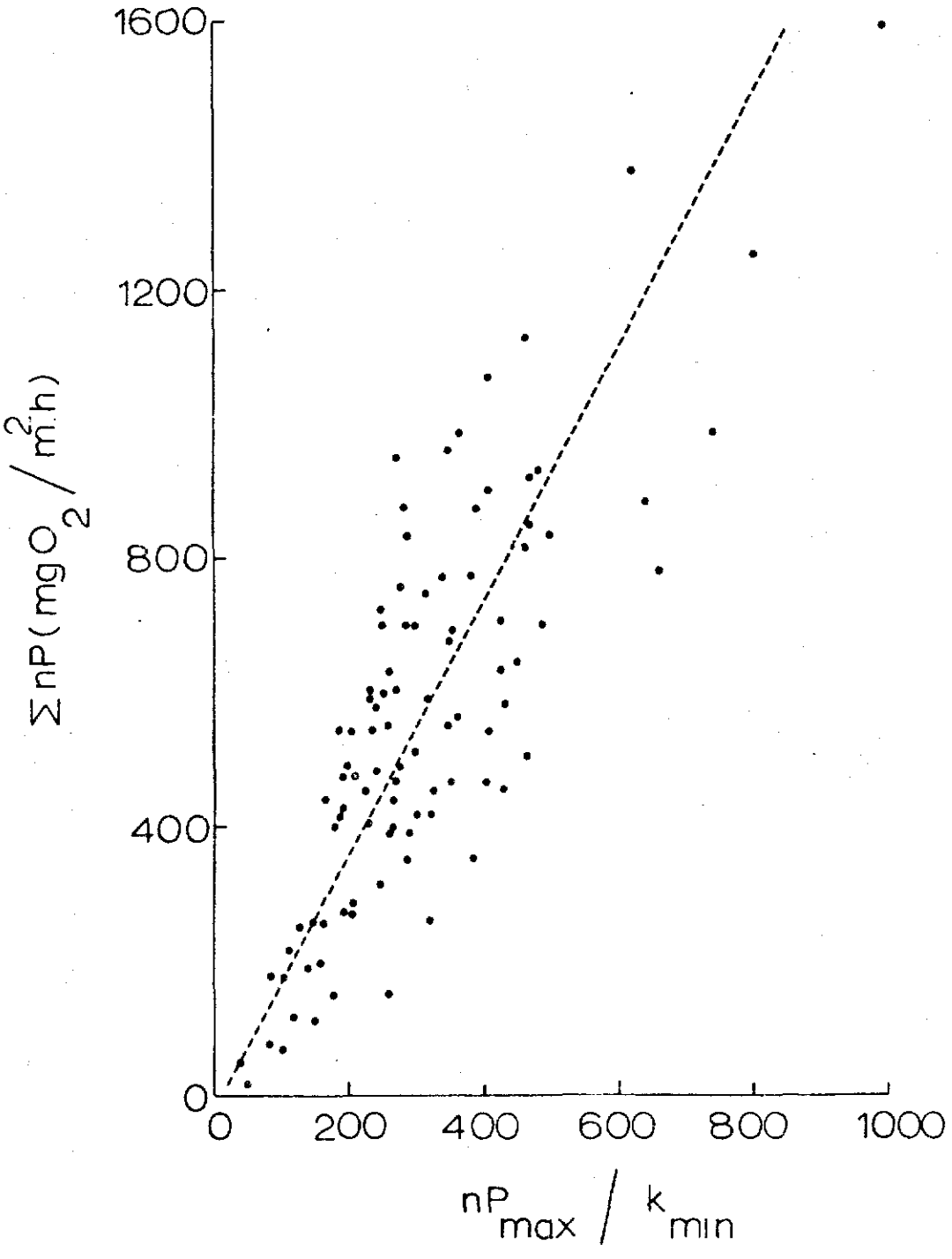


Fig. 6 Gross photosynthesis per unit area (ΣnP) as a function of the ratio nP_{max}/k_{min}. The dashed line indicates the average relationship between ΣnP and nP_{max}/k_{min}.

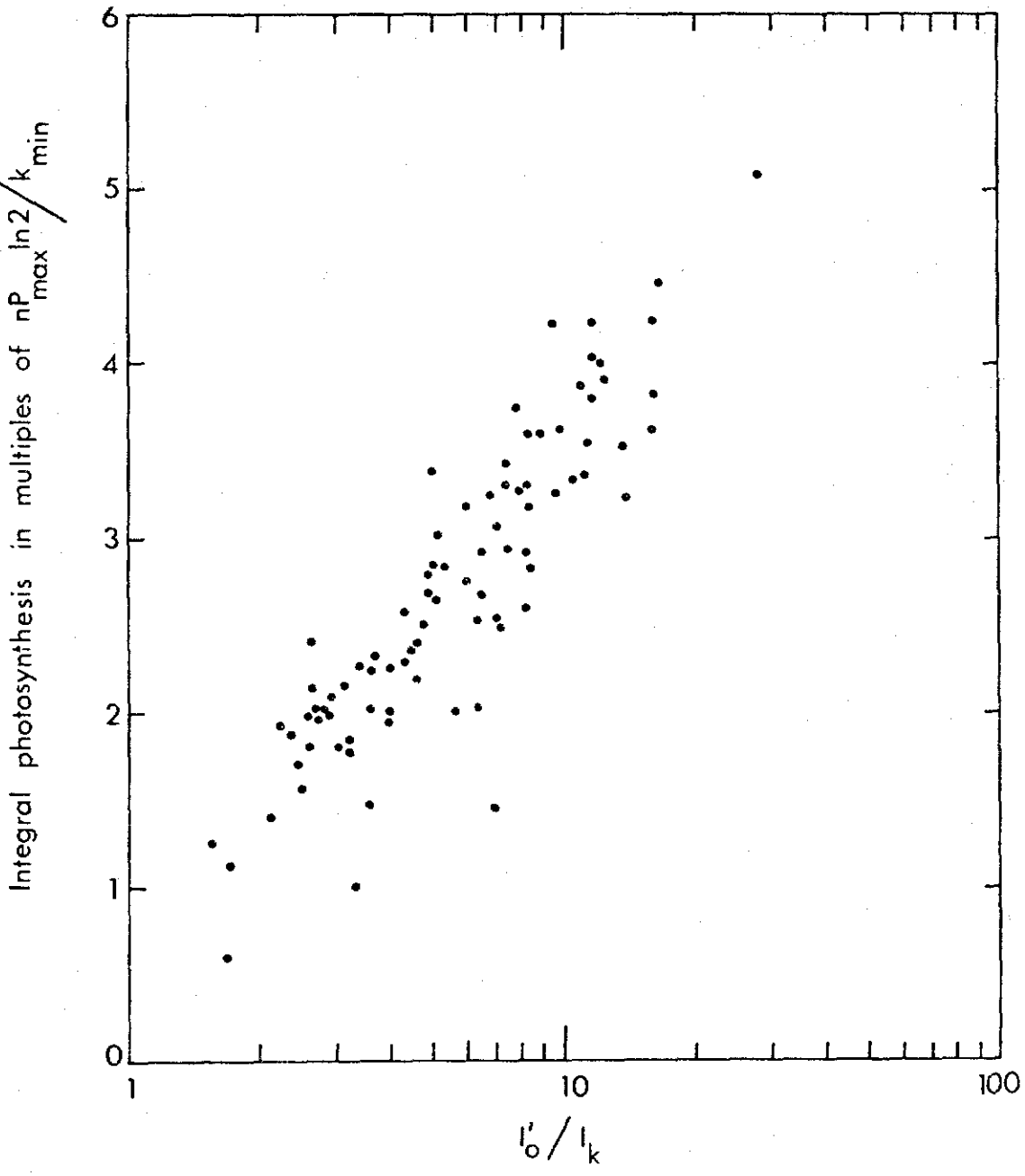


Fig. 7 Variation of integral gross photosynthesis per unit area (Σ nP), expressed in multiples of $nP_{max}.\ln 2/k_{min}$, as a function of the ratio I'_o/I_k. The latter is shown on a logarithmic scale.
A key to the symbols used is on p.1.

visible light (Talling, 1957b, 1971, p.222). Substituting k_e in the above equation gives:

$$\Sigma\, nP = \frac{nP_{max}}{k_e} \quad 2.5$$

The factor 2.5 is equivalent to the function F(I) of various authors and is equal to ln $(I'_o/0.5\ I_k)$; similar values for F(I) have been reported by Rodhe, Vollenweider & Nauwerk (1958) and Wright (1959). A rather higher value of 3.5 may be calculated from the data of Talling (1965a, p.20). The latter value refers to tropical African lakes, whereas the other references quoted refer to temperate waters. The higher value for the African lakes could arise if higher tropical I_o values overweighed a temperature-based increase of I_k. The influence of temperature on I_k is discussed in section V, part 1b.

SECTION V: FACTORS AFFECTING HOURLY RATES OF GROSS PHOTOSYNTHESIS PER UNIT AREA (Σ nP)

In this section seasonal variations in Σ nP are interpreted in terms of the variation in the components of the photosynthesis-depth profile (see the previous section), their interaction and their response to environmental factors.

1. The light-climate

The distribution of photosynthetic rate with depth for a vertically homogeneous population is determined by the light-climate in the water column, the light-saturation behaviour (I_k) and light-inhibition response of the phytoplankton and the gradient (K') of the light-limited region of the photosynthesis/light intensity curve.

The underwater light-climate is determined by the intensity of surface-incident solar radiation and the extent of its penetration into the water column.

The results of measurements of (a) surface-incident solar radiation (I_o) during exposure periods, (b) I_k and K' and (c) underwater light penetration, are presented below. Factors responsible for their seasonal variation and their influence on areal gross productivity (Σ nP) are discussed.

a) Surface-incident solar radiation

Introduction

Solar radiation supplies the energy required to drive the photosynthetic process and directly and indirectly determines water temperature; it may, therefore, be considered the master environmental factor determining photosynthetic productivity.

Local conditions of altitude, topography and degree of atmospheric pollution may modify the amount and spectral composition of the solar

radiation reaching the lake surface. The proportion of direct sunlight which is reflected from the water surface depends on the angle of incidence. According to Ruttner (1963), in temperate latitudes approximately 2.5% of noon sunshine in summer, and 14% in winter, is reflected. Of the diffuse light on average 6% is reflected. Here an average surface loss of 10% of total (i.e. diffuse plus direct) radiation has been assumed (Talling, 1957a).

Total solar radiant energy covers a spectral wavelength range from 300-3,000 nm. Photosynthetically-available radiation (Ph.A.R.), in the spectral region 400-700 nm, accounts for approximately 50% of total radiant energy; here a fractional value of 0.46 has been used, in accordance with Talling (1957a).

Results and Discussion

The mean surface-incident light intensity (I_o, units kerg (Ph.A.R.)/cm^2.sec) during each productivity measurement is shown in Fig. 3.

It was shown earlier that most of the variation in areal hourly productivity (Σ nP) could be accounted for without reference to I_o, by the ratio nP_{max}/k_{min} (Fig. 6). This suggests that Σ nP was relatively insensitive to changes in surface light intensity, within the range of intensities encountered during productivity measurements. A similar conclusion was reached by Harvey et al (1935), Steemann Nielsen (1952a, 1954, 1958) and Steemann Nielsen & Jensen (1957), using graphical illustrations of the vertical displacement and enlargement of the photosynthesis-depth profile with increasing I_o. Such insensitivity is implied in mathematical models of the photosynthesis-depth integral which show that the relationship between Σ nP and I_o is not linear, but logarithmic (Tamiya et al, 1953; Oorschot, 1955; Talling, 1955, 1957b, 1971; Shuler & Affens, 1970). In addition, light-saturation of photosynthesis (measured by I_k) usually begins at light intensities well

below I_o (Fig. 3); this results in further non-linearity between Σ nP and I_o variations. Mathematical models (e.g. Talling, 1957b) show that Σ nP is related to the ratio ln $(I_o/0.5\ I_k)$. Σ nP is, therefore, most sensitive to changes in I_o when I_o is less than I_k and becomes increasingly less sensitive as I_o increases above I_k. Theoretically, a critical I_o exists (but is unlikely to occur in nature) above which further increase in Σ nP will be offset by increased surface inhibition losses.

At Loch Leven, values of I_k showed a general increase with increase in water temperature (described in (b) below). As a result, lower winter I_o values tended to be associated with lower I_k values. The ratio $I_o/0.5\ I_k$ was, therefore, less variable than I_o itself, and I_o was almost always greater than I_k during productivity measurements (Fig. 3). These features can explain why midday hourly rates of areal gross productivity (Σ nP) appeared relatively insensitive to prevailing surface light intensities (I_o).

The <u>duration</u> of incident radiation, on the other hand, is shown later (section VI) to exert a relatively large influence on <u>daily</u> rates of areal gross productivity.

b) <u>Light-saturation behaviour (I_k) and the gradient (K') of the initial linear, light-limited region of the photosynthesis/light intensity curve</u>

The characteristic "I_k" was introduced by Talling (1957a) to express the onset of light-saturation of photosynthesis in relation to light intensity. It is defined as the light intensity at which an extrapolation of the initial linear region of the photosynthesis-light intensity curve reaches the light-saturated rate. As such it may be estimated from the curves relating photosynthetic rate and light intensity as illustrated in Fig. 8. Photosynthesis involves both photochemical and enzymatic processes. The slope of the initial part of the rate/intensity curve is a function of the photochemical part of photosynthesis whereas the horizontal portion

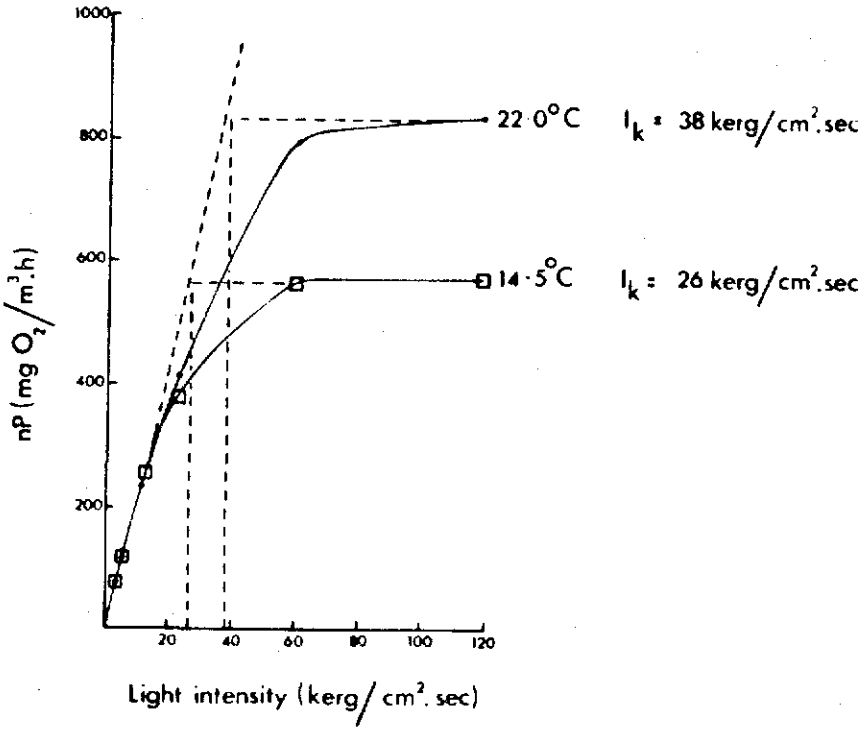


Fig. 8 The relationship between photosynthetic rate and light intensity, measured in the laboratory using fluorescent illumination at two temperatures. The dashed line shows the method of calculation of I_k. The water sample (collected 8.9.70) had an original temperature of 13.4°C and contained 80 mg chlorophyll a/m^3; the dominant algal species was Stephanodiscus rotula.

of the curve represents the maximum rate of the enzymatic processes. The I_k characteristic may, therefore, be regarded as describing the ratio between the enzymatic and photochemical processes of photosynthesis.

Talling (1960a) showed that I_k could usually be approximated by the light intensity at which photosynthetic rate reached 0.75 of the light-saturated rate (nP_{max}). As pointed out by Talling (1965a), this method leads to a more objective determination of I_k than extrapolation of the initial linear region of the rate/intensity curve, and was the method adopted here. Calculations of I_k by the two methods, using photosynthesis/intensity curves from field and laboratory exposures, were often very close. For example, for the data presented in Fig. 8, I_k values calculated as the light intensity at 0.75 nP_{max} are 27 and 38 kerg (Ph.A.R.)/cm^2.sec at 14.5 and 22.0°C respectively. Equivalent values obtained by extrapolation of the linear portion of the curves are 26 and 38 kerg (Ph.A.R.)/cm^2.sec respectively.

I_k values calculated from field exposures varied seasonally between 4 and 54 kerg (Ph.A.R.)/cm^2.sec (Fig. 9). Eighty seven per cent of a total of 84 I_k determinations gave values less than 40 kerg (Ph.A.R.)/cm^2.sec, thus falling within the range of published values of I_k for temperate lakes (Talling, 1957a; Ichimura, 1960a). Values of I_k greater than 40 kerg (Ph.A.R.)/cm^2.sec at 15-20°C, which were sometimes recorded at Loch Leven, are less common.

Temperature exerted a marked influence on I_k (Fig. 10). A linear relation between log I_k and temperature is roughly indicated. The correlation coefficient between log I_k and temperature is 0.65 and the calculated linear regression line indicates an average temperature coefficient (Q_{10}) of 1.98. Considerable scatter of the experimental points exists, and may in part be attributed to seasonal variation in species composition of the mixed natural populations to which the I_k

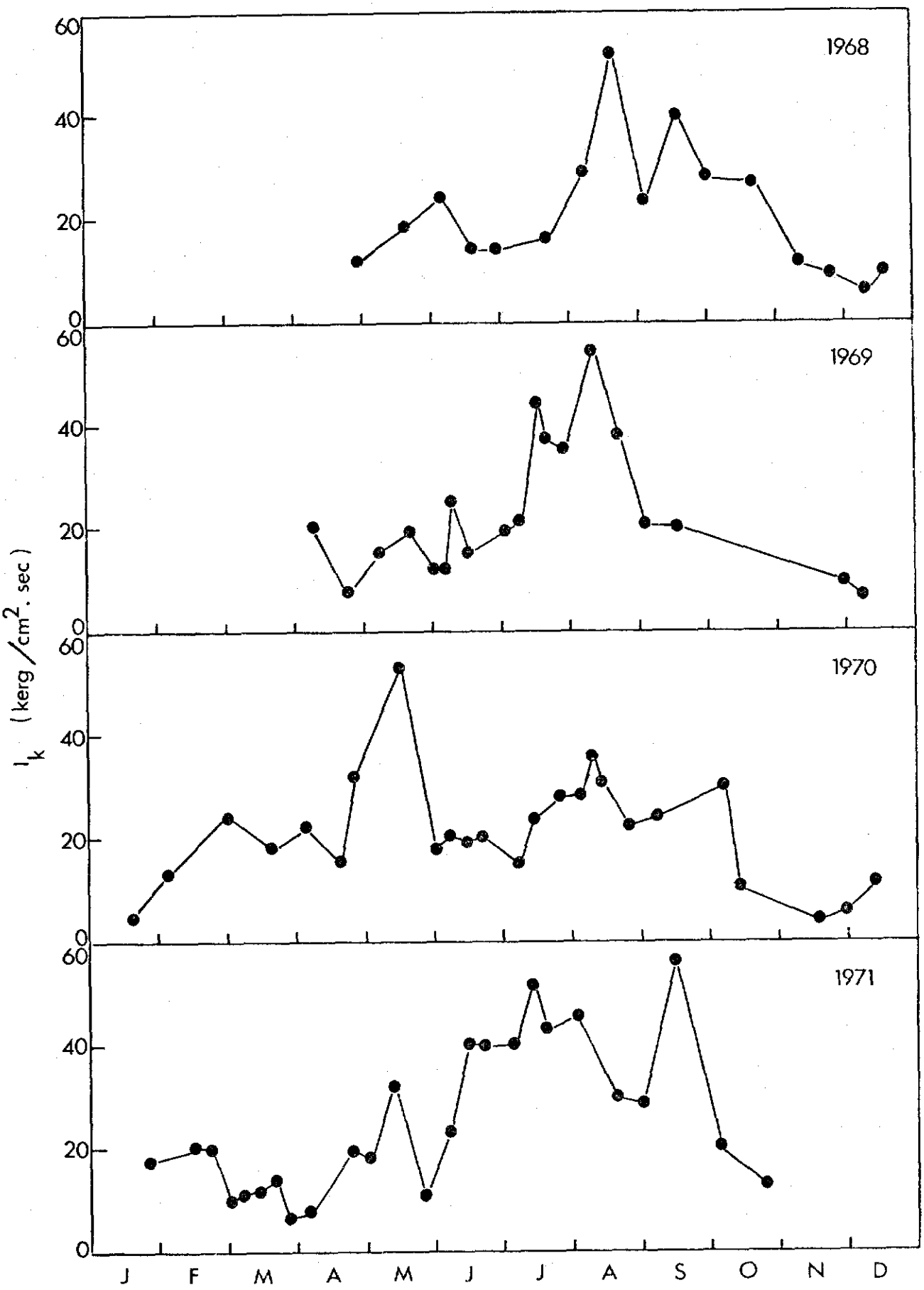


Fig. 9 Seasonal variation of I_k(kerg(Ph.A.R.)/cm^2.sec).

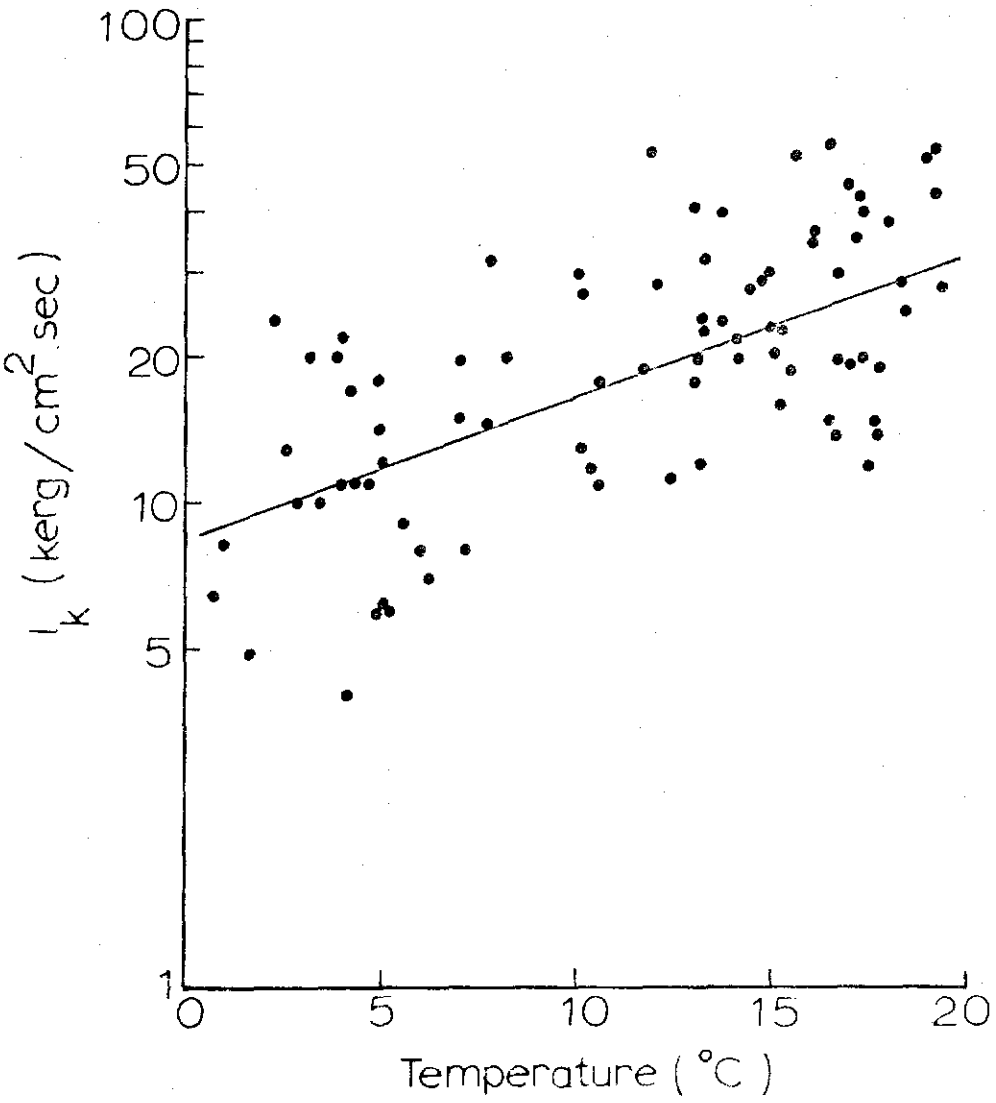


Fig. 10 Values of I_k (logarithmic scale) measured at Loch Leven, in relation to ambient water temperature. Calculated regression line is log y = 0.030x + 0.935, correlation coefficient (r) = 0.65, N = 84. Q_{10} = 1.98.

values refer; Ryther (1956a) and Aruga (1965) have shown that different species may differ in their response to light intensity and therefore yield different I_k values at the same temperature.

The effect of temperature on I_k for the same parent suspension is demonstrated in the results of the laboratory exposure illustrated in Fig. 8. Here the increase in I_k with temperature is equivalent to a Q_{10} of 1.73. Adaptation time to the higher temperature may have been too short for the photosynthetic potential at 22°C to be fully realised and may account for the rather low Q_{10} value.

Temperature dependence of I_k has also been illustrated by Talling (1957a), Wright (1959) and Steemann Nielsen & Hansen (1961), and can explain why light-saturation was recorded in Loch Leven in winter months when incident light intensity was relatively low. Steemann Nielsen & Hansen (1961) have also attributed low I_k values in winter to shade adaptation. For Loch Leven this possibility is examined later (p.74). The temperature relations of I_k largely reflect the temperature dependence of P_{max} described later (cf. Figs. 10 and 30). Fig. 11 shows that variations in I_k are to a large extent accounted for by variations in P_{max}. This suggests that the slope of the initial light-limited region of the photosynthesis/intensity curve shows little seasonal variation and is largely independent of temperature. The latter feature is also illustrated in Fig. 8 and has been demonstrated by Talling (1957a), Aruga (1965) and Steemann Nielsen & Jørgensen (1968). Temperature would not be expected to influence the rate of photosynthesis at low (sub-saturating) light intensities, since here the rate of the overall process of photosynthesis is determined by temperature-insensitive photochemical processes.

The correlation between I_k and P_{max} variations shown in Fig. 11 is not perfect; i.e. the ratio P_{max}/I_k, which is equivalent to the initial gradient of the photosynthesis/light intensity curve, is not constant.

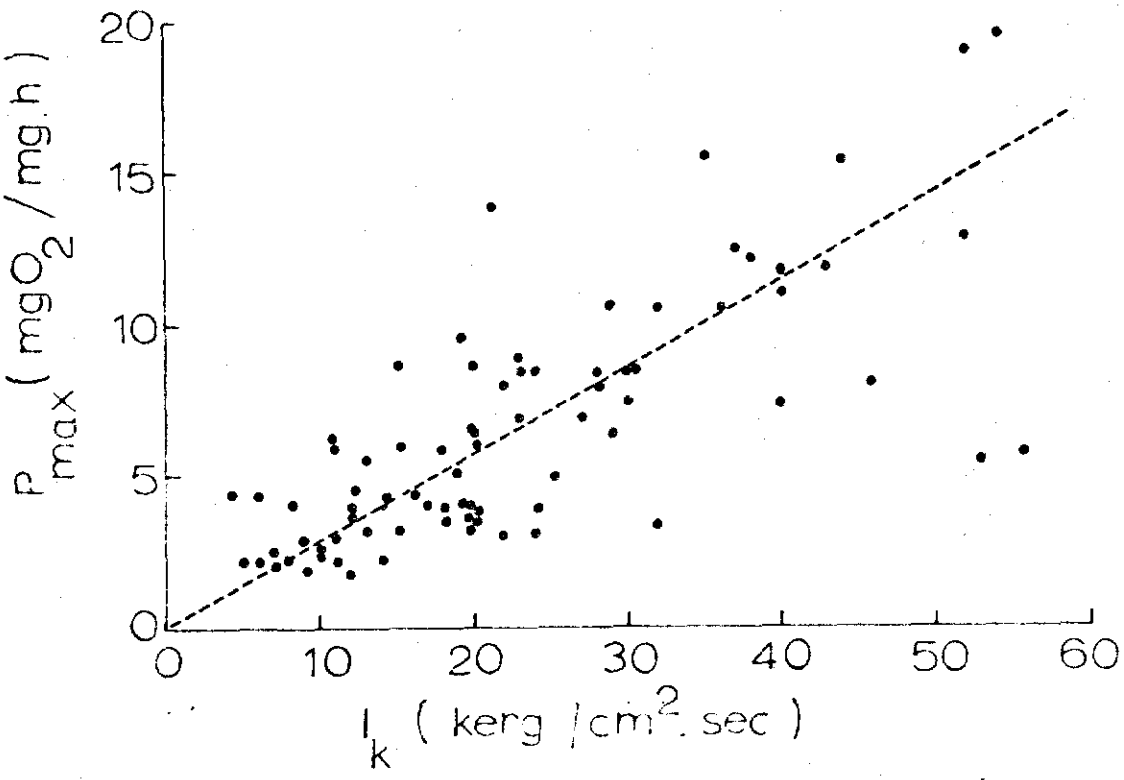


Fig. 11 The relationship between the light-saturated rate of photosynthesis per unit content of chlorophyll a (P_{max}) and the characteristic (I_k) denoting onset of light-saturation. The light intensity units of I_k refer to photosynthetically-available radiation (Ph.A.R.). The broken line indicates the average value of the ratio P_{max}/I_k.

Assuming no errors in P_{max} and I_k estimation, variation in this gradient (denoted K' by Talling, 1957a,b) implies some variation in the rate of photosynthesis per mg chlorophyll *a* at constant sub-saturating light intensity. This rate, and the gradient K', depend on quantum efficiency and on light absorption by unit concentration of chlorophyll *a* in the algal suspensions. I_k variations will be less related to P_{max} variations to the extent that quantum efficiency and light absorption vary. A reduction in either quantum efficiency or light absorption would cause an increase in I_k for constant P_{max}. I_k may remain constant despite a decline in P_{max} if K' also declines.

Clearly, from the following equation (Talling, 1957b) describing the photosynthesis-depth integral (Σ nP), increase in I_k will tend to decrease Σ nP.

$$\Sigma \text{ nP} = \frac{\text{nP}_{max}}{1.33\ k_{min}} \ln(I_o/0.5\ I_k)$$

A key to symbols is on p.1.

Since I_k is involved in the above equation as a logarithmic function its influence on Σ nP is likely to be relatively less than that of P_{max}. The relative influence of I_k fluctuations on integral photosynthesis per unit area (Σ nP) will depend on whether change in I_k is brought about by change in the maximum rate of enzymatic processes (given by P_{max}) or by change in quantum efficiency and/or light absorption (given by $K' = P_{max}/I_k$). If I_k increases due to a reduction in quantum efficiency or light absorption but P_{max} remains unaltered, Σ nP will decrease in proportion to reduction of the term, $\ln(I_o/0.5\ I_k)$. If on the other hand I_k increases due to increase in P_{max} its reductive influence on Σ nP will be offset by the tendency for increased P_{max} values to increase Σ nP. If P_{max} and I_k both decrease then the reduction in Σ nP due to reduction in P_{max} will be offset to a small extent (because I_k is

involved as a logarithmic term) by parallel reduction in I_k.

Variation in the rate of photosynthesis per mg chlorophyll a at a given sub-saturating light intensity is normally reported to be small. Steemann Nielsen (1961) found a rate of 0.50 to 0.57 mg C/mg.h at 1 klux (incandescent light) for Chlorella vulgaris grown at either 3 or 30 klux (20°C). Jørgensen (1964b) and Steemann Nielsen & Jørgensen (1968) record an average value of 0.5 mg C/mg.h at 1 klux (incandescent light) for a number of planktonic green algae and diatoms grown under a variety of conditions of light and temperature.

At Loch Leven, values of the ratio P_{max}/I_k (K') varied from 0.11 to 1.13 mg O_2/mg chlorophyll a.h at 1 kerg (Ph.A.R.)/cm^2.sec. The average value of the ratio P_{max}/I_k, indicated as a broken line in Fig. 11, was 0.35 mg O_2/mg chlorophyll a.h at 1 kerg (Ph.A.R.)/cm^2.sec. Assuming a photosynthetic quotient of 1.0 and taking 28.6 x 10^{-2} cal/cm^2.h (i.e. 3.3 kerg/cm^2.sec) as equivalent to 1 klux of incandescent light (Steemann Nielsen & Hansen, 1961), the corresponding carbon-based rate of photosynthesis per mg chlorophyll a at 1 klux (incandescent) is 0.43 mg C/mg.h. This average value is close to the average values quoted above from the literature. For the phytoplankton of Blelham Tarn and Windermere Talling (1966) found an average rate of photosynthesis at 1 klux (fluorescent light and visible daylight) of 2 mg O_2/mg chlorophyll a.h. Taking 4.1 kerg/cm^2.sec as equivalent to 1 klux of fluorescent or visible light (Talling, 1966), the corresponding average Loch Leven value is 1.44 mg O_2/mg chlorophyll a.h.

Exceptions to the rather constant rate of photosynthesis per mg chlorophyll a at 1 klux are reported in the literature and have been reviewed by Steemann Nielsen & Jørgensen (1968). The rate may be reduced by poisons (Weller & Frank, 1941), proncunced nutrient deficiency (Jørgensen, 1970) or light shock (Steemann Nielsen, 1962b). Due to

differences in pigment composition different species may show different rates of oxygen production per mg chlorophyll a at 1 klux (Steemann Nielsen, 1961). Due to mutual shading by the single pigment molecules inside a chloroplast the chlorophyll content per cell may also have some effect (Steemann Nielsen, 1961); higher values would be expected for cells poor in pigments. Some support for this is provided by Talling (1966), who recorded lower rates of oxygen production per mg chlorophyll a at 1 klux in samples of deeper origin with increased chlorophyll a per cell.

Changes in the ratio P_{max}/I_k at Loch Leven showed no obvious seasonal pattern and did not appear to be correlated with changes in any measured environmental factor, or with species composition.

It is possible that fluctuations in the ratio P_{max}/I_k are merely reflections of errors in estimation of P_{max} and/or I_k. If fluctuations in this ratio reflect 'real' changes in quantum efficiency and/or light absorption then the cause(s) of such changes are unknown in this instance.

c) Underwater light penetration

The spectral variation of light extinction

The seasonal variation of light extinction in four spectral regions, expressed as vertical extinction coefficients (k values), is shown in Fig. 12. Blue light, having the highest k value, is extinguished most rapidly at all seasons; rapid extinction of blue light is characteristic of waters with a high content of suspended particulate material or of dissolved yellow substances (Kalle, 1938; Sauberer, 1945, 1962; Talling, 1971). The k values for red, green and orange light were usually rather similar to each other, but the minimum vertical extinction coefficient (k_{min}) over the visible spectrum usually lay in the orange spectral region (at about 590 nm).

The significance of the spectral modification of irradiance with depth for the photosynthesis of phytoplankton in a water column was

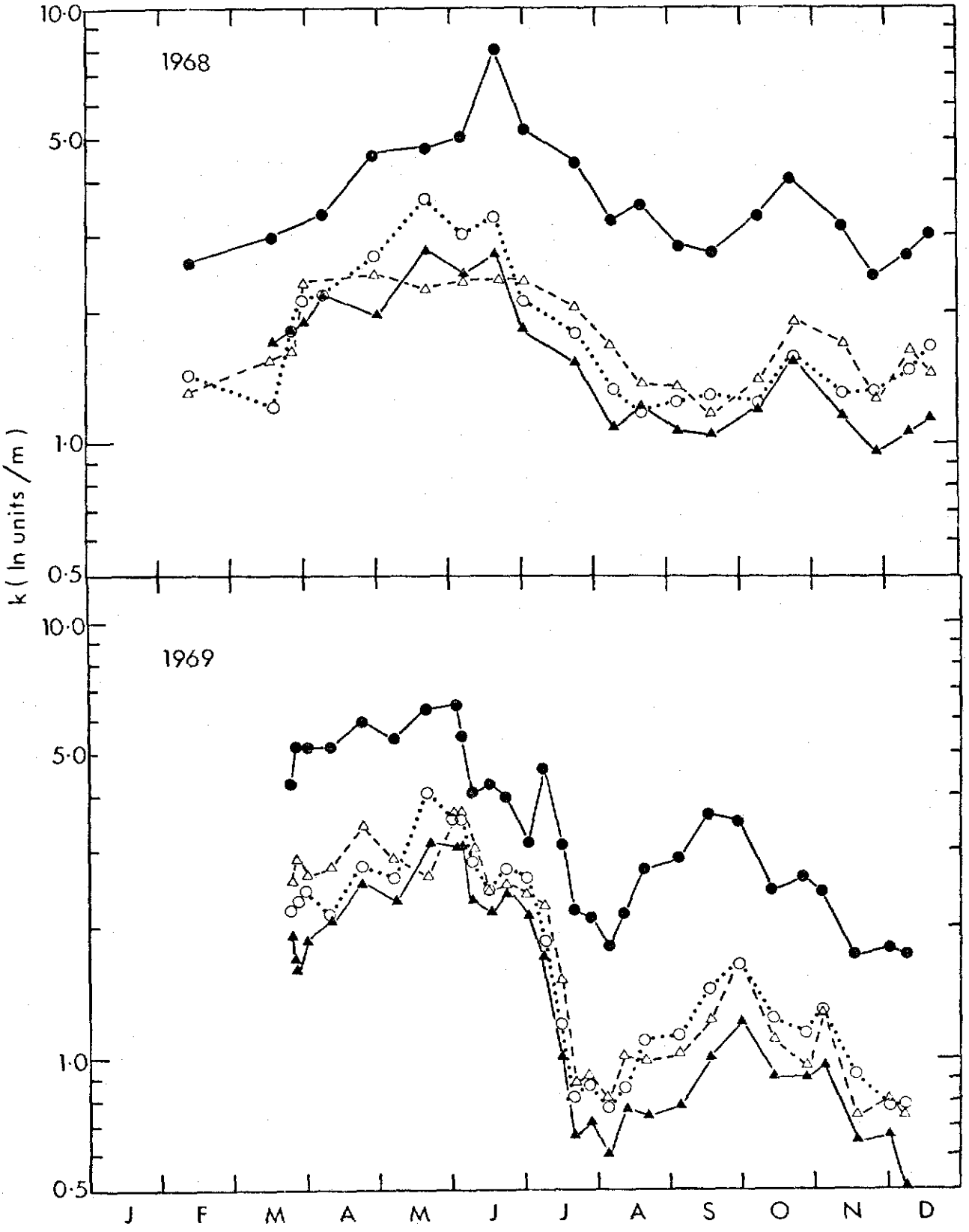


Fig. 12 (above and opposite) Seasonal variation of light extinction in four spectral regions during 1968-71. Extinction is expressed by the vertical extinction coefficient (k), shown on a logarithmic scale, and was measured in the red (O···O), orange (▲—▲), green (△--△) and blue (●—●) spectral regions using Schott colour filters RG 1, OG 2 + BG 18, VG 9 and BG 12 respectively.

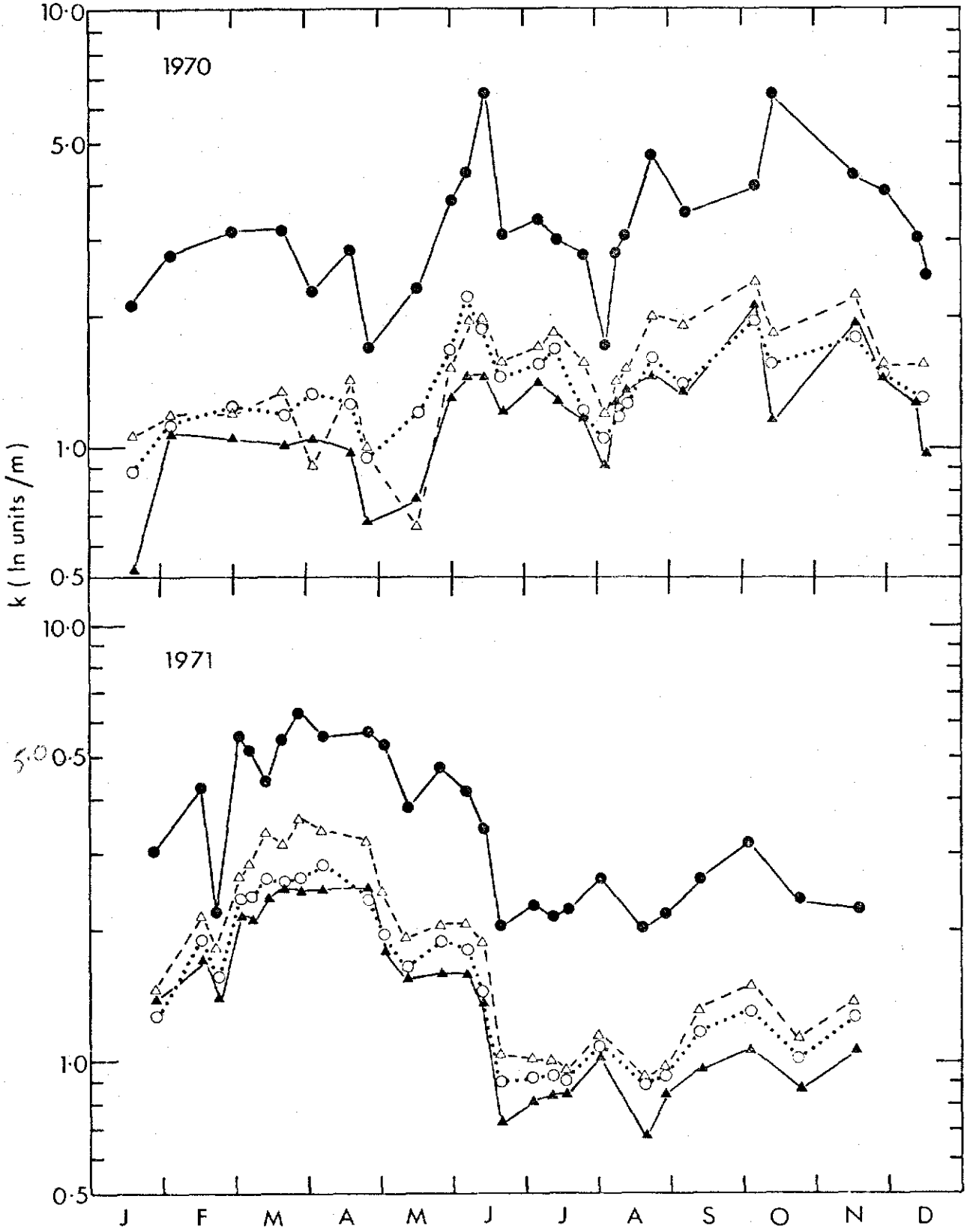


Fig. 12 (cont'd)

considered by Sakamoto & Hogetsu (1963) and Smith (1968), but such effects are still ill-defined (Talling, 1970). Here the spectral data have been used only for the calculation of underwater light intensities and not for a study of the physiological role of different wavelengths. The energy flux in the spectral region 400-700 nm has been taken as an operating basis for photosynthesis analysis.

The euphotic zone

The depth of the euphotic zone (z_{eu}), defined as the depth at which 1% of subsurface radiation (400-700 nm) is found, was estimated using the average relationship

$$z_{eu} = 3.7/k_{min}$$

found by Talling (1965a) to apply to a number of lakes in Africa and in England (Talling, 1971). Values of z_{eu} obtained in this way were in good agreement (Fig. 13) with those calculated from the depth-distribution of light intensity by the '3-block' method (see Methods section). A tendency for estimates of z_{eu} as $3.7/k_{min}$ to be higher than those based on the '3-block' method is apparent; although the discrepancy is normally small, the former method occasionally gave values for z_{eu} up to 34% higher than the latter method. The '3-block' method is itself an approximation, being based on measurements with filters of rather broad spectral transmission. Ideally, measurements of underwater light transmission over narrow spectral bands, achievable with a spectroradiometer, are required accurately to describe the spectral variation of underwater light, which is a prerequisite for accurate determination of absolute light intensities at different depths. The laboratory and field spectroradiometer data of Talling (1970) revealed details of optical "fine structure" not detectable with a photocell and broad band filters; in particular, in laboratory measurements on Loch Leven water, a sharp peak near the red absorption maximum for chlorophyll a was recorded. A less

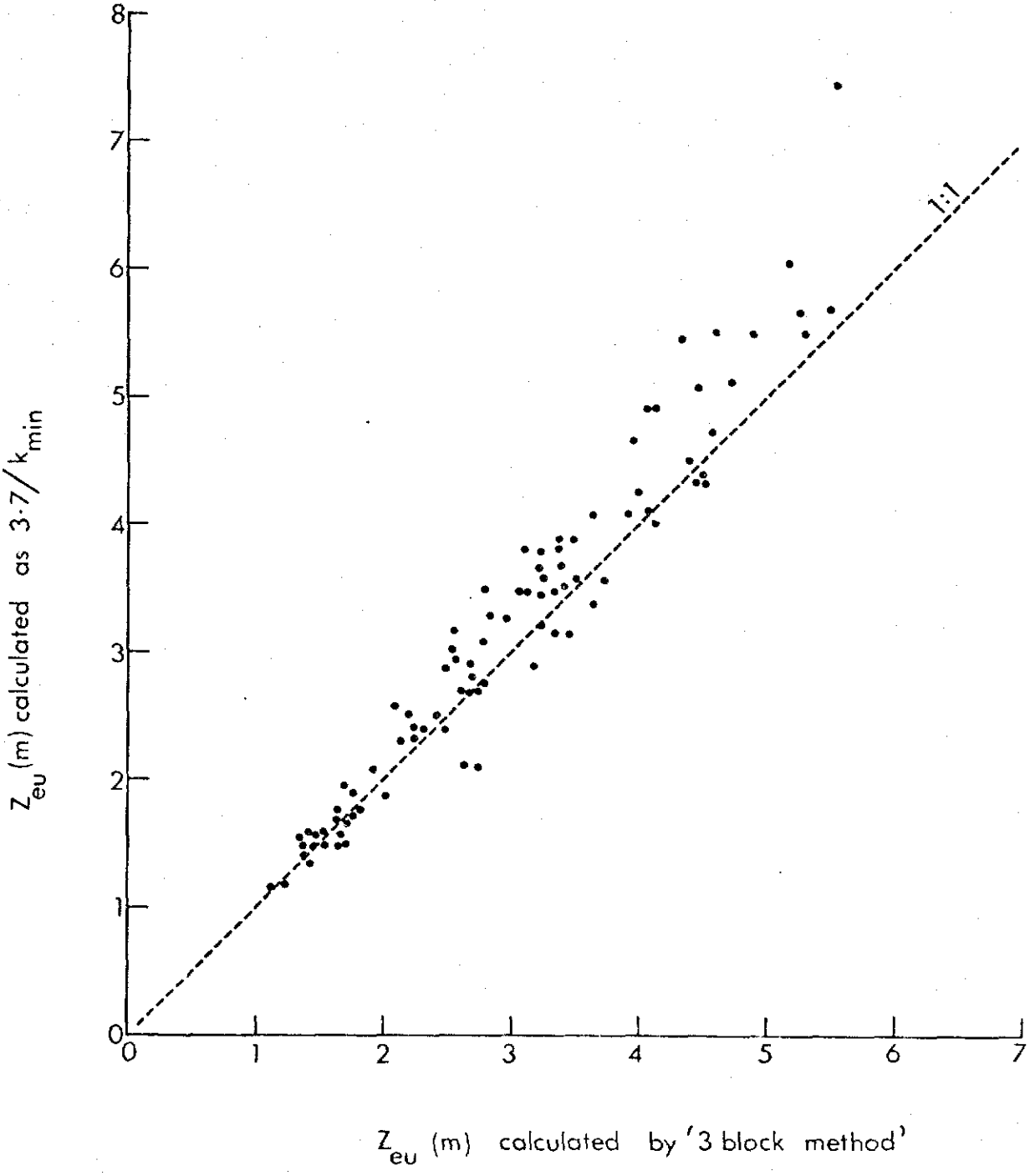


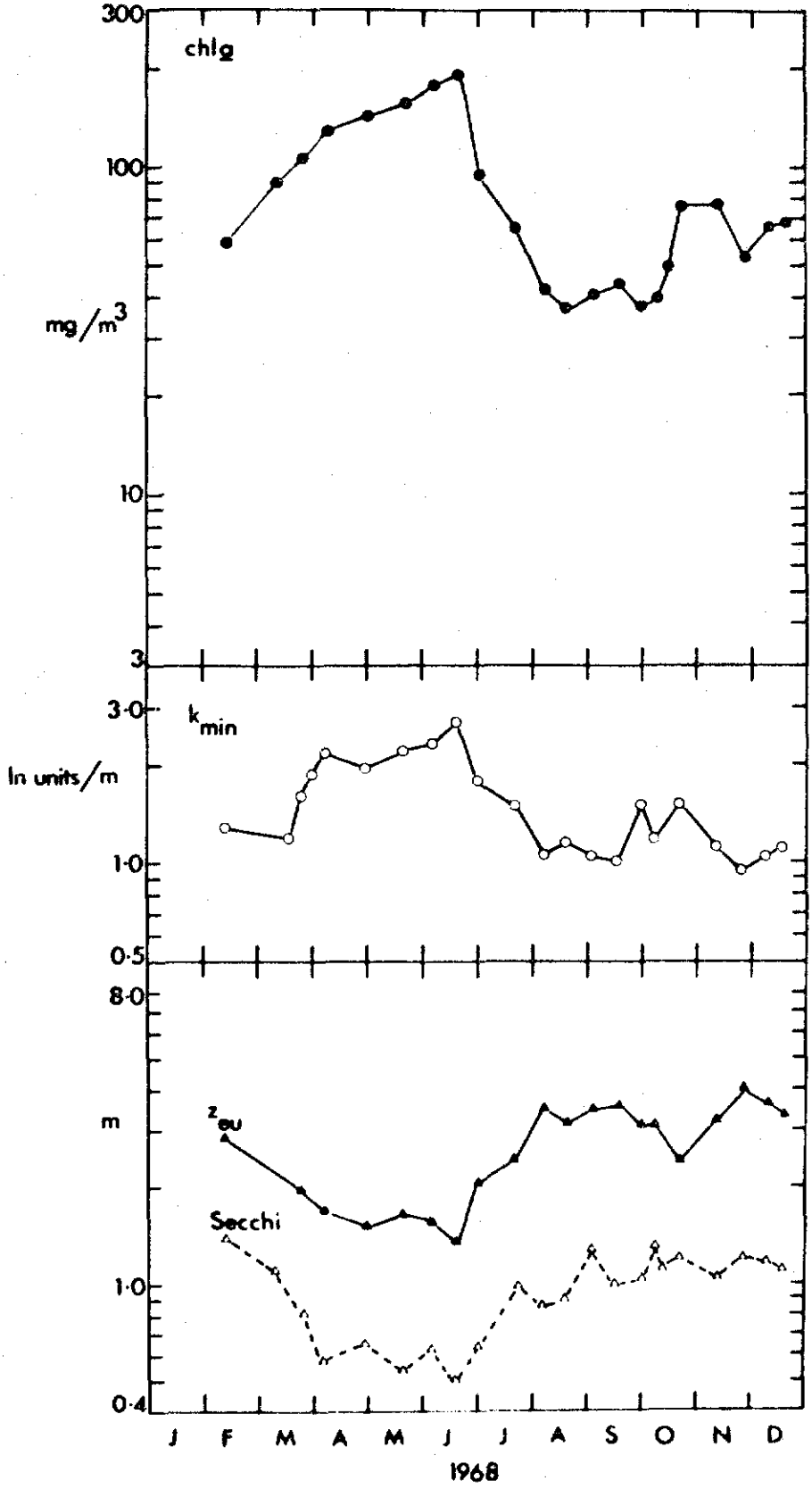
Fig. 13 The depth of the euphotic zone (z_{eu}) calculated as $3.7/k_{min}$ in relation to values obtained by the '3-block method'(Talling, 1957a). The inserted line corresponds to a 1 : 1 relationship.

marked peak in this region is also seen in the laboratory spectroradiometer data for Loch Leven water obtained in the present study and described later (p.49, Fig. 19). Spence, Campbell & Chrystal (1971) also noted an extinction peak at 675 nm in their in situ spectroradiometer measurements of underwater light intensity at Loch Leven.

In view of these complexities it is difficult to decide, where significant discrepancies between the two sources of z_{eu} exist, which (if either) approximates the true value. Values of z_{eu} calculated as $3.7/k_{min}$ have been used throughout this study.

Euphotic depth (z_{eu}) showed marked seasonal changes within the range 1.2 to 7.5 m, with a tendency for lower values to be associated with higher population densities (Fig. 14). The relatively poor light transmission at Loch Leven, largely due to its phytoplankton crops, may have been a reason for the scarcity of submerged rooted macrophytes there during most of the study period. Morgan (1970), in a review of changes in the fauna and flora of Loch Leven between 1891 and 1966, records that submerged and emergent macrophytes were formerly much more abundant and provides some circumstantial evidence that the decline of submerged forms may have been caused by competition for light by phytoplankton crops, which increased as a result of nutrient enrichment of the loch. Recently an increase in submerged macrophytes, first suspected in 1970 and becoming more noticeable in 1971, was confirmed in a survey in 1971 by Jupp, Spence & Britton (1974). During the summer of 1971 phytoplankton crops declined (possibly, in part, due to the reappearance in the zooplankton of the herbivorous filter-feeding crustacean, Daphnia) to the lowest level recorded since 1967 (Fig. 14). It is tempting to suggest that increased water clarity, associated with the reduction in the phytoplankton crop, may have caused the macrophyte increase. Certainly according to Secchi

Fig. 14 (following 4 pages) Seasonal changes during 1968-71 in (a) phytoplankton population density, as assessed by chlorophyll *a* (chl *a*) concentration, (b) the minimum vertical extinction coefficient (k_{min}), (c) euphotic depth (z_{eu}), and (d) Secchi disc transparency.



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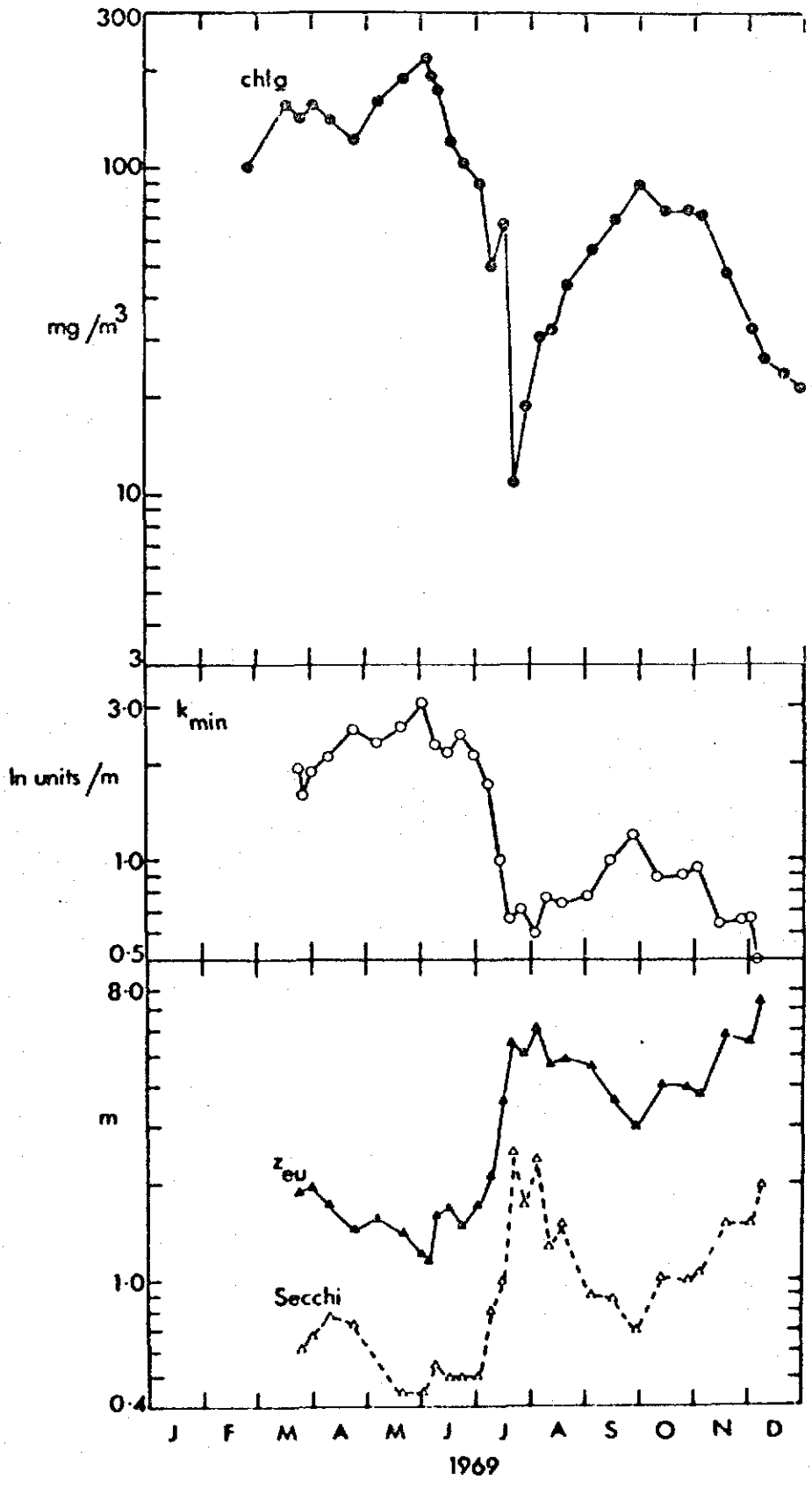
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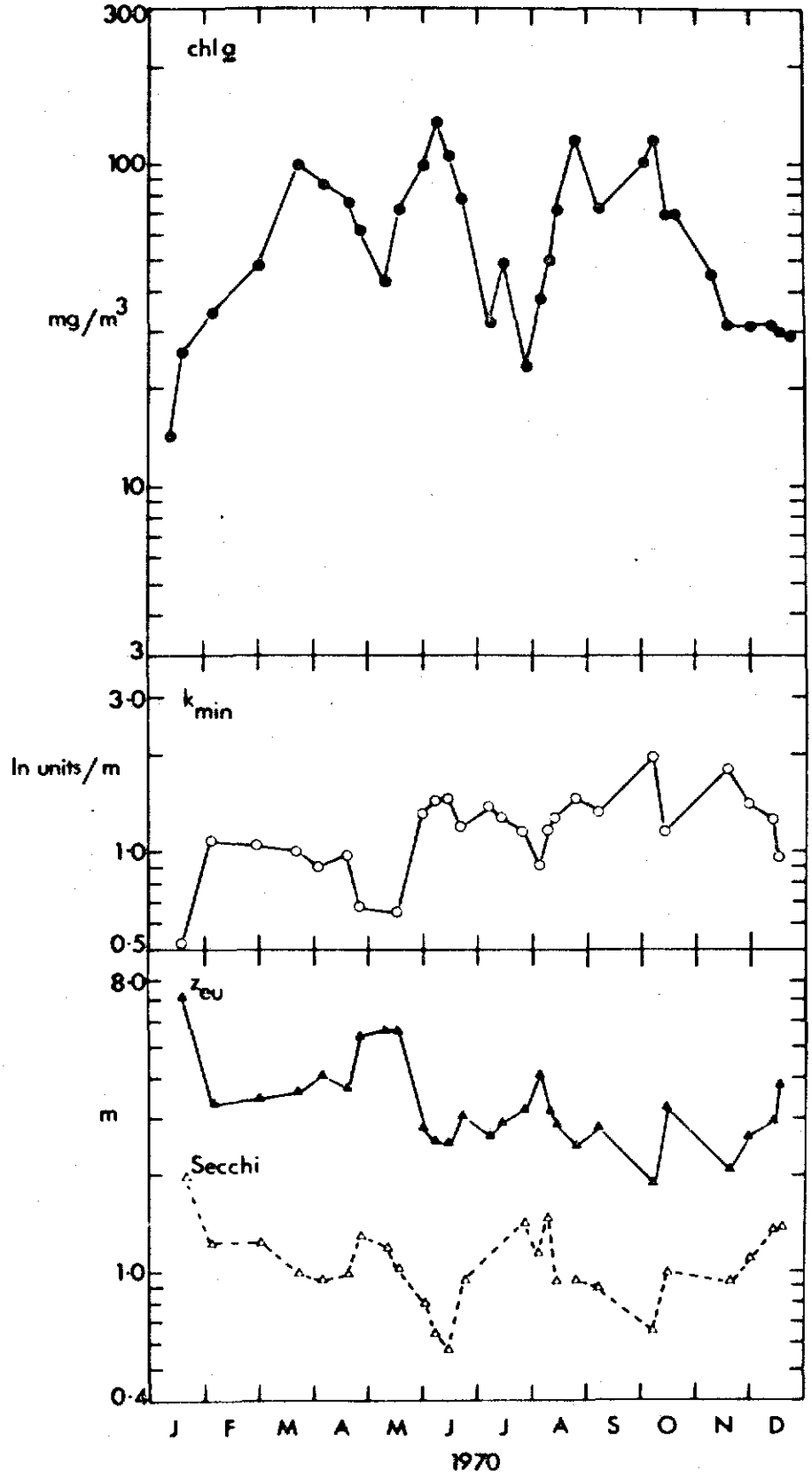
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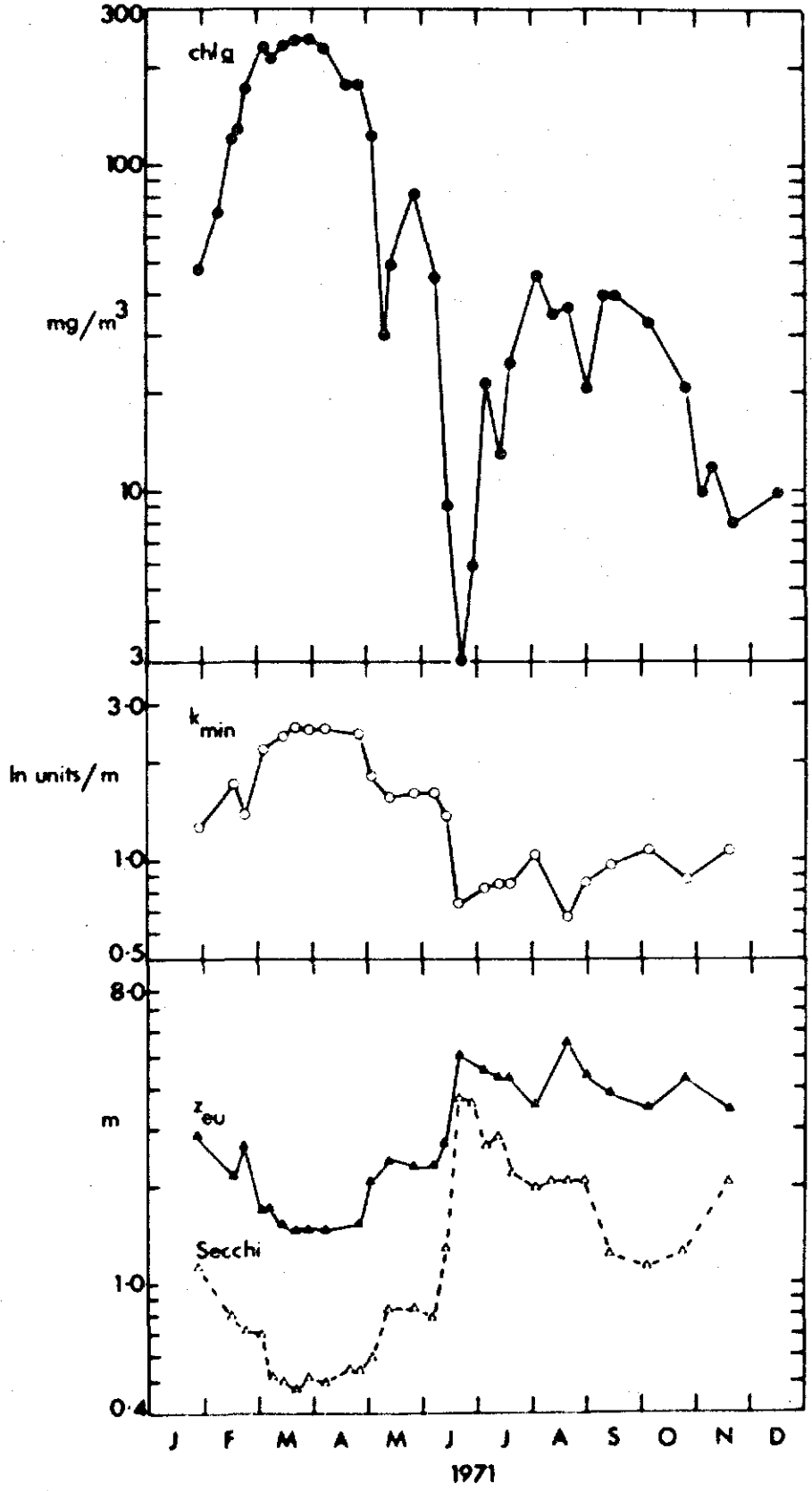
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disc readings the water was clearer in June and July 1971 than at any other time since 1967 (Fig. 14). However, calculations of the depth of the euphotic zone (as $3.7/k_{min}$) indicate that in earlier years (Fig. 14) the water had, at times, been as clear or clearer than at any time during 1971. This is also true if the euphotic depths calculated by the '3-block' method (not shown in Fig. 14) are used. However, the timing of periods of clear water in relation to daylength, water temperature and nutrient supply may be critical for macrophyte development; the month of June had clearer water in 1971, whether determined by Secchi disc transparency or euphotic depth, than any June period of earlier years.

Secchi disc transparency

Measurements of water transparency using a Secchi disc show similar seasonal trends to those of the euphotic zone, z_{eu} (Fig. 14). As indices of transparency the two types of measurement are most divergent when the water is relatively clear. In Fig. 14 the Secchi disc data indicate that the clearest water over the 4 year period occurred during June 1971 - the period during which the lowest phytoplankton crops were recorded. The z_{eu} data, however, show greatest water clarity in December 1969. Furthermore, the z_{eu} data indicate clearer water at other times in earlier years than in June 1971. Similar 'anomalies' between Secchi and z_{eu} data are often found in Windermere (Talling, pers. comm.).

In Fig. 15 Secchi disc readings are plotted against z_{eu}; scatter increases as transparency increases, but the calculated linear regression line indicates that an approximate estimate of z_{eu} can be obtained by multiplying the Secchi disc reading by 3. Different Secchi to euphotic depth conversion factors are to be found in the literature; Verduin (1956b) used a factor of 5; Riley (1941) a

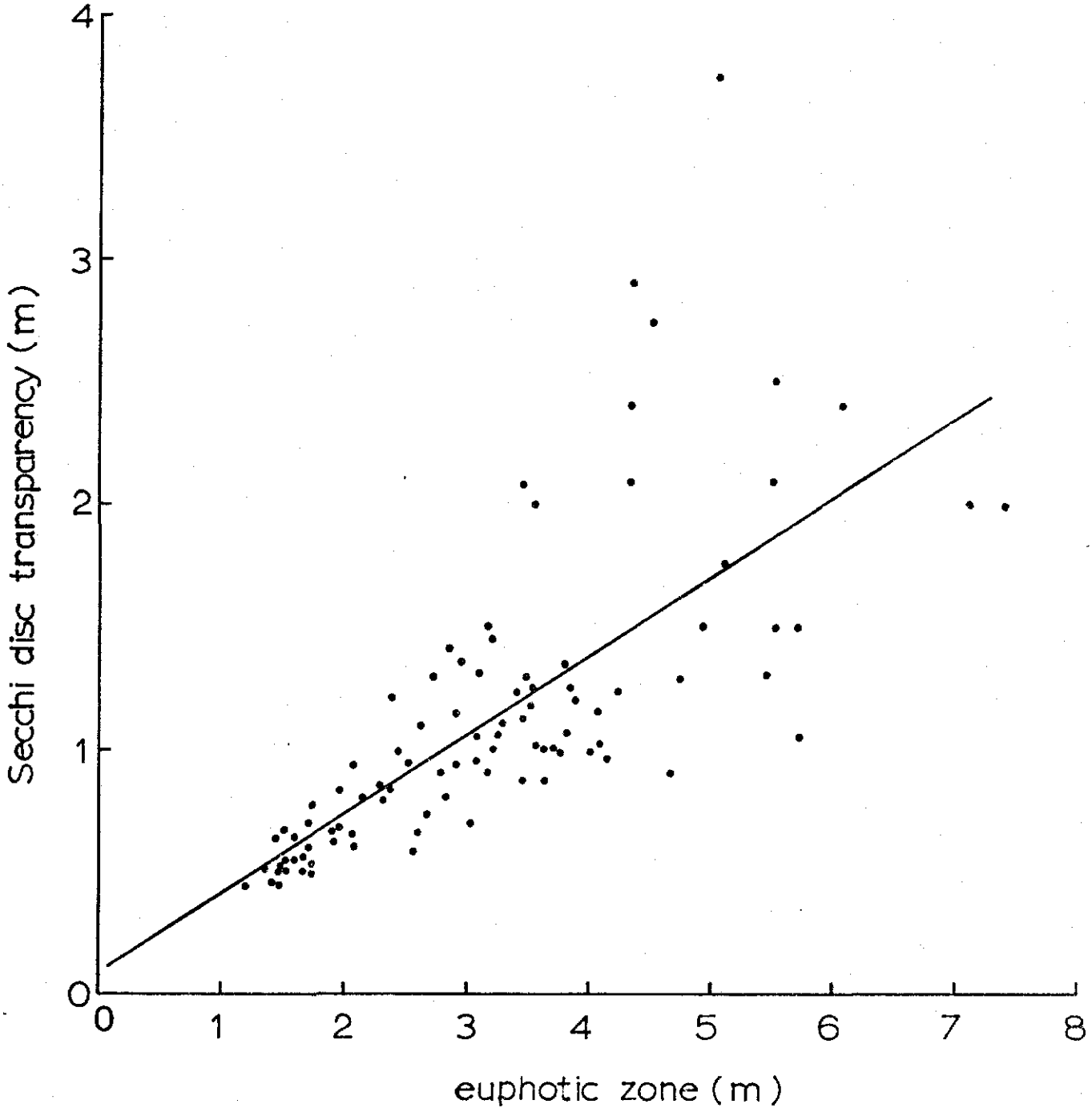


Fig. 15 The relationship between Secchi disc transparency and the depth of the euphotic zone (z_{eu}). Calculated regression line is y = 0.324x + 0.089, correlation coefficient (r) = 0.73, N = 95.

factor of 3; Rawson (1950) found that the factor decreased as transparency increased. Published conversion factors (e.g. that of Verduin, 1956b) are often based on estimates of z_{eu} gauged from underwater light data of unspecified wavelength (using a photocell without filter) and are therefore not strictly comparable to the Loch Leven factor, where z_{eu} is based on k_{min}. Talling (pers. comm.) computing z_{eu} in the same way as here, finds a conversion factor of between 2 and 3 to be applicable to a range of lakes in England and Africa.

The relation between Secchi disc transparency and k_{min}, indicated in Fig. 16, is approximately hyperbolic and closely resembles that described by Ichimura (1956). In agreement with Vollenweider (1960) and Ahlgren (1970), a linear relationship between the logarithm of the Secchi disc reading and the logarithm of k_{min} was apparent (Fig.17).

The theory and interpretation of Secchi disc measurements have been discussed by Strickland (1958) and Tyler (1968). Vollenweider (1969) has emphasised that the relationship between Secchi disc readings and values of z_{eu} or k_{min} is dependent on local conditions and on the observer and that it is, therefore, impossible to compare results from different localities and authors absolutely. According to Vollenweider (1969) this is due to the fact that Secchi disc readings probably do nct refer to the 'vertical extinction coefficient' but rather to the 'horizontal', or more likely to the turbidity integral above the disc.

Self-shading (the influence of phytoplankton density on underwater light penetration)

Changes in phytoplankton density (as mg chlorophyll a/m^3) followed similar seasonal trends to those of the minimum vertical extinction coefficient (k_{min}) and appear inversely related to changes

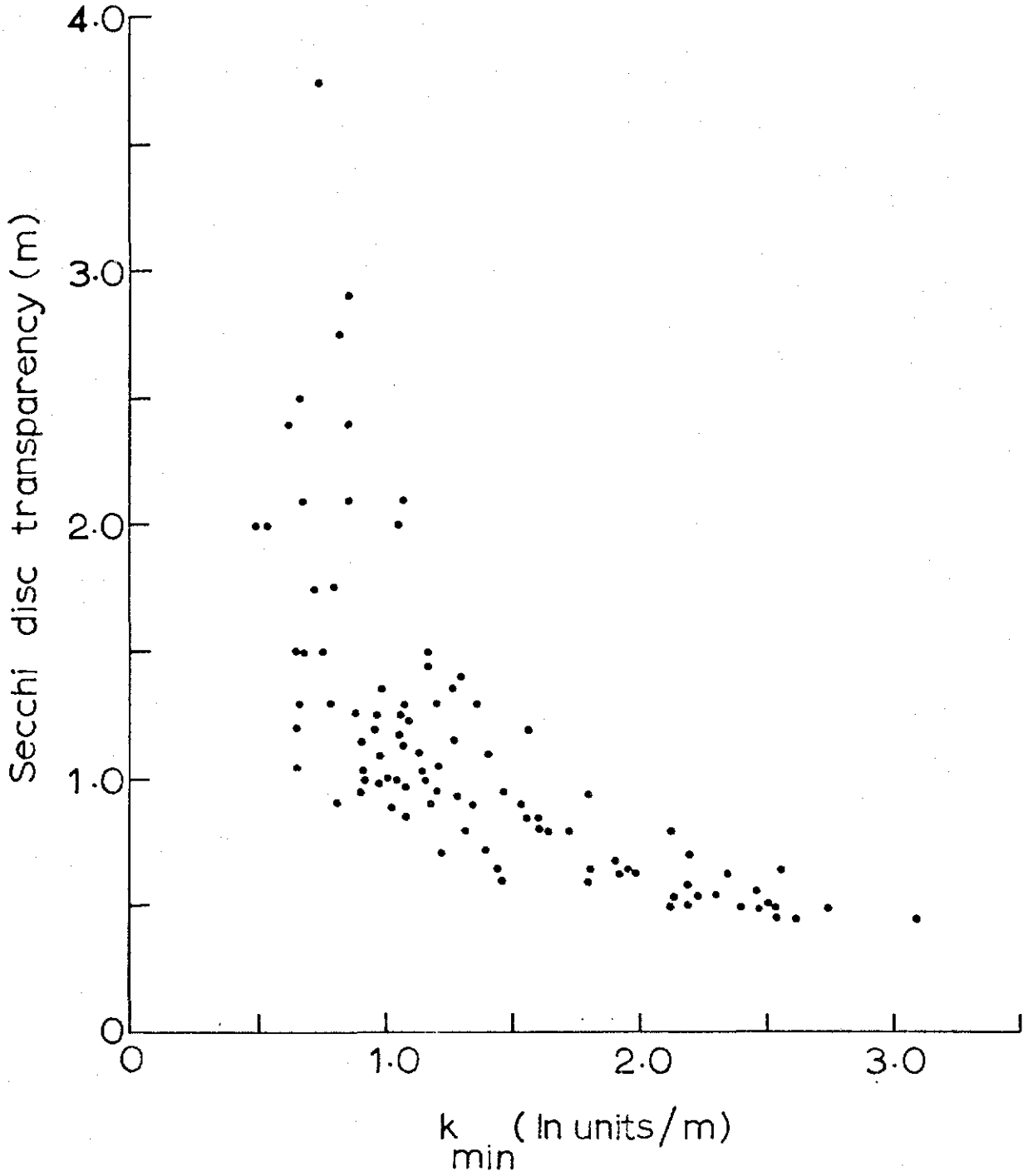


Fig. 16 The relationship between Secchi disc transparency and the minimum vertical extinction coefficient (k_{min}).

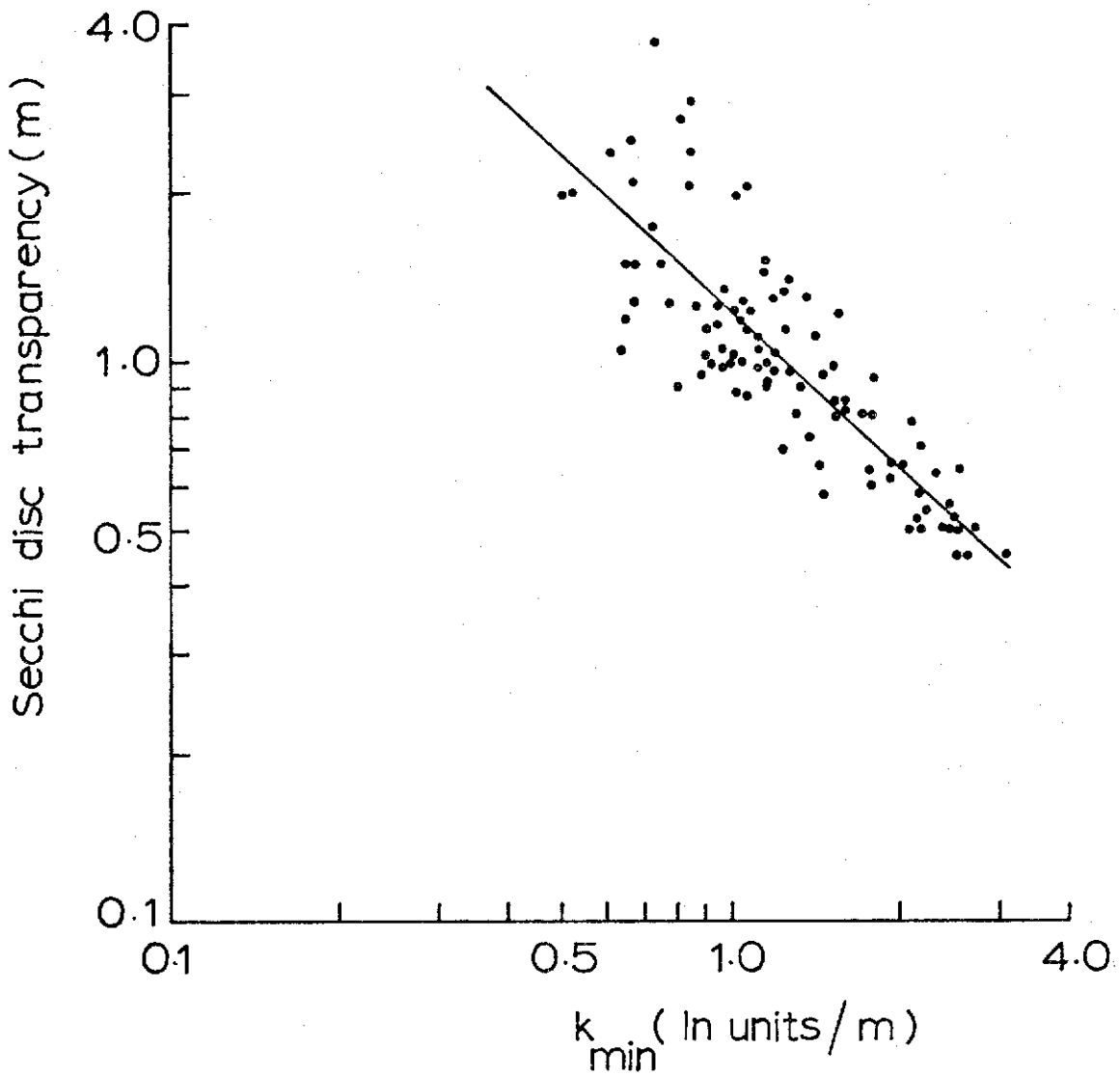


Fig. 17 The relationship between the logarithm of the Secchi disc reading and the logarithm of the minimum vertical extinction coefficient (k_{min}). Calculated regression line is log y = -0.914 log x + 0.091, correlation coefficient (r) = -0.84, N = 96.

in Secchi disc transparency and euphotic depth (Fig. 14). This suggests that the phytoplankton itself exerts a dominant influence on underwater light penetration. Inverse correlations between phytoplankton density and light penetration have often been described. A clear example is that of Talling (1960b) who discussed some earlier references and intrcduced the term 'self-shading' to describe the phenomenon. Other examples include those of Talling (1971) and Ganf (1972).

A strong positive correlation between population density and k_{min} is indicated in Fig. 18. The correlation coefficient is 0.84. From the calculated linear regression line in Fig. 18, the mean increment in k_{min} associated with unit increase in population density (i.e. the k_s value of Talling, 1960b) is 0.0086 $\pm$ 0.0011 ln units per mg chlorophyll a/m^2. This value is lower than some published values of k_s (of the order of 0.02) found by Talling (1960b) for Asterionella formosa in Windermere and by Ganf (1972) for the blue-green alga dominated phytoplankton of Lake George. Lower k_s values (of the order of 0.01) were found by Talling (1971) and Steel (1972) and attributed to the relatively large cell sizes of the dominant phytoplankton species concerned (Ceratium hirundinella and Stephanodiscus astraea, respectively).

Light extinction by phytoplankton is due to scattering and absorption by pigments. In larger cells relatively more of the cell pigment is likely to be ineffective in intercepting light than in smaller cells. Furthermore, the surface area available to intercept and scatter light will be less for larger cells than for an equal weight of smaller cells. Because of these "sieve effects" (Rabinowitch, 1951, p.715) large cells might be expected to have a smaller influence on light penetration (and therefore tc give a smaller k_s value) than

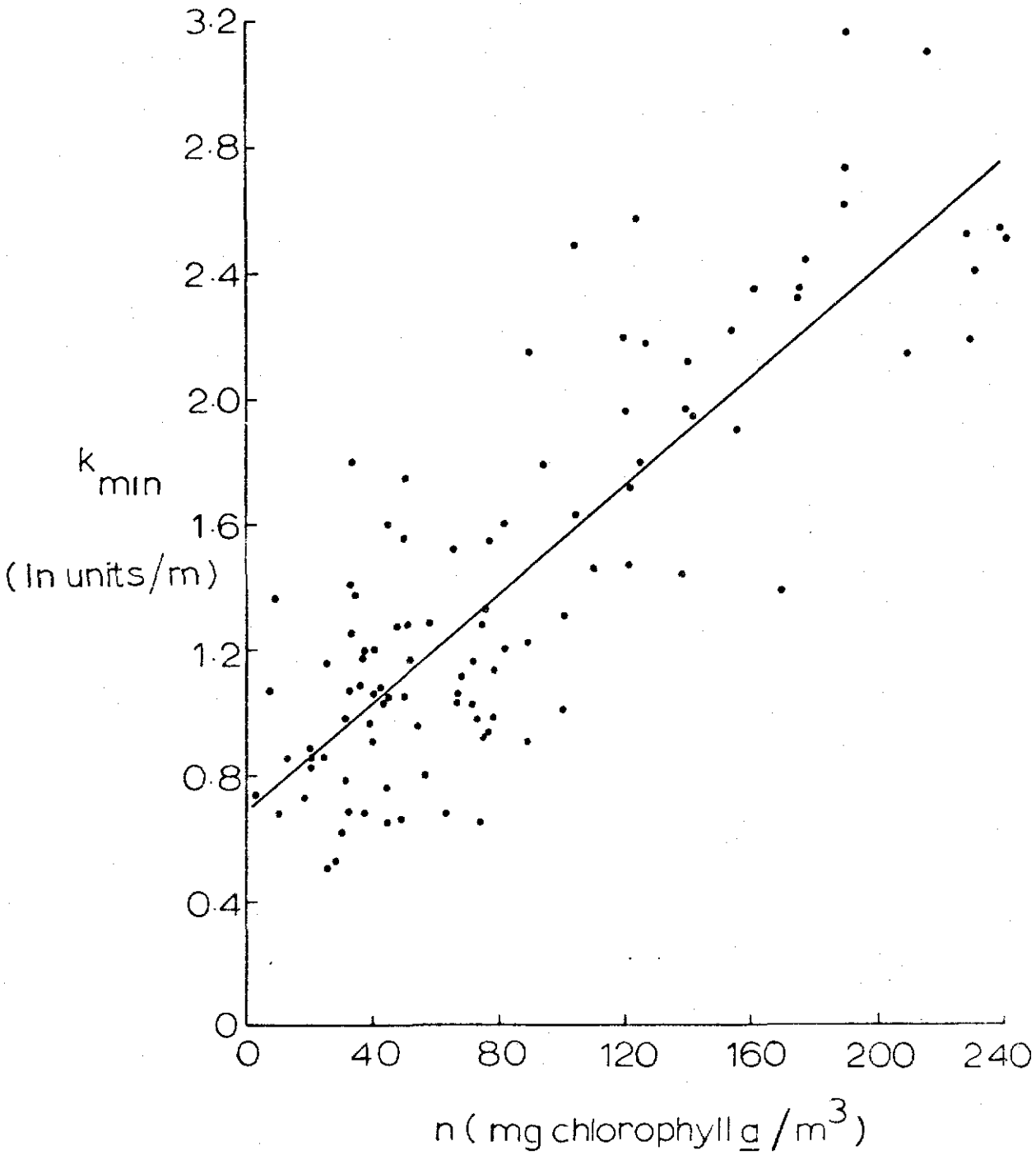


Fig. 18 The relationship between the minimum vertical extinction coefficient (k_{min}) and population density (n) expressed as the concentration of chlorophyll a in the water column. Calculated regression line is $y = 0.0086x + 0.69$, correlation coefficient (r) = 0.84, N = 101.

smaller cells. The studies of Jerlov & Kullenberg (1953), on the influence of particle size (within the range 1-12 μm diameter) on light scattering, support the view that larger particles would be expected to allow greater light penetration than an equivalent weight of smaller particles.

With the exception of the second half of 1971, the algae at Loch Leven were dominated by nannoplankton species, small enough to pass through a normal phytoplankton net of pore size ca 45 μm (Bailey-Watts, 1973, 1974). The low k_s value recorded for the loch was, therefore, unexpected in the light of the above published observations and theoretical considerations. Despite the small-celled nature of the Loch Leven phytoplankton it appears to be characterised by a k_s value lower than those reported for much larger algae (Talling, 1960b; Ganf, 1972). Aggregation of cells into larger units, which might be expected to reduce k_s, did not occur at Loch Leven and therefore cannot explain this anomaly. Furthermore, the larger algae (e.g. Asterionella formosa, Pediastrum boryanum), which were retained by the phytoplankton net, and dominated the late 1971 crops, were not associated with lower k_{min} values per unit of chlorophyll a than the smaller algae (e.g. Synechococcus sp., Cyclotella pseudostelligera, Oscillatoria redekei) dominant in earlier years.

The k_s value for Loch Leven was calculated from seasonal changes in k_{min} and chlorophyll a over a 4 year period; as such it is an average value, incorporating contributions to the chlorophyll a estimates of many different (albeit small-sized) species, as well as contributions to k_{min} of possibly variable amounts of non-algal light extinction. The magnitude of these possible sources of variation in the relationship between chlorophyll a concentration and k_{min} is

indicated by the scatter of points in Fig. 18. Differences in pigmentation of similar sized species of different algal groups and differences in pigment content of individual species, induced by their light or nutrient history, could presumably affect the relationship between population density, expressed as chlorophyll a, and light extinction. Some evidence for this is provided by Steemann Nielsen (1962a).

Little seasonal variation in the background colour of the water itself, imparted by dissolved substances, was revealed in routine spectrophotometer measurements of filtered lake water in the 400-700 nm spectral range.

In a shallow, wind-exposed water body, such as Loch Leven, one may expect to find wind-disturbed sediment material in the water column in amounts dependent on wind strength and sediment type. Variation in the amount of non-algal particulate material present in the water column probably accounts for much of the scatter in Fig. 18. Underwater light measurements could not be made on very stormy days, when the influence of sediment material would be expected to be greatest. However, measurements made under calm conditions may well have been influenced by sediment material introduced into the water column during previous stormy conditions.

The average background level of non-algal light extinction, indicated in Fig. 18 by the intercept of the regression line on the y axis, is low relative to light extinction by phytoplankton at high crop densities. On average, a crop density equivalent to 240 mg chlorophyll a/m^3 accounts for an increment in k_{min} of 0.07 ln units/m. This is equivalent to 75% of total light extinction in the water column. Pure water, with an extinction coefficient of 0.15 at 590 nm (Hutchinson, 1957, p.382), accounts for 5% of total light extinction

whilst non-algal particulate material and dissolved substances together account for the remaining 20%. The percentage of total light extinction attributable to algae declines with decline in crop density and for any given crop density may be estimated from Fig. 18.

From Fig. 18 the average background level of non-algal light extinction at Loch Leven is equivalent to a k_{min} of 0.69 ln units/m; this is lower than the corresponding value of 2.1 ln units/m given by Ganf (1972) for Lake George. Laboratory spectroradiometer studies of light extinction in these two lake waters was carried out by Talling (1970). These showed that, at the wavelengths appropriate to k_{min}, i.e. 590 nm for Loch Leven and 650 nm for Lake George, very little difference existed between the extinction coefficients of the two waters when all particulate material had been removed by filtration. Though Talling's results refer to single sampling dates only, they suggest that the higher level of non-algal light extinction in Lake George is due to a larger amount of particulate non-algal material suspended in the water column, rather than to a higher content of dissolved coloured material. Lake George might be expected to have a higher non-algal particulate content than Loch Leven because: (a) it is shallower (mean depth 2.25 m) and therefore its sediments are more accessible to wind disturbance; and (b) its sediments are very flocculent muds (Ganf, 1972) and are therefore more easily stirred up into the water column than are the sandy sediments, or the less flocculent muds, of Loch Leven.

In co-operation with Dr. J. F. Talling, further laboratory measurements with a spectroradiometer (ISCO, Nebraska, U.S.A., Type SR) were carried out on a sample of Loch Leven water, collected 11.3.69, when Diatoma elongatum, Cyclotella pseudostelligera and Oscillatoria redekei dominated the phytoplankton. The general

experimental procedure was the same as that used by Talling (1970) in his earlier spectroradiometer study of Loch Leven water. Different phytoplankton densities were prepared from the parent suspension by dilution with filtrate. Results, expressed as increments of extinction coefficient (Δk) relative to distilled water, are plotted against wavelength in Fig. 19. These detailed laboratory measurements of spectral variation in light transmission confirm the observation, based on field measurement using broad spectral band colour filters, that k_{min} is located in the orange spectral region, at about 590 nm.

From the spectroradiometer data, k_s may be calculated, without interference from changing species composition and levels of non-algal light extinction, from the values of Δk at 590 nm found for different chlorophyll a concentrations, prepared from the same parent suspension. These are plotted in the inset to Fig. 19. The best straight line was fitted by eye and k_s calculated to be 0.0066 ln units per mg chlorophyll a/m^2. In order to approximate to field data, where the average light path length is about 20% longer (Jerlov, 1951; Steemann Nielsen, 1962a), the 'laboratory' k_s value was multiplied by 1.20, giving a value of 0.0079 ln units per mg chlorophyll a/m^2. This value is very close to the value (0.0086) determined from field measurements and provides additional evidence that the k_s value of Loch Leven phytoplankton is less than 0.02.

Since population density (n) is a major factor controlling light penetration, interpretation of seasonal changes in integral photosynthesis per unit area (ΣnP), in relation to k_{min} and z_{eu}, is incorporated in the following section, where the influence of phytoplankton biomass on ΣnP is discussed.

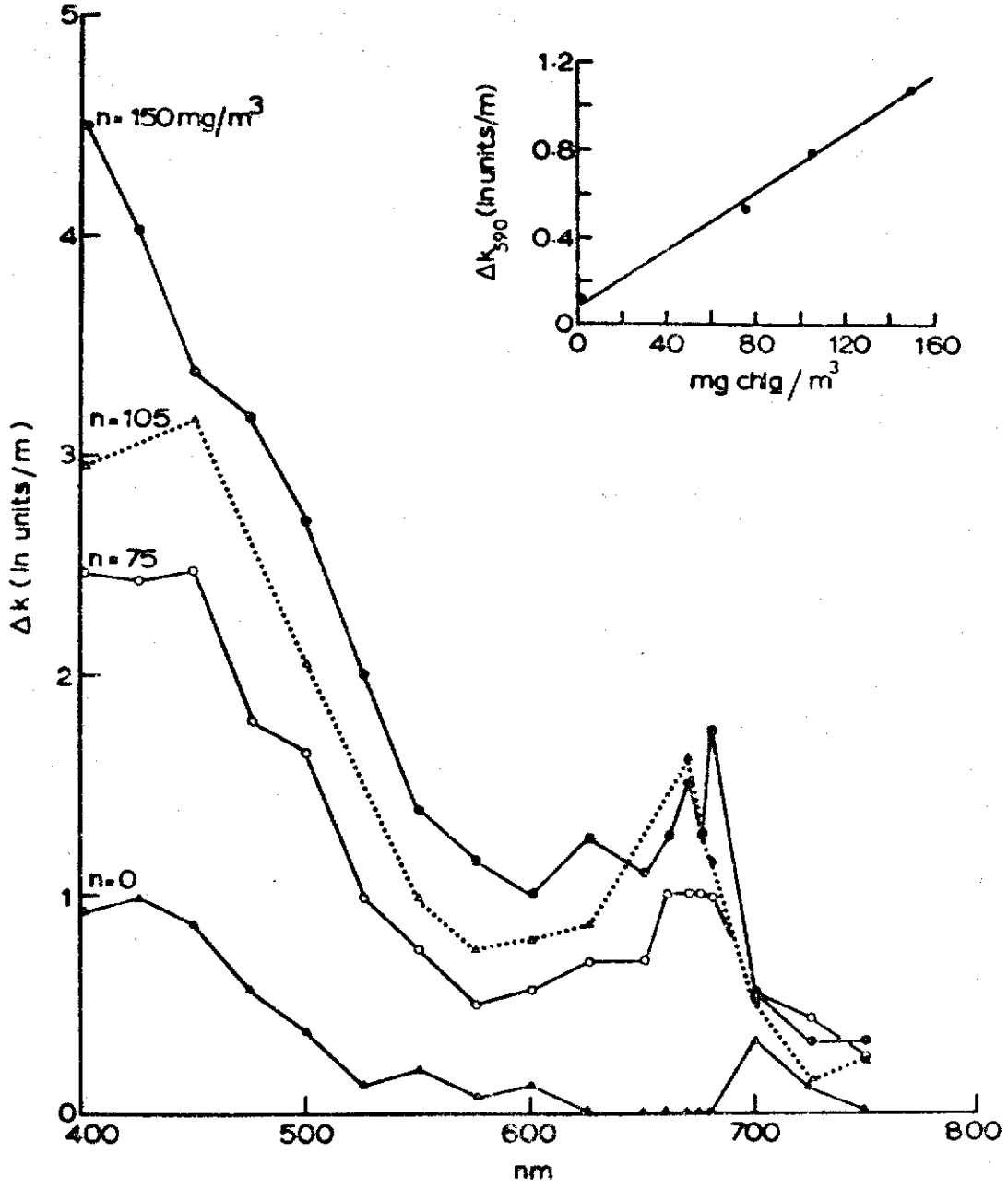


Fig. 19 Laboratory spectroradiometer measurements of light extinction in Loch Leven water, collected 11.3.69 (dominant algal species: Diatoma elongatum, Cyclotella pseudostelligera, Oscillatoria redekei). Phytoplankton density (n), in mg chlorophyll a/m^3, was varied by using filtrate and a mixture of lake water with filtrate. Extinction is expressed by vertical extinction coefficients, in ln units/m, which are increments (Δk) above the values for distilled water. The inset shows increments in the vertical extinction coefficient at 590 nm (Δk_{590}) in relation to chlorophyll a concentration. The best straight line was fitted by eye.

2. Population density

The chlorophyll a content of the water was used as the index of phytoplankton population density. Chlorophyll a is restricted to the algal part of the suspended organic material. It therefore provides a more reliable index of phytoplankton biomass than do measurements of dry weight, particulate carbon, phosphorus etc. which also include any non-algal material present in the water. The chief disadvantage of chlorophyll a as an indicator of live phytoplankton biomass is the difficulty of distinguishing between the pigment in intact cells and its breakdown products in dead or dying cells. Methods are available (Lorenzen, 1967; Moss, 1967a,b) which, though not entirely satisfactory (see below), do give some indication of changes in the relative contribution of breakdown products (phaeo-pigments) to the chlorophyll a estimates. Work with algal cultures has shown that the chlorophyll a content per cell volume varies between species and is influenced by various environmental factors, including the light intensity and nutrient conditions under which the cells are grown (Bogorad, 1962; Fogg, 1965). On the whole, however, variation in pigment content in nature is probably less than can be produced in culture (Moss, 1970) and a chlorophyll a measurement probably gives at least as good an estimate of total phytoplankton biomass as other more time-consuming methods (e.g. cell counts and total cell volume estimation).

The advantages of chlorophyll a over other methods of biomass estimation have resulted in its extensive use in limnology as a quick and fairly simple method of estimating total phytoplankton abundance.

a) Seasonal variation of total phytoplankton density, estimated as chlorophyll a

Seasonal changes in population density (n), estimated as chlorophyll a extracted in 90% methanol, are shown in Fig. 20. Very

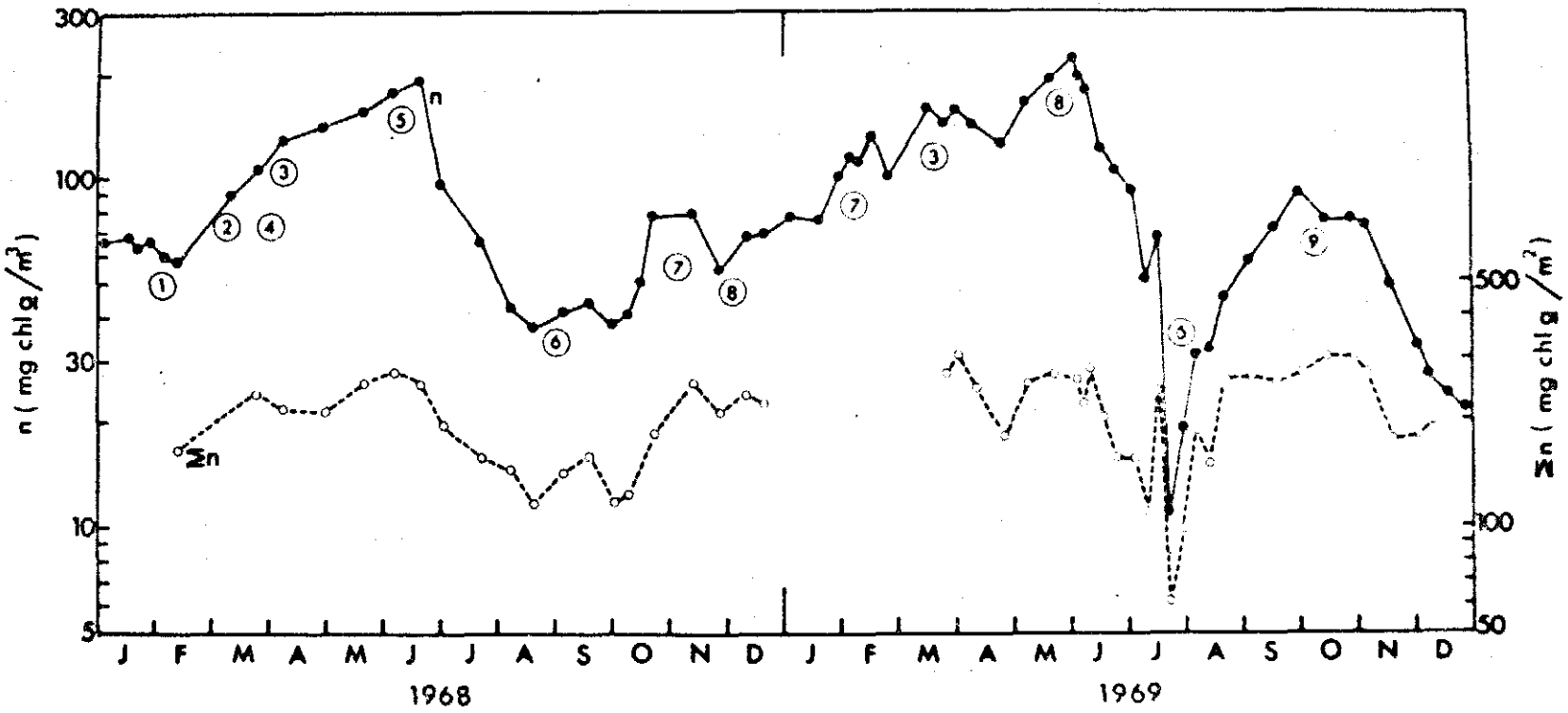
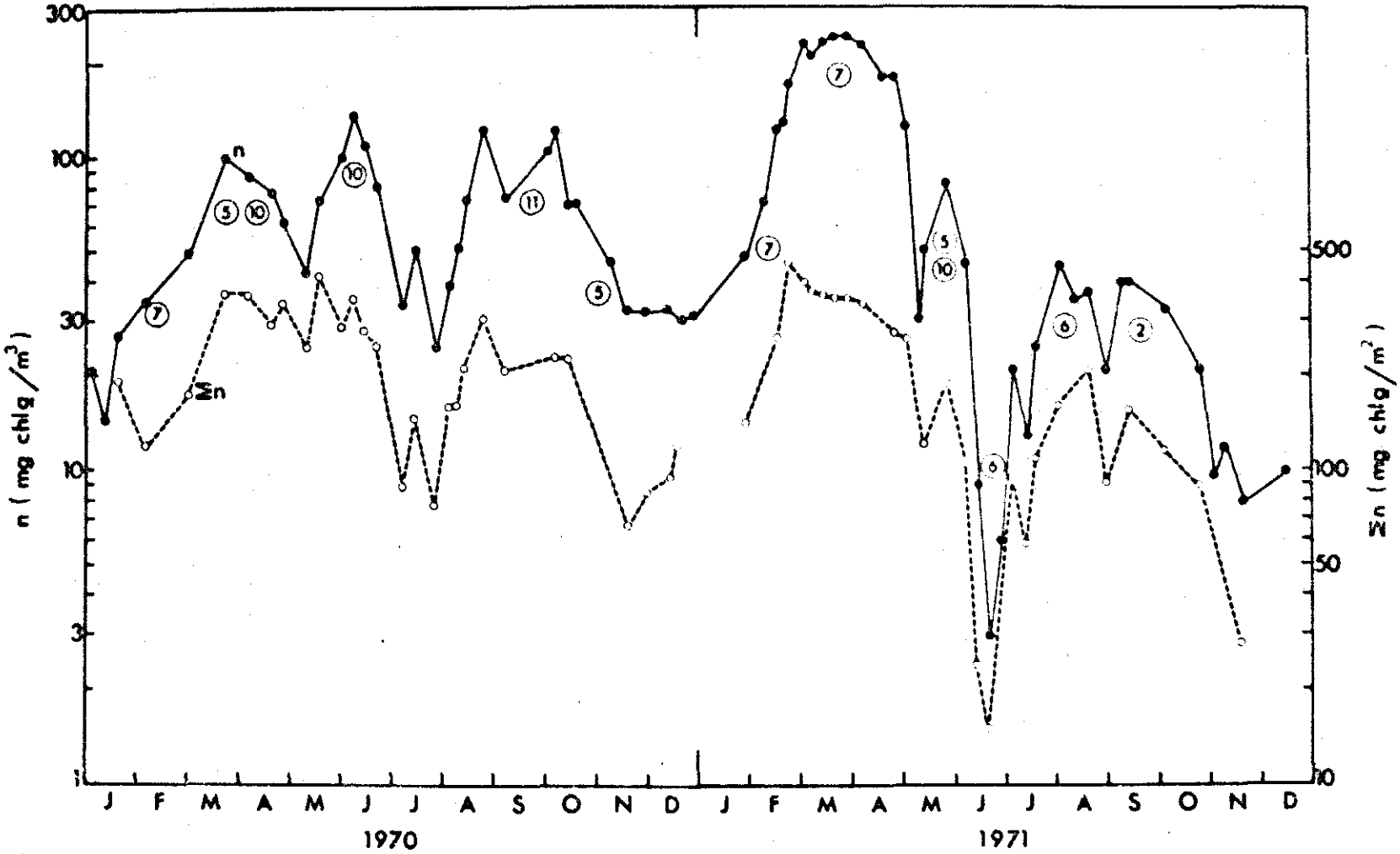


Fig. 20 (above and below) Seasonal changes in population density (n), estimated as chlorophyll a and the chlorophyll a content per unit area of the euphotic zone (Σ n). Values of n were obtained from 0.5 m depth samples but are representative of the whole water column (cf. Fig.22). Encircled numbers indicate dominant algal species identified by Bailey-Watts (1973, 1974): (1) Synedra ulna and Synedra rumpens; (2) Asterionella formosa; (3) Diatoma elongatum; (4) Stephanodiscus hantzschii; (5) Synechococcus n.sp.; (6) mixed Chlorophyceae (including species of Ankistrodesmus, Scenedesmus, Tetrastrum, Chodatella, Pediastrum, Raphidonema, Dictyosphaerium, Micractinium); (7) Cyclotella pseudostelligera; (8) Oscillatoria redekei; (9) Dictyosphaerium pulchellum; (10) Steiniella sp.(?); (11) Stephanodiscus rotula.


300
100
30
10
3
1
n (mg chlg /m3)
Σn
n
Σn
(7)
(5) (10)
(10)
(11)
(5)
(7)
(7)
(5)
(10)
(6)
(6)
(2)
500
100
50
10
Σn (mg chlg /m2)
J F M A M J J A S O N D J F M A M J J A S O N D
1970
1971

similar chlorophyll a values were obtained using 90% acetone (Fig. 21). Discrepancies between the use of the two solvents were most marked when green algae were dominant, when methanol was the superior extractant. Dominant algal species are indicated in Fig. 20. These are taken from a fuller description of qualitative changes in the phytoplankton given by Bailey-Watts (1973, 1974).

The chlorophyll a values in Fig. 20 are uncorrected for phaeopigment interference (discussed in (b) below) and refer to the 0.5 m water sample used in productivity measurements. Integrated water samples over approximately 3.9 m (the mean depth of the loch) obtained with a weighted plastic tube (Lund, 1949) always gave very similar chlorophyll a values to those obtained for the 0.5 m sample (Fig. 22). Samples from discrete depths also gave very similar chlorophyll a values (Bailey-Watts, 1973). There is thus good evidence that the phytoplankton population is generally uniformly distributed with depth throughout the year. Seasonal changes in the population density of the 0.5 m water sample may therefore be assumed to be representative of changes throughout the whole water column.

Rodhe (1958) found in Lake Erken that horizontal redistribution of a spatially heterogeneous population by wind-induced water movements often exerted a dominant influence on temporal fluctuations of phytoplankton quantity recorded at a single station. At Loch Leven, however, exhaustive surveys by Bailey-Watts (1973) invariably failed to reveal any evidence of significant horizontal variability of phytoplankton distribution. Fig. 23 shows the typical relationship invariably obtained in comparing phytoplankton crop densities from different stations. Changes at a single open water station are therefore assumed to be representative of the loch as a whole and to be due to variation in the balance between rates of phytoplankton production

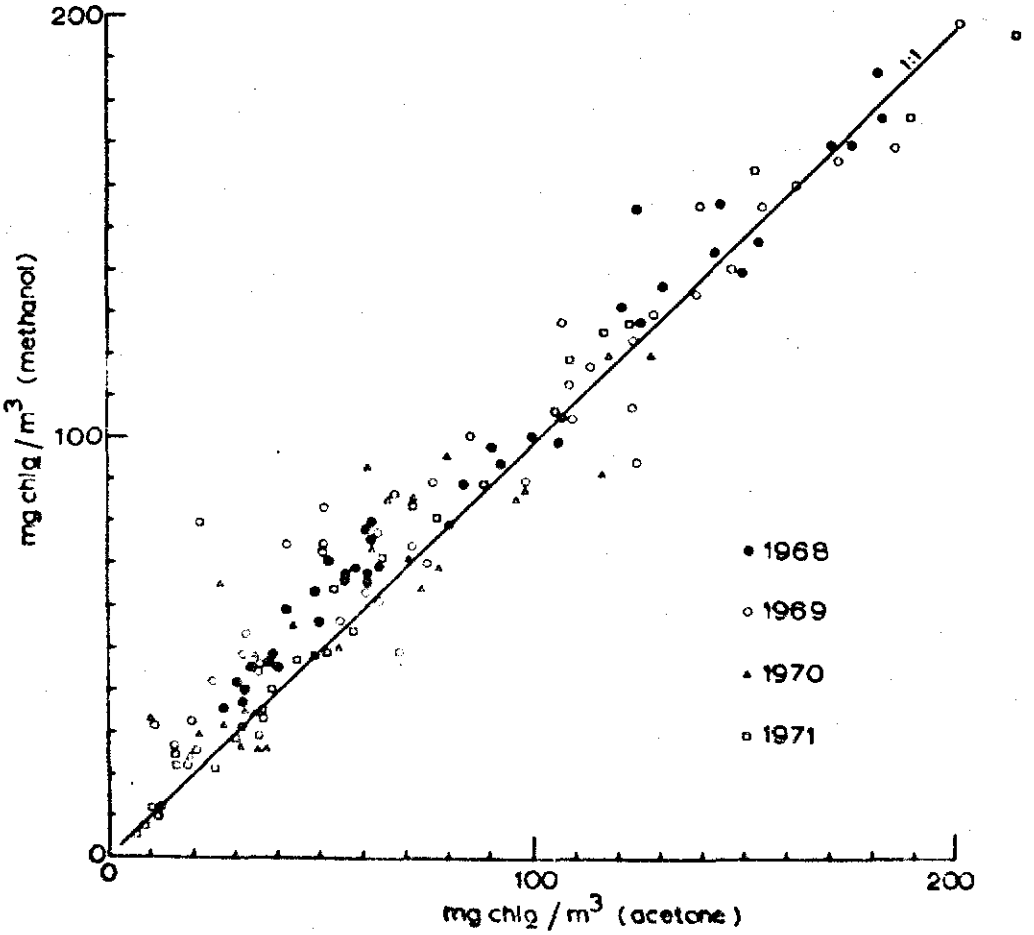


Fig. 21 Comparison of chlorophyll a concentrations obtained with 90% methanol and 90% acetone solvents.

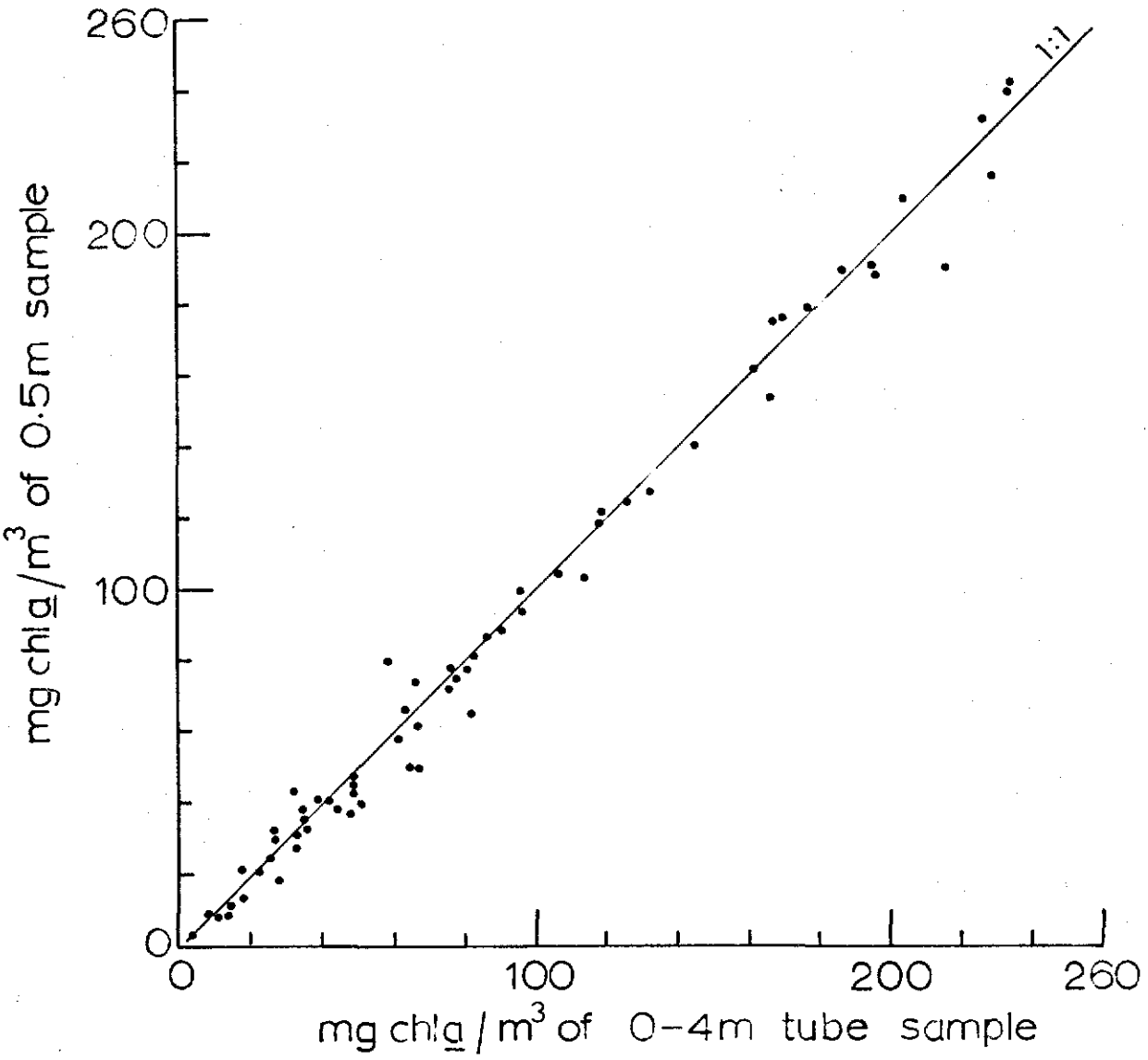


Fig. 22 Comparison of chlorophyll a concentrations in 0.5 m depth samples with 0-4 m (tube-integrated) samples.

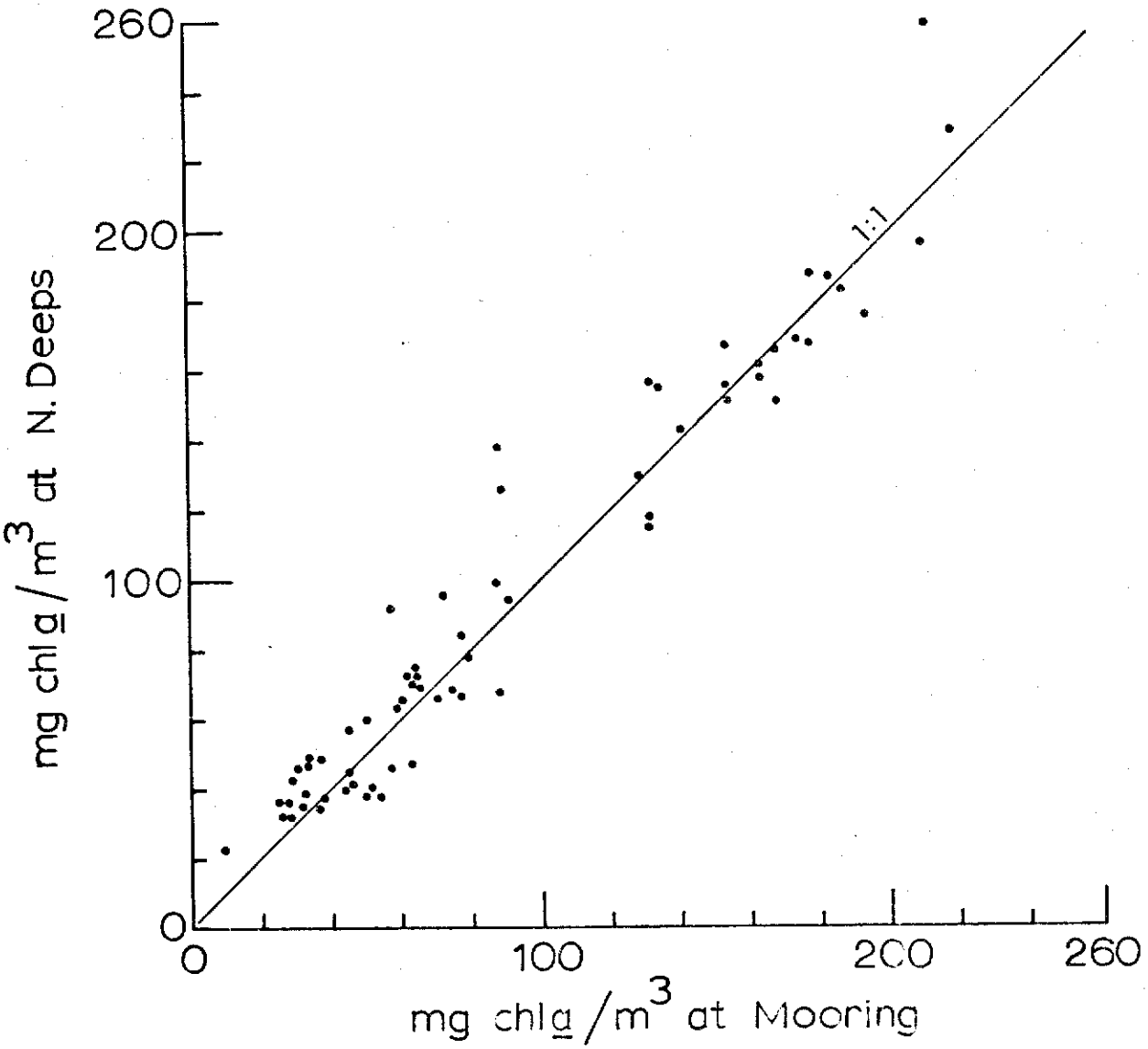


Fig. 23 Comparison of chlorophyll a concentrations at the Mooring (station 1) and North Deeps (station 3).

and depletion (as by sinking, grazing and outflow losses), rather than to horizontal transport of water masses.

Fig. 20 shows that population densities were generally high over most of the year and even mid-winter crops were considerable. In the early months of the year population density increased reaching a maximum in spring or early summer. This was followed by a sharp decline to a mid-summer minimum when crops were generally lower than in mid-winter. A second maximum, usually lower than the first, occurred in autumn. During the final two to three months of the year population densities usually declined. Although in gross outline a seasonal pattern was repeated year by year, there was considerable variation in successive years in the timing and magnitude of the maximum and minimum crop densities and in their species composition. The dominant species occurring over similar periods of successive years often belonged to different taxonomic classes (Bailey-Watts, 1973, 1974).

As indices of phytoplankton biomass, chlorophyll a values have been used as a basis for classifying the trophic nature of waters. According to Vollenweider (1968), for practical purposes the maximum plankton density that develops during the year is the most useful basis for such comparisons being, according to Ruttner (1963), more or less directly related to the nutritive status of the water. In terms of chlorophyll a per unit volume various workers have arbitrarily differentiated between various levels of trophy. Sakamoto (1966) provides the following classification from observations on Japanese waters:

Eutrophic	5-140 mg/m^3
Mesotrophic	1-15 mg/m^3
Oligotrophic	0.3-2.5 mg/m^3

Talling (1961) distinguishes between values of less than 1 mg/m^3 in unproductive regions, 1-30 mg/m^3 in moderately productive waters and greater than 30 mg/m^3 in very productive regions.

In both these classification schemes, therefore, Loch Leven (chlorophyll *a* range 3-240 mg/m^3) would be considered eutrophic and productive.

b) Degradation products of chlorophyll *a*

The pathways which decomposition of chlorophylls may follow are summarised by Yentsch (1965) as follows:

$$\text{chlorophyll} \xrightarrow{-\text{ phytol}} \text{chlorophyllide} \xrightarrow{-\text{ Mg}} \text{phaeophorbide}$$

and/or

$$\text{chlorophyll} \xrightarrow{-\text{ Mg}} \text{phaeophytin}$$

Treatment with dilute mineral acid causes loss of magnesium. Phytol is removed by the enzyme chlorophyllase, and probably also by strong acid.

Chlorophyll degradation products probably do not occur in living intact algal cells (Yentsch, 1965) but they are present in decaying material (Patterson & Parsons, 1963) and in zooplankton faeces (Currie, 1962). Since these degradation products have visible absorption spectra similar to those of chlorophylls, their presence can lead to overestimation of chlorophyll concentration in algae and to underestimation of photosynthetic rates per unit of chlorophyll.

In a shallow, wind-exposed water body such as Loch Leven turbulence may be expected to reduce the settling rate of decaying algae and to enhance the introduction of sediment material into the water column. Attempts were therefore made to estimate the amount of degraded pigment included in the chlorophyll *a* estimates. The

methods of Lorenzen (1967) and Moss (1967a,b) were used. The underlying principles and procedures involved were described earlier (see Methods section). Strictly, both methods measure the total concentration of magnesium-free chlorophyll *a* derivatives (i.e. phaeophytin *a* plus phaeophorbide *a*), but results are expressed as phaeophytin *a*.

Results

Seasonal changes in the Lorenzen acidification ratio (665_o:665_a) and the Moss (430:410) ratio (determined on acetone extracts) are shown in Fig. 24 in relation to seasonal changes in chlorophyll *a* concentration (determined on methanol extracts).

A decrease in either of these ratios indicates an increase in the relative contribution of phaeophytin *a* to the chlorophyll *a* estimate. This could arise if the absolute concentration of phaeophytin *a* increased or if the algal population density (i.e. 'true' chlorophyll *a*) decreased. An apparent decrease in algal density may be recorded if pigment extraction from the cells is incomplete. A tendency for acetone extraction to be less efficient than methanol, especially when green algae were dominant, was noted earlier. Since estimates of phaeophytin interference are based on acetone extracts, this could lead to overestimation of the proportion of phaeophytin in methanol extracts.

The Moss and Lorenzen ratios show similar seasonal trends in rise and fall, with lower ratios tending to coincide with periods of lower crop density.

Lorenzen (1967) found a maximum acidification ratio of 1.7 for a purified extract of chlorophyll *a*. This agrees with the ratio expected from published specific absorption coefficients of chlorophyll *a* and phaeophytin *a* at 665 nm in acetone (e.g. Vernon, 1960). Fig. 24 shows that on a number of occasions acidification ratios higher than

Fig. 24 (following 4 pages) Seasonal changes in (a) the concentration of chlorophyll *a*, determined from 90% methanol extracts, (b) the Lorenzen (1967) acidification ratio, 665_o:665_a, and the Moss (1967a,b) 430:410 ratio, determined from 90% acetone extracts.

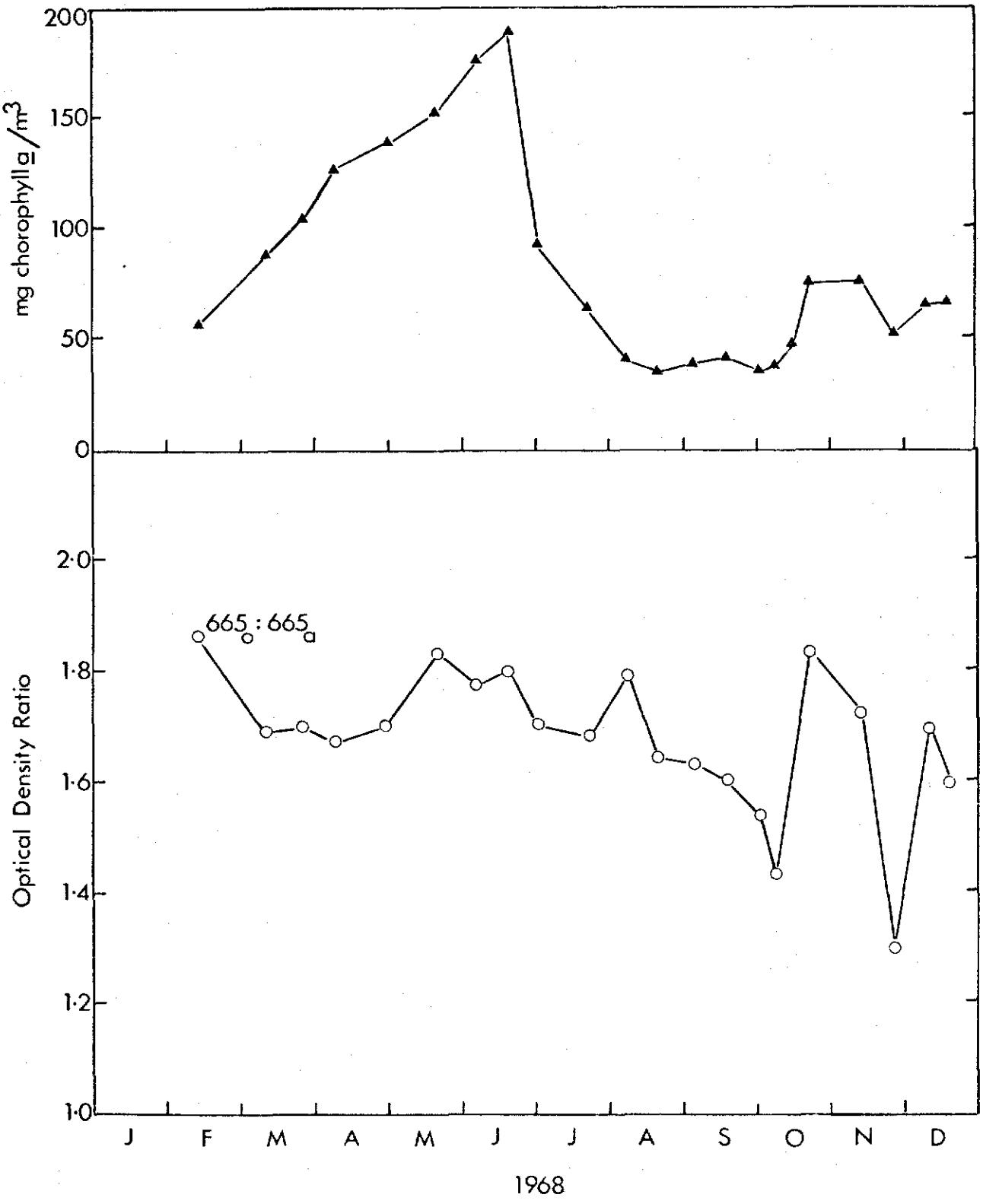
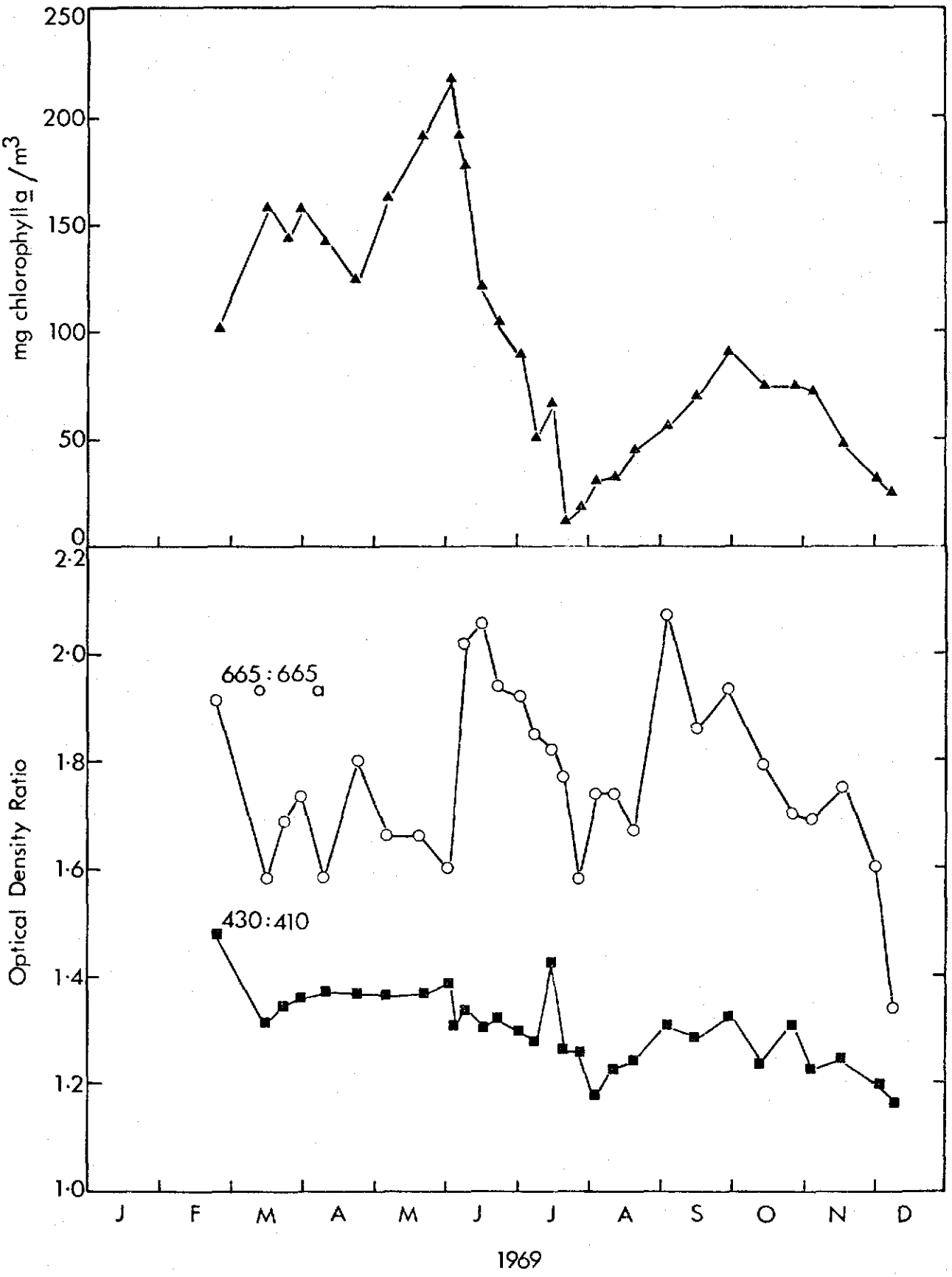


Fig. 24 Legend opposite.


250
200
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100
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mg chlorophyll a /m3
2·2
2·0
1·8
1·6
1·4
1·2
1·0
Optical Density Ratio
665 : 665a
430 : 410
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A
M
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J
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S
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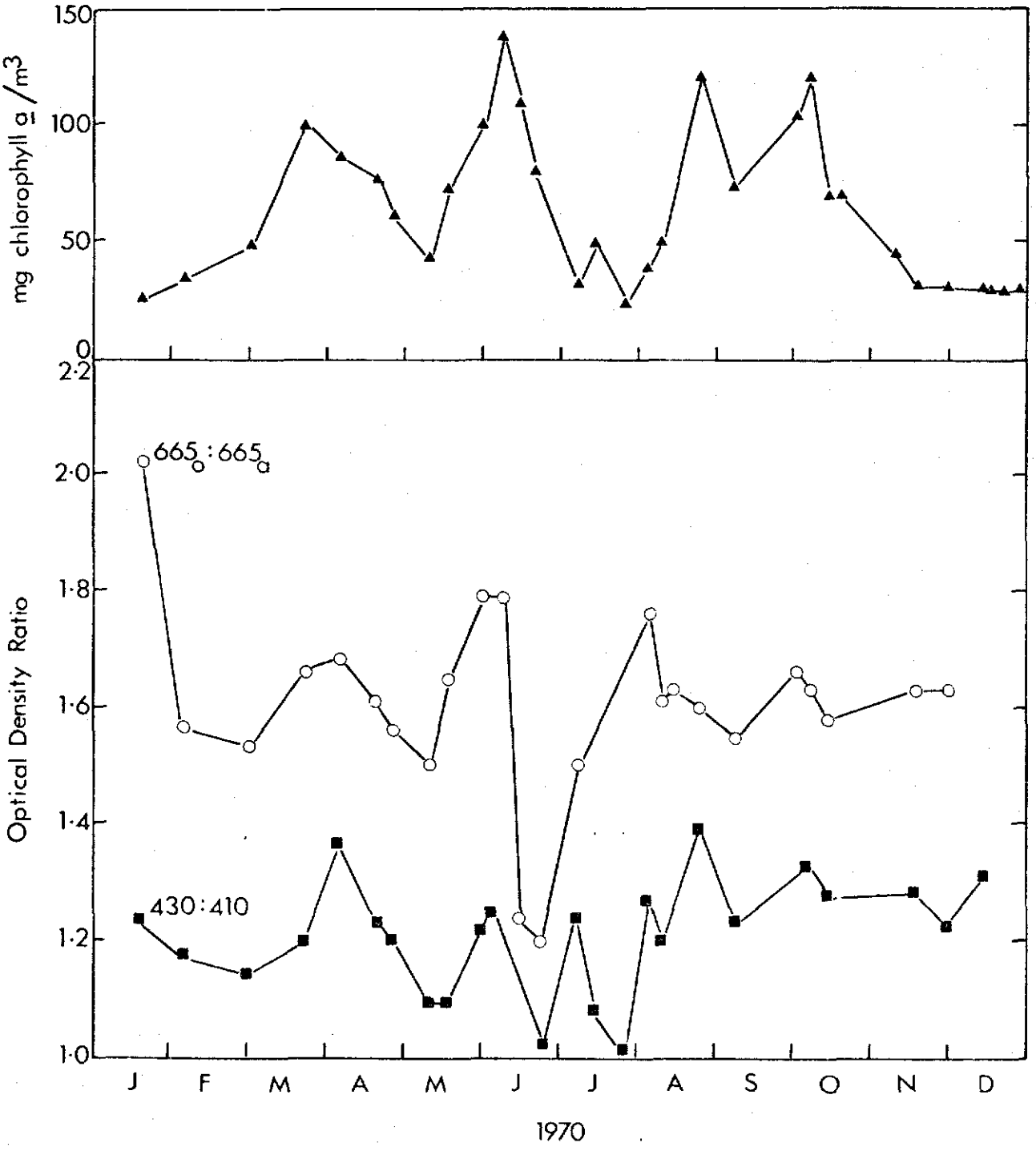
1969

Fig. 24 (cont'd)


150
100
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mg chlorophyll a /m3
2·2
2·0
1·8
1·6
1·4
1·2
1·0
Optical Density Ratio
665o : 665a
430 : 410
J
F
M
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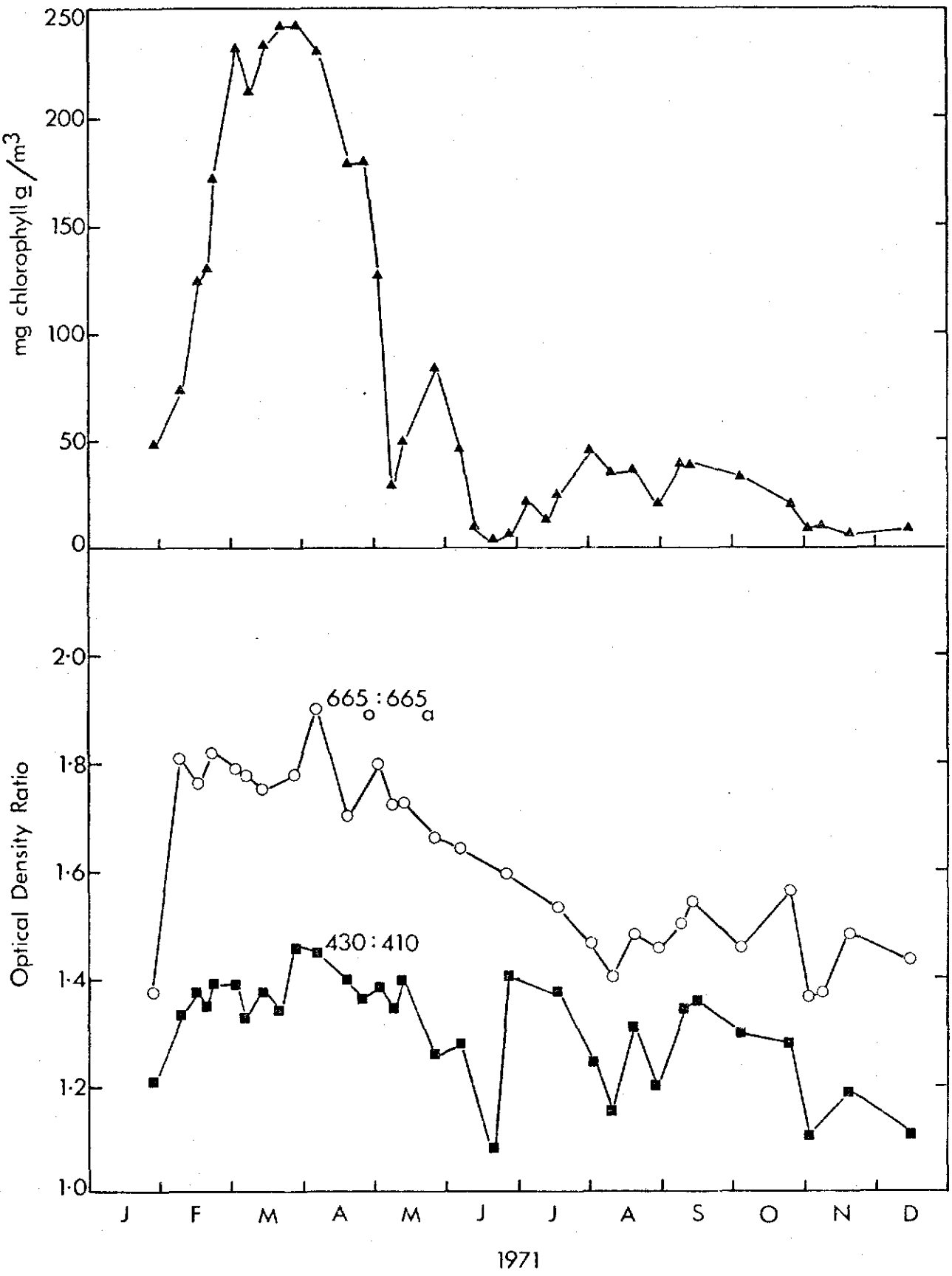
Fig. 24 (cont'd)


250
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mg chlorophyll a /m3
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1·8
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1·4
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1·0
Optical Density Ratio
665o : 665a
430 : 410
J
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1971

Fig. 24(cont'd)

1.7 (up to 2.1) were recorded for Loch Leven. The reason for this apparent anomaly is uncertain. Interference from other pigments and their degradation products might provide an explanation but was not investigated. It was noticed, however, that the value of the acidification ratio was dependent on the length of time elapsing between acidification and re-reading of the optical density of the extract. Routinely, the optical densities at 665 and 750 nm of the acidified extract were taken within 1-2 minutes of acidification. However, it was noticed that optical density (O.D.) at both 665 nm and 750 nm tended to drift upwards for about 20 minutes after acidification. The increase at 665 nm was greater than that at 750 nm. Consequently, the corrected 665 nm reading (i.e. $O.D._{665}-O.D._{750}$) also increased with time, leading to a progressive reduction in the acidification ratio. This time factor was also noted by Bailey-Watts (1973) and Marker (1972). Bailey-Watts (1973) found that if the extract was neutralised after acidification its corrected O.D. at 665 nm remained stable at approximately the value reached in the unneutralised extract after 20 minutes. The mechanism of the effect of pH on the O.D. and stability of pigment extracts is not clear. It is notable that Lorenzen (1967) made no mention of the effect and did not include a neutralisation step in his procedure. An influence of pH on the absorption spectrum of phaeophytin a in methanol was recorded by Livingston et al (1953).

An approximate idea of the percentage of 'true' chlorophyll a in the extracts was obtained, using the formula given by Lorenzen (1967), on the assumption that acidification ratios of 1.7 or above indicate 100% chlorophyll a. The relationship between % chlorophyll a and the acidification ratio is shown graphically by Golterman (1969, Fig. 7.11). Most acidification ratios at Loch Leven were above 1.6,

which is equivalent to ca 90% 'true' chlorophyll a. The lowest acidification ratio observed, 1.2, is equivalent to 40% 'true' chlorophyll a.

Most of the 430:410 ratios were greater than 1.2 which, according to the calibration curve prepared by Dr. A. E. Bailey-Watts, corresponds to ca 93% 'true' chlorophyll a. The lowest 430:410 ratio observed, 1.0, corresponds to 84% 'true' chlorophyll a.

Both methods suggest that estimates of chlorophyll a which are uncorrected for phaeophytin interference rarely overestimate 'true' chlorophyll a by more than about 15%. However, absolute values of 'true' chlorophyll a obtained by the two methods were often different. Theoretical objections to the Moss method are discussed by Yentsch (1970) and Marker (1972). Because of the uncertainties and discrepancies outlined above, chlorophyll a values presented here, and used in the calculations of photosynthetic rates per unit chlorophyll a, are uncorrected for phaeo-pigment interference. The evidence available suggests, however, that phaeo-pigments usually made a minor contribution to the chlorophyll a values. In general, phaeo-pigment interference increased as crop density decreased.

c) Chlorophyll a concentration in relation to areal gross productivity

In Fig. 25, hourly rates of gross areal photosynthesis (Σ nP) have been plotted against corresponding measurements of phytoplankton density (expressed as mg chlorophyll a/m^3). A wide scatter of points exists suggesting that in this lake phytoplankton biomass, expressed per unit volume of water, is a poor index of gross productivity per unit area.

Because of self-shading, population density (n) influences both the horizontal and vertical components of the photosynthesis-depth profile as follows:

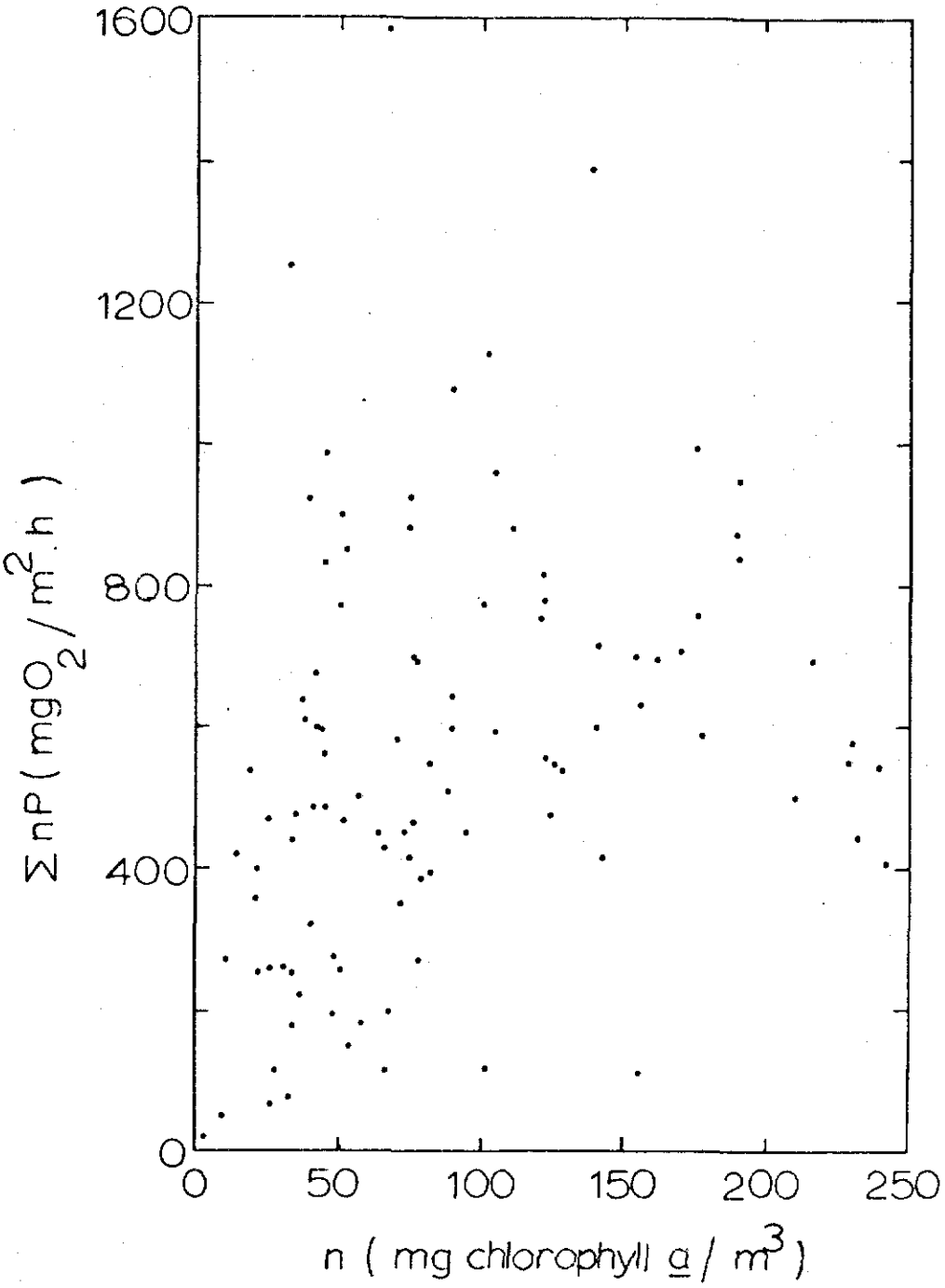


Fig. 25 Gross photosynthesis per unit area (Σ nP) in relation to population density (n) estimated as chlorophyll a.

(1) as one of the determinants of the light-saturated rate of photosynthesis per unit volume of water (nP_{max}), population density (n) influences the maximum horizontal extent of the profile;

(2) as the major determinant of underwater light penetration, expressed in terms of k_{min}, population density also influences the vertical extent of the profile.

Thus, population density affects both components of the ratio nP_{max}/k_{min} shown earlier (Fig. 6) to be the chief determinant of the area-based integral rate (ΣnP). Increase in population density (n) leads to an increase in both nP_{max} and k_{min} with the result that nP_{max}/k_{min} (and consequently ΣnP) can be expected to show less marked variation than n itself. In other words, increase in population density, whilst increasing the horizontal extent of the profile, also decreases its vertical extent so that changes in the area of the profile are not likely to be linearly related to those of population density.

Self-shading thus tends to oppose the expected increase in productivity per unit area with increasing population density.

The extent to which population density determines the light-saturated rate of photosynthesis per unit volume of water (nP_{max}) is shown in Fig.26. Clearly the relationship is not close, implying relatively wide variations in the light-saturated rate of photosynthesis per unit population (P_{max}) and providing an additional explanation for the poor relationship between gross productivity per unit area and standing crop of phytoplankton. Variation in P_{max} and its possible causes are discussed later (section V, part 3).

d) Chlorophyll a content per unit area in the euphotic zone in relation to areal gross productivity

The phytoplankton at Loch Leven was normally distributed uniformly

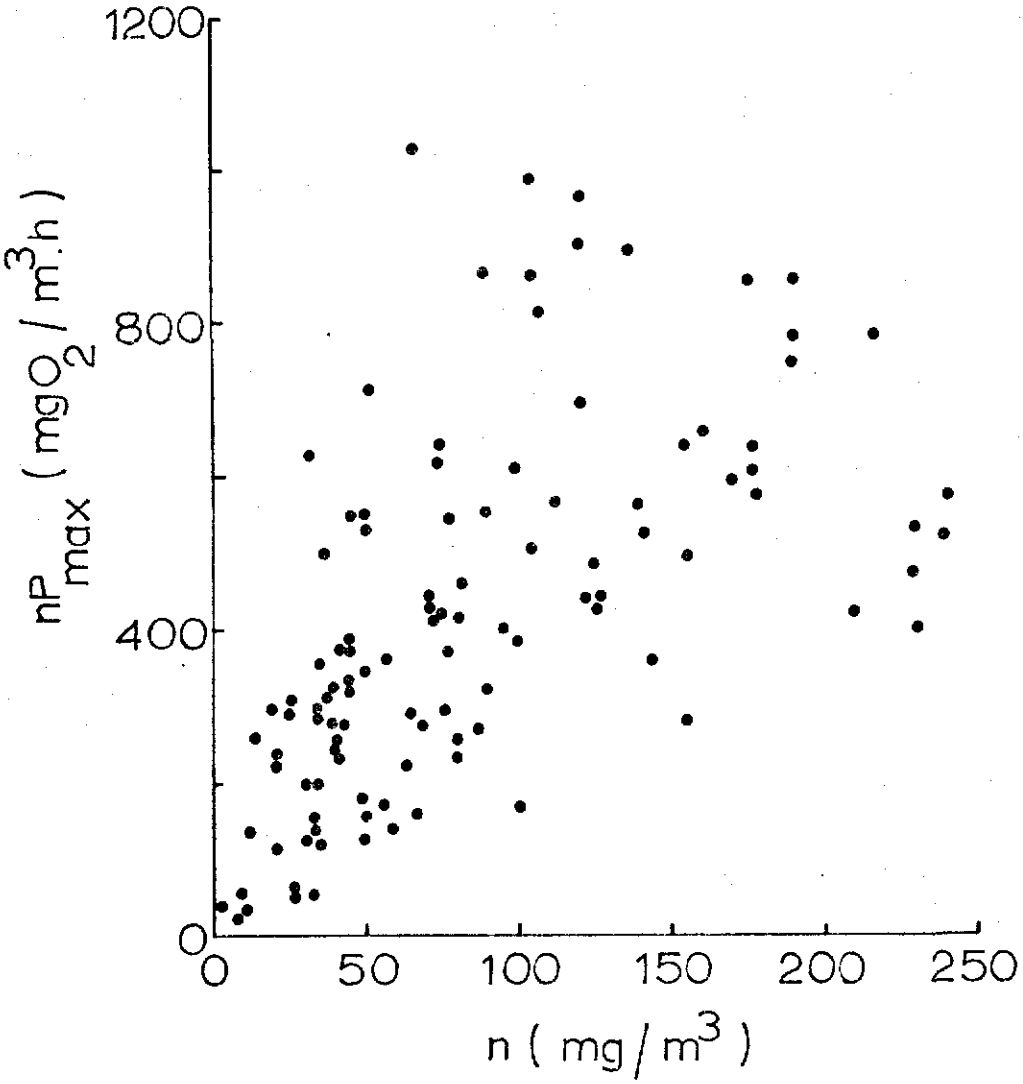


Fig. 26 The relationship between the light-saturated rate of photosynthesis per unit volume of water (nP_{max}) and population density (n), estimated as chlorophyll a.

with depth (Bailey-Watts, 1973,1974). The total amount of chlorophyll a in the euphotic zone under a square metre of water surface (Σn) was therefore calculated as the concentration of chlorophyll a per m^3 in the 0.5 m sample multiplied by the depth (in metres) of the euphotic zone.

Over the 4 year period the chlorophyll a content of the euphotic zone (Σn) ranged from 15 to 456 mg/m^2. Fig. 20 shows that seasonal fluctuations of Σn were usually less marked than those of n, particularly at higher population densities (above about 100 mg chlorophyll a/m^3).

This feature is illustrated more directly in Fig. 27 which shows that the increase in Σn with increase in n became progressively less as n increased. The values of the euphotic population content (Σn) show a tendency to approach an upper limit above which further increase in population density (n) would yield no further increase in Σn.

This relationship between euphotic cover by phytoplankton (Σn) and its population density (n) is easily understood as an expression of self-shading, discussed earlier (p.45); increase in population density (n) tends to reduce the depth of the euphotic zone (z_{eu}) and consequently to bring about a less than proportionate increase in Σn, which is the product of n and z_{eu}.

As a consequence of self-shading the chlorophyll a content of the euphotic zone (Σn) cannot theoretically exceed an upper limiting value (Σn_{max}) which will be achieved when the algae reach densities where they are the sole cause of light extinction in the water column. The magnitude of the maximum possible chlorophyll a content of the euphotic zone (Σn_{max}) depends on the effect on light extinction of unit increase in population density (i.e. on the value of k_s).

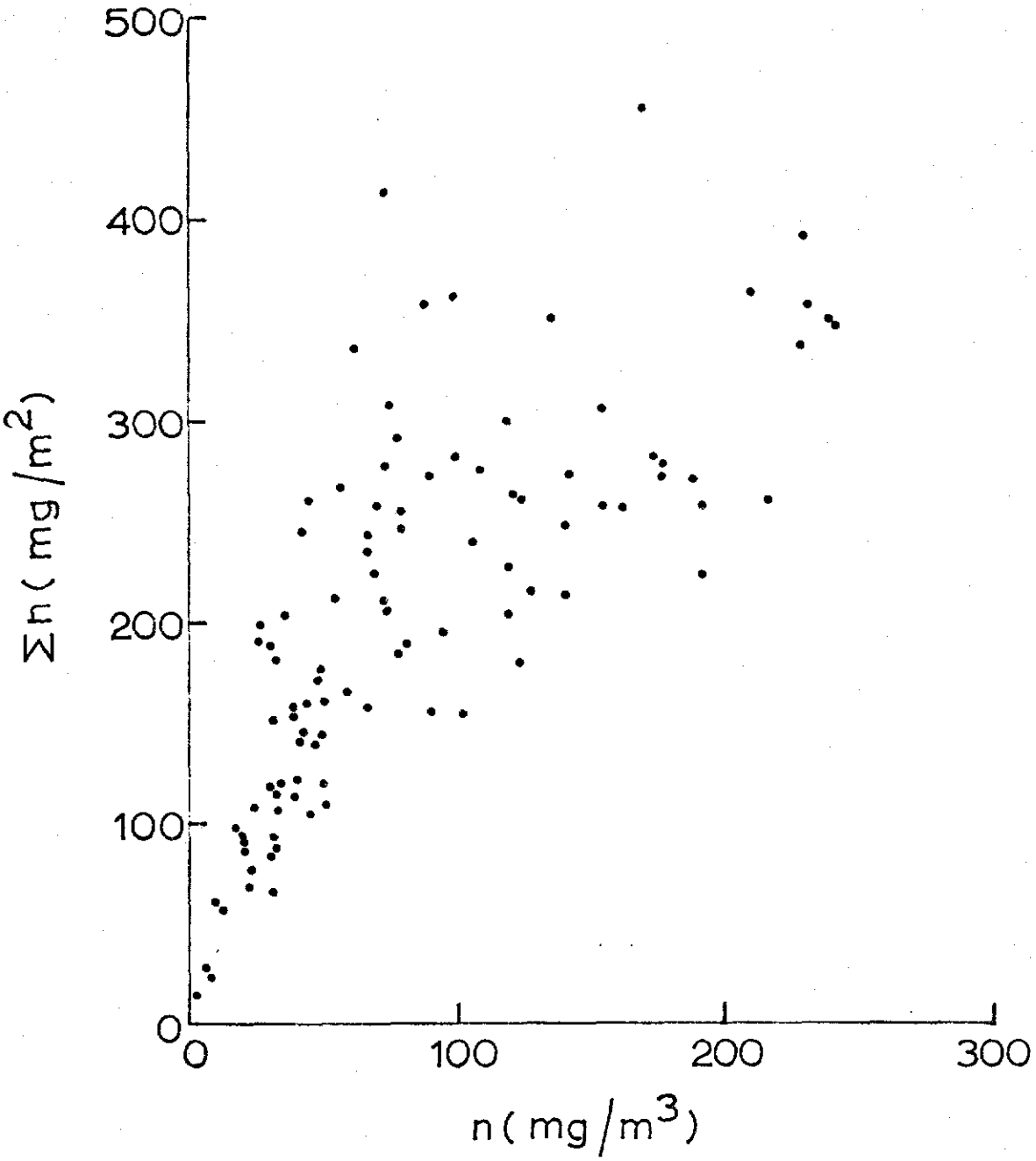


Fig. 27 The relationship between the chlorophyll a content per unit area in the euphotic zone (Σ n) and the chlorophyll a concentration per unit water volume (n).

If Beer's Law is obeyed, when light extinction is due solely to the phytoplankton, k_{min} will be equal to the product of population density (n) multiplied by k_s (the increase in k_{min} for unit increase in population density),

$$\text{i.e. } k_{min} = nk_s.$$

Ifthe euphotic zone is defined in terms of k_{min}, as $3.7/k_{min}$, Σ n will be given by $3.7\ n/k_{min}$ and the theoretical upper limit of Σ n will be equal to $3.7/k_s$. For Loch Leven, with an estimated average k_s value of 0.0086 ln units per mg chlorophyll a/m^2, the theoretical upper limit for Σ n is 430 mg chlorophyll a/m^2. This value is higher than most published values of Σn_{max} (Steemann Nielsen, 1957, 1962a; Talling, 1965a, 1970) and is a direct consequence of the relatively low k_s value estimated for Loch Leven. A higher value of Σn_{max}, 800 mg/m^2, was deduced by Steemann Nielsen (1962a) from laboratory experiments with Chlorella; the corresponding upper limit deduced for diatoms was 400 mg/m^2. In addition, Steemann Nielsen (1962a) found that a Chlorella culture which had become yellowish due to nutrient-deficiency, gave a much lower Σn_{max} value (350 mg/m^2) than that given by healthy cultures. These results emphasise both the importance of species composition and degree of pigmentation in determining the maximum upper limit of the euphotic crop (Σn_{max}), and by implication k_s.

In nature, light extinction can never be solely due to phytoplankton (unless the cells formed a literally solid mass) because some light is always extinguished by pure water itself; in addition, in most natural waters dissolved coloured matter, as well as non-algal particles, also contribute to varying degrees to total light extinction. This being so, and remembering that at the highest algal crop densities at Loch Leven on average 25% of light extinction was due to background

sources other than algae, it is evident that the theoretical upper limit of Σn (i.e. Σn_{max}) of 430 mg chlorophyll *a*/m^2 is unlikely to be reached; it will be approached as light extinction due to phytoplankton increases relative to background light extinction. A number of the Σn values measured at Loch Leven do approach Σn_{max} fairly closely. Assuming that the value of k_s is correct, this suggests that the algae on occasion approached densities where they were practically the sole extinguishers of light in the water column.

In Fig. 27 values of Σn appear to approach a ceiling value of about 340-400 mg chlorophyll *a*/m^2 as population density (n) increases. Owing to the scatter of points in Fig. 27 it is impossible to determine precisely what this ceiling value is, or how closely it is approached. Since no extended plateau is evident in Fig. 27 it is possible that further increase in n could still yield a significant increase in Σn. To this extent, then, phytoplankton density, even at the highest densities occurring in the loch, remained a limiting factor for gross productivity per unit area.

In Figs. 20 and 27 it will be noticed that the highest values of Σn are not invariably given by the highest values of n. This, and the overall scatter of points in Fig. 27, is not unexpected considering that:-

1. The k_s characteristic may vary between species and may depend on degree of pigmentation in individual species.
2. Measurements of k_{min} (and hence Σn) will be influenced by seasonal variations in background light extinction; the value of n required to produce a given value of Σn will increase as background light extinction increases.

Variations in 1 and 2 have been discussed earlier (p. 47) as factors causing variation in the relationship between k_{min} and chlorophyll *a* illustrated in Fig. 18.

Assuming no errors in estimates of z_{eu} or n, the values of Σ n reached in the loch can give an indication of the possible magnitude of k_s. Earlier (p. 46) it was reported that the average value of k_s at Loch Leven was lower than the value 0.02 ln units per mg chlorophyll a/m^2 often assumed representative of phytoplankton; this finding was inconsistent with published and theoretical evidence which had suggested that a k_s value lower than 0.02 was likely to be characteristic of very much larger cells than those dominating Loch Leven crops. Many of the observed Σ n values at Loch Leven were considerably higher than the theoretical upper limit of 185 mg/m^2 possible with a k_s of 0.02. In order to achieve the observed values of Σ n, k_s must, therefore, be less than 0.02.

A Σ n value of 350 mg chlorophyll a/m^2 (an average upper value for Loch Leven) would equal the theoretical upper limit of Σ n for a k_s of 0.011. Since, as discussed earlier, Σ n can never in fact equal, but can only approach, its theoretical upper limit, the value of k_s for an observed Σ n of 350 mg/m^2 is likely to be less than 0.011. This conclusion tends to reinforce the earlier observation that despite the small-celled nature of Loch Leven phytoplankton it was nevertheless characterised by a relatively low k_s value. An important implication of a low k_s value is that, potentially, it allows a higher euphotic population to be reached and therefore favours higher productivity per unit area.

The highest euphotic chlorophyll a contents found at Loch Leven exceed many of the maximum values (180–325 mg/m^2) measured in other densely populated lakes (Aruga & Monsi, 1963; Sakamoto, 1966; Talling et al, 1973) or indirectly estimated as theoretical maximum values (Steemann Nielsen, 1957, 1962a; Talling, 1965a, 1970). Similarly high values (372 and 377 mg/m^2) were found by Talling (1971) in a dense

population of Ceratium in Esthwaite Water, a productive English lake. Very much higher values, 650 and 900 mg/m^2, have been reported by Aruga (1966) and Ahlgren (1970) respectively. Some high values may, however, be due to overestimation of the depth of the euphotic zone (cf. Steemann Nielsen, 1962a).

Self-shading was suggested earlier as a reason for the poor correlation found between population density (n) and areal gross productivity (Σ nP). The chlorophyll a content of the euphotic zone (Σ n) incorporates the self-shading effect and therefore might be expected to show a closer relationship with Σ nP than does n. However, the poor correlation between Σ nP and Σ n, illustrated in Fig. 28, shows that even when self-shading is taken into account biomass remains an unreliable index of gross productivity per unit area.

A further cause of the 'uncoupling' of biomass and its gross rate of productivity is the variation in its photosynthetic capacity (P_{max}). This is discussed below.

3. The specific rate of gross photosynthesis per unit population measured at light-saturation (photosynthetic capacity, P_{max})

Photosynthetic capacity (P_{max}), together with population density (n), determines the maximum horizontal extent of the photosynthesis-depth profile.

Values of P_{max}, as mg O_2/mg chlorophyll a.h, were calculated from the light-saturated rates of photosynthesis per unit volume of water(nP_{max}, mg O_2/m^3.h) divided by the corresponding concentration of chlorophyll a per unit volume of water (n, mg/m^3).

Seasonal changes in photosynthetic capacity (P_{max}) are shown in Fig. 29. Over the 4 year period values covered an approximately

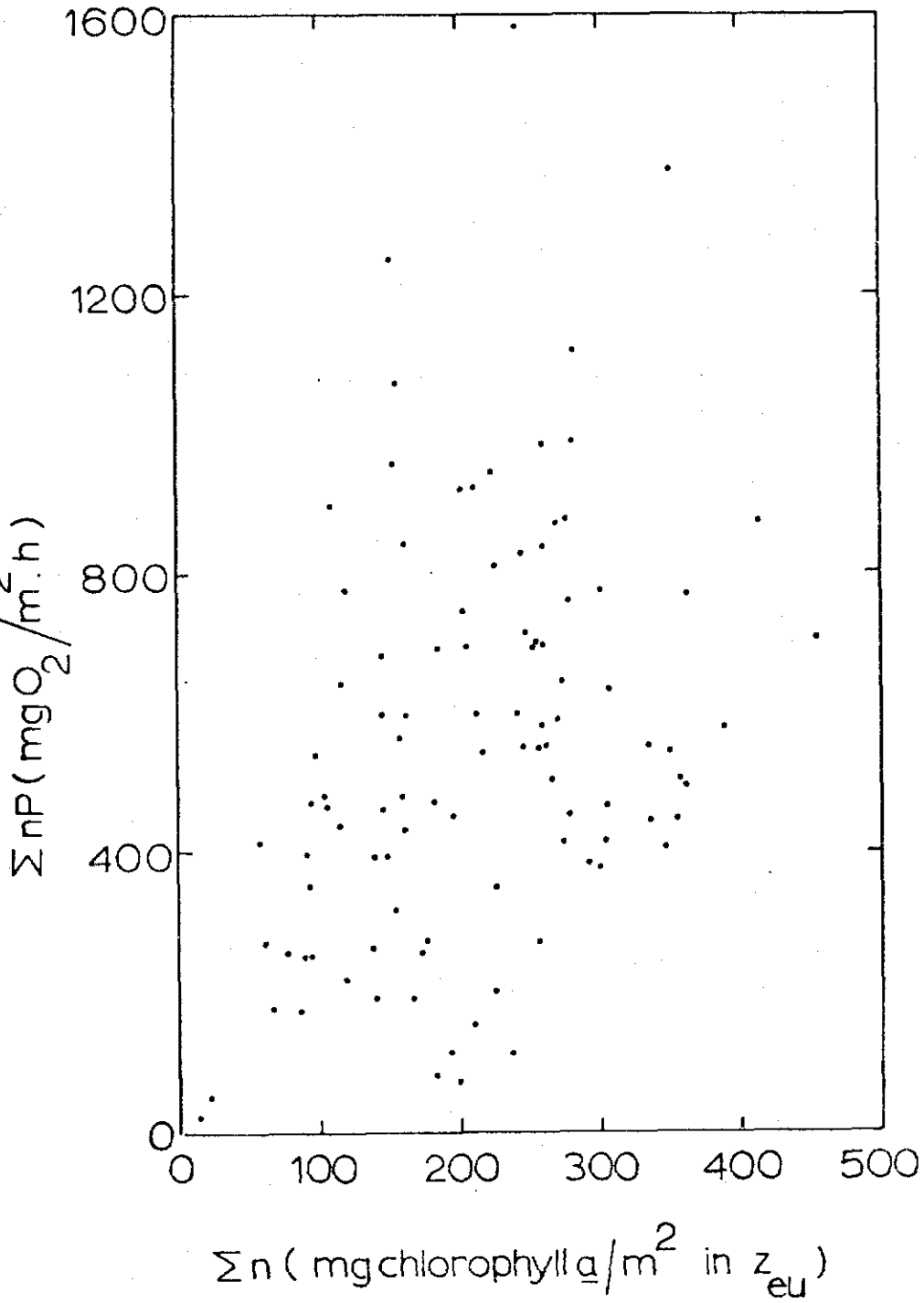


Fig. 28 Gross photosynthesis per unit area (Σ nP) in relation to the chlorophyll a content per unit area of the euphotic zone (Σ n).

Fig. 29 (following 4 pages) Seasonal changes during 1968-71 in population density (n) estimated as chlorophyll *a*, light-saturated rate of photosynthesis per mg chlorophyll *a* (P_{max}), pH and water temperature.

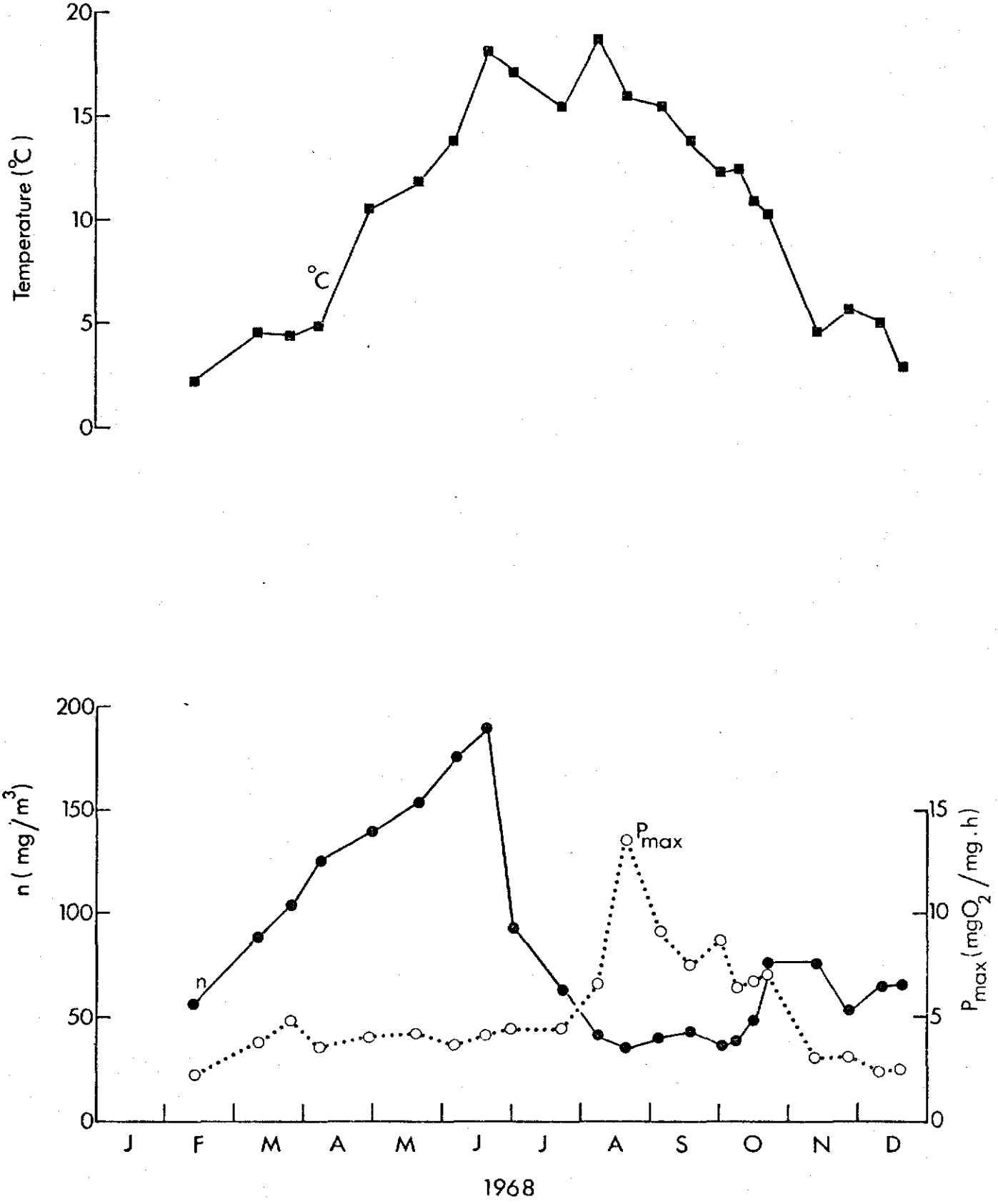
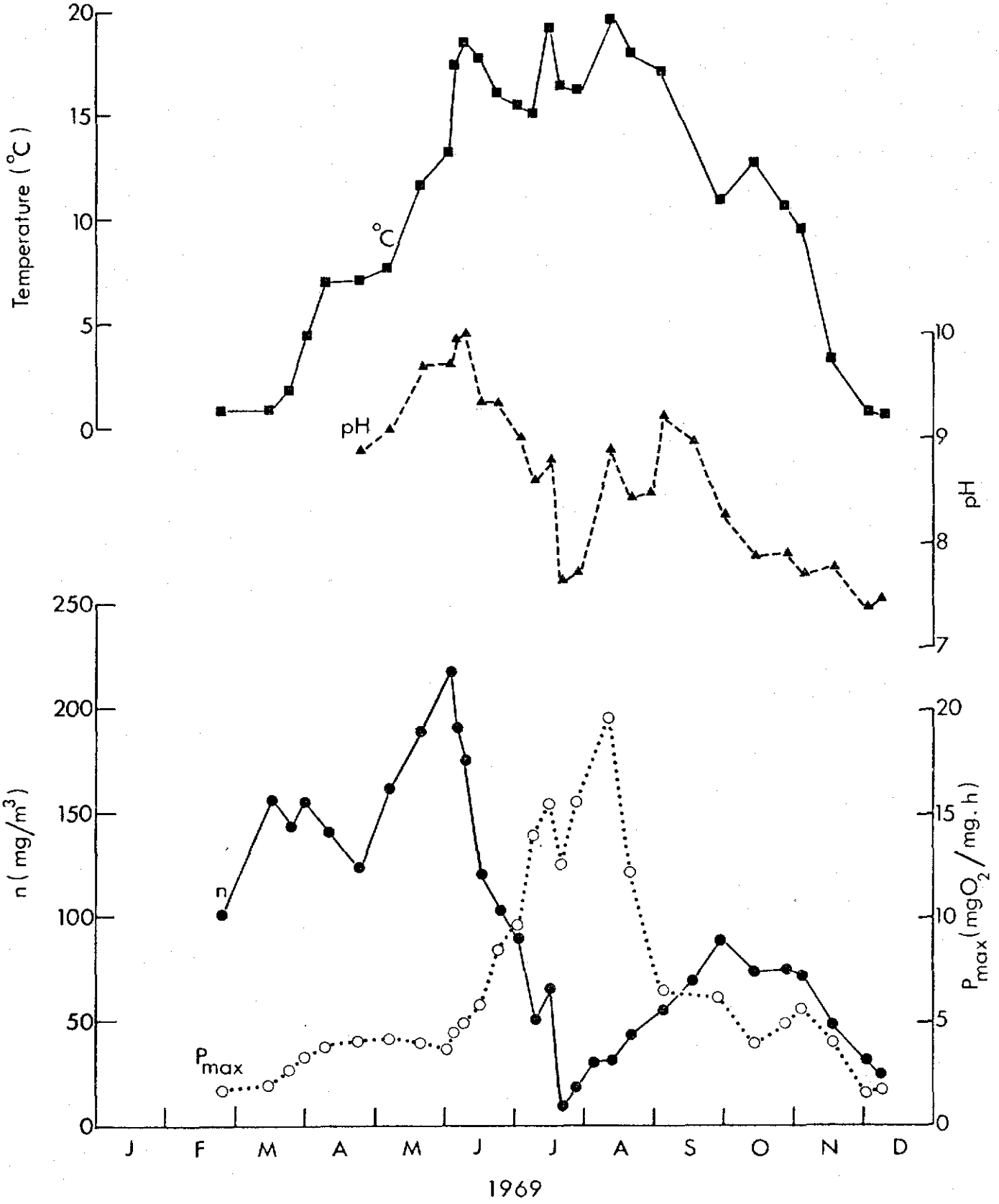


Fig. 29 Legend opposite


Temperature (°C)
20
15
10
5
0
°C
pH
10
9
8
7
pH
250
200
150
100
50
0
n (mg/m³)
n
Pmax
20
15
10
5
0
Pmax (mgO2 / mg. h)
J
F
M
A
M
J
J
A
S
O
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1969

Fig. 29 (cont'd)

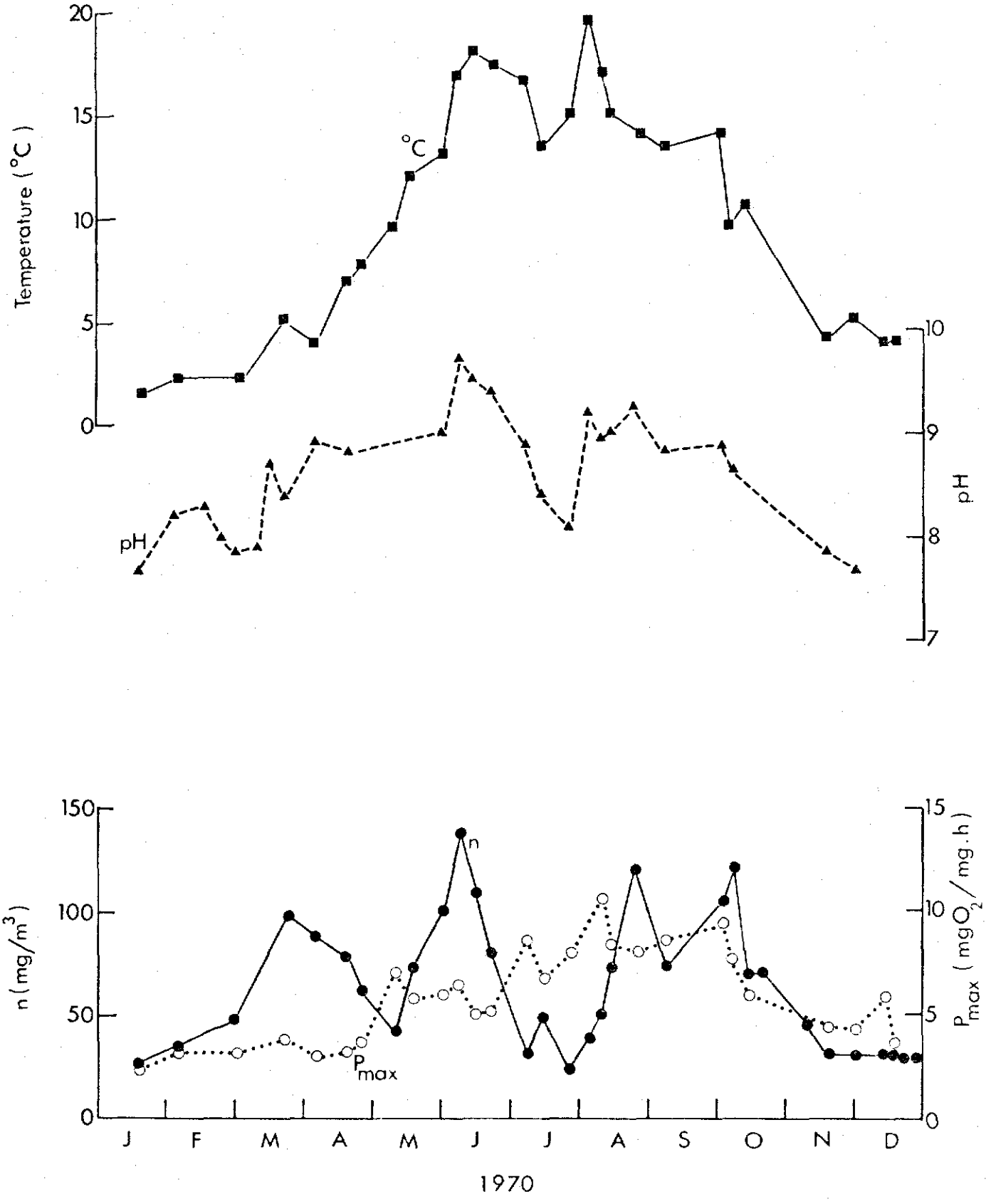

Temperature (°C)
°C
pH
pH
20
15
10
5
0
10
9
8
7
n (mg/m³)
Pmax (mgO₂/mg.h)
n
Pmax
150
100
50
0
15
10
5
0
J F M A M J J A S O N D
1970

Fig. 29 (cont'd)

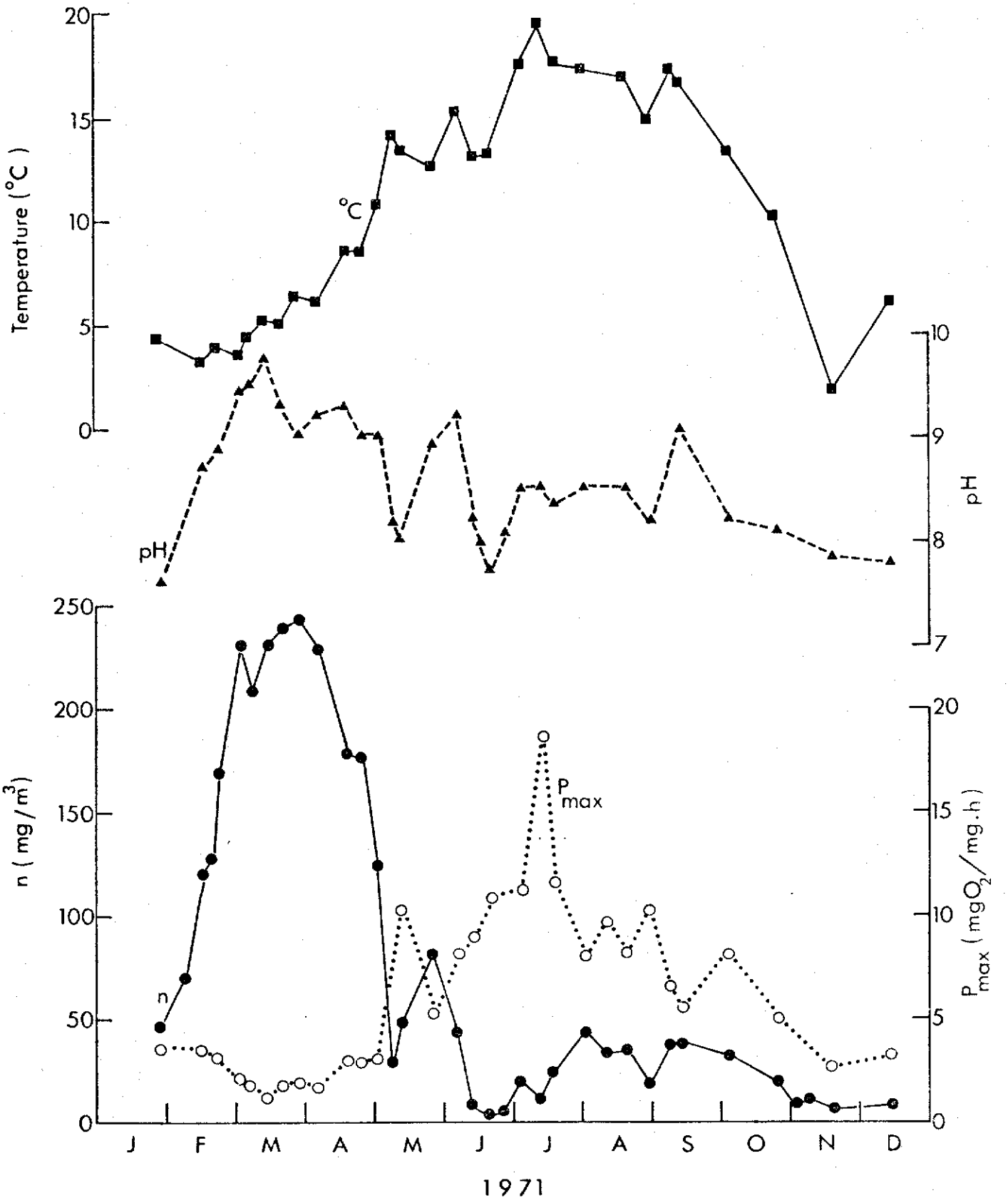

Temperature (°C)
°C
pH
pH
n (mg/m³)
n
Pmax
Pmax (mgO2/mg.h)
J F M A M J J A S O N D
1971

Fig. 29 (cont'd)

tenfold range from 1.6-19.6 mg O_2/mg chlorophyll a.h. Assuming a photosynthetic quotient of 1.0, these are equivalent to a range of 0.6-7.7 mg C/mg chlorophyll a.h. Photosynthetically inactive populations were never recorded. Wide variation in P_{max} was implied earlier when the ill-defined relationship between nP_{max} and n was described (Fig. 26). Most of the values of P_{max} recorded at Loch Leven fall within the range of values so far encountered in natural waters. Strickland (1960) quotes a range of 1-10 mg C/mg chlorophyll a.h, Talling (1961) a range of 3-15 mg O_2/mg chlorophyll a.h and Ichimura (1968) a range of 5-10 mg C/mg chlorophyll a.h.

Artificially high values could result from incomplete extraction of chlorophyll, whilst low values will result if phaeo-pigments are included in the chlorophyll estimates.

Although photosynthetic rates per unit of biomass vary widely within and between lakes, they have been found to be reasonably uniform for restricted areas or periods (Gessner, 1949; Talling, 1965a), for different species (Edmondson, 1955; Ryther, 1956b) and for cells with different nutrient histories (Fleischer, 1935) or light histories (Ryther, 1956b). Based on their measurements with natural and cultured populations of marine phytoplankton and on other published data, Ryther & Yentsch (1957) considered P_{max} values to be sufficiently constant (at ca 3.7 mg C/mg chlorophyll a.h) to justify the use of an empirical equation for estimating areal productivity from chlorophyll and light data. Further work (Ryther & Yentsch, 1958) appeared to support their use of a constant P_{max} value. However, as discussed by Strickland (1960), there is no obvious reason why the enzyme-mediated reactions of photosynthesis should be independent of temperature, illumination, nutrient levels and species composition. In general, therefore, a constant value for P_{max} is not to be expected.

Seasonal variations in photosynthetic capacity (P_{max}) at Loch Leven were examined in relation to:- (a) water temperature, (b) population density and species composition, (c) the conditions of illumination experienced during previous growth periods (light history), (d) pH and CO_2 supply, (e) dissolved oxygen levels, and (f) nitrogen and phosphorus supply. Results and discussion are presented below.

a) P_{max} in relation to water temperature

Introduction

Within the range of temperatures tolerated by living organisms the rate of most enzymatic processes is increased by a rise in temperature. The temperature coefficient (Q_{10}) - i.e. the increase in rate, as a multiple of the initial rate, produced by a 10°C rise in temperature - is often between 2 and 3 for biological as well as chemical processes.

The rate of photosynthesis per unit biomass under saturating light intensities (P_{max}) is determined by enzymatic and therefore potentially temperature-sensitive processes, whereas at sub-saturating light intensities photochemical (temperature-insensitive) reactions control the overall rate of the whole photosynthetic process.

Temperature is, therefore, an obvious factor to consider as a possible cause of the seasonal variations in P_{max}. In addition, temperature may well exert an indirect influence on photosynthetic capacity, to the extent that this is sensitive to nutrient supply, through an influence on the rates of regeneration of nutrients (CO_2, NO_3-N, PO_4-P, etc.) by bacterial and zooplankton activity.

Results

The maximum range of water temperature encountered at Loch Leven was from 0°C (ice cover) to 20°C. Seasonal variations in subsurface

water temperature are illustrated in Fig. 29. Temperature was normally uniform (to within 0.5°C) within the euphotic zone and for most of the year throughout the whole water column as well. During summer months (June and July) intermittent temperature stratification occurred over the deeper parts of the loch. The depth of the thermocline varied from 6 to 10 m. Temperature difference between top and bottom (20 m) rarely exceeded 5°C (pers. obs. and I. R. Smith, pers. comm.). Intermittent ice cover occurred during the winter, between November and February, but continuous ice cover rarely exceeded a fortnight.

Changes in photosynthetic capacity (P_{max}) appear broadly correlated with those of water temperature (Fig. 30). An increase in temperature was associated with an increase in photosynthetic rate. The correlation coefficient between log P_{max} and temperature is 0.78 and the calculated linear regression line indicates an average temperature coefficient (Q_{10}) of 2.2 over the temperature range 0-20°C. Possible fluctuations in Q_{10} over narrower temperature ranges are obscured by the fairly extensive scatter of points in Fig. 30.

Departure from the overall trend of increase in P_{max} with increase in temperature was observed during certain periods which may be identified from Fig. 29. Between April and July 1968, and between April and early June 1969, P_{max} remained more or less constant despite a temperature increase of ca 14°C. During May and June 1970, P_{max} showed little change as temperature increased from 8°C to 17°C. In February and March 1971, P_{max} declined whilst temperature remained constant and continued to decline when temperature increase began during March. Except in 1971, the subsequent increases in P_{max} occurred over periods of relatively constant temperature.

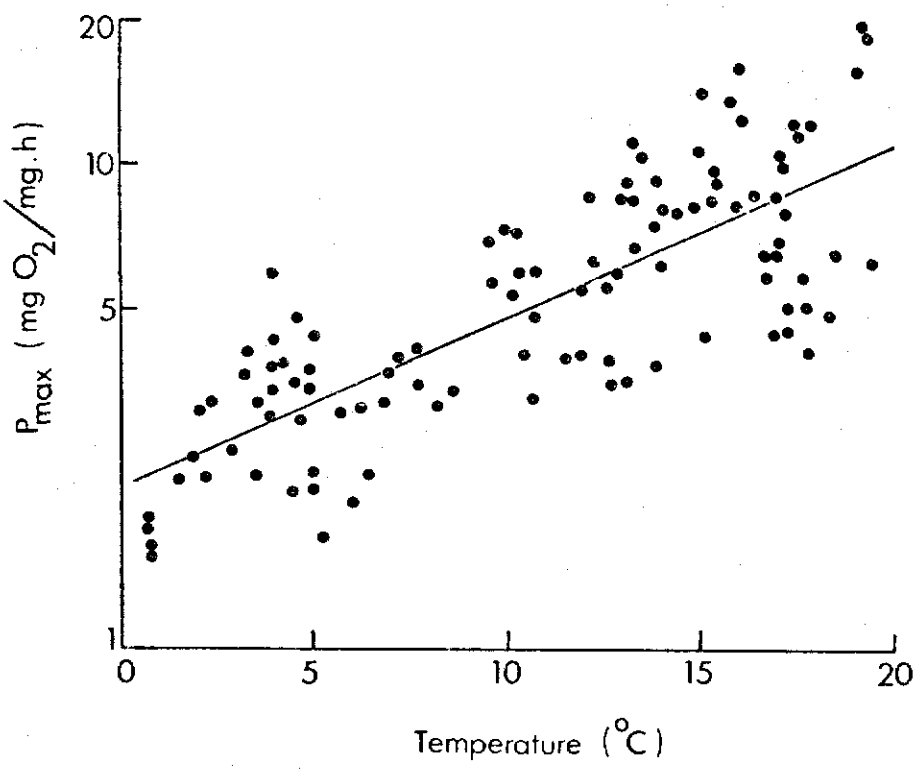


Fig. 30 The light-saturated rate of gross photosynthesis per unit of chlorophyll a (P_{max}, logarithmic scale) measured in Loch Leven, in relation to ambient water temperature. Calculated regression line is log y = 0.035x + 0.337, correlation coefficient (r) = 0.79, N = 107. Q_{10} = 2.2.

Discussion

Q_{10} values similar to the Loch Leven value have been recorded in other work on photosynthesis of cultured and natural populations of planktonic algae measured in the field (Talling, 1957a) or in the laboratory (Talling, 1966). Many other authors have observed seasonal changes in P_{max} which they have attributed to temperature changes, or have demonstrated such an influence in the laboratory (e.g. Ichimura, 1960a; Aruga, 1965). English (1959) found low P_{max} values (0.6 mg C/ mg chlorophyll a.h) in arctic waters and attributed these to low water temperature (-1.5°C). Similar low values (average, 0.8 mg C/ mg chlorophyll a.h) were found by Horne et al (1969), at a temperature near 1°C, in an area of inshore Antarctic sea. In general, P_{max} values seem to be higher in tropical than in temperate waters (Talling, 1957c, 1961, 1965b; Prowse & Talling, 1958; Steemann Nielsen & Hansen, 1959a; Talling et al, 1973). Such differences have been attributed to climatically based differences in temperature (Talling, 1965b). However, Talling (1957c) and Talling et al (1973) noted that P_{max} values in tropical waters were sometimes higher than those of temperate waters, even at similar temperatures. They therefore conclude that differences in P_{max} between tropical and temperate waters may be only partially temperature-based.

Other work has shown that the difference between P_{max} values at different temperatures is often less than would be expected for a Q_{10} of 2. Talling (1960a) measured photosynthetic capacities of 29-32 mg O_2/mg chlorophyll a.h at 14-15°C in a culture of Chaetoceras affinis. Such rates are more common at higher temperatures in tropical waters. Steemann Nielsen & Hansen (1959a) presented evidence that the photosynthetic activity per mg chlorophyll a of arctic plankton measured at 2°C was practically the same as that of temperate plankton measured

at 15°C. However, they base their observation on an assumption, which they do not substantiate, that the chlorophyll content per unit of organic matter is the same at different latitudes. Jørgensen & Steemann Nielsen (1965) and Steemann Nielsen & Jørgensen (1968) showed that the photosynthetic capacity of Skeletonema costatum was practically the same at 8°C as at 20°C when the cultures had been previously grown at these temperatures. When a culture which had been grown at 20°C was immediately transferred to 8°C its photosynthetic capacity decreased to about one-third of the value at 20°C. Verduin (1956a) found photosynthetic capacity on a cell volume basis was as high at 0-3°C as at 18-23°C.

Many examples of apparent temperature insensitivity are documented for cold blooded animals (see reviews by Bullock, 1955,1958). Some species maintain a relatively constant rate of metabolism, independent of temperature, compensating for temperature differences, associated with season or latitude, by homeostatic mechanisms of various kinds. These phenomena are generally regarded as reflecting some form of compensation, rather than a fundamental insensitivity of metabolism to temperature; the physiological mechanisms and properties of compensation are, however, still debated. In some cases altered enzyme activity is apparently involved.

Steemann Nielsen & Hansen (1959a) and Jørgensen & Steemann Nielsen (1965) suggested that the ability of algae to maintain high rates of photosynthesis despite decreases in temperature, was due to an increase in the concentration of enzymes relative to pigment concentration at low temperatures. Some support for this suggestion was provided by the experiments of Jørgensen (1968) with Skeletonema costatum, which showed that the protein content of cells adapted to 7°C was twice as high as in cells adapted to 20°C. Additional supporting evidence is

given by Morris & Farrell (1971).

During certain periods at Loch Leven, identified above, P_{max} appeared to be insensitive to accompanying increases in water temperature, i.e. P_{max} was as high at low temperatures as at higher temperatures. Subsequently, however, P_{max} did increase in the absence of any further temperature increase. This suggests that the maintenance of a constant P_{max} value during a period of temperature increase need not, in this instance, involve a compensatory mechanism (e.g. of increased enzyme/pigment ratios) in the cells present at the lower temperatures. Rather, it seems likely that the potential increase in rate due to temperature is offset by an equal and opposite effect of factors tending to depress P_{max}. These factors are discussed below.

b) P_{max} in relation to population density and species composition

Results and Discussion

During all four periods when P_{max} did not behave as expected on temperature grounds, population density was increasing (Fig. 29). This suggests that the factors depressing P_{max} at constant temperature (in 1971) or preventing its increase with temperature (in 1968, 1969 and 1970) were population density-dependent factors. This suggestion is further supported by the observation that subsequent decline in population density (n) was accompanied by an increase in photosynthetic capacity (P_{max}), the clearest example being that of 1969. Except in 1971 this increase in P_{max} as population density declined occurred over periods of relatively constant temperature. The increase in rate appeared to be due to removal of a depressive factor rather than to temperature enhancement. The algae themselves, when growing at high population density, appeared to be producing conditions unfavourable to their own photosynthetic activity.

It was noted earlier that degraded chlorophyll was relatively more abundant when crops were low. Any correction for phaeophytin interference would therefore tend to accentuate the observed inverse relationships between population density and photosynthetic capacity.

Bubbles were very rarely seen in the experimental bottles after exposure. Loss of oxygen in gaseous form was therefore unlikely to have led to underestimation of P_{max}.

Another possible methodological reason for low measurements of P_{max} at high crop density was the restriction of the zone of saturating light intensities to a narrow depth band. When population density was low, and the water in the loch therefore clearer, the range of light intensities which could saturate photosynthesis occurred over a wider depth range and the chances of locating an experimental bottle within this range were correspondingly greater. When population densities were high, light extinction rapid, and the depth range of saturating light intensities narrow, the chances of detecting the true light-saturated rate of photosynthesis were smaller. The close vertical spacing of experimental bottles in this study (see Methods section) minimised this potential source of error. Furthermore, parallel measurements of the rate of light-saturated photosynthesis in the laboratory tank were always in close agreement with those estimated from the in situ photosynthesis-depth profiles.

The depression of photosynthetic capacity observed in dense crops does not appear to be an artefact caused by the above possible sources of underestimation of gross photosynthesis or overestimation of chlorophyll a. Enhanced photorespiration, which may cause underestimation of gross photosynthetic rates, is discussed later (p.103).

A declining population might have been expected to show symptoms of senescence, such as a reduced capacity for photosynthesis. It is

notable that at Loch Leven declining populations appeared to be in a healthier physiological condition than increasing populations, at least in terms of their gross photosynthetic activity.

Talling (1957a, 1966) drew attention to the maintenance of relatively high photosynthetic rates by Asterionella in Windermere beyond the period of exponential growth into the phase of catastrophic decline. Intracellular storage or elimination of photosynthetic products by respiration or extracellular production were offered as possible explanations (Talling, 1966). Extracellular release of photosynthetic products was not investigated at Loch Leven and therefore cannot be discounted as at least a partial explanation. The carbon content of phytoplankton is normally between 40 and 60% of the ash-free dry weight (Vollenweider, 1969). Higher values, up to 75%, can occur if the cells accumulate fat (Strickland, 1960). Data on the carbon content of Leven phytoplankton were obtained for only one of the periods (July 1970) when an increasing P_{max} accompanied a declining population (see Table 5). During this period there was no evidence for intracellular storage of photosynthetic products. The carbon content of the algae was more or less constant at approximately 50% of the ash-free dry weight.

Community respiration rates, expressed per unit content of chlorophyll a, increased as crop density declined (see section VII, Fig. 45). However, as discussed in sectionVII, it is likely that at least part of the apparent increase in algal respiration rates as crop density declined was due to an increase in the relative contribution of zooplankton and bacteria to community respiration rates.

A high rate of photosynthesis in a declining population would not be anomalous if the decline in crop density was due to external factors which did not affect algal physiology directly (e.g. sinking, grazing

and outflow losses). On the basis of production:biomass ratios (which are analogous to P_{max}), Dickman (1969) distinguished two types of factors which may limit algal crops. He considered that populations which are limited by 'resource' factors (e.g. nutrients, light, temperature) are likely to have low production:biomass ratios, whereas those limited by 'cropping' factors (e.g. grazing, flushing, disease) tend to have high ratios.

Fluctuations in total population density (chlorophyll a) represent the average of all fluctuations of all component species and may mask marked differences in population density changes of individual species. This point, perhaps sometimes overlooked, has been clearly emphasised and discussed by Moss (1969) using a hypothetical model. Thus, when chlorophyll a content as a whole is declining individual species may well be increasing and vice versa; this is known to occur at Loch Leven (Bailey-Watts, 1973, 1974). Apart from possible differences in the photosynthetic capacity of cells in active growth and those in apparent senescence, different species may inherently have different photosynthetic capacities due to differences in pigment composition or different surface area:cell volume ratios. Relatively little comparative work appears to have been published on the photosynthetic capacity of individual species, either measured in the field or in the laboratory. Aruga (1965) found differences in photosynthetic capacity at the same temperature, between cultures of Chlorella ellipsoidea, Scenedesmus sp. and Anabaena cylindrica and a suspension of a natural population of Synedra sp. Aruga considered, however, that the differences he observed might be dependent on the conditions under which the algae had been grown, rather than on species differences per se. Findenegg (1971) considered that the large variations he observed in the photosynthetic activity of phytoplankton from thirty

alpine lakes was to some extent due to differences in species composition. Findenegg expressed photosynthetic activity on a unit carbon basis (and not, as here, on a chlorophyll a basis). His conclusion that Chlorophyceae tend to be more active than diatoms could merely be a reflection of the tendency of Chlorophyceae to be richer in chlorophyll a than other algal groups (see e.g. Strickland, 1960; Bursche, 1961).

The possibility remains that changes in species composition could account for some of the P_{max} variations observed at Loch Leven. Such an explanation cannot account, however, for the inverse relationship described in early 1971 when a single species, Cyclotella pseudostelligera, was more or less dominant throughout the period concerned.

The recent application of autoradiography to primary production studies seems to be a promising new approach to the problem of measuring photosynthetic rates of individual phytoplankton species in mixed natural populations. Using this technique Watt (1971) was able to demonstrate that a species may contribute significantly to the biomass but not to primary productivity, and that the contribution of nannoplankton species to primary production is usually greater than their biomass proportion would suggest. This last finding confirms earlier reports (Rodhe et al, 1958; Findenegg, 1965) that nannoplankters are, per cell volume, more active in photosynthesis than net-plankters. Malone (1971) came to the same conclusion when photosynthetic rates were expressed per unit of chlorophyll a. The higher photosynthetic capacity of smaller cells is generally held to be due to their higher surface area:volume ratio being more favourable for nutrient uptake.

For most of the study period the Leven phytoplankton was dominated by nannoplankton, there being a notable absence of net-plankters (Bailey-Watts, 1973, 1974). When, in late 1971, significant quantities of net-plankters did occur, the photosynthetic capacity of cells retained by the phytoplankton net was compared with that of cells passing through the net. No significant differences between the two fractions was revealed on the two occasions when this was examined. It therefore seems unlikely that major fluctuations in P_{max} were due to seasonal changes in cell size of the phytoplankton.

Inverse relationships between population density and photosynthetic capacity have been described elsewhere (e.g. Manning & Juday, 1941; Wright, 1959, 1960; Verduin, 1960; Abeliovitch, 1967; Ganf, 1969, 1972; Ahlgren, 1970; Findenegg, 1971). Various factors associated with increase in population density may be responsible for limiting photosynthetic capacity. These include nutrient-depletion, decrease in water clarity leading to 'shade' adaptation, and build-up of pH or oxygen to inhibitory levels. These possibilities are discussed in subsequent sections.

The inverse relationship, between population density (n) and its capacity for gross photosynthesis at light-saturation (P_{max}), tended to oppose the expected decrease in productivity per unit area (Σ nP) with decrease in standing crop, and thus tended to reinforce the self-shading effect in reducing variation in areal gross production.

c) P_{max} in relation to light history (the conditions of illumination to which the algae were exposed, prior to measurement of their photosynthetic rate)

Introduction

Adaptation to different light intensities, analogous to the 'sun' and 'shade' adaptation of higher plant leaves (Rabinowitch, 1951), has

been demonstrated for unicellular algae in culture by a number of authors, including Sargent (1940), Myers (1946), Steemann Nielsen, Hansen & Jørgensen (1962) and Jørgensen (1969). The general features associated with adaptation vary somewhat between species and involve various degrees of change in chlorophyll a per cell, photosynthetic capacity (P_{max}), light-saturation behaviour (I_k), cell size and respiratory rate. The present discussion considers only changes in P_{max} and I_k, for which comparable Loch Leven data are available. Typically, cells grown at high light intensities ('sun'-type cells) are reported to have a higher rate of photosynthesis per unit of chlorophyll a at light-saturation than cells grown at lower light intensities ('shade'-type cells). 'Sun'-type cells achieve light-saturation at a higher light intensity (and therefore have a higher I_k value) than 'shade'-type cells.

In natural phytoplankton populations 'sun'/'shade' type adaptation has been invoked by some authors to explain differences in photosynthetic behaviour of cells obtained from different depths (Steemann Nielsen & Hansen, 1959a, 1961; Ryther & Menzel, 1959; Ichimura, 1960a; Talling, 1966) or different seasons (Gessner, 1944, 1949; Ichimura, 1960a; Steemann Nielsen & Hansen, 1961), 'shade'-type cells being characteristic of deep water or winter months.

Observations by Ichimura (1960a), Steemann Nielsen, Hansen & Jørgensen (1962), Steemann Nielsen & Park (1964) and Jørgensen (1964a) suggest that planktonic algae can adapt fairly rapidly (within 1-4 days) to a new light intensity.

Results and Discussion

As in other shallow, well-mixed lakes (cf. Ichimura, 1960a), no evidence of depth-differentiation into 'sun' and 'shade' forms was found at Loch Leven (see Methods section).

'Sun'/'shade' adaptation, induced by seasonal change in the average light conditions in the water column, was explored as a possible cause of the observed seasonal variations of P_{max} and I_k.

The mean daily light intensity (i) experienced by an algal cell freely circulating in a mixed water column is determined by total daily irradiance, underwater light penetration and the depth of the mixed layer.

At Loch Leven isothermal mixing normally extends to all depths. Under these conditions the mean depth of the mixed layer (z_m) is equal to the mean depth of the loch ($\bar{z}$). As Talling (1971) points out, the effective mixed depth (z_m') over which, on average, cells circulate, is slightly greater than the mean depth. This is because mean depth is unduly influenced by shallow marginal areas of small total volume. As recommended by Talling (1957b, p.144), the effective surface area of the loch was taken as the area described by the depth contour (in this case 1 m) which approximately divided the photosynthesis/depth profile in half. Using this value, and the morphometric data for the loch given by Smith (1974), the effective mixed depth (z_m') was calculated as total loch volume/effective surface area, yielding a value of 4.8 m.

The following expression was used to give a relative index of the mean daily light intensity (i) experienced by Loch Leven phytoplankton:

$$i = \frac{\Sigma I_o \cdot z_{eu}}{z_m'}$$

where ΣI_o = 10-day mean daily surface incident radiation

z_{eu} = depth of euphotic zone

z_m' = effective depth of mixed layer

In contrast to similar expressions for the mean light intensity for circulating cells proposed by Riley (1957) and Rabe & Benoit (1962),

the above expression does not take into account the logarithmic decline of light intensity with depth and therefore does not define absolute light intensities. Consequently, although the units of i are strictly those of light intensity, they do not have any meaning as such. They are here assigned relative units and used as relative indices of average cell illumination. Even an absolute value for mean daily light intensity (i) could not be meaningfully compared with the light intensity supplied by fluorescent tubes in the laboratory experiments described later. The above expression for i gives mean values which incorporate a fluctuating diurnal cycle of incident radiation and a period of complete darkness. The laboratory light intensity was continuous and constant. The physiological effects of a given mean daily underwater light intensity may be very different from those of an equal, but constant, light intensity supplied continuously in the laboratory.

A dimensionless measure of the light-climate experienced by an algal cell circulating in a mixed water column was derived by Talling (1971). Talling's solution incorporates light-saturation behaviour (I_k) in a logarithmic measure of daily irradiance. I_k was not incorporated in the present expression since the influence of light-history (measured as i) on I_k was to be investigated.

Reynolds (1972, 1973) used an expression for the mean daily period of exposure of a lake population to light. This was derived from the product of daylength and the percentage of the isothermal volume which was illuminated. Reynolds' expression differs from that used at Loch Leven in that it takes account only of the duration of incident light, but not of its intensity.

Seasonal changes in i and P_{max} are shown in Fig. 31. Since I_k and P_{max} show similar seasonal trends (cf. Figs. 9 and 29), a very similar

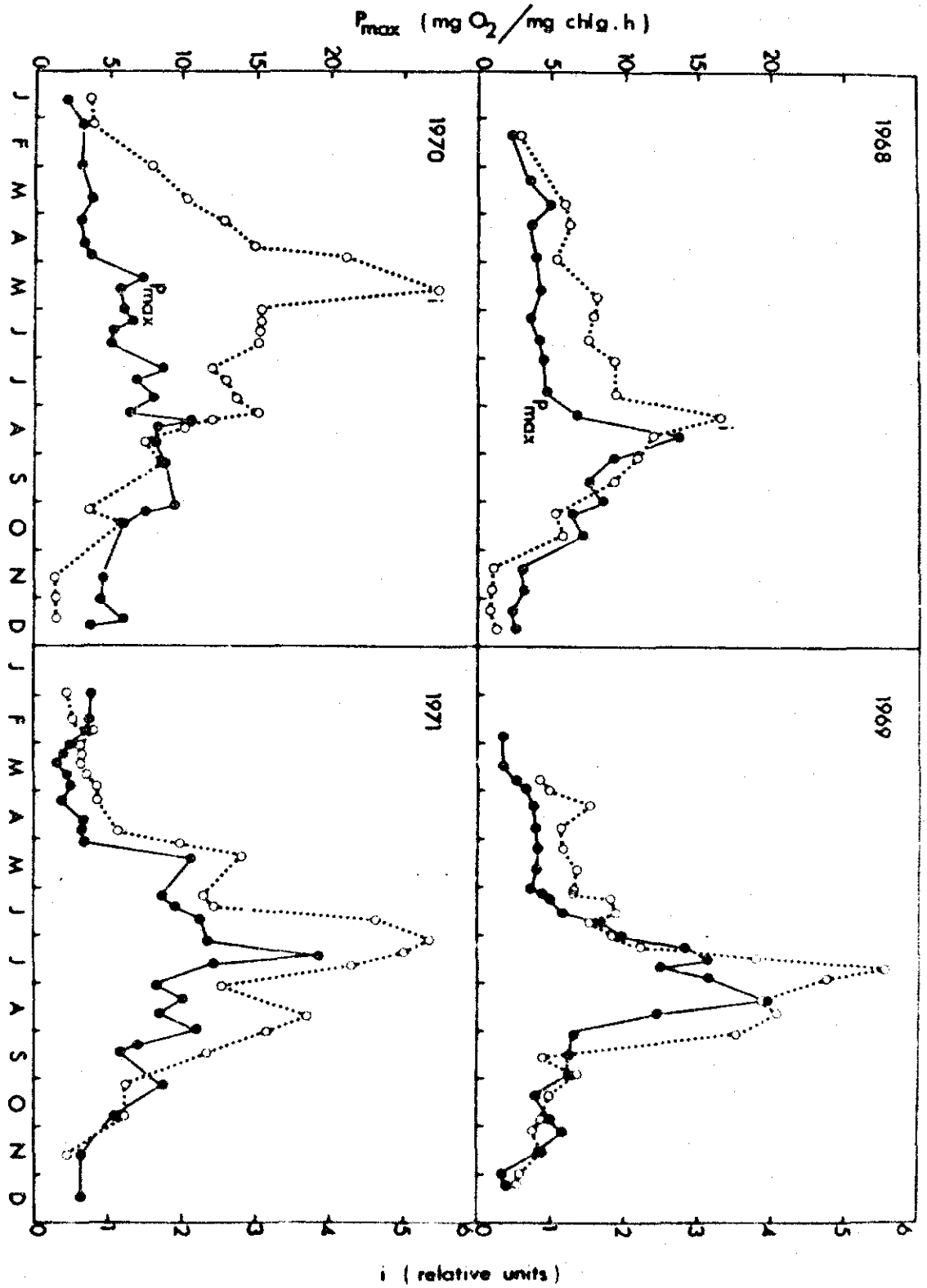


Fig. 31 Seasonal changes in photosynthetic capacity (P_{max}) and a relative index of mean daily light intensity (i) experienced by Loch Leven algae circulating in a mixed water column of depth 4.8 m.

pattern would have been obtained if i and I_k had been plotted.

With the notable exception of the first six months of 1970, overall seasonal trends in P_{max} largely paralleled those of i. The correlation coefficient between the 97 P_{max} and i determinations available is 0.69. The 82 I_k and i determinations yield a correlation coefficient of 0.74. However, the overall correlations of P_{max} and i (and of I_k and i) need not reflect causal relationships; I_k and P_{max} changes were shown earlier (Figs. 10 and 30) to be highly correlated with temperature. Since temperature and i show generally similar seasonal trends (cf. Figs. 29 and 31) a similar degree of correlation of P_{max} and I_k with i is inevitable.

If the correlation with temperature is assumed to indicate a causal relationship, then the evidence for 'sun'/'shade' adaptation may be only apparent. Whilst a causal relationship between temperature and P_{max} (or I_k) does not preclude an influence of light history, it does make such an influence difficult to demonstrate unequivocally from seasonal data - a dilemma not uncommon in ecology.

It was mentioned earlier that during particular periods P_{max} variations appeared to be independent of those on temperature. During these periods an inverse relationship between population density (n) and P_{max} was apparent.

The possibility was investigated that reduction in the depth of the euphotic zone (z_{eu}) accompanying population increase, might induce 'shade' adaptation (lower P_{max} values) in the phytoplankton and that increase in z_{eu}, as the phytoplankton population declined, might correspondingly lead to 'sun' adaptation (and higher P_{max} values).

However, the depth of the euphotic zone is not the only factor determining the underwater light-climate experienced by the phytoplankton. This is also determined by total daily irradiance (ΣI_o) which is a

seasonally variable characteristic (Fig. 44). Thus, a reduction in z_{eu} could be offset, in terms of i, by an increase in ΣI_o or vice versa.

Therefore, during the inverse relationship periods, when temperature appeared to be unimportant in controlling P_{max}, evidence was sought of a relationship between P_{max} and light history (i). The constant P_{max} during April and May 1969 was associated with a relatively constant level of i and the subsequent increase in P_{max} (June-August), approximately paralleled the increase in i. A similar, but less 'clear-cut', relationship between P_{max} and i is seen during the 1968 inverse relationship period (April to August). These observations suggest that 'sun'/'shade' adaptation may have played some part in determining P_{max} in 1968 and 1969 but again a dilemma of interpretation arises because population density-dependent factors, other than reduced z_{eu}, may have operated, in parallel to i, to depress P_{max} as population (n) increased.

Evidence against the general involvement of 'sun'/'shade' behaviour in inverse relationships between n and P_{max} is suggested by evidence from 1970 and 1971. In 1970 the constant P_{max} during the population increase in May and June was associated with a marked decline in i. The reduction in P_{max} as population density increased between January and March 1971 was not associated with any decrease in i.

Overall, evidence from seasonal data of an influence of light history on P_{max} and I_k is inconclusive.

Pretreatment of samples in light or darkness in the laboratory

A number of observations were made on the influence of continuous light or dark pretreatment of samples on their subsequent photosynthetic capacity and chlorophyll a content.

Method

1 l samples were incubated, in cottonwool-stoppered 2.5 l 'Pyrex' conical flasks, on a glass shelf above a pair of 65/80 W 'daylight' fluorescent tubes. These provided a light intensity of 7.5 klux (38 kerg/cm^2.sec) at the base of the flasks. The flasks were shaken by hand periodically to maintain cells in suspension. During the night a small amount of sedimentation occurred. The dark pretreatment flasks were covered with several layers of black cloth and incubated at the same temperature (20-22°C) as the light pretreatment flasks. An isolated experiment (17.3.70) studied only dark pretreatment and was carried out at 9-10°C.

All samples experienced a period of darkness of approximately 3 hours during transport from the loch to the laboratory. On arrival at the laboratory the 'initial' chlorophyll a content (n) and photosynthetic capacity (P_{max}) of the sample were determined. Photosynthetic rate was measured in the laboratory tank (described in the Methods section) using 3 hour exposure periods. Two or three light intensities were used to ensure that light-saturation was achieved. The preliminary 3 hour dark period did not appear to have any significant effect on the 'initial' chlorophyll a content or photosynthetic capacity of the sample when compared with a parallel sample measured earlier in the loch.

Sub-samples were taken at 18-24 hour intervals and their chlorophyll a content and photosynthetic capacity were determined.

Results are summarised in Figs. 32 and 33.

Light pretreatment (Fig. 32): results and discussion

A decline in the light-saturated rate of photosynthesis per mg chlorophyll a (P_{max}) occurred in all samples when exposed to 7.5 klux continuous fluorescent illumination.

Fig. 32 Effect of light pretreatment (7.5 klux fluorescent illumination) on the chlorophyll a content and photosynthetic capacity(P_{max}) of samples collected on 25.6.70 (●—●), 29.6.70 (▲--▲), 21.7.70 (■···■), 14.8.70 (○—○) and 27.8.70 (△--△). Temperature, 20-22°C.

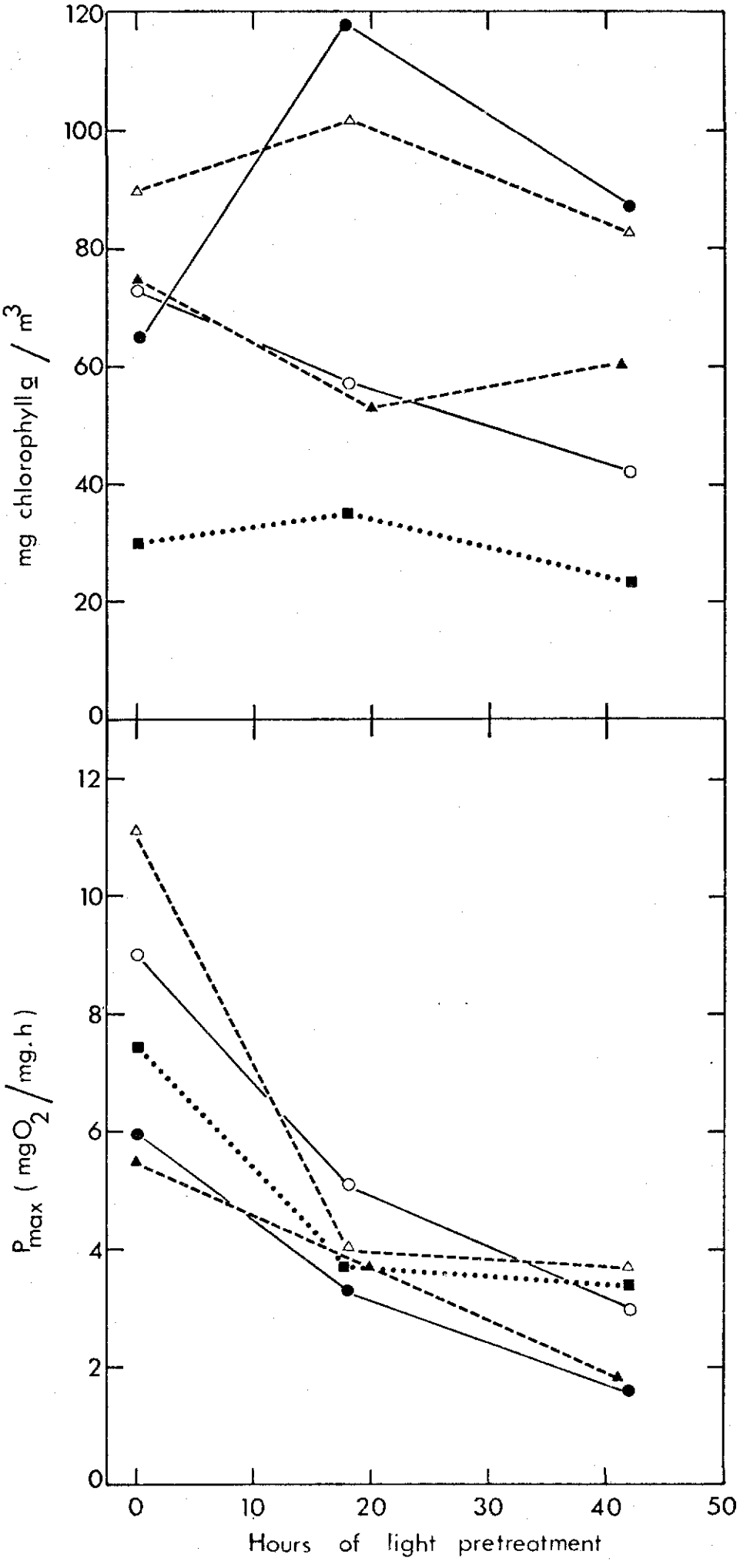


Fig. 32 Legend opposite.

In general the rate of decline of P_{max} was more rapid during the first 18 hours of exposure than in the following 24 hours. The maximum percentage decrease in P_{max} after 42 hours exposure was 71% (25.6.70 sample).

Changes in chlorophyll *a* content varied between samples. Three out of five samples showed an increase (usually slight), in chlorophyll *a* content per unit volume of water after 18 hours illumination, followed by a decrease in the following 24 hours. Two samples showed a decrease during the first 18 hours followed by either a slight increase or a further decrease in the following 24 hours.

Similar patterns of light inhibition, usually attributed to photo-oxidative destruction of enzymes and inactivation or destruction of chlorophyll *a*, have been described by Steemann Nielsen (1952b, 1962b) and Steemann Nielsen & Jørgensen (1962) using light intensities of 30-100 klux and by Kok (1956) using a light intensity "well over 10 klux". Different species exhibit different degrees of sensitivity to light inhibition (Jørgensen, 1964a). Incandescent illumination was used by all these authors. According to Steemann Nielsen & Hansen (1961, p. 598), 1 klux (incandescent light) is equal to 28.6×10^{-2} cal/cm^2.h. Therefore, 1 klux (incandescent light) is equal to 3.3 kerg/cm^2.sec. A light intensity of 38 kerg/cm^2.sec (7.5 klux), as provided here by fluorescent tubes, is equivalent to 12 klux of incandescent light. Thus, the light intensity causing inhibition in the Loch Leven experiments was considerably lower than the light intensities employed by the authors quoted above. However, since these authors did not investigate the effects of lower light intensities this does not necessarily reflect a greater sensitivity of Loch Leven plankton to light inhibition. Furthermore, light inhibition effects are dependent on length of exposure as well as on light intensity (see e.g.

Steemann Nielsen, 1952b). Therefore, inhibition at a low light intensity in the relatively long exposures used in the Leven experiments is compatible with inhibition in shorter exposures at higher light intensities.

The inhibitory effects of 30 klux reported by Steemann Nielsen (1962b) were confined to Chlorella cells grown at a low light intensity (3klux) and then illuminated for a short time (3 h) at 30 klux; longer exposure (ca 35 h) of these 3 klux-grown cells induced 'sun' adaptation and increased photosynthetic capacity. In contrast, the present results show no evidence of 'sun' adaptation in exposures of up to 42 hours even though the light intensity supplied in the laboratory was probably higher than the mean light intensity previously experienced by the phytoplankton in the lake. Absolute comparison of laboratory and field light intensities is not valid for reasons given earlier.

Since the light intensity in the laboratory was probably higher than the average light intensity in the lake, it seems unlikely that the decline in photosynthetic capacity could be due to 'shade' adaptation.

Poor nutritional conditions were reported by Saijo & Ichimura (1962) to increase susceptibility to light inhibition. The results of nutrient enrichment experiments using Loch Leven water (p.120) supported this observation.

Dark pretreatment (Fig. 33): results and discussion

On each occasion an increase in the chlorophyll a content of the sample, of between 16 and 33%, was observed after 18-24 hours of incubation in darkness. Further dark incubation led to a decrease in chlorophyll a content.

Fig. 33 Effect of dark pretreatment on the chlorophyll a content and photosynthetic capacity (P_{max}) of samples collected on 29.6.70 (▲--▲), 21.7.70 (■•••■), 14.8.70 (o—o), 27.8.70 (△--△) and incubated at 20-22°C. Sample collected 17.3.70 (□••□) incubated at 9-10°C.

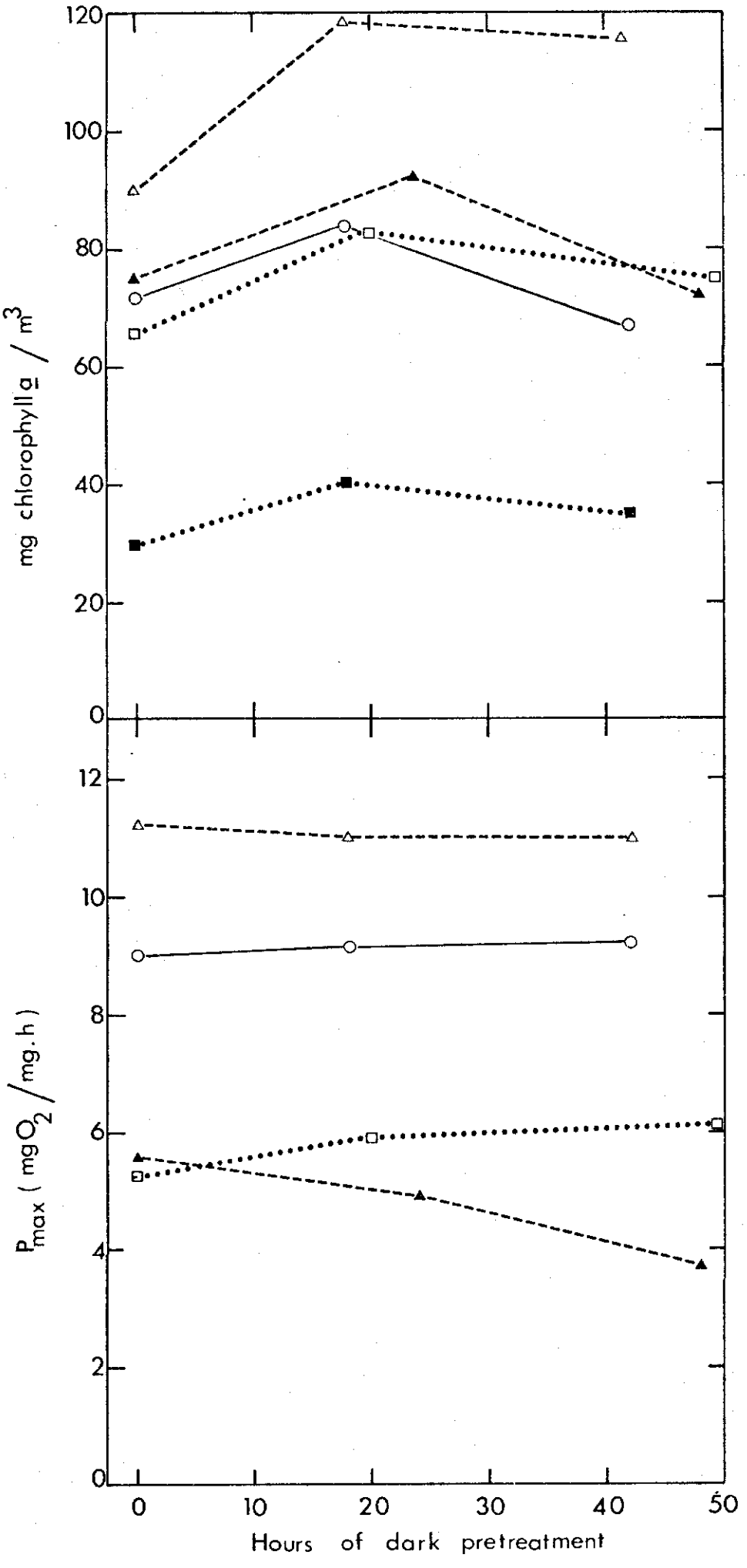


Fig. 33 Legend opposite.

In general dark pretreatment of up to 50 hours had very little effect on the photosynthetic capacity (P_{max}) of the samples investigated. The sample (29.6.70) showed a 33% decrease in P_{max} after 48 hours of darkness.

Literature reports on the influence of dark storage are variable. Yentsch & Reichert (1963) found that P_{max} increased as a function of the duration of dark storage, whilst Yentsch & Lee (1966) report a decrease in P_{max} following a period of darkness. Talling (1966) found that dark pretreatment led to an increase in the photosynthetic activity of surface samples from Blelham Tarn; this was attributed to reversal of a depression produced by prior illumination in nature. Dark reactivation after strong illumination has been described by Steemann Nielsen (1962b). For populations from Windermere, Talling (1966) found no pronounced increase in photosynthetic activity following darkness; on one occasion a decline in activity was observed and attributed to the senescent nature of the population concerned. In the experiments reported here, the sample showing a reduction in P_{max} after dark storage was taken during a phase of population decline and may have been senescent also; the samples which showed no decrease in capacity were all taken from the lake when population density was increasing.

Decline in photosynthetic capacity on dark storage is not generally considered equivalent to the decline occurring when 'sun' cells are transferred to a low light intensity. Transformation of 'sun' cells to 'shade' cells requires an input of energy; Steemann Nielsen, Hansen & Jørgensen (1962) found that cells of Chlorella vulgaris grown at a high light intensity showed less change in photosynthetic behaviour after dark storage than after a period at low light intensity.

As well as reversing depression due to high light intensity *per se*, dark storage could be expected to relieve inhibition due to nutrient depletion or increased pH and oxygen levels produced by photosynthesis in the light. Variability in the response of samples of different origin to dark storage is, therefore, not surprising and most likely reflects differences in the previous light and nutrient histories of the samples, as well as their species composition.

The lack of any increase in photosynthetic capacity of the Leven samples possibly suggests that they had not previously suffered unfavourable light and nutrient conditions.

d) P_{max} in relation to pH and CO_2 supply

Introduction

In natural waters, increased pH, due to photosynthetic CO_2 depletion, has often been correlated with reduced rates of photosynthesis in dense algal crops (Steemann Nielsen, 1955a; Bartsch & Allum, 1957; Wright, 1960; Ahlgren, 1970).

The influence of pH on growth and photosynthesis has been more clearly established from laboratory work with uni-algal cultures. Thus Felföldy (1960, 1965) found that photosynthesis by *Chlorella vulgaris* was inhibited above pH 9 but that *Scenedesmus* continued to photosynthesise up to pH 10.8. There is evidence that blue-green algae may grow best under alkaline conditions (Fogg, 1956; Jackson, 1964). Moss (1973) found distinct differences in the maximum pH tolerated by eutrophic and oligotrophic groups of freshwater algae and considered pH to be an important factor determining algal distribution patterns. Shapiro (1973) also found evidence of pH/CO_2 control of algal quality.

The influence of pH on photosynthetic capacity at Loch Leven is discussed below on the basis of seasonal observations and laboratory experiments.

Seasonal change in pH and its relationship to seasonal change in population density and photosynthetic capacity of the phytoplankton

pH values, measured near midday, were generally uniform throughout the euphotic zone and varied seasonally between 7.5 and 10.0 (Fig. 29). As illustrated by Talling & Talling (1965, Fig. 3) and Talling (1970, Fig. 2) the pH of natural waters at air-equilibrium rises with increasing alkalinity. At Loch Leven titration alkalinity showed relatively little seasonal variation within the range 1.0-1.6 m-equiv./l. Air equilibration at 17°C of samples of alkalinity 1.1 and 1.5 m-equiv./l produced final pH values of 8.1 and 8.3 respectively. These observed air-equilibrium pH values agree closely with those expected on theoretical grounds (see Stumm & Morgan, 1970, pp.128-129; Thomas & Trussell, 1970, Fig. 3). Change in alkalinity was not therefore sufficient to account for the observed seasonal pH changes. These show fluctuations both above and below air-equilibrium and are largely a consequence of the biological activities occurring in the water column and at the sediment-water interface. Of the various biological reactions that may alter pH, many of which are discussed by Goldman et al (1972), CO_2 changes, due to photosynthesis and respiration, usually play a dominant role.

Increase in pH generally accompanied increase in algal population density (Fig. 29). The photosynthetic removal of inorganic carbon, at a rate faster than it can be replaced by community respiration and atmospheric equilibration, causes a readjustment of the CO_2-HCO_3^--CO_3^{2-} buffer system with consequent increase in pH. (The equilibria involved in the CO_2-HCO_3^--CO_3^{2-} buffer system are described later.) As population density declined the pH of the water decreased. Values below the air-equilibrium pH value were recorded, indicating that when population density was low, community respiratory processes produced CO_2 at a

rate faster than it could be assimilated by photosynthesis or lost to the atmosphere. Four periods were identified earlier from Fig. 29, during which an inverse relationship between population density (n) and photosynthetic capacity (P_{max}) could be recognised. Seasonal changes of population density generally paralleled those of pH. This suggested that the limitation of photosynthetic capacity, observed at high population densities, may reflect an adverse influence of conditions associated with high pH values. Laboratory experiments which support this idea are described below.

Laboratory experiments on the influence of pH on photosynthetic capacity (P_{max})

The experiments were carried out during March 1971. Prior to the experiments, water temperature had remained relatively constant but photosynthetic capacity (P_{max}) had been declining as population density (n) and pH increased (Fig. 29). Increased pH was suspected as the factor responsible for the inverse relationship between n and P_{max}. The effect on P_{max} of lowering pH (by blowing in CO_2-rich lung air) was measured in the laboratory. Alteration of pH by CO_2 changes does not alter titration alkalinity, which was 1.22 m-equiv./l in each experiment. In one experiment only, pH was increased by addition of a small amount of NaOH, with consequent increase (not measured) in alkalinity. Changes in total and free CO_2 concentrations with pH change are illustrated in Table 3. The method of calculation of these values is described later (p.88). In view of the possible effects of oxygen concentration on photosynthesis, discussed later (p.104), it should be noted that the lung air additions caused very little change in dissolved oxygen concentration (less than 1 mg/l). The chlorophyll *a* content of each sample was determined and its photosynthetic capacity, with and without various amounts of added CO_2, was measured in the

Table 3. Changes in concentration of total CO_2, free CO_2 and OH^- ion at different pH values for constant titration alkalinity (1.22 m-equiv./l). Temperature 5°C.

pH	OH^- m-equiv./l	Total CO_2 mg/l	Total CO_2 µmol/l	Free CO_2 mg/l	Free CO_2 µmol/l
6.90	<0.001	67.10	1525	21.960	499
7.60	<0.001	57.95	1317	4.270	97
8.15	<0.001	54.66	1242	1.220	28
8.50	<0.001	53.68	1220	0.549	12
8.70	<0.001	53.07	1206	0.354	8
9.00	0.002	52.37	1190	0.134	3
9.30	0.004	51.07	1161	0.073	1.7
9.75	0.011	46.55	1058	0.018	0.4
10.25	0.033	39.17	890	0.005	0.1

illuminated laboratory water bath. Bottles were exposed for 3 h at loch temperatures.

The results of five such experiments are shown in Fig. 34. P_{max} increased with reduction in pH from 9.8 to 8.0 in each experiment. The maximum percentage increase in P_{max} due to CO_2 addition was 78% (16.3.71 experiment). When pH was increased with NaOH on 19.3.71 from 9.30 to 9.80 and 10.25, P_{max} was reduced by 54% and 77% respectively. The highest P_{max} value recorded on 19.3.71, at pH 8.50, was 588% higher than P_{max} measured at pH 10.25 on the same day.

These experimental results lend support to the earlier suggestion, based on seasonal observations, that CO_2 depletion was directly or indirectly (through its effect on pH) responsible for reduced P_{max} values in dense crops.

The % increase in P_{max} due to CO_2 addition was reduced when pH was reduced below 8.0. This may have been due to an initial 'shock' effect of a sudden large change in the pH/CO_2 environment, or to a direct inhibitory effect of high CO_2 and/or low pH. Toxic effects of high CO_2 concentrations have been reported by Steemann Nielsen (1953, 1955b) and Fogg & Than-Tun (1960).

Variation in the concentration of total dissolved CO_2 and free CO_2 at different pH values

Total dissolved CO_2 exists in three forms, free dissolved CO_2 as such (of which a small proportion hydrates to form carbonic acid), bicarbonate and carbonate ions. These CO_2 fractions are in equilibrium determined by the following equations:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \qquad (1)$$

$$CO_2 + OH^- \rightleftharpoons HCO_3^- \qquad (2)$$

$$H_2CO_3^* \rightleftharpoons H^+ + HCO_3^- \qquad (3)$$

$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+ \qquad (4)$$

$$H_2O \rightleftharpoons H^+ + OH^- \qquad (5)$$

$H_2CO_3^*$ refers to free CO_2 as such, plus H_2CO_3.

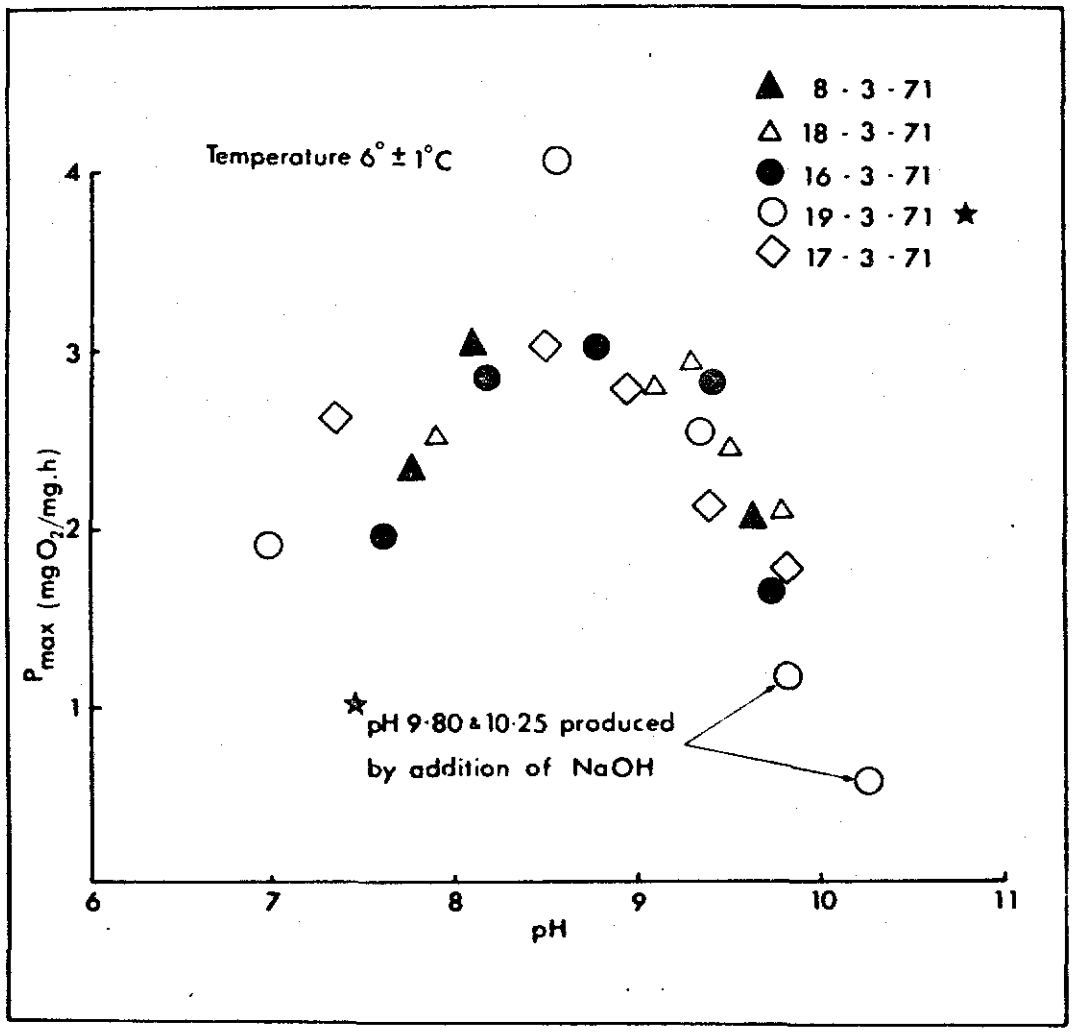


Fig. 34 Results of five laboratory experiments on the effect of lowering pH (by CO_2 in lung air) or increasing pH for two points only on 19.3.71 on light-saturated rate of photosynthesis per mg chlorophyll a (P_{max}). Dominant species: Cyclotella pseudostelligera.

The CO_2-HCO_3^--CO_3^{2-} equilibrium system and its pH relationships, first established by Faurholt (1924), are discussed in detail by Hutchinson (1957), Skirrow (1965) and Stumm & Morgan (1970). The dissociation constants and the rate constants involved are reviewed by Kern (1960). The ionisation reactions (3) and (4) are virtually instantaneous but the hydration/dehydration reactions (1) and (2) are relatively slow (seconds).

As a result of the above equilibria, at any given temperature, the molecular proportions of the various CO_2 fractions vary with pH. As illustrated by Hutchinson (1957, Fig. 186), below pH 6 most of the total CO_2 exists as free CO_2; between pH 7 and pH 9 bicarbonate is dominant; above pH 9.5 carbonate becomes significant and becomes the dominant CO_2 fraction above pH 10.5. Absolute concentrations of the various CO_2 forms, and of total CO_2, may be calculated from pH and alkalinity using the dissociation constants of carbonic acid and the ionic product of water appropriate to the temperature and ionic strength of the medium concerned. The derivation of the calculation procedure is given by Dye (1952) together with tables and nomographs to simplify the mathematics involved. The hydroxide ion concentration (determined by pH and temperature) is subtracted from the titration alkalinity and the remainder, used in the calculations, is assumed to be due to the anions of carbonic acid. Nomographs constructed by Dr. R. B. Wood from the tables of Dye (1952) and Karlgren (1962) were used here to calculate total CO_2, free CO_2 and hydroxide ion concentrations in the samples used in the pH experiments described above.

Results for a selection of the pH values used in the laboratory experiments are shown in Table 3. They illustrate the decline in total CO_2 concentration, and proportionately greater decline in free CO_2 concentration, which accompanies increased pH at constant alkalinity.

As total CO_2 declines and pH rises there is a shift in the molecular proportions of the remaining inorganic carbon away from free CO_2 towards a predominance of HCO_3^- and CO_3^{2-}. This accounts for the relatively greater changes in free CO_2, as compared with those of total CO_2. Increase in pH due to CO_2 uptake does not alter titration alkalinity (unless carbonates are precipitated) since the charge balance, which defines alkalinity, is maintained. The hydroxide ion component of the alkalinity, however, increases with increase in pH, as the data in Table 3 illustrate. Assuming anions of other weak acids to be absent, it is clear that if all the dissolved inorganic carbon were removed by photosynthesis then titration alkalinity would be due entirely to hydroxide ions. The pH of a solution with hydroxide ion concentration equal to a given titration alkalinity can be determined from the ionic product of water at the appropriate temperature (values of which are given by Hutchinson, 1957, Table 11, p.211), or read off from nomographs such as those of Dye (1952). The pH at which alkalinity is due solely to hydroxide ions may be considered the 'ceiling' pH for the water body concerned in that pH cannot be increased further due to photosynthetic CO_2 uptake. At this 'ceiling' pH value net photosynthesis in an enclosed system is impossible (since no CO_2 in any form remains). In an open system at the 'ceiling' pH, photosynthesis will be limited to that which can be sustained by CO_2 diffusing to the water from the atmosphere. The rate of CO_2 influx then becomes the rate-determining factor forphotosynthesis.

Since the ionic product of water varies with temperature, so also does the 'ceiling' pH value for a given alkalinity. For Loch Leven, when alkalinity is 1.2 m-equiv./l the 'ceiling' pH is 11.4 at 15°C and 11.8 at 5°C. When alkalinity is 1.4 m-equiv./l the 'ceiling' pH is 11.5 at 15°C and 11.9 at 5°C. Clearly the highest pH values (up to pH

10) reached in Loch Leven are below the theoretical upper limit imposed by its alkalinity. This suggests that at pH 10 some carbonate alkalinity remains and/or that some anions (other than HCO_3^-, CO_3^{2-} and OH^-) are contributing to the alkalinity.

The following 'pH drift' experiment was carried out to determine whether Loch Leven algae were capable of increasing the pH of the loch water to values greater than 10, and if so whether they could survive such high pH values.

'pH drift' experiment to determine the maximum pH Loch Leven algae could produce in loch water

A net-tow concentrate of Loch Leven phytoplankton, containing species of Pediastrum, Staurastrum and Asterionella, was obtained on 3 August 1971. Net-plankters, present in the second half of 1971, had been virtually absent in earlier years studied. To this extent results obtained using the above net concentrate may not be representative of the phytoplankton of earlier years. The concentrated suspension, which had a chlorophyll a concentration of 740 mg/m^3, a titration alkalinity of 1.4 m-equiv./l and an initial pH of 7.85, was siphoned into a 200 ml 'Pyrex' stoppered reagent bottle and incubated under continuous fluorescent illumination (6.5 klux) at 15°C in a Fisons controlled environment growth cabinet (model 140 G2, Fisons Scientific Apparatus Ltd., Loughborough, Leicestershire, England). The pH of the sample was recorded at intervals, a procedure which introduced a small air bubble equivalent to the volume of liquid displaced by the pH electrode. The electrode was removed from the suspension between pH readings.

Results are shown in Fig. 35. The pH/time curve appeared to reach a plateau at pH 11.3. After no further increase in pH had occurred for 15 hours, the pH of the suspension was reduced to its

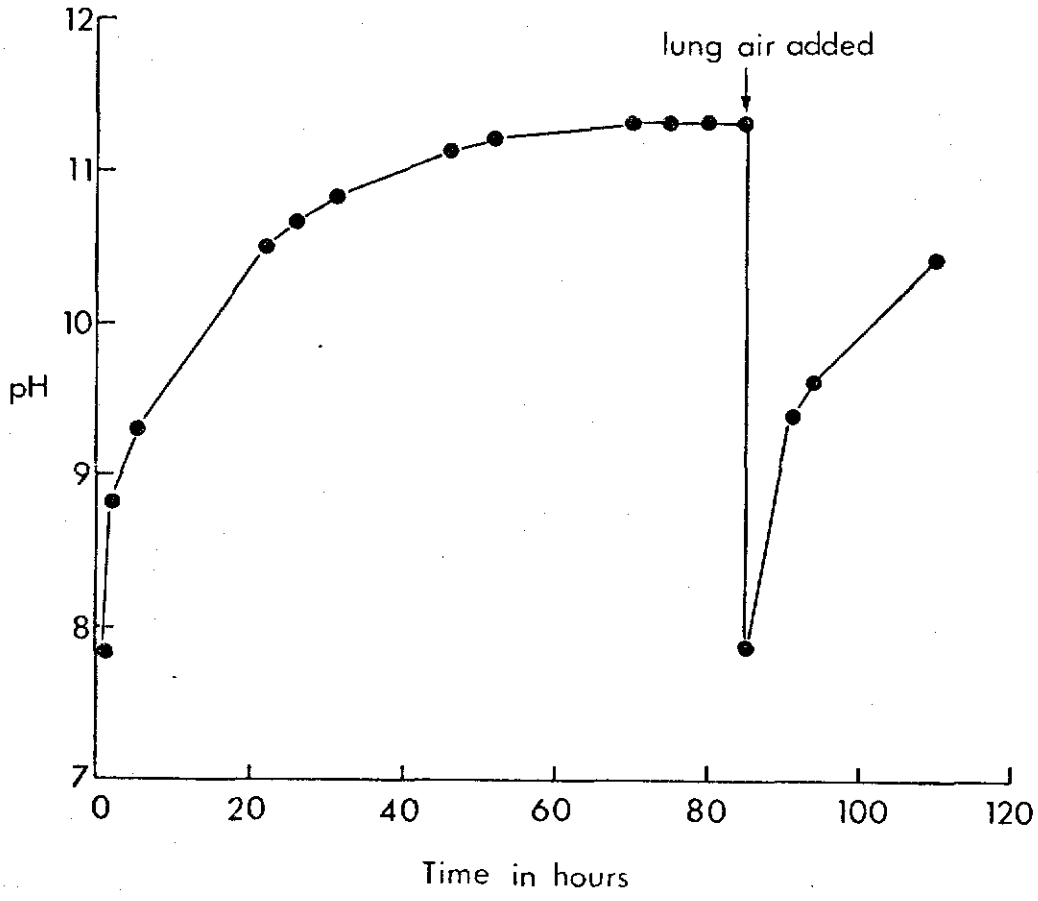


Fig. 35 Change in pH with time in a concentrated suspension of Loch Leven phytoplankton (740 mg chlorophyll a/m^3) incubated at 6.5 klux fluorescent illumination and 15°C. Lung air added after 85 hours. Dominant algae: Pediastrum, Staurastrum, Asterionella.

its original value, without change in total alkalinity, by aeration with CO_2-rich lung air. Incubation was then continued as before whereupon pH increased again, reaching values well above the air-equilibrium pH value ($\sim$8.1). The increase in pH was not therefore due solely to loss of CO_2 from the super-saturated solution to the air-bubble enclosed in the bottle, but must be attributed to continued photosynthetic activity by the algae. This shows that the particular algae used in the experiment could survive a pH of 11.3 for 15 hours. The results do not reveal, however, whether photosynthetic capacity was completely unaffected by the experimental treatment. Some detrimental effect of continuous illumination per se, quite apart from any pH effect, might be expected in view of the results reported in the section on light-history effects.

The maximum pH (11.3) reached in this experiment closely approached the theoretical 'ceiling' pH value (11.5) for an alkalinity of 1.4 m-equiv./l and a temperature of 15°C. There are a number of possible reasons why this 'ceiling' pH value was not actually reached:-

1. The rate of CO_2 influx from the air bubble trapped in the bottle, together with CO_2 released in respiration, may have been sufficient to balance the rate of gross photosynthetic CO_2 uptake at pH 11.3.
2. The algae may have been incapable of taking up inorganic carbon (in any form) from the low concentration (12 mg/l, total CO_2) remaining at pH 11.3. Most of this carbon is present as CO_3^{2-} and probably unusable.
3. High pH may have inhibited metabolism generally.
4. The increase in carbonate ions at higher pH values may have led to precipitation of $CaCO_3$, with a consequent lowering of alkalinity and its associated upper pH limit. An attempt was made to investigate this possibility by following the pH change with time due to

photosynthesis by algae suspended in a sodium bicarbonate solution of the same alkalinity as loch water. Because of the greater solubility product of Na_2CO_3 compared with $CaCO_3$, precipitation of carbonates should be eliminated when Ca^{2+} is replaced by Na^+.

Results of several such experiments were inconclusive, however, since the maximum pH reached in the bicarbonate solution was always lower than that achieved by a parallel sample in loch water. This may have been due to the generally less favourable chemical environment of the bicarbonate solution ultimately limiting photosynthesis in the rather long incubation periods used.

Enrichment with nitrate and phosphate at the start of the experiment did not increase the final pH value reached in loch water or bicarbonate solution.

5. The remaining non-hydroxide alkalinity at pH 11.3 may have been due to anions other than HCO_3^- and CO_3^{2-}. It will be shown later that this is a likely possibility.

The results of the 'pH drift' experiment suggest that the maximum pH (10) recorded in the loch was not determined by the inability of the algae to photosynthesise at higher pH values, nor could it be entirely attributed to the possible presence of non-carbonate, non-hydroxide alkalinity at pH 10.

In the open lake, influx of CO_2 from the atmosphere and from respiratory processes in the aphotic zone, would slow the rate of pH increase compared with that occurring in an enclosed algal suspension. Furthermore, at high pH values, the rate of CO_2 influx may be accelerated due to reaction (2) above, in which CO_2 reacts directly with OH^- (Bolin, 1960). Thus photosynthetic CO_2 uptake may ultimately itself restrict further CO_2 depletion and pH rise, by producing conditions more favourable for CO_2 influx from air to water. The high

degree of turbulent mixing at Loch Leven, which results from its shallowness and wind-exposed situation, also tends to favour gaseous equilibration with the atmosphere (Kanwisher, 1963). These factors would all tend to prevent the 'ceiling' pH actually being reached in the loch.

Investigation of non-carbonate, non-hydroxide alkalinity

The above estimations of total and free CO_2 concentrations (Table 3) assumed that the loch water behaved as a simple carbonate solution, with total alkalinity due solely to the anions of carbonic acid (carbonate alkalinity) plus hydroxide ions (hydroxide alkalinity). In fact, titration alkalinity also measures the concentrations of anions of acids weaker than carbonic acid; these may include silicates, borates, sulfides, phosphates and the anions of organic acids. These non-carbonate, non-hydroxide buffer components are normally present in much lower concentrations than the carbonate species. Also, being weaker acids than carbonic acid, a smaller proportion of their total concentrations are ionised, and present as the acid-titrating species, at any given pH value. Their contribution to total alkalinity is, therefore, usually minor at low pH values but increases, as degree of ionisation increases, at higher pH values.

Total dissolved CO_2 can be measured directly, e.g. by vacuum extraction of an acidified sample, followed by manometric (e.g. Slyke & Neill, 1924) or conductimetric (e.g. Milburn & Beadle, 1960) measurements, or by infrared gas analysis (e.g. Tregunna & Thomas, 1968). For accurate indirect calculation of dissolved CO_2 from alkalinity, it is necessary to correct titration alkalinity, not only for its OH^- component, but also for other non-carbonate weak acid anions. Such correction, particularly important at high pH, could be achieved if the concentrations of the non-carbonate species

were measured and their degree of ionisation calculated, from their dissocation constants and the pH of the water sample. The necessary measurements and calculations would, however, be time-consuming. A more convenient method involves a step-wise potentiometric titration procedure, originally proposed by Gran (1952) and applied to CO_2 determination in seawater by Dyrssen (1965) and Edmond (1970). Recently, Talling (1973) has applied the procedure in a simplified form to fresh waters. The derivation of the basic relationships involved in the Gran titration procedure for CO_2 estimation are described in detail by Edmond (1970) and Stumm & Morgan (1970, pp. 155-158). The Gran method has several advantages over the conventional pH-alkalinity method of CO_2 estimation (e.g. that of Dye, 1952):-

1. Most non-carbonate buffer components do not change their extent of ionisation markedly in the pH region (ca 4.5-7.5) used for the CO_2 calculations. Their presence does not, therefore, interfere with total CO_2 estimation by the Gran method, which is based on the difference between two end-points.

2. The transformation of potentiometric titration curves into straight lines, by the Gran procedure, allows precise end-point location by simple extrapolation. Titration methods using indicators are less precise. Indicators change colour at particular pH values whereas the true end-point pH,at which all CO_3^{2-} and HCO_3^- has been converted to H_2CO_3, depends on the molarity of the resulting acid solution. The pH at the end-point of an alkalinity determination increases with decreasing total CO_2 concentration (Stumm & Morgan, 1970). Also, a subjective element (with consequent risk of error) is involved in a visual assessment of indicator colour change.

3. Absolute calibration of the pH meter or mV meter is unimportant in the Gran method since this is based on relative changes in a stepwise titration.

Potentiometric (pH) titrations of Loch Leven water, followed by graphical Gran transformations of the results, were used to check the accuracy of CO_2 estimations by the conventional pH-alkalinity method at differing initial pH values. The working procedure was essentially as described by Talling (1973). Samples of known volume (30-50 ml) were titrated with 0.25 N HCl from a 0.5 ml 'Agla' micrometer syringe burette (Wellcome Reagents Ltd., Beckenham, Kent, England) or with 0.01 N HCl from a 10 ml 'Jencons' vertical free-piston burette (Jencons (Scientific) Ltd., Hemel Hempstead, Herts., England). Magnetic stirring was used, except during pH readings. The antilogarithmic Gran functions, F_1 and F_2, were calculated from at least three pH readings (to 0.02 unit) in each of the ranges 8.0-6.5 and 4.4-3.4, according to equations (1) and (2) of Talling (1973). F_1 and F_2 values were plotted against v (titrant volume in ml) as illustrated for two samples in Fig. 36. From v_2 (the titrant volume at the F_2 end-point) total alkalinity (V_2), in m-equiv./l, was calculated from:

$$V_2 = v_2 \frac{1000}{V_s} \cdot n$$

where V_s = sample volume (ml) and n = normality of acid used.
From v_1 (the titrant volume at the F_1 end-point) V_1, in m-equiv./l, is given by:

$$V_1 = v_1 \frac{1000}{V_s} \cdot n$$

(V_1 = normality of sample corresponding to Gran F_1, v_1 end-point)

Then,

$$\text{total } CO_2 \text{(mmol/l)} = V_2 - V_1$$

$$= \frac{1000}{V_s} n(v_2 - v_1)$$

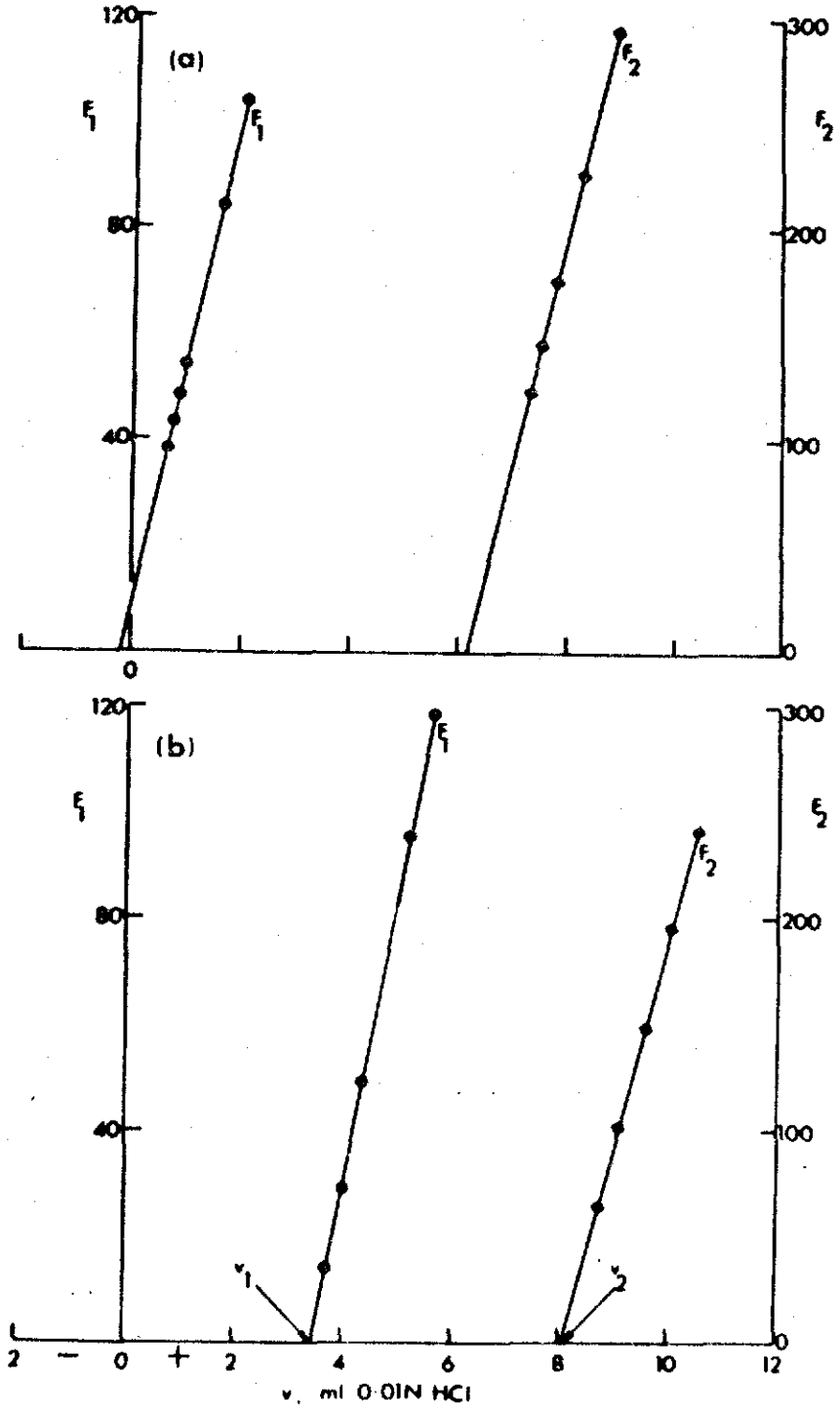


Fig. 36 Gran plots of pH titrations with HCl of Loch Leven water with initial pH 7.9(a) or 10.2(b).

The samples used to illustrate Gran plots in Fig. 36 had different initial pH values and show the effect of increasing pH due to photosynthetic CO_2 withdrawal on the intercept v_1. v_1 is a measure of free CO_2 excess ($-v_1$) or deficit ($+v_1$) relative to the classical (phenolphthalein) end-point (Mackereth, 1963). During the period of the investigation of this aspect the loch itself did not yield particularly high pH values. In order that a wide range of values (within the range previously encountered at the loch) could be studied, higher pH values were produced in the laboratory without change in total alkalinity, by photosynthesis of dense net phytoplankton samples. Samples titrated on 8.10.71, 14.8.72, 16.8.72 and 18.8.72 had had their pH increased in this way.

Total CO_2 concentrations calculated from Gran plots are compared in Table 4 with parallel estimations made by the conventional pH-alkalinity method. The two methods agreed closely at lower pH values, but a tendency for the pH-alkalinity method to give values up to 14% higher than the Gran method was observed at higher pH values.

These results show that the loch water does not behave as a simple carbonate solution at high pH (above about pH 8.5), when a non-carbonate, non-hydroxide component forms a significant contribution to total alkalinity. Neglect of this component can lead to overestimation, by calculation, of CO_2 concentrations.

The nomographs constructed by Dr. R. B. Wood, and used here to calculate CO_2 concentrations from pH and alkalinity, are strictly valid only for waters with ionic strength of 0.001 or less. The approximate ionic strength of Loch Leven water was estimated, using equation 23 of Stumm & Morgan (1970, p.150), as 0.0025. The first and second dissociation constants of carbonic acid (K_1, K_2) and the dissociation constant of water (K_w), which had been used for the Wood nomographs, were

Table 4. Total CO_2 concentrations, calculated from pH and alkalinity determinations or determined from Gran plots, of samples of Loch Leven water with different initial pH values. The total titration alkalinity of the samples (V_2) is also indicated. Temperature 20°C.

Date	Total titration alkalinity (V_2) m-equiv./l	Initial pH	Total CO_2 (mg/l) calculated (pH/alk.)	observed (Gran)
12.5.71	1.240	7.90	55.8	57.0
20.5.71	1.378	8.00	61.8	60.6
20.5.71	1.347	8.25	59.2	58.9
8.10.71	1.476	9.50	57.7	53.6
14.8.72	1.481	9.82	51.8	46.5
16.8.72	1.481	9.76	52.4	46.9
18.8.72	1.468	10.20	42.5	37.0

corrected for an ionic strength of 0.0025, yielding the apparent constants, K_1', K_2' and K_w'. The correction used the Guntelberg approximation recommended by Stumm & Morgan (1970, p.149) and gave $pK_1' = pK_1 - 0.02$, $pK_2' = pK_2 - 0.09$, $pK_w' = pK_w - 0.02$. Corrected (apparent) dissociation constants were used to recalculate CO_2 concentrations, from pH and alkalinity, using the formulations and nomographs given by Talling (1973). Results showed that values obtained using corrected dissociation constants were, at most, about 3% lower than those obtained using the Wood nomographs. Neglect of a correction for ionic strength of the water does not, therefore, account for the differences observed (Table 4) between the Gran and pH/alkalinity methods of CO_2 estimation.

Discrepancies between direct CO_2 measurements and indirect estimates based on carbonate equilibria have been reported, particularly in the alkaline range, in a number of natural waters (Verduin, 1956a; Ganf, 1969; Wood, 1970; Talling, 1973). Talling (1973) points out, however, that the discrepancy was overestimated by Wood (1970) due to his neglect of hydroxide alkalinity.

In sea water, borates have long been recognised as buffer components additional to carbonates. A borate correction (estimated from the apparent dissociation constant of boric acid in sea water) is routinely employed, in addition to the hydroxide correction, in calculating CO_2 concentrations (Skirrow, 1965). The possible role of silicates in buffering of the oceans has been emphasised by Garrels (1965). Silicic acid has also been implicated by Talling (1973) as a possible source of non-carbonate alkalinity in fresh waters at high pH. Talling found, however, that correction for silicate did not remove all of the discrepancy between observed and calculated CO_2 concentrations in Esthwaite Water, and suggested that other, unknown, alkalinity components (e.g. anions of organic acids) may be involved.

If silicate is a dominant non-carbonate, non-hydroxide alkalinity component, the discrepancy between 'true' and calculated CO_2 contents may largely depend on whether high pH values result from diatom growth (with consequent silica depletion) or from growth of other, non-silica requiring, species. Discrepancies are likely to be greater in nutrient-rich waters because of the greater concentrations of possible interfering anions, and because of the greater likelihood of high pH values occurring due to the greater potential for algal growth in such waters.

The experiments described here suggest that the earlier calculations of CO_2 concentrations in the samples used to study pH effects on photosynthesis (Table 3) may be overestimates. This is particularly likely for the higher pH samples. Gran titrations were not carried out on these samples, so the degree of overestimation cannot be stated precisely. Results of Gran titrations on other samples suggest an overestimation of about 0-14% in samples of pH 8.5-10.2.

Discussion

Evidence has been presented above, based on seasonal observations and laboratory experiments, that photosynthetic CO_2 depletion with associated pH increase was responsible, at least in part, for reducing photosynthetic capacity (P_{max}) in dense crops at Loch Leven. Gran titrations showed that the extent of CO_2 depletion at high pH may have been greater than predicted by calculation.

Many published claims of direct or indirect CO_2-limitation of photosynthesis are not supported by experimental evidence (Steemann Nielsen, 1955a; Bartsch & Allum, 1957; Wright, 1960; Ahlgren, 1970). Experimental evidence of enhanced photosynthetic rates following inorganic C-enrichment of natural waters has, however, been reported (Ichimura & Saijo, 1958; Sakamoto, 1971; Schindler & Nighswander, 1970; Munawar, Verduin & Fatima, 1972). It is not completely clear,

however, from published observational or experimental evidence, whether effects of CO_2 depletion and enrichment are to be attributed to a direct effect of CO_2 concentration *per se*, or to some other effect of the associated pH change.

A rise in pH could affect algal metabolism in a number of ways:

1. Any increase in pH causes a readjustment of the CO_2-HCO_3^--CO_3^{2-} equilibrium system such that a smaller proportion of the total inorganic carbon remains as free CO_2. If the pH increase is caused by carbon removal from the system (as in photosynthesis) then the decline in free CO_2 concentration, for the same pH increase, is even greater.

If an alga is an obligate free CO_2 user, reduction in the instantaneous free CO_2 concentration could ultimately reduce its instantaneous photosynthetic rate when all available enzyme sites for CO_2 in the cell can no longer be occupied. Some authors, cited in reviews by King (1970) and Raven (1970), have attempted to measure the critical free CO_2 concentration for optimum photosynthetic rate. Results were variable (2.5-30 μ moles free CO_2/l), and depended on the species involved and on the degree of mixing during photosynthesis measurements. For Loch Leven, results in Fig. 34 showed that photosynthesis of a crop dominated by the diatom, *Cyclotella pseudostelligera*, began to decrease as pH increased above *ca* 9.0. If this effect is assumed to be due to free CO_2 depletion, rather than to an effect of pH *per se*, the corresponding critical free CO_2 concentration is approximately 3 μmol/l (Table 3).

Below the critical free CO_2 concentration, the rate of gross photosynthesis of an alga confined to free CO_2 as its direct C source will be determined by the rate at which CO_2 is supplied from the atmosphere, from non-algal respiration, and from the alkalinity reserve. The rate of CO_2 entry from the atmosphere is slow compared with the

rate at which the CO_2-HCO_3^--CO_3^{2-} equilibrium system can adjust (Skirrow, 1965). The rate at which CO_2 is supplied from the alkalinity reserve is determined by the temperature-dependent rate constants governing reactions (1) and (2) referred to earlier:

$$H_2CO_3 \rightleftharpoons CO_2 + H_2O \qquad (1)$$

$$HCO_3^- \rightleftharpoons CO_2 + OH^- \qquad (2)$$

The reaction yielding H_2CO_3 from HCO_3^- (equation 3, p. 87) is virtually instantaneous and does not limit the rate of dehydration of H_2CO_3. Reaction (1) occurs mainly at pH values below 8, whilst at pH values above 10 reaction (2) is dominant. Between pH values 8 and 10 both reactions occur and their relative importance cannot be distinguished (Kern, 1960). Calculation of the rate of CO_2 supply via (1) and/or (2), over a 3 hour exposure period, would be mathematically complex due to the progressive decline in substrate (H_2CO_3, HCO_3^-) concentration, and the necessity to take into account the rate of the back reactions also. This type of calculation has been attempted, in simplified form, by Rabinowitch (1951, chapter 27), Ganf (1969) and Goldman et al (1972). Ganf (1969) found that the rate of free CO_2 supply could not account for the rates of algal carbon increase he observed at pH 10.4, and therefore concluded that HCO_3^- must be used to some extent. Rabinowitch (1951) and Goldman et al (1972), on the other hand, found that CO_2 supply was adequate to meet the requirements of the algal crops they considered.

For Loch Leven, such calculations are complicated by the fact that its pH normally lies within the range where reactions (1) and (2) are indistinguishable. Because of this, theoretical estimates of the rate at which free CO_2 could be supplied from the alkalinity were not obtained. Consequently, it is not known whether observed rates of

photosynthesis could be accommodated on the basis of free CO_2 uptake alone, or whether direct HCO_3^- uptake must be invoked.

HCO_3^- uptake has been claimed for some algae, whilst others are apparently restricted to free CO_2 as inorganic C source. The extensive literature has been reviewed recently by Raven (1970) and Goldman et al (1972). Rigorous proof of direct HCO_3^- uptake is difficult to obtain experimentally since some free CO_2 is continually made available (at a rate which is difficult to estimate) from the bicarbonate reserve. Also, it is difficult to carry out experiments in which the concentration of a particular form of carbon is the only variable. A method devised recently by Jolliffe & Tregunna (1970) does, however, permit distinction between the effect of total inorganic carbon concentration and that of pH.

Some authors (e.g. Steemann Nielsen, 1960) claim that the ability of an alga to raise pH to about 11 is evidence of direct HCO_3^- uptake. If this is true, then the results of the pH drift experiment, in which a pH of 11.3 was reached, suggest that at least some Loch Leven algae possess this capability.

Algae able to utilise HCO_3^- would be able to continue photosynthesis, even after the concentration of free CO_2 had become extremely small. There would then be no reason to assume that reduction in P_{max} and reduction in free CO_2 are causally related. However, there is evidence for at least one fresh water alga that the rate of photosynthesis at light and carbon-source saturation may be lower when bicarbonate is used than when free CO_2 is used (Raven, 1968). Thus, even if Loch Leven algae are capable of utilising bicarbonate directly, the observed high pH effect could still reflect a form of carbon limitation.

The question of carbon limitation of phytoplankton growth has been much discussed during the current debate (Bowen, 1970; Goldman et al,

1972) on whether carbon or phosphorus is the nutrient primarily responsible for eutrophication of natural waters. The formerly accepted view, that phosphorus was generally the most important limiting nutrient, was questioned when experiments by Lange (1967, 1970, 1971) and reviews by Kuentzel (1969) and King (1970) suggested that carbon might be more important. However, the general conclusion that carbon, rather than phosphorus, is the key nutrient in eutrophication has been convincingly criticised by Sawyer (1970), Shapiro (1970), Schindler (1971) and Goldman et al (1972). Schindler et al (1971) demonstrated clearly that even in a very soft water lake, with low alkalinity reserves of CO_2, growth was primarily limited by phosphate supply. It is important to realise, however, that whilst CO_2 may never be yield-limiting, because of the infinite potential supply from the atmosphere, it could become rate-limiting. Direct or indirect C-limitation of photosynthetic rate is most likely to occur in situations where enrichment with other nutrients has already permitted dense growths of algae. Loch Leven represents one such situation; sewage ponds another (Goldman et al, 1972). The evidence of Shapiro (1973) suggests that the rate of CO_2 supply may be an important factor regulating the qualitative nature of the phytoplankton.

2. The availability of phosphorus may be affected by pH in two ways:

   a) Orthophosphate can exist in three ionic forms ($H_2PO_4^-$; HPO_4^{2-}; PO_4^{3-}). These are in equilibrium; their relative proportions are determined by pH. As illustrated by Hepher (1958, Table 4), increase in pH leads to an increase in HPO_4^{2-} and PO_4^{3-} at the expense of $H_2PO_4^-$. The ionic form of phosphorus most readily taken up by algae is still debated. However, there is evidence for Ankistrodesmus (Ullrich-Eberius & Simonis, 1970; Ullrich-Eberius, 1973) and for Chlorella (Jeanjean, Gaudin & Blasco, 1972)

that the monovalent ion may be preferred. If this is generally true of algae, higher pH values would certainly depress phosphate uptake; this might ultimately lead to a reduced capacity for photosynthesis.

b) At higher pH values, increased PO_4^{3-} concentrations may lead to the precipitation of calcium phosphate, such as Hepher (1958) describes for fish ponds in Israel. More complex precipitates, such as hydroxyapatite, are even more likely (Golterman, 1967). The tendency for high pH values to favour phosphorus precipitation may be offset to some extent by the reduction in phosphorus absorption onto ferric hydroxide in sediments at higher pH values (Ohle, 1937). As discussed later (p. 111), the increased availability of phosphorus, which may result from a reduction in pH, is less likely to have an immediate effect on photosynthetic capacity than is the accompanying increase in carbon availability; increased phosphorus availability is therefore unlikely to have caused the immediate response of P_{max} to decreased pH, illustrated in Fig. 34. The possibility that seasonal changes in P_{max} are determined by phosphorus supply is examined later (p. 117).

3. The solubility of other nutrients, e.g. iron or trace elements, may be reduced at high pH, particularly if they are poorly chelated in the loch water.

4. High pH values may affect adversely membrane permeability and active uptake mechanisms, or may inhibit internal cell metabolism.

5. Carbonate or hydroxide ions, which increase in concentration with increasing pH, may have a direct toxic effect.

6. Photorespiration in Anabaena cylindrica is reported to be stimulated by conditions of low pCO_2 and high pO_2 (Lex, Silvester & Stewart, 1972). It may be that such enhanced photorespiration caused

the apparent reduction in gross photosynthetic capacity at Loch Leven. The influence of photorespiration on measurements of gross photosynthesis is discussed in more detail in the methods section and in the section dealing with possible effects of O_2 on photosynthesis. Its influence on net production is discussed in section VII.

High pH values may also have detrimental effects on macrophytes (Wright & Mills, 1967) and zooplankton: O'Brien & de Noyelles (1972) found that cladocerans have an upper limit of pH tolerance between 10.5 and 11.0. It is possible that a factor contributing to the disappearance of macrophytes and Daphnia from Loch Leven (Morgan, 1970) was high pH values likely to have been associated with increasing algal crops.

e) P_{max} in relation to dissolved oxygen

Introduction

An inhibition of photosynthesis by molecular oxygen (the Warburg effect) has been recorded in higher plants (Björkman, 1966, 1967, 1968), algae (Warburg, 1920; Richardson, Wagner & Welch, 1969; Stewart & Pearson, 1970; Bunt, 1971) and isolated chloroplasts (Gibbs, 1970). Other earlier references to the phenomenon are cited by Turner & Brittain (1962) in a review in which five explanatory hypotheses are discussed. Of these, Turner and Brittain favoured the explanation that oxygen inactivates one or more enzymes of the carbon cycle, possibly by oxidation of -SH groups. More recently, evidence has accumulated which suggests that increased photorespiration (Jackson & Volk, 1970), with increased O_2 tension, is responsible for the Warburg effect (e.g. Zelitch, 1966; Gibbs, 1970; Lex, Silvester & Stewart, 1972).

The occurrence of photorespiration invalidates the assumption made in measuring gross photosynthesis by the oxygen light and dark bottle method, that O_2 uptake rates are the same in the light and dark bottles;

photorespiration leads to an apparent, rather than to a real reduction in gross photosynthetic capacity. Clearly a real reduction in net photosynthetic capacity does however result.

Low CO_2 concentration is reported to enhance the inhibitory effect of oxygen (Turner & Brittain, 1962; Gibbs, 1970; Gibbs et al, 1970) and to enhance photorespiration (Lex, Silvester & Stewart, 1972).

Algae vary in their sensitivity to high O_2 levels (Bunt, 1971).

Oxygen levels at Loch Leven

Seasonal changes in the oxygen content of Loch Leven water, in the euphotic zone, measured near midday, are shown in Fig. 37. Results are presented as mg O_2/l and as percentages of saturation at the prevailing water temperature. Although a wide range of oxygen saturation values, from 68-169%, were encountered over the four years, it is notable that values below 85% and above 120% were comparatively rare. Weak, transient oxygen stratification was occasionally recorded, in summer months, in water deeper than 10 m; anaerobic conditions were rare and confined to water immediately above the sediment surface, in water depths greater than 20 m.

The maintenance of oxygen levels close to 100% saturation, despite high rates of photosynthesis, is probably due to the shallowness and exposed position of the loch, which favours efficient mixing in the water column and accelerates oxygen exchange with the atmosphere.

Because of seasonal change in water temperature, highest oxygen saturation values did not always coincide with highest oxygen concentrations. Presumably, in terms of possible inhibitory effects, oxygen concentrations are more important than saturation levels.

Oxygen concentrations were generally highest during the early months of the year. This was partly due to lower water temperatures and partly to increasing rates of oxygen production per unit area

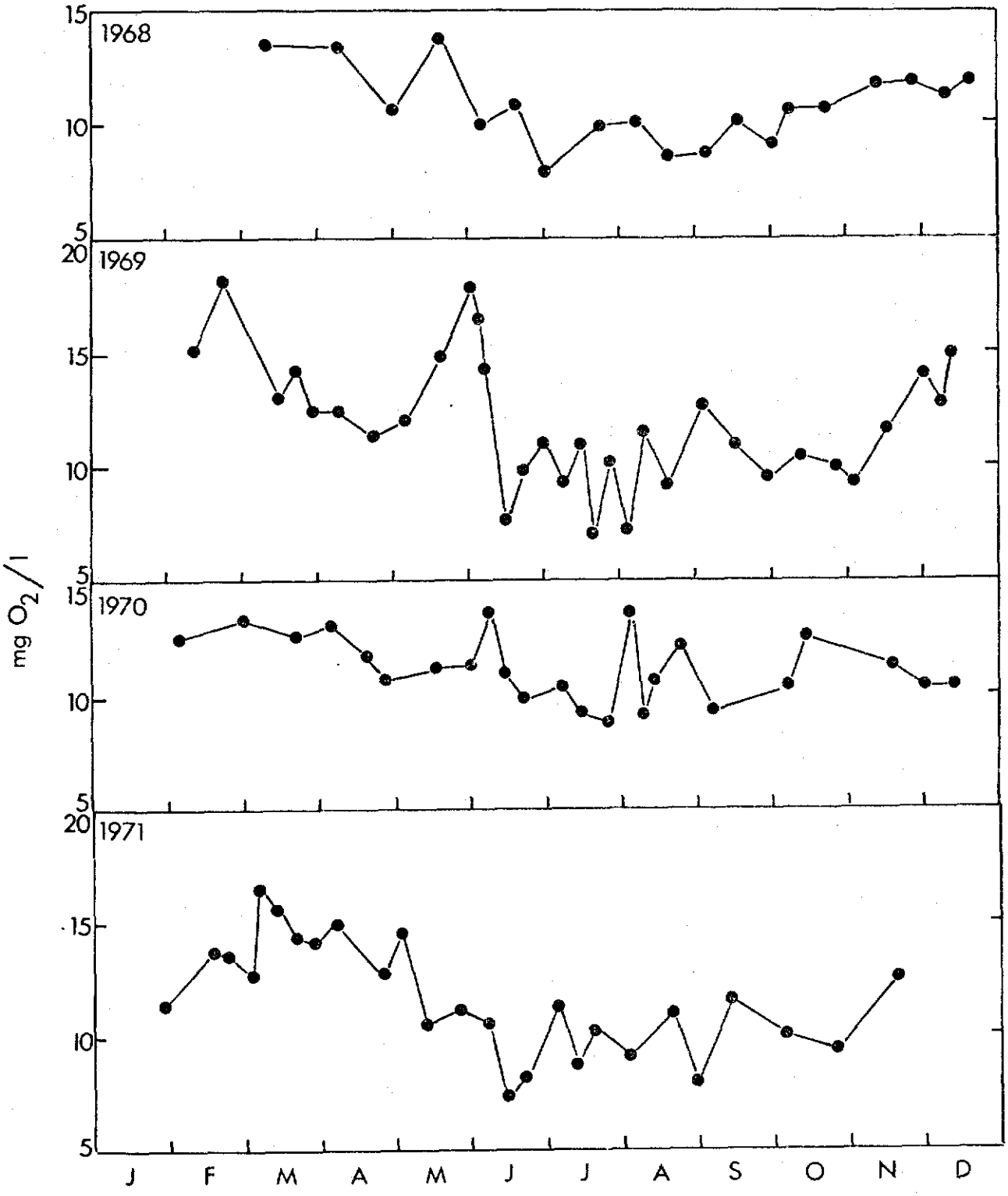


Fig. 37 Seasonal variation in dissolved O_2 concentration (above) and % O_2 saturation (opposite) at midday in the euphotic zone.

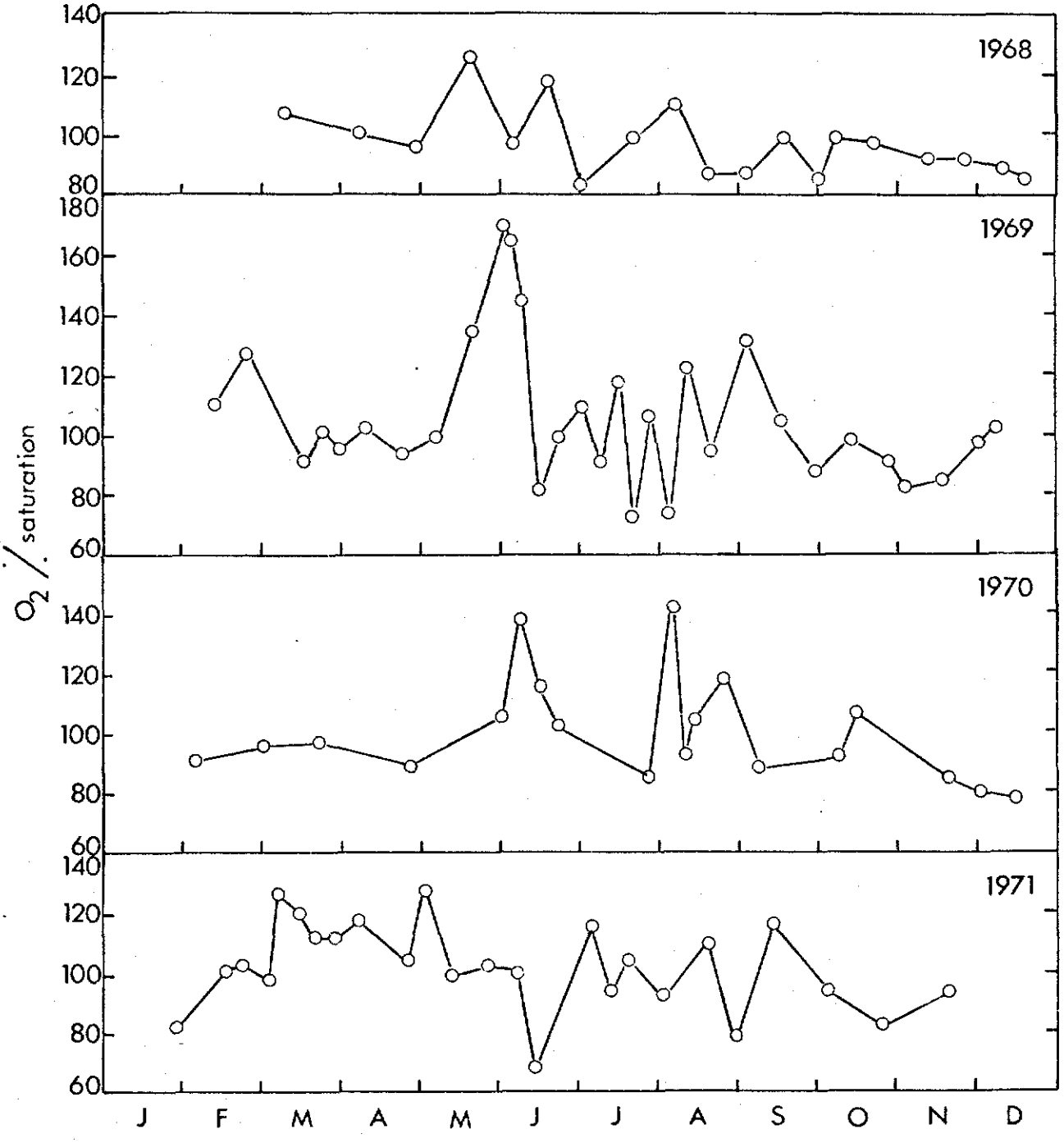


Fig. 37 Legend opposite.

during the spring increase in population density.

Thus the apparently reduced P_{max} values at high population densities, discussed earlier, were associated with relatively high dissolved oxygen concentrations as well as with high pH values (cf. Figs. 29 and 37).

Discussion

It is difficult to decide from published work whether the higher oxygen concentrations found in Loch Leven (10-15 mg/l) are of an order likely to influence photosynthetic rates. This is because, as mentioned above, algae vary in their sensitivity to oxygen and the degree of oxygen inhibition may depend on CO_2 concentration. Stewart & Pearson (1970) found that photosynthesis, at 25°C, of two species of blue-green algae was maximal when the algal suspension had been equilibrated with a gas phase containing a pO_2 of 0.1 atm. This is equivalent to a dissolved O_2 concentration of 4.2 mg/l at 25°C. Depression of photosynthesis became progressively more marked with increasing pO_2 level. O_2 concentrations in the euphotic zone at Loch Leven were always higher than 4.2 mg/l. If Stewart and Pearson's data are assumed applicable to Loch Leven conditions they would suggest that some degree of O_2 inhibition of photosynthesis may have operated at all times. However, as described earlier, it was possible to increase P_{max} by lowering pH, without significantly altering dissolved O_2 concentration. Any direct inhibitory effect of O_2 cannot therefore be the sole cause of reduced P_{max} values at high population densities. Further evidence against an inhibitory effect of oxygen was provided by an experiment described in the methods section. This showed that photosynthetic rate in a 6 h exposure was almost the same as that in a 3 h exposure, despite the considerably higher O_2 concentrations produced in the longer exposure.

f) P_{max} in relation to nitrogen and phosphorus supply

Forms of dissolved nitrogen and phosphorus present in the water and potentially available as nutrients for the phytoplankton

Inorganic nitrogen was present mainly as nitrate, and presumably (though not actually measured) as molecular nitrogen. As in other well-aerated waters (Vollenweider, 1968), no nitrite and only small traces of ammonia were detected.

The concentration of soluble organic nitrogen varied between 0.1 and 1.4 mg N/l, but 80% of the values fell within a much narrower range (0.4-0.6 mg N/l). According to Ruttner (1963), dissolved organic nitrogen concentrations are very similar in many lakes in Europe and North America at about 0.5 mg N/l - a value similar to most of the Loch Leven values. Use of dissolved organic compounds as nitrogen (and/or phosphorus) sources by algae has been claimed by several authors, cited by Hutchinson (1957), Syrett (1962) and Vollenweider (1968). However, proof of direct utilisation of organic compounds in the absence of an intermediate bacterial mineralisation stage is apparently lacking (Vollenweider, 1968). It is not known whether these compounds are utilised by Loch Leven algae. The function of dissolved organic compounds as chelators of essential cofactors and trace elements may well be more important in phytoplankton nutrition than their possible role as direct nutrient sources.

Amongst the algae the capacity to fix molecular nitrogen is largely confined to those blue-green algae which form heterocysts (Fay, Stewart, Walsby & Fogg, 1968; Stewart, 1970; Fogg, 1971). Anoxic conditions may enable non-heterocystous algae to fix molecular nitrogen, as Stewart & Lex (1970) have shown for Plectonema boryanum. Throughout the study period heterocystous blue-green algae were rare in the Loch Leven flora (Bailey-Watts, 1973); anaerobic conditions developed very

occasionally, in summer, and were confined to deep water (below about 20 m) which represents a very small proportion of the total loch volume (Smith, 1974). It is therefore unlikely that nitrogen fixation contributed significantly to the nitrogen economy of the loch. Using the N^{15} method Mr. A. Horne found no evidence of nitrogen fixation in Loch Leven (Horne, unpubl., cited by Fogg, 1971).

Nitrate was probably the principal source of inorganic nitrogen for the phytoplankton, although the possibility that ammonia was utilised, as soon as it was released by bacterial decomposition and zooplankton excretion, cannot be excluded. However, oxidation of ammonia by nitrifying bacteria was probably the major cause of the low levels of ammonia normally present.

Inorganic phosphorus was present largely as orthophosphate; polyphosphates, introduced to the loch from industry, are quickly hydrolysed (Holden, pers. comm.). The ionic form of phosphate (determined by pH-dependent equilibria) may affect its availability to the phytoplankton. This was discussed earlier in relation to the effect of pH on photosynthetic capacity.

Dissolved organic phosphorus occurred at concentrations varying from 2-24 µg P/l but, as mentioned earlier, it is not known whether the algae can utilise such compounds directly.

Seasonal changes in the concentrations of nitrate-nitrogen and phosphate-phosphorus

The changes in instantaneous nitrate and phosphate concentrations, shown in Figs. 38 and 39, reflect changes in the balance between: 1. biological consumption by phytoplankton, benthic algae, macrophytes and bacteria, plus outflow losses; 2. replenishment from inflows and remineralisation in the water column and sediments.

Changes in nitrate and phosphate concentrations cannot be

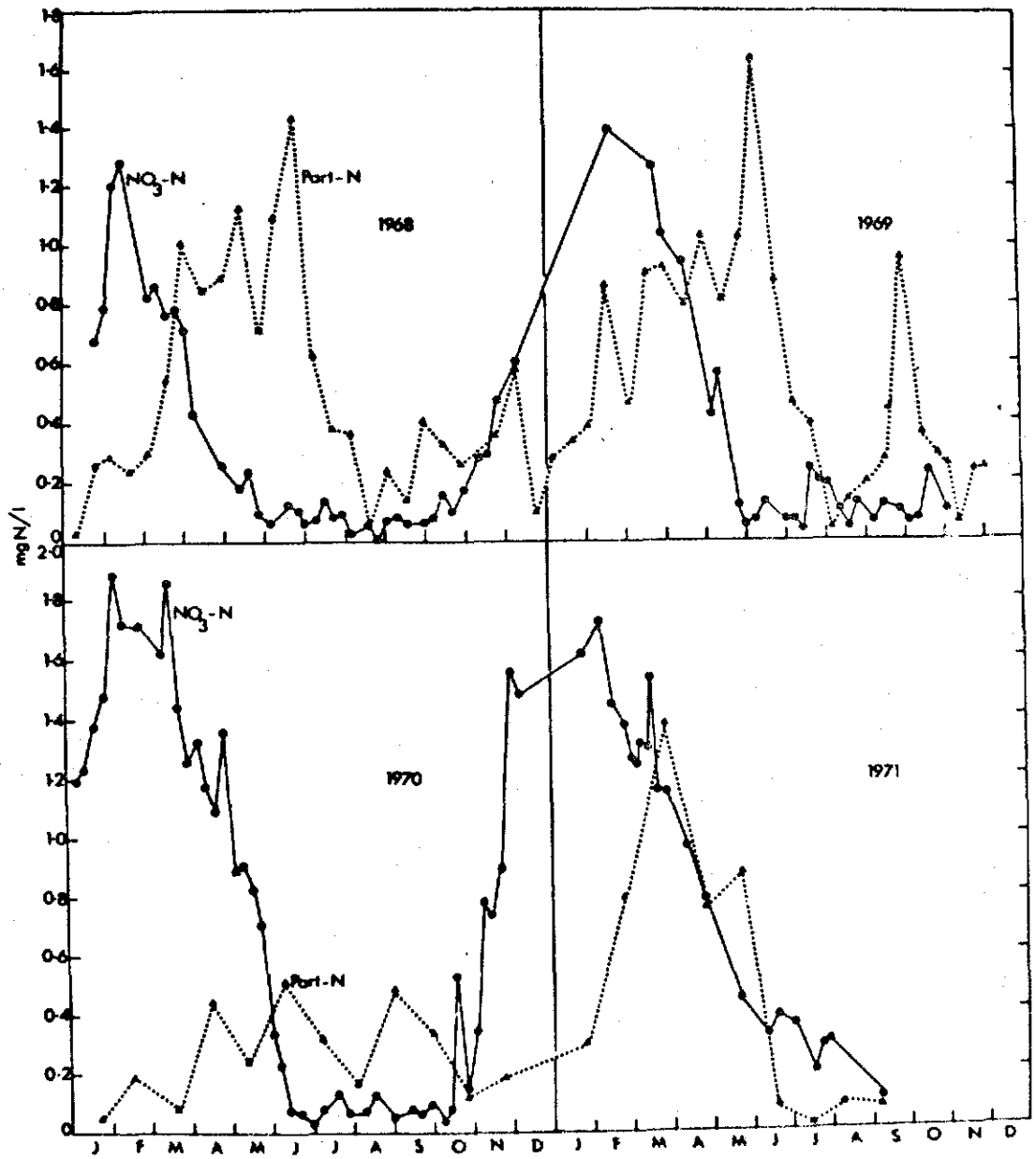


Fig. 38 Seasonal variations in nitrate-nitrogen (NO_3-N) and particulate-nitrogen (Part-N) concentrations during 1968-71.

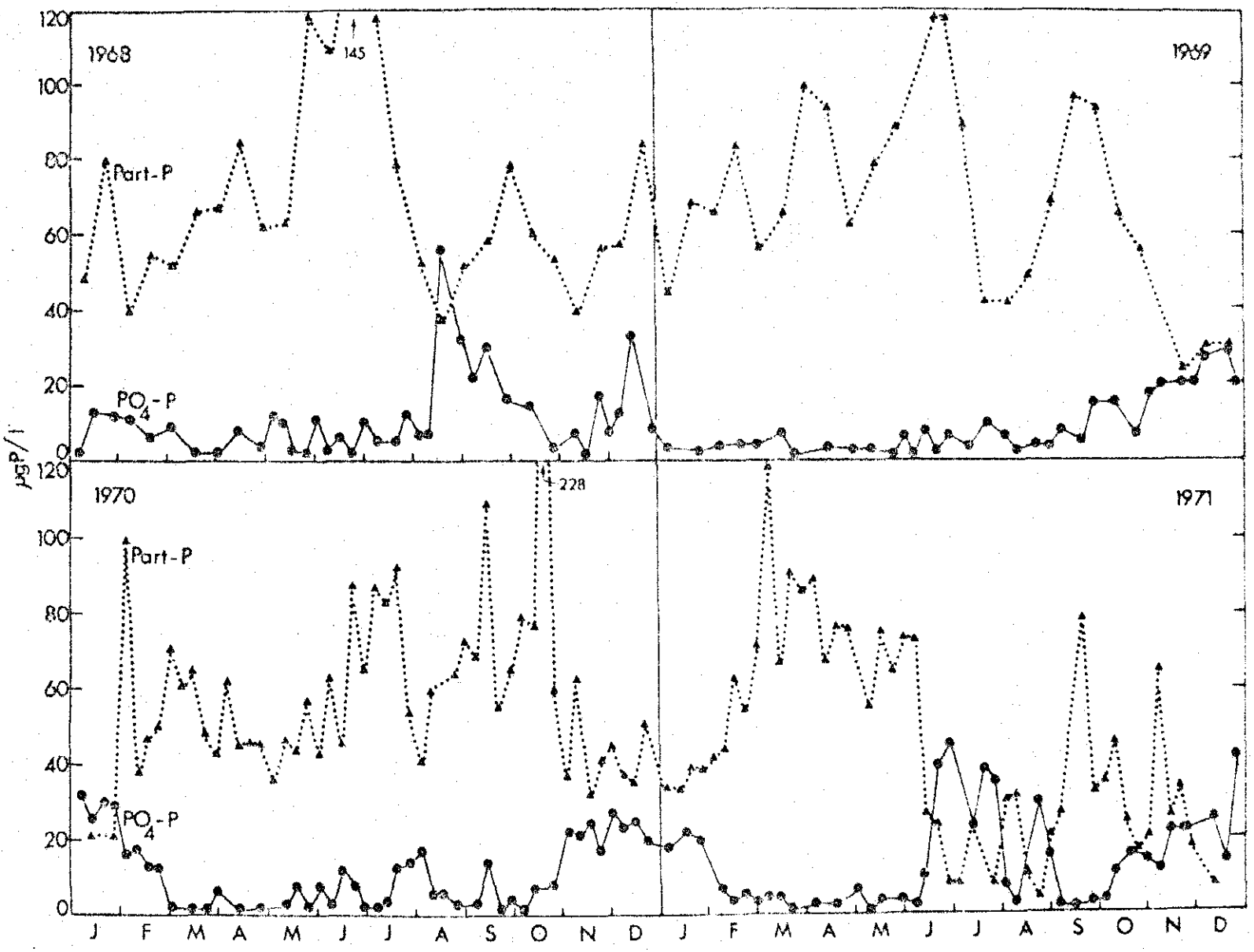


Fig. 39 Seasonal variations in phosphate-phosphorus (PO_4-P) and particulate-phosphorus (Part-P)concentrations during 1968-71.

quantitatively accounted for by accompanying changes in particulate (largely algal) nitrogen and phosphorus (Figs. 38 and 39). The observed increase in particulate nitrogen was sometimes less than (e.g. February-June 1970), and sometimes greater than (e.g. January-March 1971), the decrease in NO_3-N over the same time period; at other times (e.g. March-June 1971) decline in NO_3-N was accompanied by a decline in particulate nitrogen. The increasesin particulate phosphorus were almost always greater than the decreases in PO_4-P. The actual production of algal biomass must have been somewhat greater than observed biomass changes indicated, since some biomass must have been lost by grazing, sedimentation or to the outflow. Thus a decline in NO_3-N in excess of particulate-N increase, or accompanying a particulate-N decrease, does not necessarily imply NO_3 uptake by benthic algae or macrophytes. Where even observed biomass increase (which gives an underestimate of nutrient demand) was greater than the corresponding inorganic nutrient decline, then we must assume that inflowing and recycled nutrients were utilised immediately by the phytoplankton.

Since accurate measurements of net phytoplankton production were not obtained (see section VII), and since stream flow data are not yet available, it is not possible to evaluate the relative importance of the various factors causing changes in NO_3-N and PO_4-P concentrations in the loch.

Nevertheless, the general trends in NO_3-N and PO_4-P concentrations, in relation to those of phytoplankton density, do suggest that phytoplankton growth was often a significant factor controlling these nutrient concentrations. NO_3-N concentrations were generally high in winter and low in summer. The decline in spring was accompanied each year by an increase (though not equivalent) in phytoplankton biomass; the maximum biomass more or less coincided with the attainment of

minimal NO_3-N concentrations, except in 1971.

PO_4-P concentrations fluctuated irregularly throughout the year. Although there was a trend for PO_4-P to decrease to low levels during the biomass increase in the first half of the year, it usually reached its minimum value some time before crops reached their maximum density.

P_{max} in relation to nitrate and phosphate concentrations and in relation to the chemical composition of the phytoplankton

Introduction

Below certain critical levels, instantaneous nitrate and phosphate concentrations have been shown to reduce instantaneous growth rates of algae in culture (Ketchum, 1939; Chu, 1942, 1943; Rodhe, 1948; Gerloff, Fitzgerald & Skoog, 1950). A similar effect on instantaneous photosynthetic rate might be expected, although Ketchum (1954) points out that the same nutrient concentration may not be optimal for both growth rate and photosynthetic rate. Published critical nutrient concentrations for optimal growth rate in culture media vary with species, and are often higher than those required for optimum growth rate of algae in lake water in the laboratory (Lund, 1965). Nutrients often appear to be less available in culture media than in lake water. Conversely, the concentration of a nutrient in lake water may not always be equal to the amount available for algal uptake. Availability may be partly determined by pH, as is the case with carbon and phosphorus.

Because of the above complications, comparison of observed nutrient concentrations with published minimum concentrations permitting maximum growth rate is of little value for detection of potential nutrient limitation of photosynthetic capacity. Actual limitation by any given nutrient will also depend on whether or not other growth conditions are optimal.

The effect of nitrate or phosphate supply on P_{max} is likely to be rather different from that of another essential nutrient, carbon dioxide. CO_2 is the immediate substrate of the first enzymatic reaction of photosynthesis and as such its concentration (below a certain critical level) can be expected to have an immediate and direct effect on activity per unit amount of pre-existing enzyme. Temperature, and trace elements acting as enzyme co-factors, also exert their influence on photosynthetic rate by affecting specific enzyme activity. Since nitrogen and phosphorus compounds are not immediate substrates of photosynthesis, their effects are more likely to be on the amounts of enzymes and pigments in the cell, rather than on the activity of unit amount of enzyme. A lag, representing the time required for degradation or synthesis of enzymes, may therefore be expected in the response of photosynthetic capacity to change in nitrogen and phosphorus supply. In contrast to the effects of CO_2, those of nitrogen and phosphorus are likely to be more closely related to the previous nutrient history (and therefore chemical composition) of the population than to instantaneous concentrations at the time photosynthesis is measured.

Work with uni-algal cultures has shown that the nutrient conditions to which cells are exposed during their growth (i.e. their nutrient history) influences their chemical composition, pigment content and photosynthetic capacity. Changes in photosynthetic capacity per cell are not solely due to changes in chlorophyll *a* content; photosynthetic rate per unit chlorophyll *a* is also affected. Bongers (1956) and Thomas & Dodson (1972) found that photosynthetic capacity per unit chlorophyll *a* decreased as cells became nitrogen deficient. Calculation of the light-saturated rate of photosynthesis per mg chlorophyll *a* from the data of Jørgensen (1970, Table 2) shows that nitrogen or phosphorus deficiency both led to a depression of the light-saturated

photosynthetic rate per unit of chlorophyll a, as well as to a reduction in the rate per mg chlorophyll a at sub-saturating intensities. McAllister, Parsons, Stephens & Strickland (1961) and Antia, McAllister, Parsons, Stephens & Strickland (1963) found a decline in photosynthetic capacity of a natural mixed population, consisting mainly of diatoms, undergoing nitrogen deficiency inside a large-volume plastic sphere, suspended in the sea.

A number of authors have commented on the higher photosynthetic capacities of natural populations often observed in nutrient-rich waters as compared with nutrient-poor waters (Ichimura, 1958, 1960a; Ichimura & Aruga, 1958; Hepher, 1962; Anderson, 1964; Curl & Small, 1965; Thomas, 1970; Glooschenko & Curl, 1971; Powers et al, 1972). Although other differences between the water bodies concerned are usually ignored, such correlations have prompted some authors, notably Curl & Small (1965), to suggest that nutrient deficiency may be indicated by a reduction in photosynthetic capacity to certain specified values.

There does exist, then, a fair amount of indirect and direct evidence that nutrient deficiency can lead to a decline in photosynthetic capacity on a chlorophyll a basis. The only work to date providing evidence to the contrary appears to be that of Fogg (1959), which shows the photosynthesis/chlorophyll ratio of a Monodus culture rising with increasing nitrogen deficiency. According to Fogg (1965) a similar trend was found by Spoehr & Milner (1949) with Chlorella. However, the results of Spoehr & Milner (1949) are not strictly comparable to those of Fogg (1959) since the former authors did not measure the rate of photosynthesis directly but merely showed that "the weight of cells may increase three-fold with a corresponding decrease in the percentage of chlorophyll". Such an effect need not

reflect change in activity per unit chlorophyll but merely a change in the fate of products formed in photosynthesis, due for example to a reduction in protein synthesis or cell division rate, or reduction in respiratory rate.

The possibility that changes in photosynthetic capacity at Loch Leven may at times have been influenced by nitrogen or phosphorus deficiency is examined below. The discussion is based firstly on seasonal observations and secondly on the results of laboratory nutrient enrichment experiments.

Evidence from seasonal observations

Seasonal changes in photosynthetic capacity (P_{max}) are shown in Fig. 40 in relation to:- (1) changes in nitrate-nitrogen (NO_3-N) and phosphate-phosphorus (PO_4-P) concentrations in the water, (2) changes in the chemical composition of the phytoplankton as indicated by the ratios chlorophyll a/particulate nitrogen (chl a/part-N) and chlorophyll a/particulate phosphorus (chl a/part-P), (3) changes in population density, estimated as chlorophyll a.

Chlorophyll a was not always measured on the same day as particulate N and P; values for particulate N and particulate P on the days when chlorophyll a was measured were obtained by interpolation and used in the chl a/part-N and chl a/part-P ratios.

Table 5 shows the carbon, nitrogen, phosphorus and chlorophyll a contents of the phytoplankton, on an ash-free dry weight basis, for the periods June-October 1970 and January-March 1971. The nitrogen and phosphorus data in Fig. 40 and Table 5 were obtained from different samples; those in Fig. 40 were provided by the Pitlochry chemists, those in Table 5 by the Merlewood chemists (see methods section).

P_{max} in relation to nitrogen

Fig. 40 shows that highest P_{max} values were recorded when nitrate

Fig. 40 (following 4 pages) Seasonal variation during 1968-71 in (a) the ratio chlorophyll a/particulate nitrogen (chl a/part-N), (b) nitrate-nitrogen (NO_3-N) concentration, (c) chlorophyll a concentration, (d) the ratio chlorophyll a/particulate phosphorus (chl a/part-P), (e) phosphate-phosphorus (PO_4-P) concentration, (f) photosynthetic capacity (P_{max}).

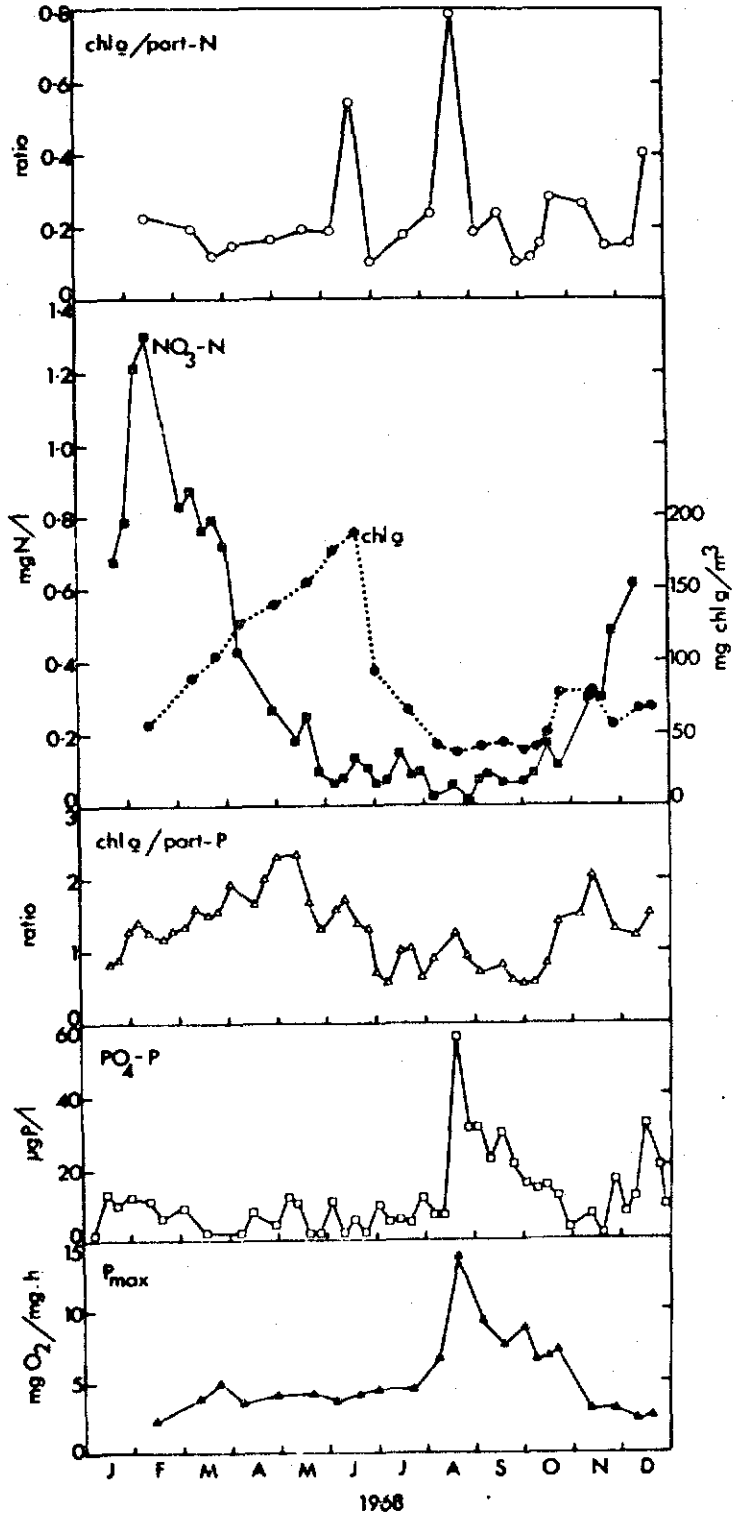


Fig. 40 Legend opposite

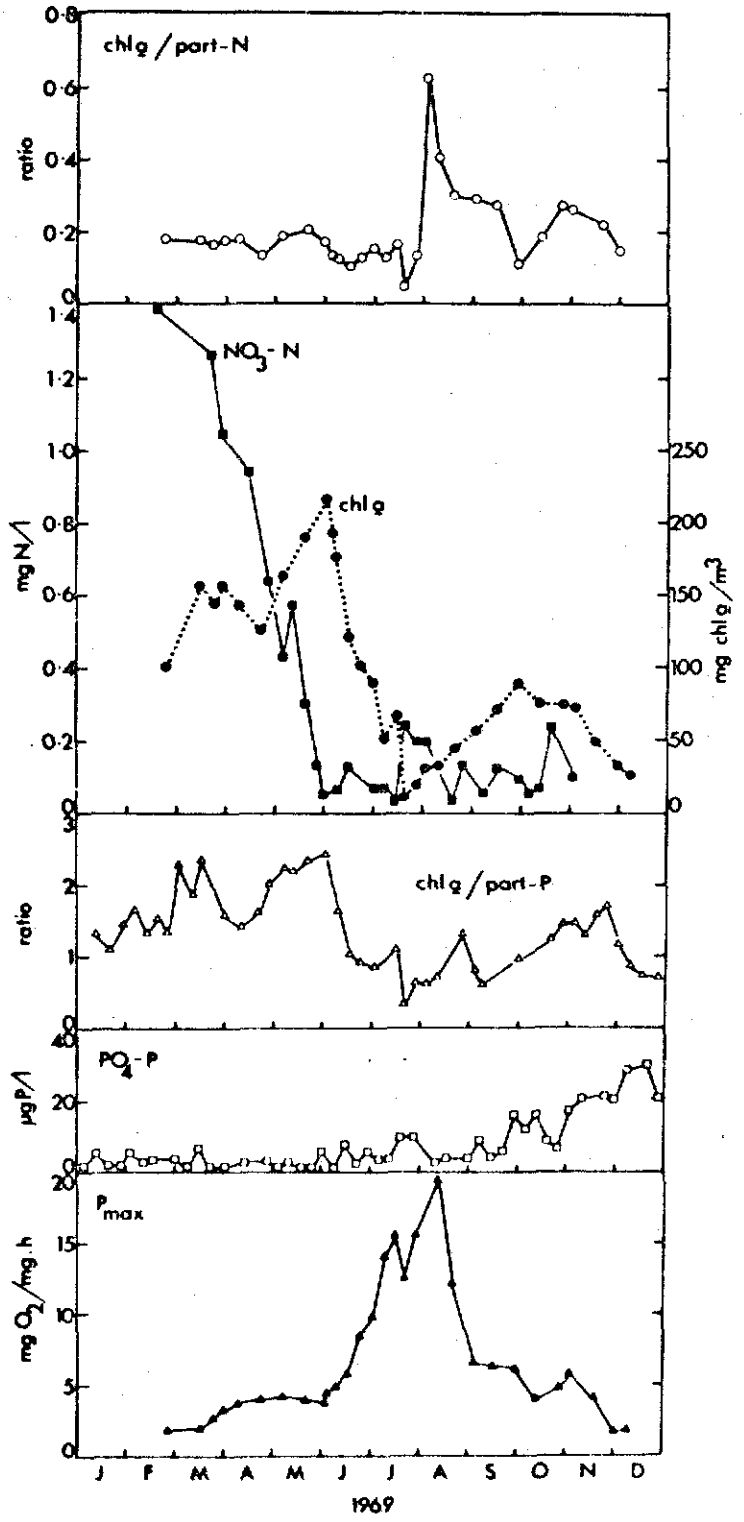

chl a / part-N
ratio
NO_3-N
chl a
mg N/l
mg chl a/m³
chl a / part-P
ratio
PO_4-P
µg P/l
P_{max}
mg O_2/mg.h
J F M A M J J A S O N D
1969

Fig. 40 (cont'd)

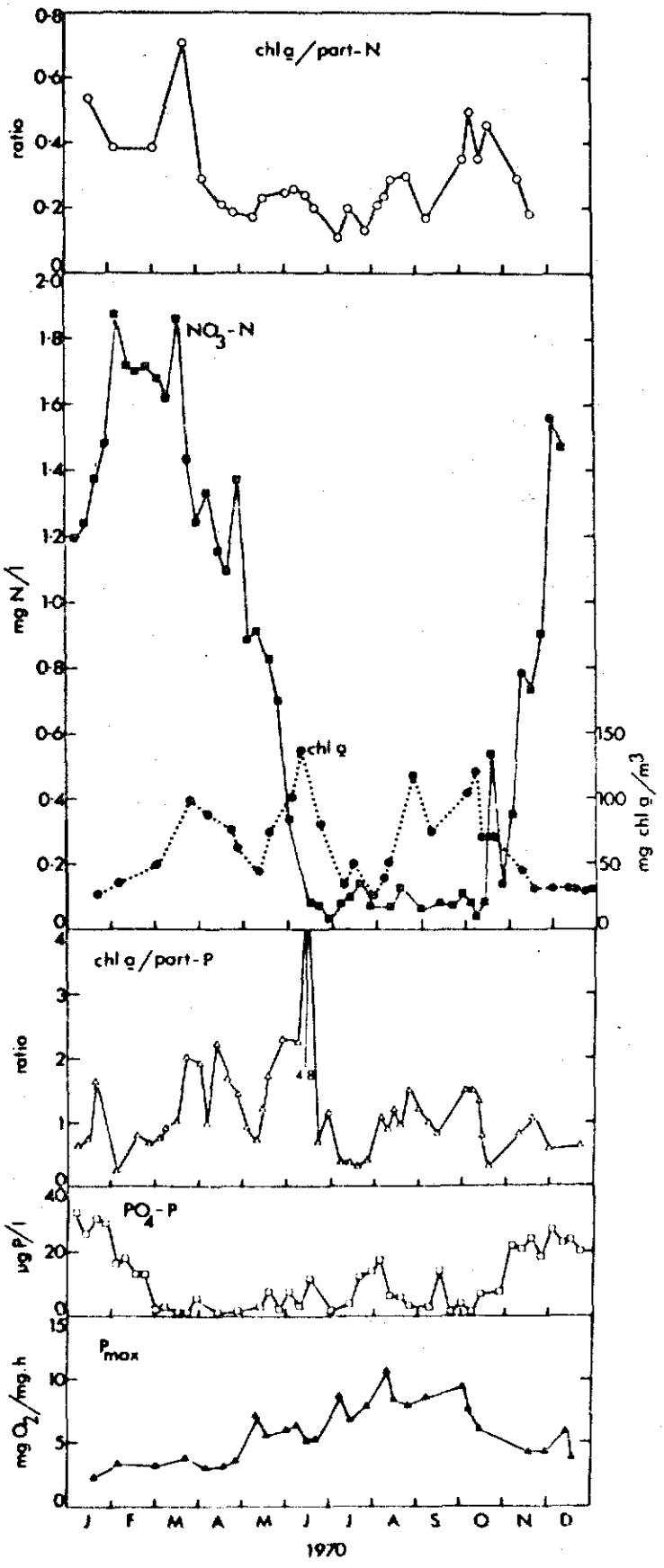

chl a / part-N
ratio
NO3-N
mg N/l
chl a
mg chl a/m3
chl a / part-P
ratio
PO4-P
µg P/l
Pmax
mg O2/mg.h
J F M A M J J A S O N D
1970

Fig. 40 (cont'd)

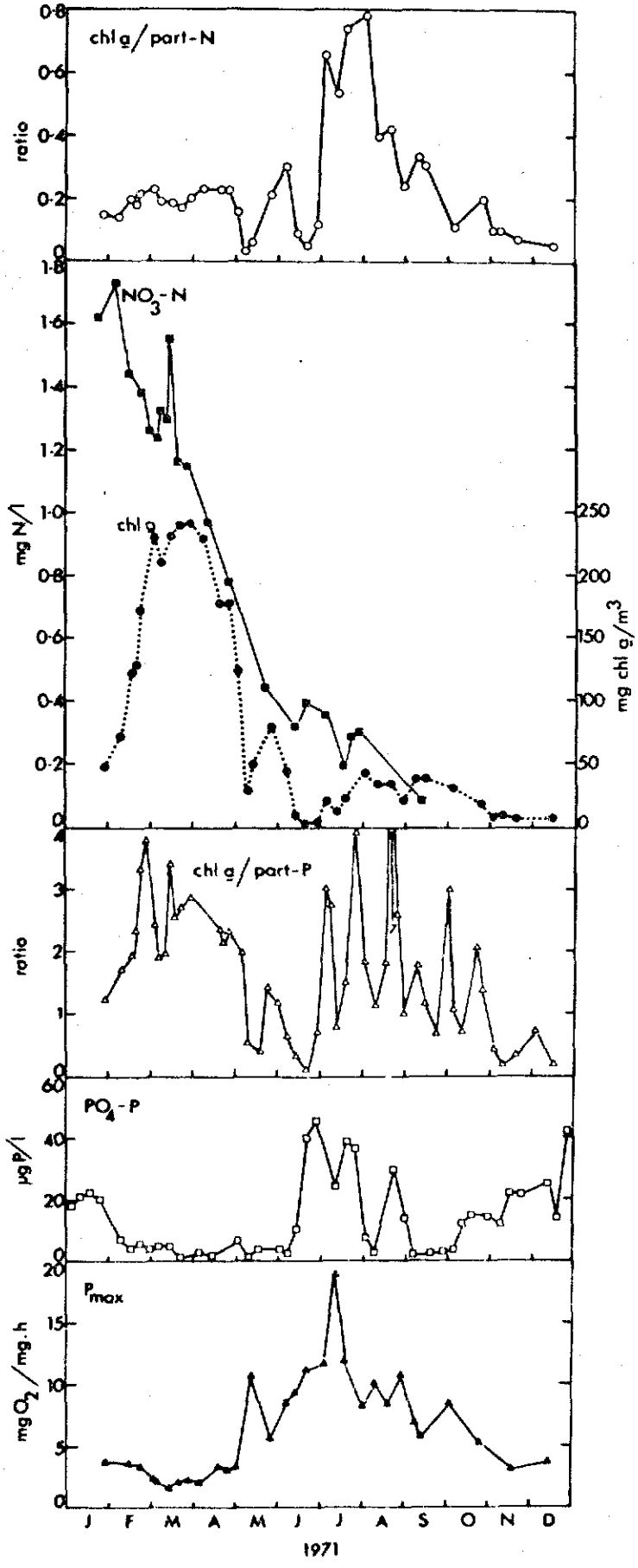

chl a/part-N
ratio
NO3-N
chl a
mg N/l
mg chl a/m3
chl a/part-P
ratio
PO4-P
µg P/l
Pmax
mg O2/mg.h
J F M A M J J A S O N D
1971

Fig. 40 (cont'd)

Table 5. The chemical composition of the phytoplankton on various dates in 1970 and 1971.

Date	% chl.*a* (ash-free dry weight basis)	% C	% N	% P	C : N : P (by weight)
22.6.70	-	55.0	7.8	0.6	100 : 14.1 : 1.2
8.7.70	-	52.7	7.5	0.7	100 : 14.2 : 1.4
27.7.70	-	52.1	7.6	0.9	100 : 14.7 : 1.8
5.8.70	1.4	51.7	6.4	0.6	100 : 12.4 : 1.2
19.8.70	1.5	49.7	6.4	1.0	100 : 12.9 : 1.9
31.8.70	1.8	51.3	7.3	0.9	100 : 14.2 : 1.8
7.9.70	1.4	43.5	5.6	0.8	100 : 13.0 : 1.8
13.9.70	1.6	45.5	6.2	1.0	100 : 13.6 : 2.1
7.10.70	2.5	50.0	7.3	1.3	100 : 14.6 : 2.6
12.10.70	1.9	42.5	6.5	1.2	100 : 15.4 : 2.9
21.1.71	2.0	49.2	6.7	1.2	100 : 13.7 : 2.4
1.2.71	-	40.8	6.7	1.1	100 : 16.5 : 2.8
16.2.71	3.1	47.9	7.7	1.0	100 : 16.1 : 2.2
19.2.71	-	46.9	8.4	1.5	100 : 17.8 : 3.2
22.2.71	2.8	48.9	8.5	0.9	100 : 17.4 : 1.9
4.3.71	2.8	50.0	8.0	0.8	100 : 16.1 : 1.6
8.3.71	1.9	44.0	7.6	0.6	100 : 17.3 : 1.5
15.3.71	3.3	58.9	8.8	0.7	100 : 14.9 : 1.2
22.3.71	2.1	51.1	7.7	0.7	100 : 15.0 : 1.4
24.3.71	2.5	52.0	7.7	0.7	100 : 14.8 : 1.3
29.3.71	2.7	53.0	7.6	0.7	100 : 14.3 : 1.2

concentrations were relatively low and, except in 1970, were associated with relatively high chl *a*/part-N ratios. It will be remembered that high P_{max} values also coincided with periods of low crop density, low pH and high water temperature (Fig. 29). Reduction of the chl *a*/part-N ratio to less than about 0.1 has been shown to accompany nitrogen deficiency in laboratory-grown uni-algal cultures (Bongers, 1956; Fogg, 1959; Manny, 1969) and in mixed natural populations contained in a large-volume plastic sphere, suspended in the sea (McAllister *et al*, 1961; Antia *et al*, 1963). Where authors gave results as part-N/chl *a*, the reciprocal (i.e. chl *a*/part-N) was calculated for direct comparison with Leven results. Except on one occasion in 1969, and on a few occasions in 1971, the chl *a*/part-N ratio at Loch Leven was greater than 0.1, suggesting, on the basis of the above published evidence, that the algae were rarely, if ever, suffering from nitrogen deficiency. This conclusion is reinforced, at least for the periods June-October 1970 and January-March 1971, by the additional data on the chemical composition of the phytoplankton presented in Table 5. Bailey-Watts (1973) has shown that measured carbon contents in Table 5 were in good agreement with those calculated from live algal cell volume using the regression equation, between $\log_{10}$ (cell carbon) and $\log_{10}$ (cell volume), found to apply to a range of algae in culture by Mullin, Sloan & Eppley (1966). This would suggest that the data in Table 5 can be assumed to reflect the chemical composition of the phytoplankton, with minimum interference from detritus, zooplankton etc. The values obtained for the carbon/nitrogen ratio, % nitrogen and % chlorophyll *a* fall within the range of values reported in the literature for algae not deficient in nitrogen (Lund, 1950; Gerloff & Skoog, 1954; Ketchum, 1954; Lund & Talling, 1957; Strickland, 1960; Parsons, 1961; Parsons, Stephens & Strickland, 1961).

It seems unlikely, from the foregoing, that nitrogen deficiency ever reduced the photosynthetic capacity of the phytoplankton at Loch Leven. This is so even during the periods, noted earlier (Fig. 29), when P_{max} did not respond to increased water temperature (April-July 1968, April-June 1969, May-June 1970) or declined at constant temperature (February-March 1971).

In marked contrast to the results obtained by other workers, referred to above, Yentsch & Vaccaro (1958) found that higher chl *a*/part-N ratios (*ca* 0.5) were typically associated with N-deficient cultures; ratios of *ca* 0.1 were characteristic of N-enriched cultures. On the basis of these results we might postulate that the high chl *a*/part-N ratios found at Loch Leven are indicative of N-deficiency. Whilst this might seem a reasonable suggestion in view of the low NO_3-N concentrations prevailing, for reasons given below it does not seem consistent with the accompanying high P_{max} values.

Increased P_{max}, such as Fogg (1959, 1965) describes for a N-deficient *Monodus* culture, depends on N-starvation reducing the cell's chlorophyll *a* content more than it reduces its photosynthetic capacity calculated on some 'ideal' index of biomass. There is no evidence for this at Loch Leven: relative to their N-content (Fig. 40) and relative to their cell volume (Bailey-Watts, 1973), the algae appear to have an increased chlorophyll *a* content when P_{max} is high (and crop density low). Values of 4-6 µg chlorophyll *a*/mm^3 cell volume were typical when crops were high and composed of relatively few species, whereas the low midsummer crops, composed of many species, often gave values greater than 30 µg chlorophyll *a*/mm^3 cell volume. Bailey-Watts (1973) has pointed out that this difference may in part result from the relatively greater inaccuracies involved in counting cells and in estimating cell volumes when crops are lower and composed of more species.

In addition, it is possible that the apparent increase in chlorophyll a content per cell-N or per cell volume, at low crop density, results from increased quantities of phaeo-pigments, which are included in the chlorophyll a estimates. As discussed earlier (p. 54), although phaeo-pigment interference could not be precisely quantified, the general trends revealed by the ratios of Moss (1967a,b) and Lorenzen (1967) suggested that phaeo-pigments were relatively more abundant when crops were lower. However, even when phaeo-pigments are taken into account, cells at low crop density still appear relatively rich in chlorophyll a (Bailey-Watts, 1973 and pers. comm.). High chlorophyll a contents per unit cell volume were not unexpected during the midsummer population density minima, since then crops were dominated by a mixture of green algae, a group generally held to be richer in chlorophyll a than other groups (Strickland, 1960; Bursche, 1961). This last point demonstrates how seasonal change in species composition can complicate attempts to interpret changes in physiological behaviour in nature from similar changes occurring in uni-algal cultures under defined growth conditions.

The tendency for higher chl a/part-N ratios to occur when water temperature was high may have reflected a selection by the environment of those species best able to exploit the prevailing conditions for photosynthesis. Whereas the photochemical part of photosynthesis is insensitive to temperature, the activity of the enzymes associated with photosynthesis increases with temperature. In order to fully utilise this potential increase in enzyme activity it may be of advantage to cells growing at higher temperatures to have relatively more photochemical apparatus (i.e. chlorophyll a) per unit photosynthetic enzyme. This is consistent with a higher chl a/part-N at higher temperatures.

There is no evidence, from seasonal data, to suggest that nitrogen played a significant role in determining P_{max} at Loch Leven.

P_{max} in relation to phosphorus

Fig. 40 shows that in 1968 and 1971 highest P_{max} values coincided with highest PO_4-P concentrations. However just as high, or higher, P_{max} values were recorded, at similar temperatures (cf. Fig. 29), in 1970 and 1969 respectively, in the presence of much lower PO_4-P concentrations. A decline in P_{max} at constant temperature (cf. Fig. 29) accompanied the decline in PO_4-P between January and March 1971 (Fig. 40). A similar decline in PO_4-P during January and February 1970 was not, however, accompanied by any change in P_{max}.

Thus, although correlations between PO_4-P and P_{max} were sometimes apparent, there is evidence that P_{max} values were not causally related to instantaneous phosphate concentrations.

Except in 1971, highest P_{max} values in summer were associated with relatively low chl *a*/part-P ratios. In general, P_{max} tended to increase as the algae became richer in phosphorus relative to chlorophyll *a*. This trend would have been accentuated if chlorophyll *a* values had been corrected for phaeo-pigment interference since, as discussed earlier, this tended to be greater when crops were low. As discussed in the previous section on P_{max} and nitrogen, chlorophyll *a* per cell volume was relatively high when crops were low in midsummer. Reduction in chlorophyll *a* per cell is not likely, therefore, to have caused the declines in chl *a*/part-P which accompanied the declines in population density.

It was noted earlier that some factor(s), associated with increase in population density, apparently delayed increase in P_{max} with temperature between April and July 1968, April and May 1969 and May and June 1970 and caused P_{max} to decline at constant temperature

in February and March 1971. For much of the time during each of these periods the chl a/part-P ratio was increasing; this might suggest that decline in phosphorus content relative to chlorophyll a content, perhaps due to phosphorus shortage during the cells' history, may have been, at least partially, responsible for limiting P_{max} during these periods. Decline in phosphorus content, on an ash-free dry weight basis, is also shown for the February-March 1971 period in Table 5. Similar data are not available for the May-June 1970 period (the data in Table 5 for 1970 refer to a later period, late June-early October).

Since chlorophyll a and phosphorus contents on an ash-free dry weight basis are not available for the whole 4 year period, conclusions regarding phosphorus deficiency and P_{max} must be regarded as very tentative because:-

1. Algae can store phosphorus (as polyphosphate bodies) in excess of their immediate requirements (e.g. Mackereth, 1953). Increase in chl a/part-P could result from utilisation of stored phosphorus without the phosphorus content per cell falling to a level sub-optimal for photosynthesis. There is no reason to expect a cell containing stored phosphorus to be any more efficient in photosynthesis than a cell with a 'normal' phosphorus content.

2. Different species may have different chlorophyll a and phosphorus contents even when all growth conditions are optimal.

3. A decrease in the chl a/part-P ratio as the population declines could arise if pigment is decomposed relatively quickly as cells die, but phosphorus remains bound, for a longer time, to particulate organic material. This was the explanation given by Ketchum et al (1958) to account for the inverse relationship they observed between the phosphorus:chlorophyll a ratio and population density. Although

for restricted periods at Loch Leven there is evidence, discussed earlier, that the particulate fraction used in the chemical analyses was largely present as live algae, this is by no means certain for the whole of the 4 year period. Fluctuations in the chl a/part-P ratio due to the reason given by Ketchum et al (1958) cannot therefore be ruled out.

Summary and discussion of seasonal evidence

P_{max} values were independent of instantaneous nitrate and phosphate concentrations and bore no clear relationship to the 'nitrogen history' of the population. The chemical composition of the algal crops gave no conclusive evidence of nitrogen deficiency. Broad correlations between the 'phosphorus history' of the population and its photosynthetic capacity suggested that phosphorus deficiency in the cells may have developed during periods of population increase, leading to a depression of P_{max}. Increase in pH, with increase in population density, may have contributed to this phosphorus shortage as discussed in the previous section on pH and P_{max}. In other words, cells could have become low in phosphorus not so much because phosphorus per se was in short supply, but because prevailing conditions of pH prevented (or reduced) its uptake by phytoplankton. Reasons are given, however, for regarding the evidence for phosphorus deficiency as suggestive, but not conclusive.

The absence of any evidence of nitrogen deficiency, or any unequivocal evidence of phosphorus deficiency, in the chemical composition or photosynthetic capacity of the phytoplankton, does not necessarily prove that the phytoplankton crops were not potentially limited from further increase by shortage of nitrogen or phosphorus. Nutrient-deficient cells may have accelerated sinking rates (Steele & Yentsch, 1960; McAllister et al, 1961; Antia et al, 1963). It is

possible, therefore, that the changes induced by nutrient deficiency in cultures (from which cells cannot be lost) may escape detection in weekly field sampling owing to the accelerated rate of loss of nutrient-depleted cells to the sediments. This is particularly likely with photosynthetic capacity since this is a characteristic which may not respond immediately to nutrient deficiency, at least in the case of nitrogen (Fogg, 1959, 1965; cf. however Goldman, 1960).

Evidence from laboratory enrichment experiments

The short- and long-term effects of nitrate and phosphate on photosynthetic capacity (P_{max}) were further investigated in laboratory enrichment experiments, using natural phytoplankton populations sampled between June and October 1970. The aims were firstly to determine whether photosynthetic capacity in the loch was limited (or enhanced) by nitrogen or phosphorus deficiency, and secondly to detect possible nutrient limitation of phytoplankton growth.

Loch water samples were collected in polyethylene containers, kept dark and transported to the laboratory within 3 h. The chlorophyll a content of the sample was determined and 1 litre subsamples were dispensed into cottonwool-stoppered 2.5 litre 'Pyrex' conical flasks. These subsamples were variously treated as follows:

a) enriched with 0.68 mg NO_3-N/l as $Ca(NO_3)_2 4H_2O$ (N)

b) enriched with 0.89 mg PO_4-P/l as K_2HPO_4 (P)

c) enriched with nitrate plus phosphate in the form and concentrations given above (N + P)

d) unenriched (control)

The nitrate and phosphate quantities added were the same as those used in medium No. 10 of Chu (1942). The initial nitrate and phosphate concentrations present in the unenriched samples may be determined from Fig. 40 and were low compared to the quantities added.

The 'initial' photosynthetic capacity of enriched and control samples was then determined using the illuminated laboratory incubator (described in the methods section) with 3 h exposure periods.

Other enriched and unenriched subsamples were incubated at 20-22°C with 'daylight' fluorescent 65/80W tubes beneath the flasks providing a continuous light intensity of 7.5 klux (38 kerg/cm^2.sec). Flasks were shaken periodically by hand to maintain cells in suspension. Later experimental series also included a dark control (flask covered by several layers of black cloth).

After 18-90 h incubation, the chlorophyll a content and photosynthetic capacity of the samples were measured.

No immediate effect of nutrient enrichment was observed on any occasion examined. This tends to confirm the suggestion based on seasonal observations, that P_{max} was not limited by instantaneous nitrate or phosphate concentrations.

Incubation of samples in the presence of extra nutrients produced changes in both population density (assessed as chlorophyll a) and in photosynthetic capacity (on a chlorophyll a basis). Results are shown in Table 6. Changes occurring in the light and dark controls are also included in Figs. 32 and 33 and were discussed in the earlier section on light history effects.

Except in the experiment begun on 25 June, the chlorophyll a content of the samples showed little, if any, increase in the light in the absence of additional nutrients. On some occasions a decline in chlorophyll a content was recorded and this was most pronounced in longer exposures. Such declines may be attributable to an inhibitory effect of light per se since they did not occur in the 'dark' controls. The lack of growth in most of the 'light' controls appeared to be due to nutrient deficiency rather than to the presence of some toxic

Table 6. Effect of nutrient enrichment on chlorophyll *a* content and photosynthetic capacity (P_{max}) of Loch Leven water samples incubated for various lengths of time in continuous fluorescent light (7.5 klux) or in darkness. The pH of the samples before and after the various treatments are given in parentheses.

Date incubation started in 1970	Incubation period(h)	chlorophyll *a* (mg/m^3)						P_{max} (mg O_2/mg chlorophyll *a*.h)					
		Nutrients added			Controls		Initial	Nutrients added			Controls		Initial
		N	P	N+P	Light	Dark		N	P	N+P	Light	Dark	
19 June	90	116	45	436	55	-	100	2.5	4.1	2.7	3.5	-	5.2(9.3)
25 June	18	172	126	172	118	-	65	3.3	3.4	3.6	3.3	-	6.0(9.3)
	42	-	-	226	87	-		-	-	3.7(9.5)	1.6(8.5)	-	
29 June	41	133	50	183	60	-	75	2.9	2.3	5.6	1.8	-	5.5(9.2)
21 July	18	57	36	63	35	40	30	6.0(8.9)	4.5(8.8)	6.4(9.0)	3.8(8.7)	-	7.5(8.3)
	42	63	21	143	23	35		5.6(9.1)	3.3(8.4)	5.7(9.6)	3.4(8.4)	-	
14 August	18	115	61	126	57	84	73	5.3(9.5)	5.2(9.3)	7.0(9.4)	5.1(9.3)	9.1(9.0)	9.0(9.0)
	42	129	42	288	42	67		3.5(9.5)	3.5(9.1)	4.0(10.0)	3.0(9.1)	9.2(8.4)	
27 August	18	-	-	144	110	119	90	-	-	6.0	4.0	11.0	11.2(9.0)

principle, since growth could be initiated by addition of nutrients.

Phosphorus did not appear to be the immediate limiting nutrient since addition of phosphate alone consistently failed to stimulate growth (at least in terms of chlorophyll a).

Addition of nitrate stimulated growth (as chlorophyll a) in each experiment. This suggests that, in the absence of a nutrient supply from inflows and bacterial recycling in sediments, nitrogen would be the critical nutrient potentially limiting biomass increase in the loch during the period investigated. The fact that addition of nitrate plus phosphate gave greater yields than did nitrate alone merely shows that once nitrogen limitation is alleviated phosphorus becomes yield-limiting.

Laboratory enrichments of this type, in which nutrient supply from sediment and inflow is cut off, can only indicate the potential of a nutrient to limit growth. Furthermore, the light intensity supplied in the laboratory is probably higher than an average algal cell would experience circulating in the water column of Loch Leven. In the loch, as discussed later (p. 153), because of self-shading, light may become insufficient to sustain further net growth before nutrients actually become limiting. A better approach to detection of actual nutrient limitation would be to monitor the effects of nutrient additions in natural populations enclosed in an open-ended polyethylene column embedded in the sediment so that contact between water and sediment, and the natural light regime, are maintained. Such an approach has been used by Goldman (1962), Brook, Baker & Klemer (1971) and Lund (1972b). Clearly, even this approach has limitations; nutrient supply from sediments may be changed by the altered turbulence characteristics in the enclosed water column. The error due to the lack of supply from inflows could, however, be calculated if nutrient

concentrations in the inflows, and flow rates, are known.

Photosynthetic capacity (P_{max}) declined in all samples after exposure to 7.5 klux continuous fluorescent illumination (Table 6; Fig. 32). This decline could be prevented by keeping the samples dark. Since in the light most samples showed no increase in chlorophyll a content (some in fact showed a decline), it seems unlikely that the accompanying decline in P_{max} could be due to the progressive development of nutrient deficiency caused by growth in a nutrient-depleted medium. It seems that the decline in P_{max} is due to an effect of light per se and, as discussed earlier, may be due to oxidative destruction of enzymes. The effect of light per se is thus not confined to the destruction of chlorophyll a described above, but also extends to a destruction or inactivation of other parts of the photosynthetic apparatus, leading to a decline in the maximum rate of oxygen evolution by the remaining chlorophyll a.

The decline in P_{max} in continuous light was not arrested by provision of extra phosphorus (Table 6). Enrichment with nitrogen alone delayed the decline in P_{max} in two experiments (29 June, 21 July). Enrichment with both nitrogen and phosphorus delayed the decline in P_{max} to a greater extent and on more occasions than did nitrogen alone. These results are interesting in that they tend to support the view expressed by Saijo & Ichimura (1962), Strickland (1965) and Pechlaner (1971) that nutrient deficiency enhances susceptibility to light inhibition. It should be pointed out, however, that the above authors do not distinguish clearly between destruction of chlorophyll a and inhibition of activity per unit of chlorophyll a.

Table 6 shows that the photosynthetic capacity of the N + P enriched sample was usually higher than that of the unenriched light-grown control sample. Taken in isolation this result suggests a

stimulation of P_{max} by N + P enrichment. Similar results were reported by Ichimura & Aruga (1958) and Ichimura (1960a) and were taken to indicate previous nutrient limitation of P_{max}. However, it is not absolutely clear in the above two papers whether the photosynthetic capacity of the 'initial' or 'raw' water is literally the initial capacity of the material when it is removed from the lake (i.e. equivalent to 'initial' capacity as used here) or whether it refers to the capacity of the unenriched raw water after exposure to the same light conditions as the enriched sample (i.e. equivalent to the 'light' control as used here).

Clearly in the Leven experiments, at any rate, nutrient enrichment did not lead to an increase in photosynthetic capacity over that observed initially in the unenriched sample before it was exposed to continuous light. The results therefore cannot be said to show that P_{max} in the loch, at the time of sampling, was limited by nitrogen or phosphorus history. Nevertheless, it is still possible that P_{max} was in fact nutrient-limited at the time of sampling but that subsequent growth, stimulated by extra nutrients, led to some other factor, associated with the increase in population density, becoming limiting to P_{max}. Thus, stimulation of P_{max} due to nutrient enrichment could have been obscured by other factors. Two obvious possible factors are - a) cell density *per se*, b) the associated pH increase. An experiment was described in the methods section which showed that cell density *per se*, within the range 50-240 mg chlorophyll *a*/m^3, had no effect on measured P_{max}. Higher densities were not investigated. Apart from the two samples with chlorophyll *a* concentrations above 240 mg/m^3, it seems unlikely that cell density *per se* reduced P_{max} in the enriched samples, through a self-shading effect within the bottles.

The pH of enriched samples increased during incubation in the light

(Table 6). P_{max} of enriched samples was therefore measured at higher pH values than that of initial samples. Subsequent experimental work in 1971 (p. 86) showed that pH values above about 9.0-9.5 did reduce P_{max}, at least at 5°C. It is possible, therefore, that the P_{max} values of the enriched samples would have been higher had the measurements been made at a lower pH value, equivalent to that of the initial sample.

Clearly, in future, shorter incubation periods and pH adjustment should be incorporated in experiments of this type.

SECTION VI: EXTRAPOLATION OF HOURLY RATES OF GROSS PHOTOSYNTHESIS TO LONGER TIME PERIODS

1. Daily gross photosynthesis per unit area

The method of calculation and its limitations

Laboratory experiments, described in the methods section, showed that the photosynthetic capacity of Loch Leven phytoplankton began to decline in exposures longer than 6 h. Direct measurement of daily integral photosynthesis per unit area, using whole day exposures, was therefore considered undesirable in this lake. The general limitations of long exposures are discussed by Soeder & Talling (1969).

Daily rates were calculated from hourly rates, measured in 3 h exposures, using the following equation (No. 5 of Talling, 1965a):

$$\frac{\Sigma\Sigma nP}{\Sigma nP} = 0.9\ \Delta t \frac{(\log \bar{I}'_o - \log 0.5\ I_k)}{(\log I'_o - \log 0.5\ I_k)}$$

where $\Sigma\Sigma nP$ = daily integral photosynthesis per unit area

ΣnP = hourly integral photosynthesis per unit area

$\bar{I}'_o$ = mean sub-surface light intensity over the daylength (Δt), in kerg (Ph.A.R.)/cm^2.sec (Fig. 41)

I'_o = mean sub-surface light intensity over the 3 h exposure period, in kerg (Ph.A.R.)/cm^2.sec

Δt = daylength (in hours)

I_k = photosynthetic characteristic (units, kerg(Ph. A.R.)/cm^2.sec) expressing the onset of light-saturation, as defined earlier

The derivation and limitations of this equation are described by Talling (1957b); implicit in its use is the assumption that photosynthetic activity at a given light intensity remains constant throughout the day. Evidence exists that this assumption may not

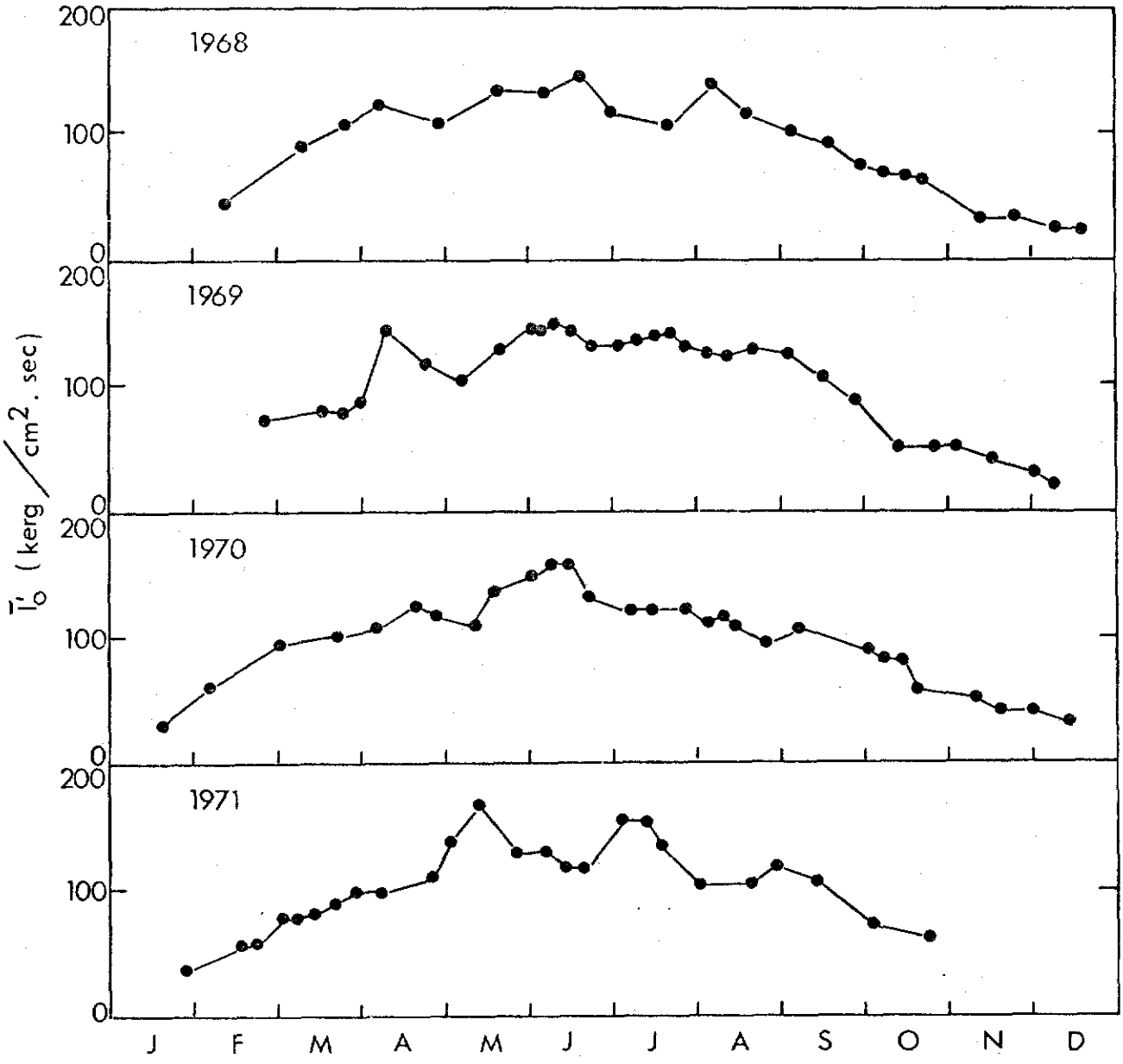


Fig. 41 Seasonal variation in the mean sub-surface light intensity ($\bar{I}'_o$) over the daylength, in kerg/cm^2.sec of photosynthetically-available radiation. Values were calculated from 10-day mean daily radiation values divided by daylength.

always be valid; a diurnal depression of photosynthetic activity per unit water volume was been recorded in marine habitats (Doty & Oguri, 1957; Yentsch & Ryther, 1957; Shimada, 1958) as well as in fresh waters (Verduin, 1957; Ohle, 1958, 1961; Ichimura, 1960b; Ganf, 1969, 1972). This daily decline is commonly attributed to photo-oxidative destruction or inactivation of chlorophyll a. Reduction in photosynthetic activity per unit of chlorophyll a, caused by photo-oxidative destruction of enzymes, may also be involved. Other possible causes of diurnal change include inherent circadian rhythms and diurnal migration or redistribution of algal cells. Progressive depletion of nutrients or build-up of pH and oxygen levels may limit photosynthetic capacity towards the end of the day in certain situations, e.g. Lake George, Uganda (Ganf, 1969, 1972). Diurnal variation is reported to be most pronounced at low latitudes (Doty, 1959) and to be enhanced by strong illumination and poor nutrient conditions (Saijo & Ichimura, 1962).

Diurnal changes at Loch Leven

Diurnal variation in photosynthetic capacity (P_{max}), pH and % oxygen saturation was investigated at Loch Leven on five occasions. Complete 24 h measurements were obtained for oxygen only. pH measurements were restricted to daylight hours. It was not possible to measure photosynthetic capacity, in the loch, in the early morning or late evening because light intensities were then too low to saturate photosynthesis. Laboratory facilities for measuring photosynthetic rate under artificial illumination were not available at the loch side. Information on possible diurnal changes is thus severely limited; such data as were obtained showed no evidence of significant diurnal variation in photosynthetic capacity or in two of the factors (pH and oxygen level) likely to induce such variation. A further point limiting the general

applicability of these results is that they were obtained when the chlorophyll a concentration in the water was only about half its maximum recorded value. Larger diurnal changes in pH, oxygen and nutrient concentration may have occurred in denser crops, with consequent effects on photosynthetic capacity. However, the efficiency of turbulent mixing at Loch Leven, in preventing the residence of algal cells for long periods at inhibitory light intensities and in accelerating gaseous exchange with the atmosphere and gaseous and nutrient exchange with the aphotic zone, may well have reduced the likelihood of significant diurnal changes developing. Ohle (1961) has shown that diurnal variation is reduced by increased water turbulence.

Results

Calculated values of daily gross photosynthesis per unit area ($\Sigma\Sigma$ nP), in g O_2/m^2.day, are shown in Fig. 42. Marked seasonal changes in daily gross productivity were recorded, within the range 0.4-21.0 g O_2/m^2.day (0.1-7.9 g C/m^2.day). The highest value recorded represents an isolated measurement but rates of 10.0-15.0 g O_2/m^2.day (3.8-5.6 g C/m^2.day) were fairly common. During most of the year rates of gross production of 5.0 g O_2(1.9 g C)/m^2.day were maintained. Values less than 5.0 g O_2(1.9 g C)/m^2.day were restricted mainly to autumn and winter months (September-March), with the exception of a midsummer minimum, of short duration, observed in 1970 and 1971.

Annual mean daily gross productivity (assessed over the 4 years) was 5.8 g O_2(2.2 g C)/m^2.day.

Discussion

At the latitude of Loch Leven, 56°N, daylength varies between 17.6 hours in June and 6.9 hours in December (Fig. 43). Clearly, from equation 5 of Talling (1965a), reproduced above, seasonal variation in

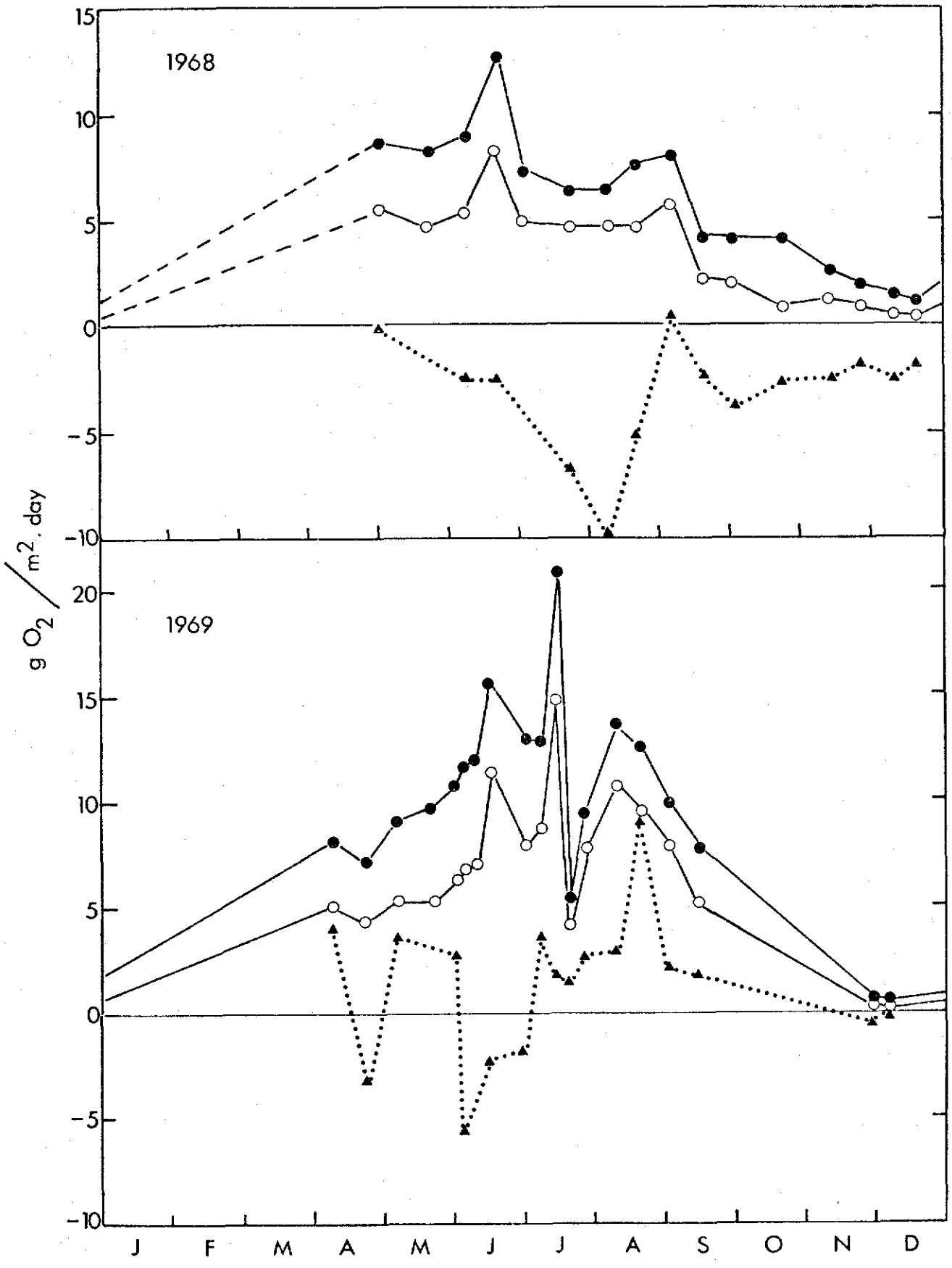


Fig. 42 Legend opposite.

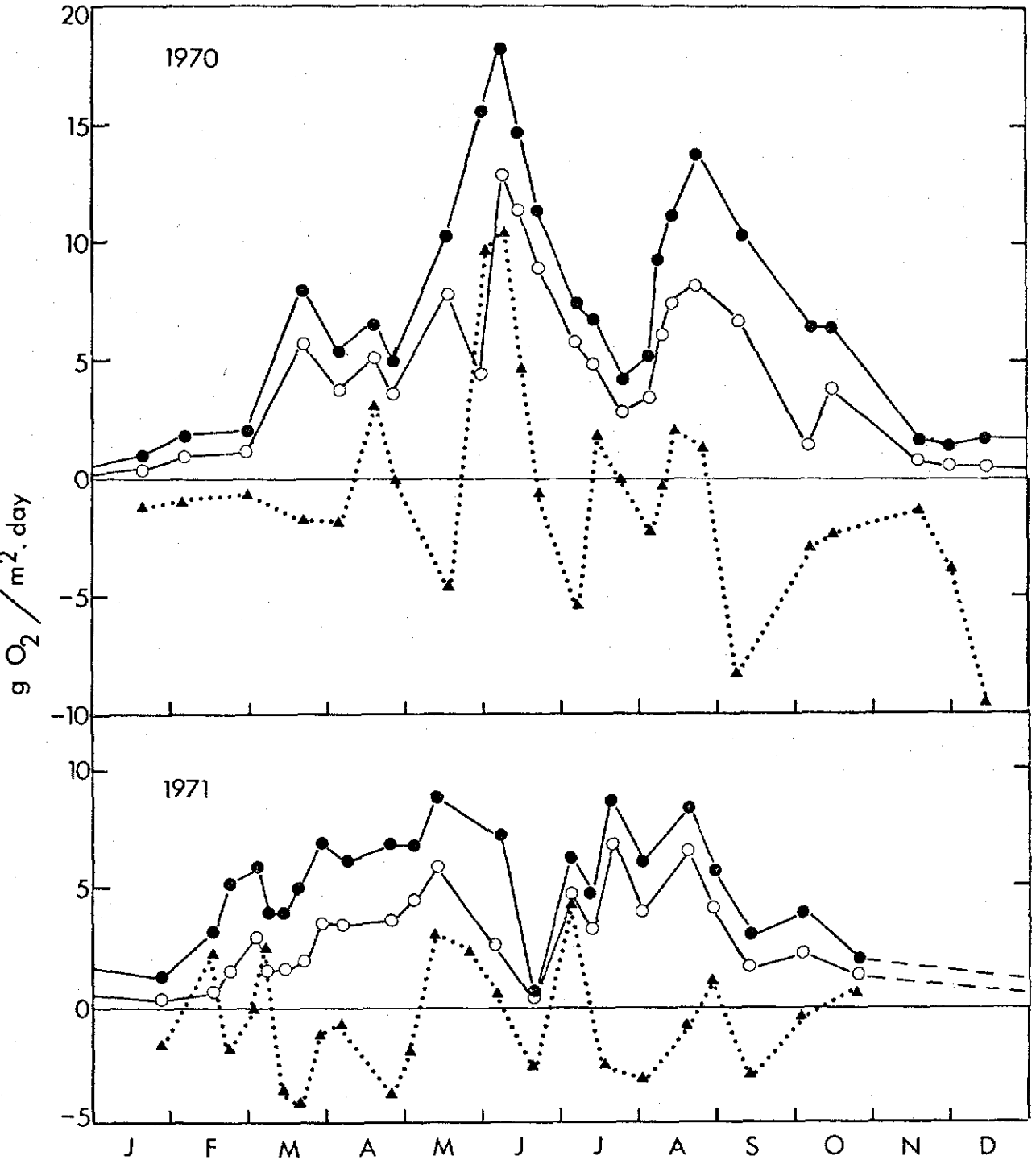


Fig. 42 (above and opposite) Seasonal variation during 1968-71 in gross (●—●) and net (▲···▲) daily photosynthetic productivity per unit area. Net daily photosynthetic productivity calculated assuming algal respiration rate to be 5% of the light-saturated photosynthetic rate is also shown (o—o). The dashed line indicates extrapolation to the beginning of 1968 and the end of 1971, which was based on the mean of the three intervening December/January values.

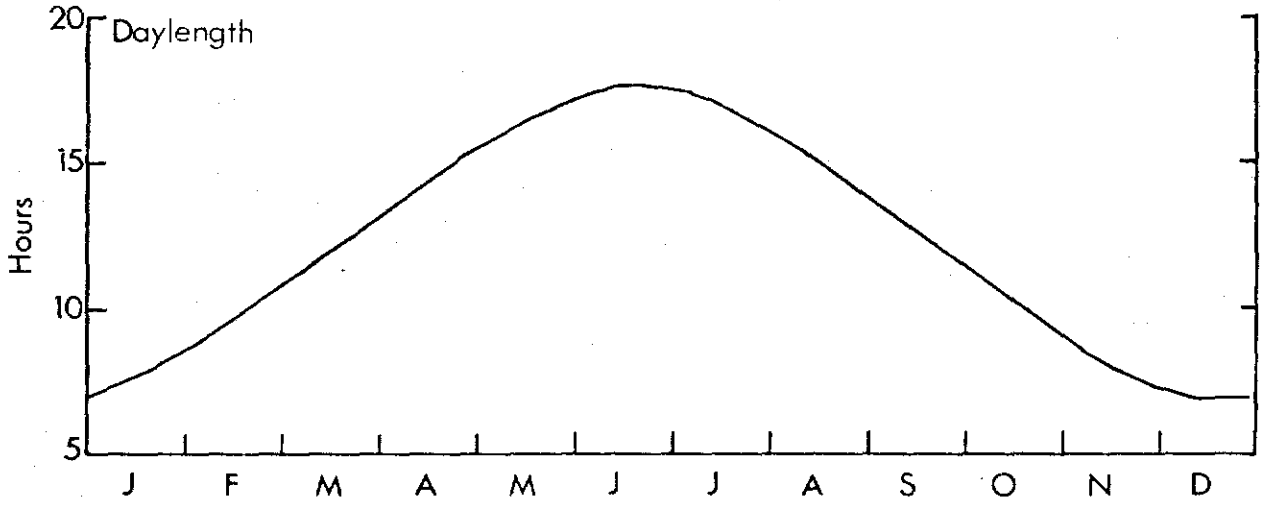


Fig. 43 Seasonal variation of daylength at Loch Leven.

daylength (Δ t) exerts a direct influence on the amount of daily gross production ($\Sigma\Sigma$ nP) possible for any given hourly value (Σ nP). As a determinant of water temperature, daily incident radiation exerts further control of $\Sigma\Sigma$ nP by virtue of the influence of temperature on photosynthetic capacity (P_{max}). Thus low daily light intensities (Fig. 44) and temperatures (Fig. 29) are major factors limiting daily gross productivity in winter months in Loch Leven, as in many other temperate lakes. However, other factors being equal, the limitations which low winter light intensities impose on daily gross productivity are likely to be reflected in a less severe limitation of net production (and therefore of potential biomass accumulation) in a shallow lake than in a deeper lake. This is because shallowness, in reducing respiration losses, can partly compensate for low winter illumination. The relative shallowness of Loch Leven is one of the factors which may contribute to the higher winter crops and earlier spring rise encountered here as compared with other deeper temperate lakes like Windermere (Talling, 1971).

Within the limits of incident light intensity and temperature imposed largely by latitude, daily rates are determined by the influence of 'local' conditions on the ratio nP_{max}/k_{min} discussed earlier and shown to be the chief determinant of hourly photosynthesis per unit area (Σ nP). 'Local' factors are clearly important in restricting gross daily production to low levels in midsummer, when daylength and water temperature are close to maximal. The mid-summer minima found at Loch Leven coincided with periods of particularly low crop densities. This observation does not invalidate the overall absence of a correlation between population density (n) and areal productivity (Σ nP) described earlier (Fig. 25); uncoupling of n and Σ nP variations is more severe at higher population densities

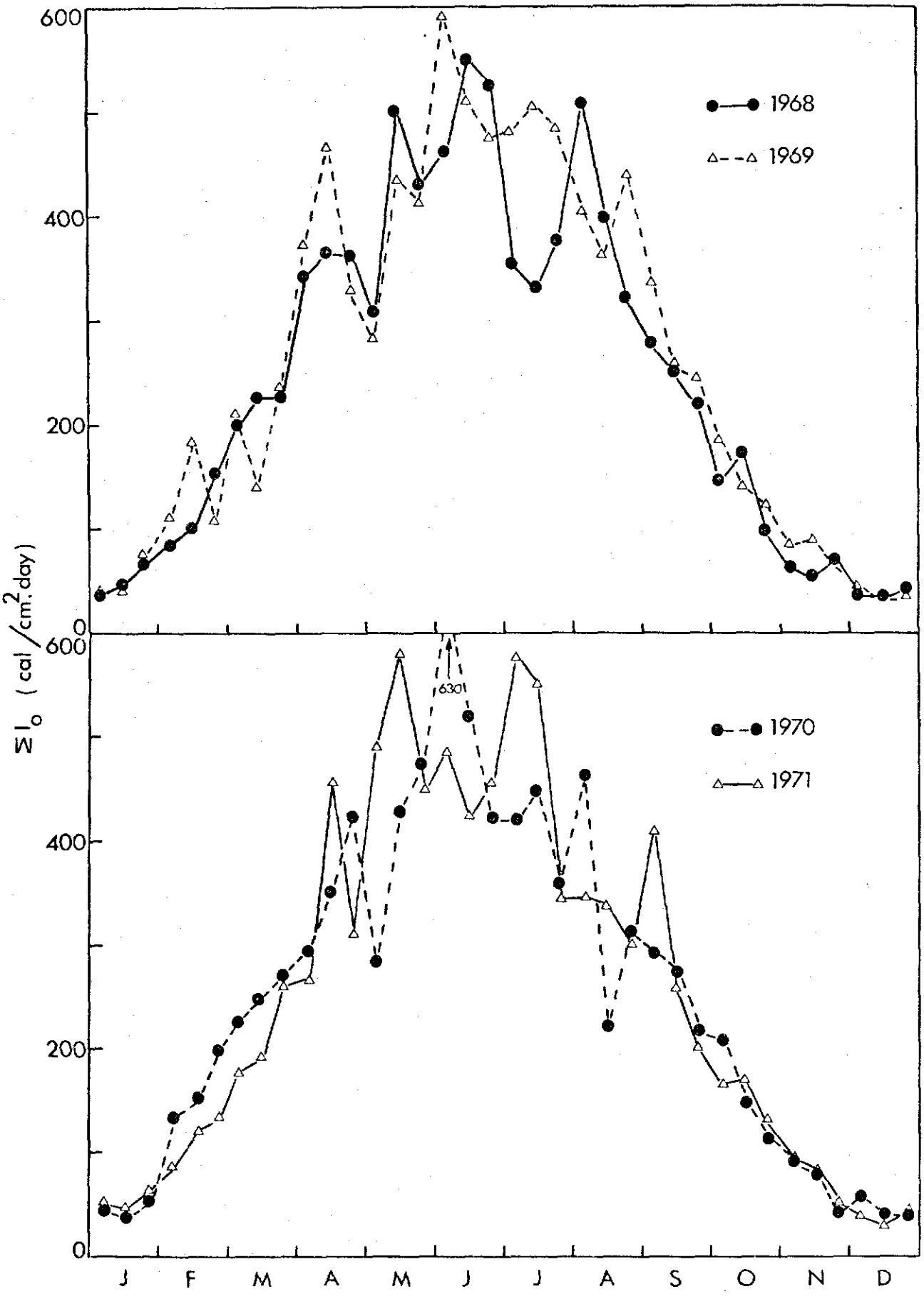


Fig. 44 Seasonal variation in total daily irradiance (ΣI_o), 10-day mean values.

owing to (i) self-shading, (ii) inverse relationships between phytoplankton density (n) and photosynthetic capacity (P_{max}).

The highest values of daily gross productivity recorded for Loch Leven lie towards the upper end of the range of values so far encountered in natural waters. According to Steemann Nielsen (1954) the most productive tropical and sub-tropical oceans yield between 0.5-3.0 g C/m^2.day. Of the thirty-five lakes in Europe and North America listed by Vollenweider (1968), only one has a maximum daily production as high as the maximum found at Loch Leven and only five have rates higher than 10.0 g O_2(3.8 g C)/m^2.day - a fairly common upper value at Loch Leven. The annual mean daily gross production rate at Loch Leven is higher than that of any of the lakes listed by Vollenweider and according to the classification scheme of Rodhe (1969) places Loch Leven in the 'eutrophic polluted' category.

In general daily rates higher than 10.0 g O_2(3.8 g C)/m^2.day seem to be commoner in tropical and sub-tropical fresh waters. Values between 10.5 and 16.0 g O_2(4-6 g C)/m^2.day were recorded by Talling (1965a) in a number of African lakes. Similarly high values have been measured in L. Mariut, Egypt (Vollenweider, 1960), in fertilised fish ponds in Israel (Hepher, 1962) and in impoundments and ponds in India (Sreenivasan, 1972). Such rates approach the maximum rates of net production (ca 14 g C/m^2.day) which have been achieved in outdoor mass cultures of algae (Tamiya, 1957).

Daily rates of gross production will clearly approach an upper limiting value as the amount of phytoplankton in the euphotic zone approaches its theoretical maximum. As discussed earlier (p. 61), this upper limit to 'photosynthetic cover' is at times closely approached at Loch Leven and is an important factor contributing to the high daily rates observed. Once this upper limit to the size of

the euphotic crop is reached, the maximum daily production limit is set essentially by the photosynthetic capacity of the algae and by daylength. Based on such considerations Vollenweider (1968) estimates that the theoretical upper limit to daily production is of the order of 10-20 g C/m^2.day.

2. Annual gross productivity and % energy conversion estimates

Annual gross production values (Table 7) were determined by planimetry of the areas under the curves of daily rates in Fig. 42. Carbon-based values were calculated assuming a photosynthetic quotient of 1.0. Table 7 also shows annual incoming radiation totals calculated by planimetric integration of the areas under the curves of seasonal changes in total daily radiation (Fig. 44). Percentage energy conversion estimates (Table 7) were calculated assuming an approximate calorific equivalent of 3.7 kcal/g O_2 (see Winberg, 1971). In order to obtain a figure for annual production for each calendar year studied the values for the beginning of 1968 and the end of 1971, which were not actually measured, have been taken as equal to the mean of the three intervening December/January values determined from the continuous curve in Fig. 42.

Mean annual gross productivity over the 4 years was estimated as 2,100 g O_2(785 g C)/m^2.year. On the basis of this value Loch Leven would be considered 'polytrophic' in the classification scheme of Vollenweider (1968) and 'eutrophic polluted' in that of Rodhe (1969).

Table 7. Annual total incident radiation, annual gross phytoplankton production and percentage energy conversion estimates for 1968-71

Year	Total incident radiation (kcal/m^2.y)	Gross productivity			Percentage of total energy converted in photosynthesis
		g O_2/m^2.y	g C/m^2.y	kcal/m^2.y	
1968	889 x 10^3	1918	720	7097	0.8
1969	949 x 10^3	2590	971	9583	1.0
1970	925 x 10^3	2310	853	8547	0.9
1971	944 x 10^3	1590	597	5883	0.6

SECTION VII: RESPIRATION RATES AND NET DAILY PHOTOSYNTHETIC PRODUCTIVITY

Introduction

In order to determine the net photosynthetic productivity of organic material which is available for the rest of the aquatic community, it is necessary to subtract, from the total gain by gross photosynthesis, the loss equivalent to the respiratory activity of the phytoplankton. This is attempted in this section.

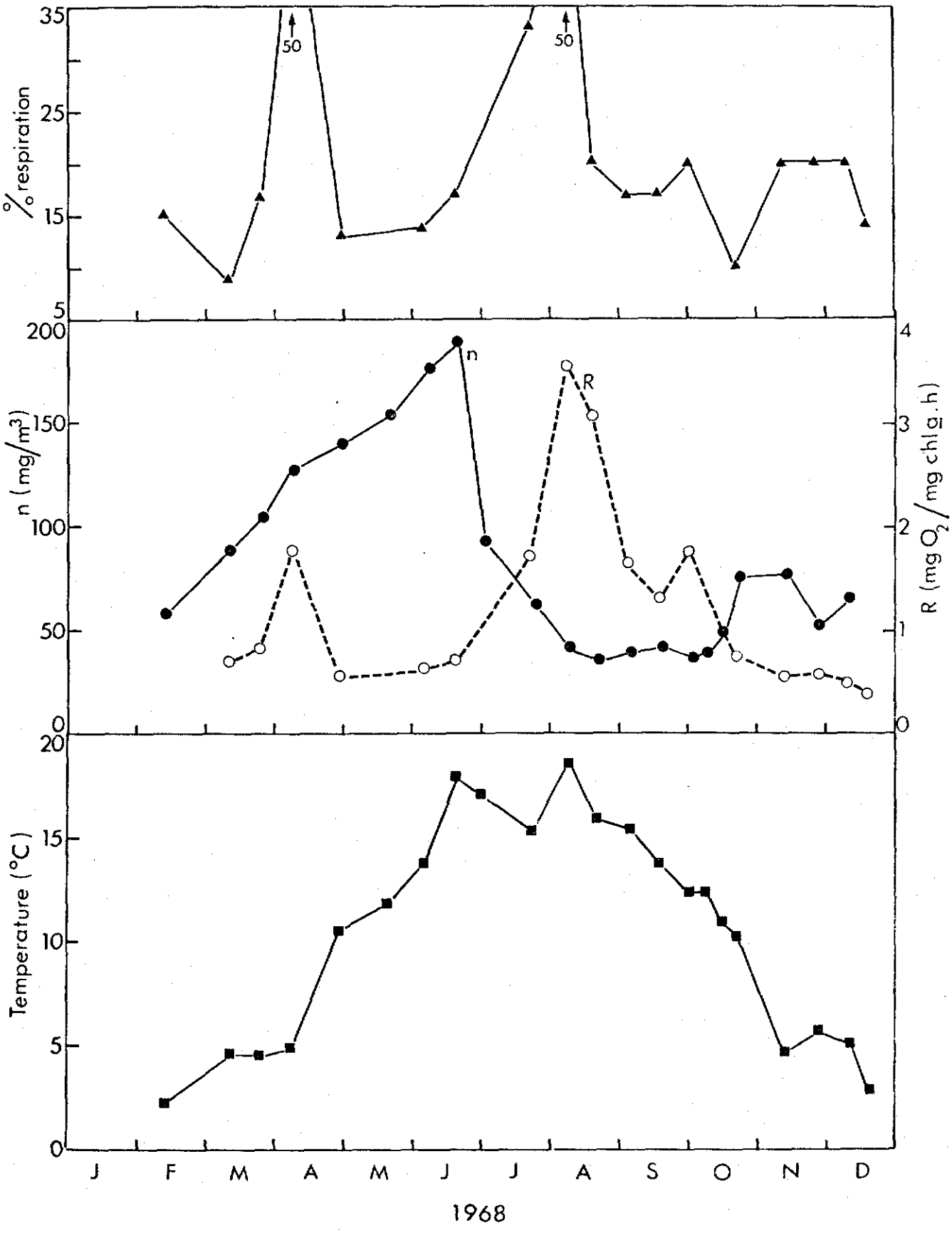
1. Respiration rates

Results

Dark respiratory oxygen uptake rates per unit volume of water were determined from the differences between initial and final oxygen concentrations in the dark bottles. The results are a measure of community respiration, including contributions from phytoplankton, zooplankton and bacteria. It was not possible to separate the phytoplankton from all zooplankton and bacteria and therefore the contribution of non-algal respiration is not known.

If the measured rates of community respiration are assumed to be largely due to phytoplankton, and are expressed per unit of chlorophyll *a*, they range from 0.1-3.9 mg O_2/mg chlorophyll *a*.h. Seasonal trends in the apparent rate of respiration per unit of chlorophyll *a*(R) were broadly similar from year to year, with a tendency for higher values to coincide with periods of low population density and high water temperature (Fig. 45). In this respect seasonal changes in R resemble those of photosynthetic capacity (P_{max}) described earlier (Fig. 29). This has the effect of reducing variation in relative respiration rate r, i.e. the ratio between respiratory rate and light-saturated gross photosynthetic rate.

Fig. 45 (following 4 pages) Seasonal variation during 1968-71 in (a) the rate of community respiration expressed as a percentage of the light-saturated photosynthetic rate (% respiration), (b) the rate of community respiration expressed per unit of chlorophyll *a*(R), (c) population density (n) estimated as chlorophyll *a*, (d) water temperature.



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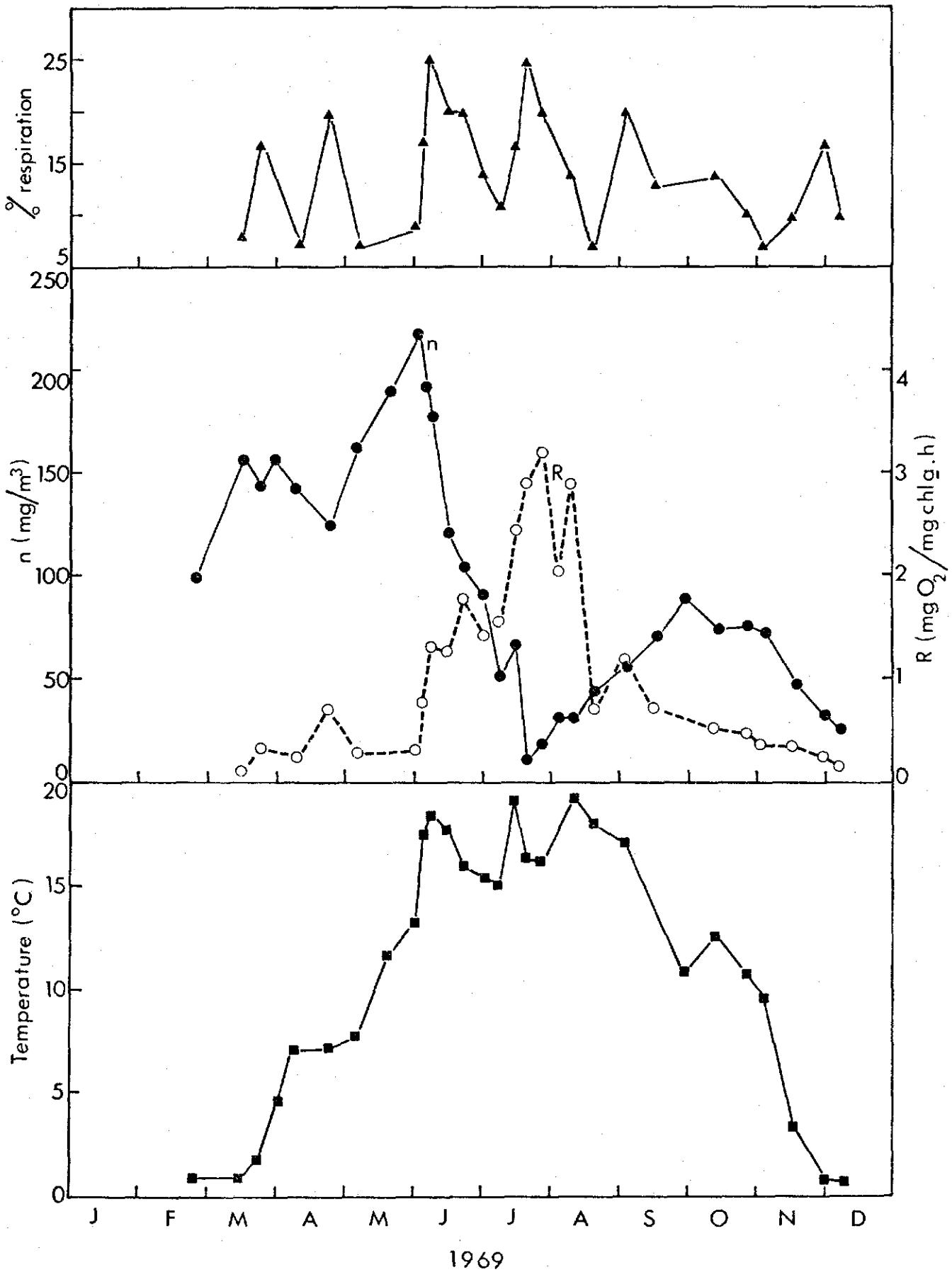
% respiration
25
15
5
n (mg/m³)
250
200
150
100
50
0
n
R
R (mg O₂/mg chl g.h)
4
3
2
1
0
Temperature (°C)
20
15
10
5
0
J
F
M
A
M
J
J
A
S
O
N
D
1969

Fig. 45 (cont'd)

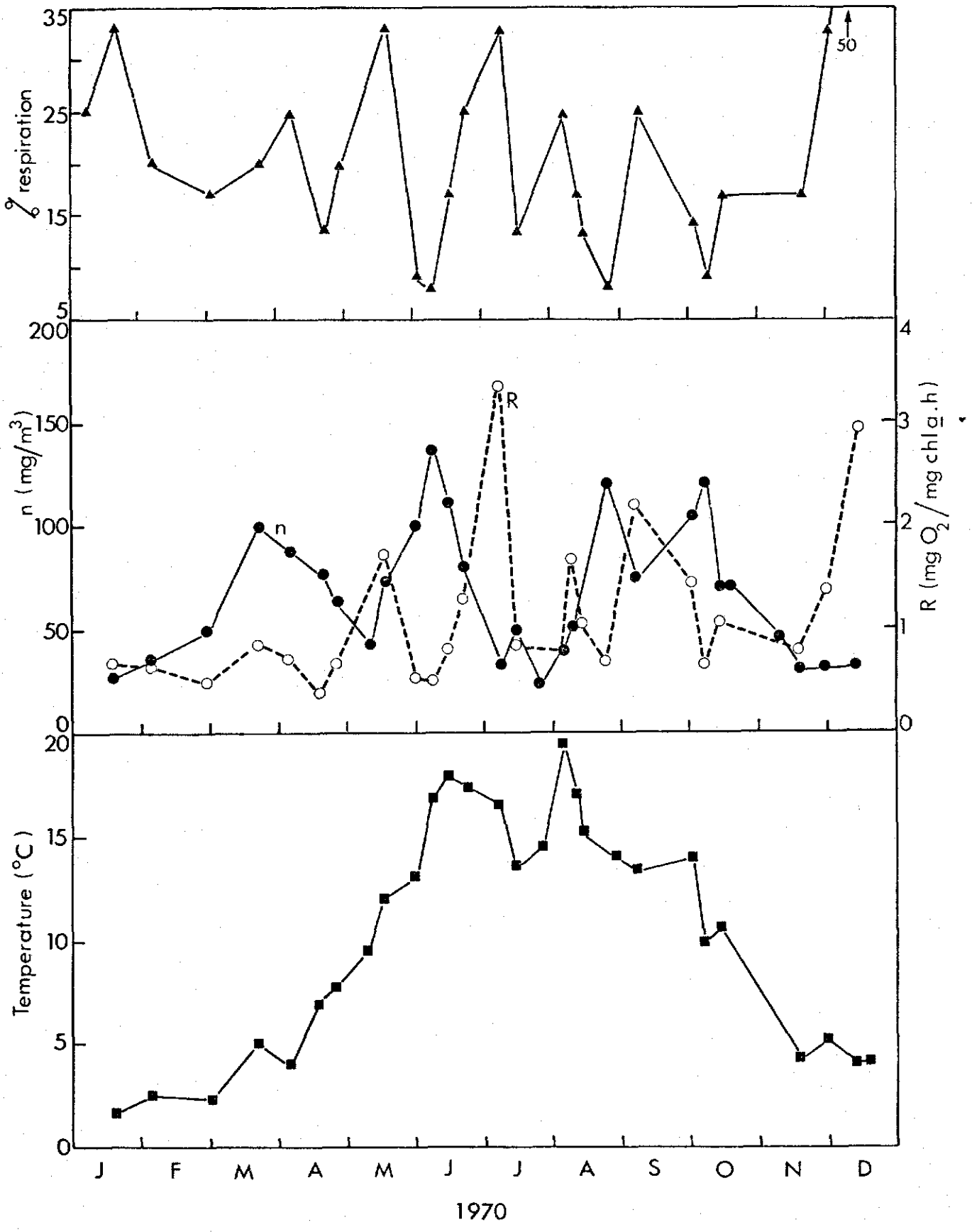
% respiration
35
25
15
5
50
n (mg/m³)
200
150
100
50
0
n
R
R (mg O₂/mg chl a.h)
4
3
2
1
0
Temperature (°C)
20
15
10
5
0
J
F
M
A
M
J
J
A
S
O
N
D
1970

Fig. 45 (cont'd)

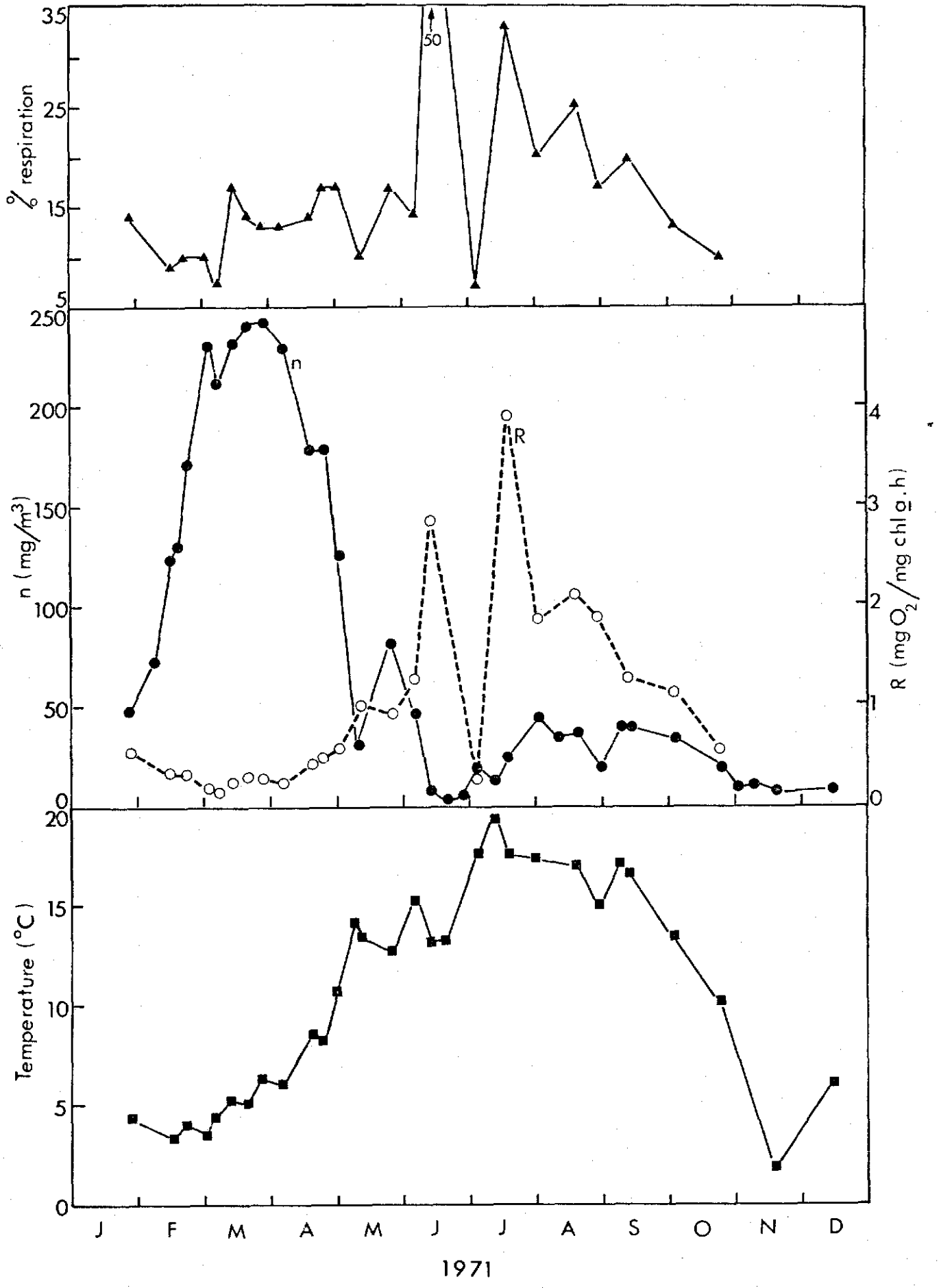
% respiration
35
25
15
5
50
n (mg/m³)
250
200
150
100
50
0
n
R
R (mg O₂/mg chl a.h)
4
3
2
1
0
Temperature (°C)
20
15
10
5
0
J
F
M
A
M
J
J
A
S
O
N
D
1971

Fig. 45 (cont'd)

The rate of respiration expressed as a percentage of the light-saturated (optimum) gross photosynthetic rate (% respiration) varied from 7-50% (Fig. 45). Eighty two per cent of all respiration rates measured were 20% or less of the optimum photosynthetic rate; 48% were 15% or less.

Overall, rates of respiration per unit of chlorophyll a(R) appear loosely correlated with water temperature (Fig. 46). The correlation coefficient between log R and temperature is 0.67 and the calculated linear regression line indicates an average temperature coefficient (Q_{10}) of 2.5.

Discussion

Assuming for the moment that community respiration rates include a negligible contribution from zooplankton and bacteria, the data in Fig. 46 suggest that temperature is probably an important factor determining algal respiration rate. However, Fig. 45 shows that the start of the main increase in respiratory rate coincided with the start of the decline in algal crop density but lagged behind the main increase in water temperature. This suggests that factors other than temperature may at times exert a dominant influence on respiratory rate. High pH values, associated with dense crops, were shown earlier to be a factor contributing to the delay in the response of P_{max} to seasonal increase in water temperature. Though not investigated experimentally, a similar inhibitory effect of high pH on respiratory activity cannot be discounted. The likelihood of such an effect is difficult to assess from the limited amount of published evidence available (Steemann Nielsen, 1955c; Gibbs, 1962); variable effects have been shown depending on species and previous growth conditions.

The increase in respiratory activity as the algal crop declines (Fig. 45) could partly account for the possible anomaly, discussed

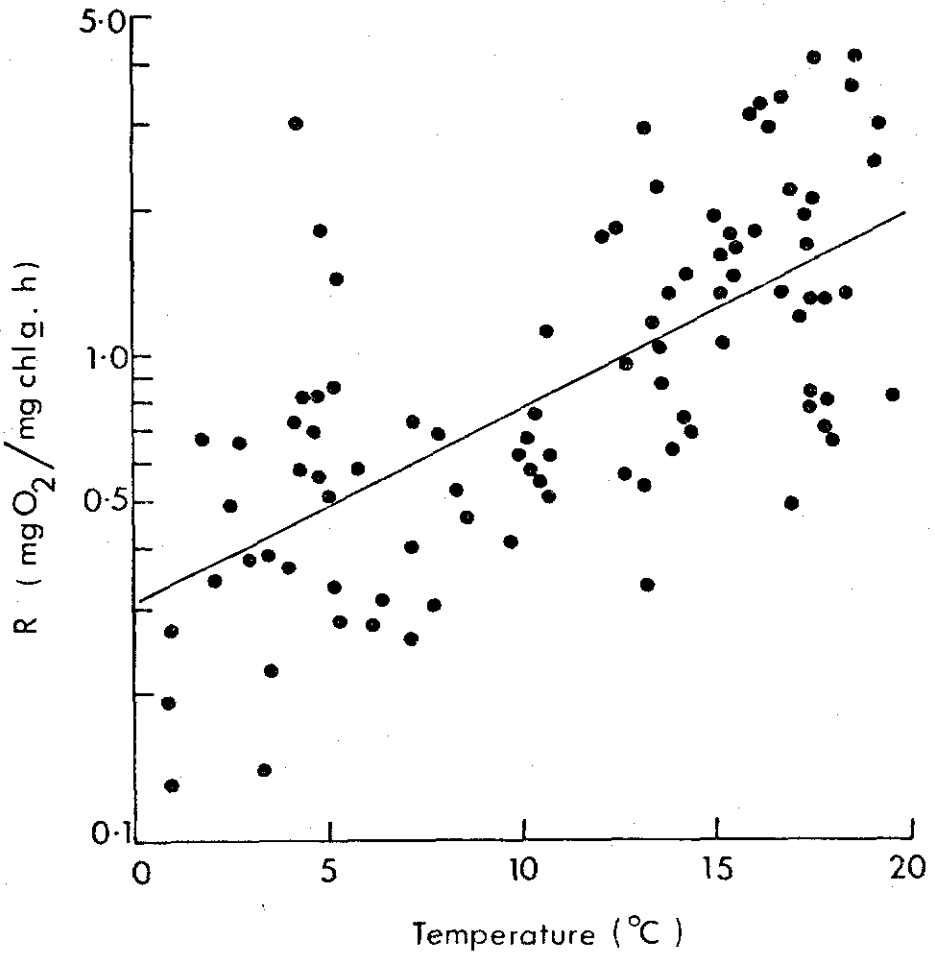

Fig. 46 Community respiration rates expressed per unit of chlorophyll a (R, logarithmic scale) in relation tc ambient water temperature. Calculated regression line is log y = 0.04x - 0.51, correlation coefficient (r) = 0.67, N = 92. Q_{10} = 2.51.

earlier (p. 71), of a declining crop having an enhanced capacity for gross photosynthesis. Whereas an increase in water temperature will increase respiratory rate at all depths in the water column, its effect on gross photosynthetic activity will be largely confined to those depths where light is sufficient to saturate photosynthesis. Photosynthesis at sub-saturating light intensities was shown (Fig. 8) to be insensitive to temperature. Thus, an increase in algal respiration rate, due to temperature increase, could reduce the rate of net production and hence of biomass accumulation, even when, as at Loch Leven, increased respiratory rate is accompanied by increased photosynthetic capacity (P_{max}). The effect will be accentuated if the increase in respiratory rate is proportionately greater than the increase in P_{max}.

In pure cultures of algae, and in some natural populations where respiration of bacteria and zooplankton is considered negligible, the rate of respiration of actively growing healthy cells is often found to be a more or less constant percentage (5-15%) of the light-saturated (optimum) photosynthetic rate (Ryther, 1954, 1959; Steemann Nielsen & Hansen, 1959b; McAllister, Shah & Strickland, 1964). Nutrient deficiency is reported to reduce both respiratory and photosynthetic activity of algae (Ryther, 1954; McAllister et al, 1964; Stewart & Alexander, 1971); photosynthetic activity is usually (though not always) the more severely affected, with the result that respiration increases as a fraction of optimum photosynthesis when cells are short of nutrients. At Loch Leven 52% of all respiration rates measured were greater than 15% of the optimum photosynthetic rate. These high respiratory fractions are not considered, in this case, to be necessarily indicative of nutrient (nitrogen or phosphorus) deficiency; this is because no consistent relationship was found

between % respiration and the nutrient history of the population as indicated by chl *a*/part-N and chl *a*/part-P ratios (cf. Figs. 40 and 45). Gessner & Pannier (1958) found that the respiratory rate of algae in culture increased with increase in O_2 tension. At Loch Leven increased respiratory rates were not associated with increased O_2 levels (cf. Figs. 37 and 45).

It is likely that at least part of the apparent increase in algal respiration rate in summer months was due to the increased abundance and respiratory activity of zooplankton and bacteria. No data exist for bacteria. Johnson & Walker (1974) found a peak in crustacean zooplankton numbers in June and July 1969; Bailey-Watts (unpublished data) recorded a maximum number of rotifers in the same months. Furthermore, even if non-algal respiration were to remain constant throughout the year, higher apparent rates of respiration per unit chlorophyll *a* would tend to occur at lower algal densities.

However, it is interesting to note that despite uncertainties about the extent of non-algal 'interference', the measured respiration rates, when expressed per unit of chlorophyll *a*, fall within the range of values reported by Ryther & Guillard (1962) for bacteria-free cultures of marine planktonic diatoms, and by Aruga (1965) for cultures of freshwater green and blue-green algae.

2. Net photosynthetic productivity

Results

Net areal photosynthetic productivity per day was calculated by subtracting respiratory oxygen consumption per unit area per 24 h from the corresponding estimate of gross areal photosynthesis per day.

Daily respiratory oxygen consumption per m^2 over 4.8 m (the effective mean depth) was calculated on the assumption that hourly respiration rates, measured near midday, remained constant over 24 h

and were the same throughout the water column. Effective mean depth was calculated on p. 76 . It was used here in preference to the mean depth, for the reasons discussed by Talling (1957b, p.144).

Daily respiratory losses frequently equalled or exceeded estimated daily photosynthetic gain, resulting in zero or negative values of net photosynthetic productivity (Fig. 42).

Discussion

The estimated values of net photosynthetic productivity appear anomalous in that zero or negative values were found even during periods when algal population densities were increasing.

Some algae are reported to utilise dissolved organic carbon for growth, either in complete darkness (Khoja & Whitton, 1971) or in dim light (Baalen, Hoare & Brandt, 1971). Earlier work on the subject is reviewed by Goldman et al (1972, pp.655-657). Uptake and utilisation of dissolved organic carbon by Leven phytoplankton was not investigated; if it occurred it could have permitted a greater increase in algal biomass than would be predicted from net photosynthesis estimates, thereby accounting for at least part of the apparent discrepancy noted above.

Underestimation of gross photosynthetic productivity, another possible reason for the discrepancy, was attributed by Golterman (1971) to the occurrence of photochemical oxidation and by Kowalczewski & Lack (1971) to the use of stationary bottles. These two source of error were investigated (see methods section) and found to be unimportant at Loch Leven. Photorespiration was not investigated. If it occurred it could have led to underestimation of gross rates of oxygen evolution. At present it is uncertain whether photorespiratory oxygen uptake is accompanied by carbon dioxide release in algae (Lex, Silvester & Stewart, 1972). If carbon dioxide is evolved with an R.Q. of 1, then although photorespiration would cause underestimation of

gross production, it could not explain the discrepancy between estimates of net photosynthetic productivity and observed algal crop changes.

The use of community (rather than algal) respiration rates in the calculations of net photosynthesis was probably a major cause of underestimation of net productivity.

Dissolved oxygen concentrations below saturation were recorded in the euphotic zone on several occasions (Fig. 37) confirming that a negative community oxygen balance did sometimes exist. However, dissolved oxygen levels (Fig. 37) and pH values (Fig. 29) were frequently above their respective air-equilibrium values when estimates of net daily oxygen production by phytoplankton were negative. This additional anomaly suggests that community respiration rates may themselves be overestimates. Underestimation of gross photosynthesis due to photorespiration could not account for this anomaly, since photorespiratory oxygen uptake would occur in the open water as well as in the experimental bottles.

Oxygen production by benthic algae might provide a partial explanation. Benthic algae occur in shallow areas (less than 3 m deep) where the sediment is sand (Bailey-Watts, 1973, 1974). Their productivity has not been measured, however, and therefore their oxygen contribution to the loch cannot be quantified.

Dark storage is reported to result in lowered rates of dark respiration by algal cultures (Kok, 1952; Hoch, Owens & Kok, 1963; Yentsch & Reichert, 1963). The effect is possibly due to the depletion of endogenous respirable reserves (Gibbs, 1962) and is distinct from the short-term after-effect of photorespiration on subsequent dark respiration measurement, such as that described by Brackett, Olson & Crickard (1953). It may be that during the night

algal respiration rate fell below the value recorded near midday. Simple extrapolation of midday hourly values to a 24 h basis may, therefore, have led to overestimation of daily respiratory losses.

Community respiration rates in 3 h exposures yielded very small changes in oxygen concentration which were sometimes almost at the limit of sensitivity of the Winkler method. The respiration rates measured in this study were therefore subject to relatively greater inaccuracies than were the measurements of gross photosynthesis. Even small errors assume greater importance when hourly respiratory rates per unit volume are extrapolated to a 24 h areal basis. Such experimental errors may account, at least in part, for the discrepancies described above. Measurement of respiration rates from longer exposures was inconvenient for routine use and of debatable value since bacterial activity is likely to be enhanced, particularly in longer exposures, by increased bacterial growth on bottle surfaces (Zobell & Anderson, 1936).

Because of these as yet unresolved uncertainties it is not possible to offer a reliable estimate of net photosynthetic productivity.

For illustrative purposes, daily net photosynthetic production rates were calculated assuming that algal respiration rate was 5% of the light-saturated photosynthetic rate. The resulting values (Fig. 42) ranged from 0.3 to 14.7 g O_2/m^2.day. Annual net production values, estimated from the areas under the seasonal curves of these net daily rates, were 1132 g O_2/m^2.y in 1968, 1643 g O_2/m^2.y in 1969, 1424 g O_2/m^2.y in 1970 and 913 g O_2/m^2.y in 1971. Estimates of net production are extremely sensitive to the value assumed for the relative respiration rate, the variability of which is largely unknown. Consequently the accuracy of the above net production values is also unknown. In fact all that can be said with any confidence is that net annual

production is likely to be less than gross annual production. The latter value is not completely useless in the study of food chain dynamics: if the food demands of the secondary producers are in excess even of gross primary production by phytoplankton, then this would indicate that these animals must also be utilising non-phytoplankton material (e.g. benthic algae or allochthonous material).

Because of self-shading gross daily photosynthesis per m^2 does not increase linearly with increase in population density. Column respiration losses, however, will increase with increase in population density. Unless the self-shading limitation of gross areal photosynthesis is offset by parallel increases in daily irradiance and/or photosynthetic capacity, 24 h column respiration losses are likely to increase in relation to daily gross photosynthesis as population density increases. Thus self-shading tends to push the population towards a position of zero net production. The above calculated net production values are all greater than zero. Thus, under Loch Leven conditions, light-limitation of net production, due to self-shading, would never be sufficient to preclude further increase in population density if algal respiration rate was always 5% of the light-saturated photosynthetic rate. However, respiration rate is likely to become a relatively greater fraction of the light-saturated photosynthetic rate when the latter is reduced by high pH or other population density-dependent factors. High pH is thus likely to reinforce the self-shading effect in limiting net production and hence the rate of biomass accumulation.

SECTION VIII: GENERAL DISCUSSION

Hourly and daily rates of gross photosynthetic productivity showed marked seasonal changes, within the ranges 0.02 to 1.59 g $O_2/m^2.h$ and 0.4 to 21.0 g $O_2/m^2.d$, respectively. Annual values ranged from 1.6 to 2.6 kg O_2/m^2. In general, gross rates were high compared to values published for other temperate waters. The higher values observed at Loch Leven approximated those more commonly recorded in tropical and subtropical waters or in mass outdoor algal cultures. Seasonal variability was, however, much more pronounced in Loch Leven than is generally observed in tropical waters.

The horizontal and vertical uniformity of temperature, nutrients and phytoplankton density at Loch Leven provided a particularly favourable situation for the analysis of factors controlling seasonal changes in productivity. The interpretation of temporal changes was simplified by the elimination of influences arising from changes in horizontal and vertical distribution of water masses. The physiological condition of the phytoplankton was also found to be the same at all depths. This simplified the procedure required for measurement of areal production. Photosynthesis-depth profiles obtained using 0.5 m water were the same as those obtained using the more laborious method in which bottles are filled with water taken from the depth at which they were to be exposed. The routine use of water from 0.5 m depth was therefore justified.

The vertical distribution of photosynthetic activity per unit volume of water was typical of the pattern often reported for phytoplankton. The area of the photosynthesis-depth profile (equivalent to the integral rate of photosynthesis per unit area) was found to be closely approximated by equation 3 of Talling (1957b). The components of the profile, identified in Talling's equation, their interaction

and response to environmental factors, formed the basis for the analysis of factors affecting gross productivity.

Talling et al (1973) distinguish three main components or groups of components which determine hourly rates of gross photosynthesis. These are: (a) population density and underwater light penetration which together determine euphotic population content per unit area; (b) the light-saturated rate of photosynthesis per unit population (photosynthetic capacity); (c) a factor, F(I), which is a function of the ratio I'_o/I_k, where I'_o is the photosynthetically-available irradiance immediately below the water surface and I_k is the irradiance defining the onset of light-saturation of photosynthesis. This factor determines the vertical displacement of the photosynthesis-depth profile. A fourth component, daylength, is also involved in determining daily rates.

Of these factor-groupings, the ratio I'_o/I_k is generally the least variable (Talling et al, 1973). This was found to be true at Loch Leven also. The fact that I_k tended to increase with increase in water temperature resulted in increased I'_o values being accompanied by increased I_k values. This had the effect of reducing variation in the ratio I'_o/I_k. The average value of the factor F(I), which equalled $\ln(I'_o/I_k)$, was rather lower for Loch Leven, and other temperate lakes, than for the tropical lakes studied by Talling (1965a) and Talling et al (1973) or for Lake Tchad (Lemoalle, 1973). This may be because higher tropical I'_o values are not totally offset by the generally higher I_k values associated with higher tropical temperatures. However, the difference between F(I) values in tropical and temperate lakes is not great, suggesting that large differences in hourly rates of photosynthesis at different latitudes are not simply related to differences in irradiance. Similarly, seasonal changes in hourly gross

photosynthesis per unit area (Σ nP) at Loch Leven were shown to be relatively insensitive to changes in I'_o. This was because light-saturation (defined by I_k) usually began at light intensities well below I'_o and because Σ nP was related to a logarithmic function of the ratio I'_o/I_k.

Duration of irradiance (daylength), on the other hand, exerted a relatively large influence on daily gross productivity ($\Sigma\Sigma$ nP). This was partly due to the direct effect of daylength *per se* and partly to the influence of daily irradiance on water temperature and hence on photosynthetic capacity (P_{max}). Thus low daily light intensities and temperatures are major factors limiting gross productivity in winter months at Loch Leven, as in many other temperate lakes.

High rates of gross photosynthesis were facilitated by the generally high chlorophyll *a* content per unit area in the euphotic zone (Σ n). This often approached its theoretical upper limit of 430 mg/m^2. This upper limit is higher than has been estimated for phytoplankton elsewhere and is a consequence of the relatively low value of k_s (i.e. the increment in the minimum vertical extinction coefficient per unit increase in population density) found at Loch Leven. The low k_s value was unexpected for algae as small as those of Loch Leven but it was nevertheless substantiated by laboratory spectroradiometer measurements and by the high values of Σ n actually reached in the loch.

Water temperature appeared to be an important general factor regulating photosynthetic capacity (P_{max}) at Loch Leven. P_{max} values ranged from 1.6 to 19.6 mg O_2/mg chlorophyll *a*.h with an overall tendency for higher values to coincide with periods of higher water temperature. An average Q_{10} of 2.2 was applicable to the seasonal data collected over the 4 year period. No evidence was found of adaptation to lower winter temperatures such as has been suggested by Steemann

Nielsen & Hansen (1959a). Highest P_{max} values approached the lower end of the range (20 ± 5 mg O_2/mg chl *a*.h) typical of tropical waters (Talling, 1965a).

During certain periods factors other than temperature exerted a dominant influence on P_{max}. During these periods an inverse relationship between population density (n) and photosynthetic capacity (P_{max}) was observed. Factors associated with high population density appeared to depress P_{max}. As a result highest P_{max} values did not coincide with highest algal contents in the euphotic zone (Σ n). This imposed a limitation on gross areal productivity and reduced its potential seasonal variation. This situation contrasts with that described by Talling *et al* (1973) in two Ethiopian soda lakes where high population densities (n and Σ n) were coupled with high photosynthetic capacities, resulting in exceptionally high gross yields. For Loch Leven, field and laboratory evidence indicated that reduction in photosynthetic capacity could be attributed, at least in part, to the high pH values (up to pH 10) produced by photosynthetic CO_2 uptake by dense crops. By means of a pH titration procedure (see Talling, 1973), the extent of CO_2 depletion at high pH was shown to be greater than predicted by classical pH/alkalinity calculations.

The presence of dense yet active populations in the Ethiopian lakes described by Talling *et al* (1973) was attributed to their high titration alkalinity (57-67 m-equiv./l) with consequent high pH buffering and large reserves of CO_2. In Loch Leven (titration alkalinity 1.0-1.6 m-equiv./l), CO_2 was not totally exhausted at the highest pH value reached (i.e. pH 10). More severe limitation of P_{max} by higher pH values was probably prevented by the efficient turbulent mixing conditions at Loch Leven. These tend to enhance rates of CO_2 influx from the atmosphere and from respiratory processes in the aphotic zone.

It was not clear from the present study whether the adverse effects of high pH resulted directly from carbon-limitation of photosynthetic rate, from enhanced photorespiration, from an inhibitory effect of high pH per se, or from an effect of pH on phosphate availability. Further work is needed to distinguish between these possibilities.

The possible influences on P_{max} of other population density-dependent factors (including nitrogen and phosphorus supply, light history and dissolved oxygen concentration) were also examined, but the available evidence was considered inconclusive. No clear relationship between P_{max} and species composition was apparent.

Respiration losses were a major uncertainty in this as in many other primary productivity studies. Zero or negative values for net photosynthetic productivity were frequently obtained when 24 h column respiration losses were subtracted from daily gross photosynthetic gains. Such results were considered anomalous since they often coincided with periods of population increase and with dissolved oxygen and pH values above those expected at air-equilibrium. Assuming that heterotrophic growth was negligible, net photosynthetic productivity must have been at least sufficient to allow for the observed increases in phytoplankton crop density. The latter give minimum estimates of net production since they do not take account of material removed by grazing, sinking or in the outflow.

Various explanations for the anomalously low net productivity estimates have been discussed (p.136), but further experimental work is needed to identify the main factors involved. In particular, the contribution of bacteria and zooplankton to measured respiratory rates, the possibility that algal respiration rates may vary diurnally or with depth, and the influence of photorespiration require investigation.

Theoretically, for a given value of daily incident radiation, the amount of gross production lost in respiration depends on: the ratio (r) of respiration rate to light-saturated photosynthetic rate, and on the ratio of effective mixed depth to euphotic depth (z_m'/z_{eu}) - which determines the relative time which the algal cells spend in darkness.

Other factors being equal, respiratory losses will decrease with decrease in the effective depth of the mixed layer. Consequently, shallow lakes potentially provide a more favourable environment for high net production and phytoplankton growth than do deeper lakes. This potential for shallowness to favour high productivity is often realised in practice. Rawson (1953) found an inverse relationship between the standing crop of net plankton and mean depth in a series of lakes in Western Canada, and Talling (1971) observed that some of the shallower English lakes (or their basins) had higher mid-winter crops and earlier spring increases than deeper lakes (or basins). In other shallow lakes, however, the potential for high productivity is not realised due to the limiting effect of some other factor or factors. Dickman (1969) has shown that the main factor limiting phytoplankton productivity in Marion Lake is the rapid rate of water renewal which dilutes the phytoplankton crop so frequently that the standing crop never builds up to high density. In other shallow waters, e.g. Rybinsk reservoir (Sorokin, 1972) productivity is limited by shortage of nutrients. In Rybinsk and other shallow lakes, including Neusiedlersee (Dokulil, 1973), Tjeukemeer (Beattie et al, 1972) and Lake Tchad (Lemcalle, 1973) high non-algal light extinction in the water column reduces the light available to phytoplankton, thereby limiting productivity. In Neusiedlersee (Dokulil, 1973) surface irradiance is also reduced by the shading effects of emergent macrophytes (Phragmites).

Deep lakes become a more favourable environment for phytoplankton growth following the development of a thermocline. This restricts vertical mixing to a shallower depth range, thereby reducing the ratio z'_m/z_{eu}, and with it the relative time spent in darkness by the algae. This reduction in the depth of the mixed layer, together with increasing daily irradiance, is a critical factor controlling the onset of spring growth in many lakes (Fogg, 1965; Talling, 1971) and seas (Strickland, 1965). Javornický (1966) found that the production of a thermocline, in a reservoir which did not normally stratify, led to a conspicuous increase in algal crops. He therefore concluded that light must have been the main factor limiting growth in previous years.

In lakes which do not stratify, variation in the ratio z'_m/z_{eu} can only be due to variation in euphotic depth (z_{eu}). Many morphometrically shallow lakes are rendered optically deep by high light extinction due either to dense phytoplankton crops, or suspended inorganic matter or both. Light extinction due to suspended inorganic material is likely to increase with decrease in mean depth. This is because the sediments of a shallow lake are more accessible to wind disturbance than those of a deeper lake. On average, background light extinction due to non-algal particles was relatively low at Loch Leven compared with that of the shallower Lake George (p. 49). There is also evidence that the non-algal component of light extinction is even higher in still shallower waters, e.g. Neusiedlersee (Dokulil, 1973), Tjeukemeer (Beattie et al, 1972) and Lake Tchad (Lemoalle, 1973) where, as mentioned earlier, it imposes a major limitation on phytoplankton productivity. Loch Leven's high productivity may in part be due to the fact that it is deep enough to prevent large amounts of sediment material being introduced into the water column. Although a decrease in the mean depth of Loch Leven would tend to increase its net productivity, due to reduced respiratory

losses, it is possible that this could eventually be offset by increased competition for light from suspended material from the sediments. This interaction between mixing-depth and turbidity has been explored theoretically by Murphy (1962).

At Loch Leven, population density was the principal determinant of euphotic depth. Because of this self-shading effect the mixed to euphotic depth ratio (z'_m/z_{eu}) increased with increase in population density, from a minimum value of 0.7 to a maximum of 4.1. Because of self-shading, gross daily photosynthesis per m^2 does not increase linearly with increase in population density. This non-linearity becomes progressively more pronounced at higher population densities. Column respiration losses, on the other hand, can increase linearly with increase in crop density. Respiratory losses will therefore tend to increase in relation to gross photosynthesis as population density increases. Further autotrophic growth of the population will be precluded once respiratory loss in the water column equals gross photosynthetic gain. In this situation, equivalent to the 'critical depth' condition of Sverdrup (1953) or the 'column compensation point' of Talling (1957b), light is the factor limiting net photosynthetic productivity. Light-limitation, due to self-shading, was considered to be an important self-regulatory factor preventing further increases in crop density in Lake George (Ganf, 1969) and Crose Mere (Reynolds, 1973).

According to Strickland (1965), sustained phytoplankton growth is not observed in nature when the ratio z'_m/z_{eu} is greater than 5. However, the value of the ratio z'_m/z_{eu} which is critical for growth will not be constant, but will vary with daily irradiance. A value which is critical in winter, when daily radiation is low, would not be sufficient to prevent growth in summer when daily radiation is higher. The maximum

crop in 1971 is thus more likely to have been light-limited than those of other years, because it occurred earlier in the year.

Calculations showed (Fig. 42) that if Loch Leven algae are assumed to respire at 5% of their light-saturated photosynthetic rate, a value often assumed characteristic of 'healthy' cells, then decreased light penetration, caused by increased crop density, would never have been sufficient to reduce net production, and hence population increase, to zero.

It is clear that the question whether light could be the critical factor limiting further biomass increase depends on the validity of the relative respiration rate assumed for algae whose growth is not limited by any other factor. This component is largely unknown and could vary in different algal species.

Relative respiration rate will be affected by any factor which has a differential effect on respiratory rate and photosynthetic rate. Net production and autotrophic growth will be reduced by any factor which increases respiratory rate more than photosynthetic rate and by any factor which reduces photosynthetic rate more than respiratory rate. Ganf (1972) found that, over a certain temperature range, an increase in temperature increased the respiratory rate of Lake George phytoplankton much more than it increased their photosynthetic rate. In this case temperature could ultimately be the critical factor limiting net production.

At Loch Leven the high pH values, and possibly other population density-dependent factors, which depressed photosynthetic capacity, may not have affected respiration. Thus relative respiration rate, r, probably increased with increase in population density, thereby reinforcing the self-shading effect in limiting net production.

It is notable that no extended periods of high total crop density, such as occur in Lake George (Ganf, 1969, 1972) or in the stationary phase of laboratory cultures, were recorded in Loch Leven. Immediately after the maximum algal concentration was reached the crop showed a rapid and drastic decline. The cessation of biomass increase and the decline in the population need not necessarily be caused by the same factors.

Whilst self-shading may contribute to the cessation of further increase in population density, it cannot be the cause of its decline. Since pH decline accompanied population decline it is unlikely that the initially high pH value was responsible for the persistent decrease in population density, unless the high pH values damaged or killed the algal cells. The fact that photosynthetic capacity (P_{max}) increased as the population declined suggests that this was not the case. Also, laboratory experiments (p. 90) showed that the particular algae in the samples investigated could survive a pH value higher than was ever reached in the loch, for at least 15 hours. Thus, like self-shading, the high pH limitation of photosynthesis may contribute to the cessation of increase of population density but cannot account for its decline.

There are many factors which could be responsible for a decrease in crop density. These have been discussed in general by Fogg (1965) and include: (1) deficiency of a mineral nutrient, trace element or organic growth factor; (2) production of autoinhibitors; (3) an increase in flushing rate causing increased outflow losses; (4) an increase in sinking rate due to a decrease in bucyancy or a reduction in turbulence; (5) increased grazing pressure; (6) parasitism; (7) reduction of light penetration due to increased non-algal light extinction; (8) a decrease in daily surface-incident radiation.

This last factor may have contributed to the declines from the 'spring' maxima in 1968, 1969 and 1970, since these were accompanied by declining values of daily irradiance (cf. Figs. 20 and 44). But the 1971 population decline occurred while daily irradiance was increasing.

Chandler (1942) attributed a decline in algal crop density in Lake Erie to an increase in non-algal turbidity. There is no evidence that this occurred at Loch Leven.

The effects of parasitism and grazing have been considered by Bailey-Watts (1973, 1974). The influence of grazing by benthic-dwelling chironomid larvae is not known, but it is unlikely that this caused the massive declines in algal crops observed. For most of the study period grazing in the plankton was considered to be insignificant. This conclusion was largely based on the fact that for the first $3\frac{1}{2}$ years of the project the zooplankton was dominated by Cyclops strenuus var. abyssorum (Walker, 1970; Burgis & Walker, 1972; Johnson & Walker, 1974), a copepod generally accepted to be a carnivore, at least in its adult stages (Fryer, 1957; Monakov, 1972). During the summer of 1971 the herbivorous cladoceran Daphnia hyalina var. lacustris became abundant, possibly causing at least the later stages of the major decline in population density in 1971. Protozoan grazing and fungal parasitism did not appear to be dominant factors controlling algal abundance (Bailey-Watts, 1973, 1974).

Smith (pers. comm.) has estimated that seasonal fluctuations in flushing rate at Loch Leven could not account for the major fluctuations observed in algal concentration.

Periods of calm conditions and reduced turbulence are rare at Loch Leven and bear no consistent relationship to algal population dynamics. It is very unlikely that reduced turbulence and consequent

increased sinking rates caused the major declines in crop density. Sinking rates may, however, increase if cells become nutrient-deficient (Steele & Yentsch, 1960).

Deficiency of any major plant nutrient, essential trace element or organic growth factor could theoretically be responsible for cessation of algal growth and decline of population density in an open system such as a lake. Consider the situation where crop density is low and nutrient concentration high relative to the needs of the crop. An increase in biomass is then stimulated, for example by increasing daylength or water temperature; this leads to a reduction in nutrient concentration. The reduced nutrient concentration is unable to support a rate of gross biomass increase sufficient to balance the loss of biomass by sedimentation, grazing and outflow, therefore the population density declines. It is important for this argument that the requirement of the higher level of crop for the nutrient is greater than can be supplied by inflow and recycling of nutrient.

In contrast to this situation, in the absence of autolysis, nutrient deficiency in a laboratory culture will only cause cessation of growth and not a decrease in crop density.

Shortage of any one of the elements necessary for plant growth could theoretically have been responsible for the population decline, but only carbon, nitrogen and phosphorus were investigated as possible limiting nutrients in the present study. Evidence that diatom crops may have been silica-limited is discussed by Bailey-Watts (1973).

Evidence has been presented which suggested that CO_2 supply may have directly or indirectly, through an effect of high pH, limited further increase in maximum crop densities at Loch Leven. However, since total CO_2 concentration increased as soon as crops began to

decline it cannot account for the continued decrease in crop density.

Nitrate and phosphate concentrations were relatively high in winter, before the start of the main increase in phytoplankton crop density. As crop density increased, with increasing daily irradiance, the instantaneous concentrations of these nutrients declined. The minimum concentration of phosphate was often reached some months before the attainment of maximum crop densities (see Fig. 40). This illustrates the fact that a low concentration of a nutrient is not necessarily indicative of a limiting rate of supply. The phosphorus demand for the subsequent increase in crop density was presumably met by supply from inflows and recycling, and possibly from stored phosphorus taken up earlier. 'Sharing out' of stored phosphorus may have occurred at Loch Leven: the ratio of chlorophyll a/particulate-P increased as cells approached their maximum density. On an ash-free dry weight basis also, phosphorus content appeared to decline as the 1971 maximum crop was approached.

The availability of phosphorus may have been reduced at high crop density, due to effects of the associated high pH values on ion uptake by cells.

In contrast to the situation described above for phosphorus, maximum crop densities more or less coincided, except in 1971, with the attainment of minimal nitrate concentrations. This difference may reflect a more limited capacity of algae to store nitrogen, or a relatively lower rate of recycling or inflow supply of N compared to P. In 1971, algal growth ceased at a nitrate concentration well above its minimum recorded value, suggesting that nitrogen deficiency did not cause the decline of that crop.

Whereas, as mentioned above, dissolved CO_2 concentrations increased

as soon as the populations began to decline, there was a considerable lag before any increase in nitrate or phosphate concentration was observed. This difference can be partly attributed to the fact that CO_2 can be replenished from the atmosphere whereas nitrate and phosphate replenishment is dependent solely on the rates of inflow and recycling. Rates of nitrogen and phosphorus supply by the inflows have not been calculated as the required stream-flow data, collected by Mr. I. R. Smith, have not yet been processed. Rates of recycling by bacteria and zooplankton are unknown, but could be calculated given inflow data and reliable estimates of net productivity. The difficulties in estimating the latter have been discussed.

The seasonal data therefore suggest, but do not prove, that the phosphate and/or nitrate supply from inflows and recycling may have been insufficient to maintain the highest crop densities found at the loch, i.e. to allow the amount of net production required to balance that which is lost in grazing, to the sediments, and by outflow.

Enrichment experiments indicated that during the particular period investigated, nitrogen would become limiting before phosphorus, if supplies from sediments and inflows are disregarded. These laboratory experiments (p.120) were designed for another purpose, and could not determine whether in nature, light (because of self-shading) would have limited population increase before any potential nutrient limitation was realised.

If the self-imposed limitations of self-shading and high pH values are the two critical factors limiting net production and maximum crops at Loch Leven, then further enrichment of the loch with nitrogen and phosphorus would not produce higher maximum crop densities, though clearly it might prolong their duration. Experimental fertilisation of columns of lake water, isolated by polyethylene film, but maintained

under the natural incident and underwater light regime, could help to determine the relative importance of light, high pH and nutrient supply in limiting crop density.

In conclusion, the generally high levels of phytoplankton biomass and gross photosynthetic productivity at Loch Leven may be attributed to the following combination of factors: a rich supply of nitrogen and phosphorus, a shallow mean depth, a wind-exposed situation favouring efficient turbulent mixing, negligible macrophyte competition, low non-algal light extinction, a fairly long retention time and relatively low grazing pressure.

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APPENDIX

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