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Brian Moss

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THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE DISTRIBUTION OF FRESHWATER ALGAE: AN EXPERIMENTAL STUDY

II. THE ROLE OF pH AND THE CARBON DIOXIDE–BICARBONATE SYSTEM

By BRIAN MOSS*

Department of Botany and Plant Pathology, Michigan State University, U.S.A.

INTRODUCTION

Contrasted levels of several common ions present in different freshwaters could help to explain the differential distribution of eutrophic and oligotrophic algae noted in Part I (Moss 1972). There are yet few experimental data, however (Lund 1965).

The availability of carbon dioxide and the effects of pH and dissolved bicarbonate and carbonate on it seem likely to be important. Carbon is a major nutrient and there is evidence that some species can use only carbon dioxide, whereas others can also directly use bicarbonate for photosynthesis (Raven 1970). Some species (Felföldy 1960) may be able to use carbonate also, though carbonate may be toxic for other species (Österlind 1949). Data are so few that no pattern of bicarbonate utilization has yet emerged. Among known bicarbonate users *Scenedesmus quadricauda* (Österlind 1949) is eutrophic (Moss 1972) and *Hydrodictyon africanum* (Raven 1968) is probably eutrophic since *Hydrodictyon* spp. are often found as noxious growths in enriched lakes (Prescott 1959; B. Moss, unpublished). *Chlorella pyrenoidosa* cannot use bicarbonate (Österlind 1951; Briggs & Whittingham 1952) or may use it slightly (Steemann Nielsen & Jensen 1958). Taxonomic problems make ecological extrapolation impossible in this case.

Bicarbonate levels increase markedly from those in the softest oligotrophic waters to those in the eutrophic waters of soft rock areas, and pH tends to increase with bicarbonate level. The availability of free CO_2 ($CO_2+H_2CO_3$) decreases, at constant bicarbonate level, with increasing pH and increases, at constant pH, with increasing bicarbonate. The combined effect is usually an overall decrease in availability of free CO_2 with increasing hardness of natural waters. Above about 1·5 m-equiv./l alkalinity there may be a slight increase in free CO_2 concentration, since pH may then increase very little with greatly increasing bicarbonate concentration. Hutchinson (1957) discusses the equilibria involved in detail.

Experiments were carried out on the effects of the pH–CO_2–bicarbonate system on growth of the algae listed in Part I, Table 1 (Moss 1972), in which authorities for nomenclature are also given. Descriptions of species as 'eutrophic' or 'oligotrophic' depend upon evidence given by Moss (1972).

* Present address: School of Environmental Sciences, University of East Anglia, Norwich NOR 88C.

METHODS

General methods are given in detail in Part I (Moss 1972). Series of media with different pH values were made by addition of dilute hydrochloric acid or sodium bicarbonate to the standard medium (Table 3, Moss (1972)). The major ion composition of this medium (exclusive of Na^+ and HCO_3^-) was K^+ 6·73 mg/l (14·83 mg/l for media in which diatoms were grown), Ca^{++} 6·78 mg/l (16·78 mg/l in media for *Euglena gracilis, Trachelomonas grandis, Chlamydomonas reinhardii*, when ammonium was supplied as a nitrogen source), Mg^{++} 2·46 mg/l, SO_4^{--} 9·74 mg/l, Cl^- 0·064 mg/l (31·0 mg/l in media for the euglenoids and *C. reinhardii*), PO_4-P 0·89 mg/l, NO_3-N 4·75 mg/l, NH_4-N 5·23 mg/l (for the three species listed above only), and SiO_3-Si 4·93 mg/l (for diatoms only). Trace elements and vitamins were supplied at low microgram levels (see Moss 1972) and 3 mg/l tetrasodium ethylene diamine tetraacetic acid were added. Media were autoclaved at 1·05 kg/cm^2 (15 lb/in.^{2}) for 15 min and allowed to stand for at least 3 days before inoculation. After this time the pH, which rose during autoclaving, had fallen to a steady value, and was measured with a Corning Model 84 pH meter to the nearest 0·01 ($\pm$0·02) unit. The initial pH values given in the tables are of the media just after inoculation.

Growth was measured as increase in cell or coenobium numbers over a suitable period (at least a week) of the logarithmic phase. At least three, and sometimes as many as ten, counts were made at suitable intervals and logarithmic growth curves were plotted. Results are expressed as doublings per day ($1/g$ where g is generation time in days). Counting technique is described by Moss (1972). Statistical reproducibility of the values for generation time is shown, for sixteen of the organisms used, in Table 4 of Part I (Moss 1972). The precision of individual counts always lay within $\pm 20\%$ of the mean with a 95% probability.

Incubation conditions are given in the tables with the individual results. The experiments were carried out in 250 ml or 500 ml foam-stoppered flasks which were manually swirled once per day.

Carbonate and bicarbonate levels were determined on samples of the media, prepared, autoclaved, and allowed to stand exactly as those used in the experiments, by titration with standardized hydrochloric acid at 20° C to the end point of phenolphthalein (8·46) and of a methyl red-bromocresol green indicator (4·5) (Mackereth 1963). Free CO_2 (including H_2CO_3) was calculated from the Henderson–Hasselbalch equation:

$$pK_1' = pH - \log \frac{[HCO_3^-]}{[CO_2 + H_2CO_3]}$$

where pK_1' is $-\log K_1'$, and the dissociation constant

$$K_1' = \frac{[H^+][HCO_3^-]}{[CO_2 + H_2CO_3]}.$$

In these calculations, $pK_1' = 6{\cdot}38$ (Hutchinson 1957, p. 656).

The titration measures concentration of weak acid salts rather than the carbonates specifically. In the media used the anions hydroxide and ethylene diamine tetraacetate (and for two diatom species, silicate) were the only non-carbonate weak acid salts. Only 2·05 mg/l of ethylene diamine tetraacetate were present, and, at most, 0·025 m-equiv. (0·425 mg)/l of OH^-. Corrections for these anions were therefore not made in calculating bicarbonate and carbonate levels.

RESULTS

Tables 1, 2 and 3 show the growth rates of thirty-four species of algae in relation to pH, and Fig. 1 shows the extremes of pH between which species of known distribution grew in culture. Fig. 2 shows the relationships of free CO_2, bicarbonate, carbonate and pH

Table 1. *Effects of initial pH on growth of species of Cyanophyta, Cryptophyta and Euglenophyta (growth measured as doublings per day* (1/g) *or as estimated biomass (Gr.) after stated number of days of growth; growth conditions* 21±1° *C, 2·4 klux, 15-h day)*

Gloeocapsa sp.	pH	3·65	3·9	4·75	6·2	6·3	6·95	7·35	7·8
	1/*g*	0	0	0	0	0	0·13	0·15	0·22
	pH	8·1	8·43	9·05	9·25	9·3	9·35	9·38	
	1/*g*	0·18	0·21	0·11	0·05	0	0	0	
Gloeotrichia sp.	pH	3·65	3·9	4·75	6·2	6·3	6·95	7·35	7·8
	Gr.27	0	0	0	0	+	++	+++	++++
	pH	8·1	8·43	9·05	9·25	9·3	9·35	9·38	
	Gr.27	++++	++++	++++	++++	+++	++	++	
Tolypothrix distorta var.	pH	3·75	3·95	4·9	5·55	6·45	6·9	7·4	7·75
	Gr.27	0	0	0	0	+	++	+++	+++
	pH	8·0	8·45	8·95	9·15	9·2	9·3	9·35	
	Gr.27	+++	+++	+++	+++	+++	+++	++	
Cryptomonas ovata var.	pH	3·8	4·05	4·85	6·5	7·0	7·8	8·15	8·55
	1/*g*	0	0	0·55	0·43	0·57	0·59	0·18	0·27
	pH	9·1	9·3	9·35	9·37	9·4			
	1/*g*	0	0	0	0	0			
Euglena gracilis	pH	3·65	3·9	4·75	6·3	6·4	6·95	7·35	7·8
	1/*g*	0·75	0·79	0·79	0·71	0·60	0·68	0·65	0·63
	pH	8·1	8·43	9·05	9·25	9·3	9·35	9·38	
	1/*g*	0·64	0·40	0·55	0·58	0·49	0·47	0·46	
Trachelomonas grandis	pH	3·65	3·9	4·75	6·3	6·4	7·8	8·1	8·43
	1/*g*	0	0	0	0·05	0·03	0·16	0·10	0·02
	pH	9·05	9·25	9·3	9·35	9·38			
	1/*g*	0	0	0	0	0			

Table 2. *Effects of initial pH on growth of species of Bacillariophyta, Chrysophyta and Pyrrophyta (growth measured as doublings per day* (1/g) *or estimated biomass (Gr.) after stated number of days of growth; growth conditions* 21±1° *C, 2·4 klux, 15-h day)*

Eunotia sp.	pH	3·9	4·6	6·9	7·0	7·2	7·35	7·5	7·75
	1/*g*	0·27	0·30	0·34	0·40	0·37	0·37	0·15	0·11
	pH	8·0	8·35	8·65	8·8	8·95	9·1	9·25	
	1/*g*	0·08	0	0	0	0	0	0	
Nitzschia palea	pH	3·9	4·6	6·9	7·0	7·2	7·35	7·5	7·75
	Gr.8	0	0	+	++	++	++	+++	+++
	pH	8·0	8·35	8·65	8·8	8·95	9·1	9·25	
	Gr.8	++++	++++	++++	++++	++++	++++	+++	
Peridinium cinctum f.*	pH	3·4	3·65	3·9	4·9	7·55	7·6	7·9	8·2
	1/*g*	0	0	0	0	0·04	0·10	0·05	0·20
	pH	8·5	9·0	9·2	9·3	9·35			
	1/*g*	0·13	0·04	0·01	0·04	0			
Synura petersenii	pH	3·35	3·55	3·9	4·9	7·55	7·6	7·9	8·2
	Gr.15	0	0	0	0	+++	+++	+++	++
	pH	8·45	8·9	9·15	9·2	9·25			
	Gr.15	+	0	0	0	0			

* For discussion of nomenclature of this species see Moss (1972)

Table 3. *Effects of initial pH on growth of species of Chlorophyta (growth measured as estimated biomass (Gr.) after stated number of days or as doublings per day* (1/g) ±2 *standard deviations where data available (see Moss* 1972)*; growth conditions* 21±1° *C,* 2·4 *klux,* 15*-h day, except where stated)*

Chlamydomonas reinhardii	pH	3·5	3·8	4·6	6·9	7·4	7·8	8·1	8·3
	Gr.8	0	0	+++	+++	+++	+++	+++	+++
	pH	8·5	8·65	8·8	9·0	9·1	9·25		
	Gr.8	+++	+++	+++	+++	+++	+++		
Closterium acerosum	pH	8·9	9·0	9·05	9·07	9·08	9·1	9·2	9·25
	1/*g*	0·64	0·60	0·60	0·67	0·62	0·33	0·26	0·13
	±2σ	0·13	0·12	0·12	0·14	0·13	0·07	0·05	0·03
	pH	9·3	9·3						
	1/*g*	0	0						
Cosmarium botrytis	pH	3·65	3·92	4·75	5·3	6·4	7·0	7·25	7·4
	1/*g*	0	0	0	0·01	0·16	0·16	0·23	0·28
	±2σ				0·001	0·014	0·014	0·02	0·025
	pH	7·45	7·55	7·9					
	1/*g*	0·26	0·27	0·31					
	±2σ	0·023	0·024	0·028					
C. botrytis 23±1° C, 4·8 klux	pH	6·4	7·2	8·09	8·25	8·35	8·5	8·8	9·0
	1/*g*	0·16	0·21	0·22	0·31	0·24	0·36	0·31	0·30
	±2σ	0·014	0·019	0·02	0·028	0·022	0·011		
	pH	9·2							
	1/*g*	0·27							
	±2σ	0·024							
Cosmarium sp.	pH	3·45	3·8	4·48	5·6	5·8	6·3	7·2	7·45
	1/*g*	0	0	0·14	0·21	0·31	0·17	0·30	0·31
	±2σ	0	0	0·028	0·042	0·06	0·034	0·061	0·06
	pH	7·65	8·58	8·8	9·1				
	1/*g*	0·24	0·25	0·13	0·09				
	±2σ	0·049	0·05	0·026	0·018				
Desmidium swartzii	pH	3·75	3·95	4·9	5·55	6·45	6·9	7·4	7·75
	1/*g*	0	0	0	0	0·043	0·07	0·08	0·04
	±2σ					0·012	0·19	0·022	0·011
	pH	8·0	8·45	8·95	9·15	9·2	9·3	9·4	
	1/*g*	0·07	0	0	0	0	0	0	
	±2σ	0·019							
Eudorina californica	pH	3·35	3·6	3·9	5·05	7·55	7·65	7·9	8·2
	1/*g*	0	0	0	0	0·5	0·5	0·35	0·38
	±2σ					0·07	0·07	0·05	0·06
	pH	8·5	8·6	9·15	9·2	9·25			
	1/*g*	0·51	0·37	0·43	0·25	0			
	±2σ	0·075	0·05	0·06	0·04				
Gonatozygon monotaenium	pH	3·55	3·85	4·9	5·5	6·9	7·05	7·5	8·0
	1/*g*	0	0	0·34	0·42	0·47	0·52	0·48	0·50
	±2σ			0·01	0·013	0·015	0·017	0·015	0·016
	pH	8·4	8·85	9·3	9·35	9·4			
	1/*g*	0·47	0·18	0·13	0·08	0			
	±2σ	0·015	0·006	0·004	0·003				
Haematococcus droebakensis	pH	3·3	3·6	3·85	4·85	7·0	7·55	7·85	8·15
	1/*g*	0	0	0	0·15	0·58	0·58	0·58	0·58
	±2σ				0·04	0·16	0·16	0·16	0·16
	pH	8·45	9·15	9·18	9·2	9·25			
	1/*g*	0·58	0·59	0·61	0·37	0·36			
	±2σ	0·16	0·16	0·17	0·10	0·10			
Mesotaenium kramstai	pH	3·55	3·85	4·9	5·5	6·9	7·05	7·5	8·0
	1/*g*	0	0	0	0·42	0·52	0·54	0·61	0·67
	±2σ				0·024	0·03	0·03	0·04	0·04

Table 3 (*contd*)

Mesotaenium kramstai	pH	8·4	8·85	9·3	9·35	9·4	9·4	9·4	
	1/*g*	0·69	0·70	0·76	0·81	0·78	0·76	0·75	
	±2σ	0·04	0·04	0·04	0·05	0·05	0·04	0·04	
Micrasterias americana	pH	3·55	3·8	4·5	5·8	6·25	6·4	6·9	7·4
	1/*g*	0	0	0·04	0·16	0·19	0·19	0·19	0·17
	±2σ			0·005	0·02	0·02	0·02	0·02	0·02
	pH	7·6	8·5	8·6	8·8				
	1/*g*	0·05	0·08	0·06	0				
	±2σ	0·006	0·01	0·007					
M. denticulata	pH	3·7	3·95	4·95	5·7	6·95	7·25	7·65	8·1
	1/*g*	0	0	0·07	0·08	0·04	0·05	0·04	0
	pH	8·3	8·65	9·1	9·35	9·4			
	1/*g*	0	0	0	0	0			
Micrasterias thomasiana	pH	3·75	3·95	4·9	5·55	6·45	6·9	7·4	7·75
	1/*g*	0	0	0·14	0·12	0·11	0·14	0·06	0
	pH	8·0	8·45	8·95	9·15	9·2	9·3	9·4	
	1/*g*	0	0	0	0	0	0	0	
Oedogonium cardiaceum	pH	3·45	4·15	4·9	5·95	6·8	7·55	7·95	8·1
	Gr.15	0	0	0	+	+++	+++	++	++
	pH	8·15	8·85	9·0	9·2				
	Gr.15	++	++	+	+				
Pandorina morum	pH	3·6	3·8	4·34	5·03	6·45	6·6	6·8	7·3
	1/*g*	0	0	0	0·49	0·57	0·54	0·49	0·51
	±2σ	0	0	0	0·035	0·04	0·04	0·035	0·036
	pH	7·53	7·55	7·6	7·7	8·1			
	1/*g*	0·51	0·51	0·60	0·60	0·66			
	±2σ	0·036	0·036	0·043	0·043	0·048			
P. morum	pH	6·4	7·2	8·19	8·25	8·37	8·5	8·82	9·0	9·2
23±1° C,4·8	1/*g*	0·40	0·37	0·37	0·36	0·40	0·41	0·44	0·46	0·34
klux	±2σ	0·03	0·026	0·026	0·026	0·03	0·03	0·032	0·33	0·025
Pediastrum duplex	pH	3·5	3·6	3·95	4·9	7·0	7·6	7·85	8·15	
	1/*g*	0	0	0	0	0·39	0·39	0·39	0·44	
	±2σ					0·06	0·06	0·06	0·065	
	pH	8·5	9·05	9·2	9·25	9·3				
	1/*g*	0·44	0·36	0·23	0·10	0				
	±2σ	0·065	0·05	0·03	0·014					
P. tetras	pH	3·55	3·8	4·65	5·35	6·05	6·8	7·4	7·65	
	1/*g*	0	0	0·43	0·6	0·6	0·42	1·45	1·42	
	pH	8·15	8·45	9·0	9·15	9·25	9·35	9·4		
	1/*g*	1·45	1·35	0·16	0·05	0·05	0	0		
Pleurotaenium trabecula	pH	3·45	3·8	4·48	5·6	6·0	6·0	6·1	7·1	
	1/*g*	0	0	0·083	0·093	0·17	0·14	0·14	0·14	
	±2σ			0·045	0·05	0·09	0·075	0·075	0·075	
	pH	7·55	7·75	8·5	8·65	8·95				
	1/*g*	0·24	0·19	0·07	0·07	0				
	±2σ	0·13	0·10	0·04	0·04					
Roya anglica	pH	3·55	3·85	4·9	5·5	6·9	7·05	7·5	8·0	
	1/*g*	0	0·37	0·42	0·40	0·55	0·52	0·41	0·36	
	±2σ	0	0·05	0·055	0·052	0·072	0·069	0·054	0·047	
	pH	8·4	8·85	9·3	9·35	9·4				
	1/*g*	0·27	0	0	0	0				
	±2σ	0·036								
Scenedesmus quadricauda	pH	3·55	3·85	4·9	5·5	6·9	7·05	7·5	8·0	
	1/*g*	0	0	0·08	0·11	0·08	0·10	0·09	0·09	
	±2σ			0·009	0·011	0·009	0·01	0·009	0·009	
	pH	8·4	8·85	9·3	9·35	9·4				
	1/*g*	0·16	0·15	0·08	0·09	0				
	±2σ	0·017	0·016	0·009	0·009					

Table 3 (*contd*)

Ulothrix fimbriata	pH	3·65	3·95	4·95	6·05	6·5	7·2	7·55	7·9
	Gr. 8	0	0	0	+++	+++	+++	+++	++++
	pH	8·25	8·55	9·0	9·2	9·3	9·35	9·38	
	Gr. 8	++++	++++	++++	++++	++++	++++	++++	
Volvox aureus	pH	3·3	3·55	3·85	4·8	7·0	7·55	7·85	8·2
	1/*g*	0	0	0	0	0·31	0·31	0·35	0·31
	$\pm 2\sigma$					0·20	0·20	0·22	0·20
	pH	8·45	9·0	9·15	9·2	9·25			
	1/*g*	0·18	0	0	0	0			
	$\pm 2\sigma$	0·11							
Zygnema circumcarinatum	pH	3·6	3·9	4·8	5·65	6·3	7·15	7·4	7·9
	Gr. 15	0	0	+	+	+	+++	+++	+++
	pH	8·2	8·65	9·1	9·3	9·35	9·4		
	Gr. 15	+++	+++	+++	0	0	0		
Z. cylindricum	pH	3·6	3·85	4·9	5·8	6·45	7·2	7·85	7·95
	Gr. 15	0	0	0	+	+	++	++	++++
	pH	8·3	8·65	9·15	9·35	9·4			
	Gr. 15	++++	++	++	0	0			
Z. peliosporum	pH	3·6	3·95	4·75	5·4	6·45	7·2	7·8	7·95
	Gr. 15	0	0	+	++	++	++	+++	+++
	pH	8·3	8·65	9·15	9·3	9·35	9·4		
	Gr. 15	++	++	0	0	0	0		

in the experimental media. No particular pattern was found in the minimum pH tolerated. Most of those species for which the minimum pH was established to within about 1 unit would not grow at pH values lower than 4·5–5·1, though the exact minima lay somewhere above pH 3·8. The oligotrophic *Desmidium swartzii* and the eutrophic *Ulothrix fimbriata* had higher minima, between 5·55 and 6·45 and between 4·95 and 6·05 respectively. The lower limits for the oligotrophic *Eunotia* sp. and *Roya anglica*, and the eutrophic *Euglena gracilis* lay below pH 3·9, between 3·55 and 3·85, and below 3·65 respectively. No case can be made for group differences between the oligotrophic and eutrophic species. The three blue-green algae would not tolerate low pH, the minima for growth lying between 5·55 and 6·45 for *Tolypothrix distorta* var. *symplocides*, between 6·2 and 6·3 for *Gloeotrichia* sp., and between 6·3 and 6·95 for *Gloeocapsa* sp. Requirements for relatively high pH by blue-green algae have been noted elsewhere (Holm-Hansen 1968).

Distinct differences were found in the maximum pH tolerated by the eutrophic and oligotrophic groups (Fig. 1). Temperature varied by a few degrees between experiments (see Tables 1–3) and pH varies with temperature independently of concentration of added bicarbonate. However, pH varies by only about 0·1 unit per 20° C change in temperature (Golterman 1969, p. 137). The experimental differences observed, therefore, cannot be accounted for by temperature differences. Most oligotrophic species would not grow at pH values above 8·85, and the actual maxima recorded were 8·6 or less. Maxima for *Micrasterias denticulata* and *M. thomasiana* lay between pH 7·65 and 8·1, and 7·4 and 7·75 respectively. Two apparent exceptions from the oligotrophic group were *Cosmarium* sp. and *Gonatozygon monotaenium*. The former may not be a real exception since eutrophic species do occur in oligotrophic lakes (Moss 1972) and classification of this organism was based only on its isolation from a bog lake. Growth of *G. monotaenium* decreased markedly above pH 8·4 and was meagre at pH 9·3 (Fig. 3). This contrasts with growth of typical eutrophic algae where very high rates were maintained between pH 8·4 and 9·3 or above.

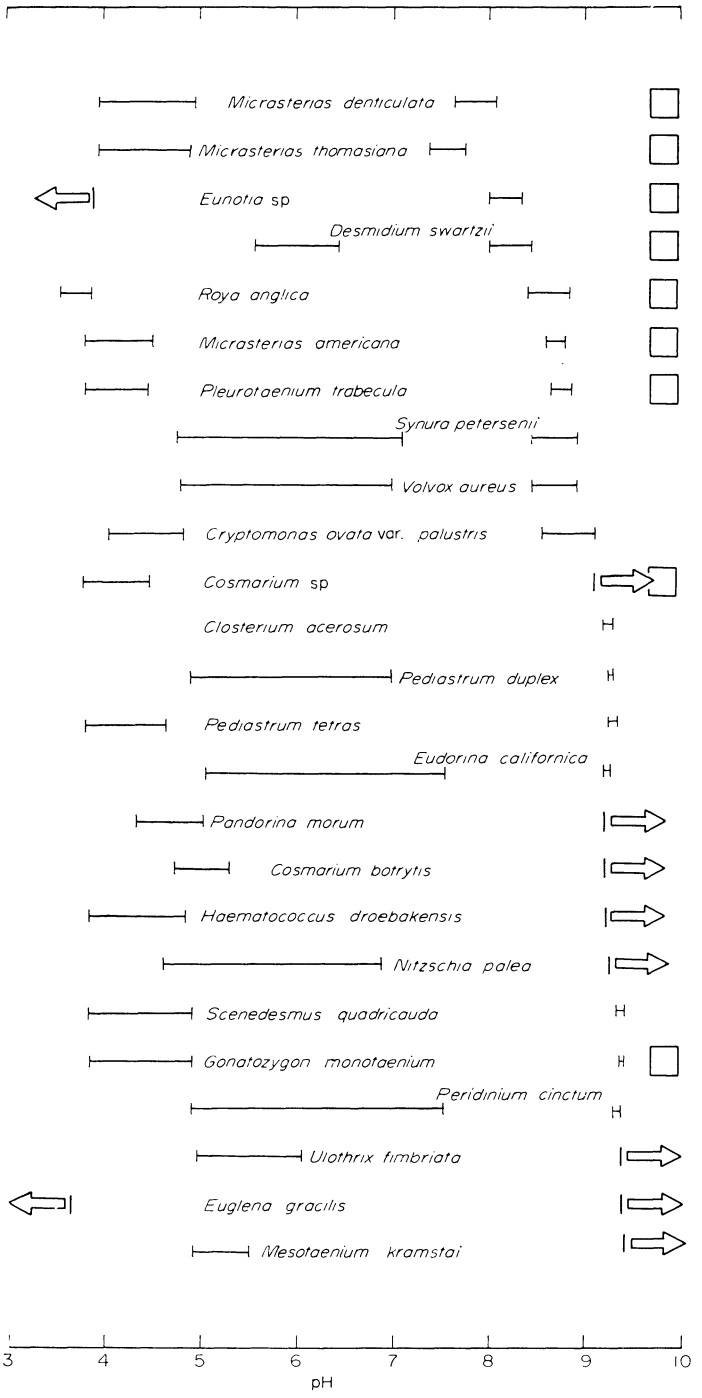

FIG. 1. Extremes of pH between which twenty-five species of algae of known natural distribution grew in standard medium. Square symbols indicate oligotrophic species (see Part I, Moss 1972). Precise limits lie within the range indicated by the bars. Arrows show that the precise limit was not determined but lay at a greater or lower pH than that indicated by the short vertical line.

Exceptions in the eutrophic group include *Synura petersenii*, *Volvox aureus* and *Cryptomonas ovata*, where growth was maintained only up to pH 8·45, 8·45, and 8·55 respectively. These species behaved similarly to those of the oligotrophic group. The three blue-green algae grew at pH values above 9 and, though *Gloeocapsa* ceased to grow between pH 9·25 and 9·30, the upper limits were probably above 9·4 for the other two species. Two *Zygnema* spp. behaved as eutrophic algae and one as an oligotrophic type.

To establish whether cessation of growth in the oligotrophic species at high pH was a function of the pH–carbonate system, or merely of increase in dissolved ionic content, the effects of adding sodium chloride on the growth of two typical oligotrophic species and on *Cosmarium* sp. were studied (Fig. 4). A level of 420 mg/l $NaHCO_3$ was more than sufficient to suppress growth of *Pleurotaenium trabecula* and *Micrasterias americana*, but the former tolerated 420 mg/l NaCl with no decrease in growth rate and 1000 mg/l NaCl

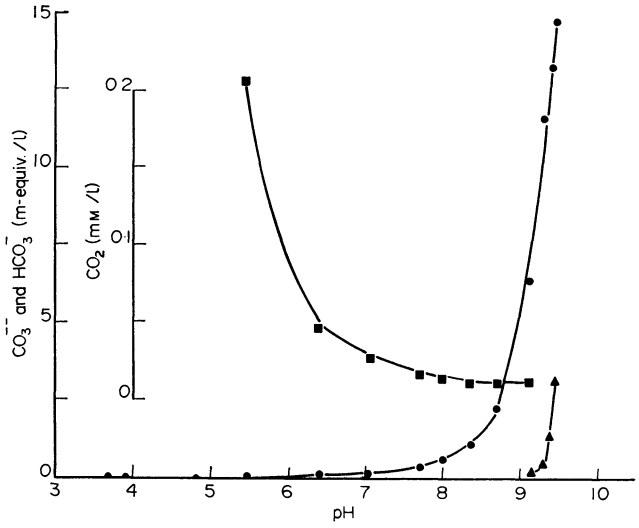

FIG. 2. Amounts of free CO_2 (■), bicarbonate (●) and carbonate (▲) in relation to pH in the experimental media.

with only a minor, probably insignificant, decrease. A level of 420 mg/l NaCl decreased growth rate of *M. americana* by about half and 1000 mg/l NaCl by about two-thirds. *Cosmarium* sp. grew less well with 420 and 1000 mg/l NaCl, though the decrease was probably insignificant, and continued to grow with 840 mg/l $NaHCO_3$. The total ionic content of a hard water lake with 3 m-equiv./l HCO_3^- would be about 315 mg/l and of one with 1·5 m-equiv./l HCO_3^- about 158 mg/l (Rodhe 1949). Total ionic content was therefore unlikely to be the operative factor in preventing growth of oligotrophic species at high pH.

Effects of pH on growth could be expressed through decreased solubility at high pH of transition metal trace elements (Hutchinson 1957). Trace elements in this work were well chelated with EDTA and precipitates did not appear at high pH in media in which oligotrophic species failed to grow. The possibility was examined, however (Fig. 5). Sets of media with pH 7·6–7·7 (HCO_3^- 0·24 m-equiv./l) and pH 8·65–8·70 (HCO_3^- 2·5 m-equiv./l) were used. The standard trace element mixture (Moss 1972) at normal and three-fold

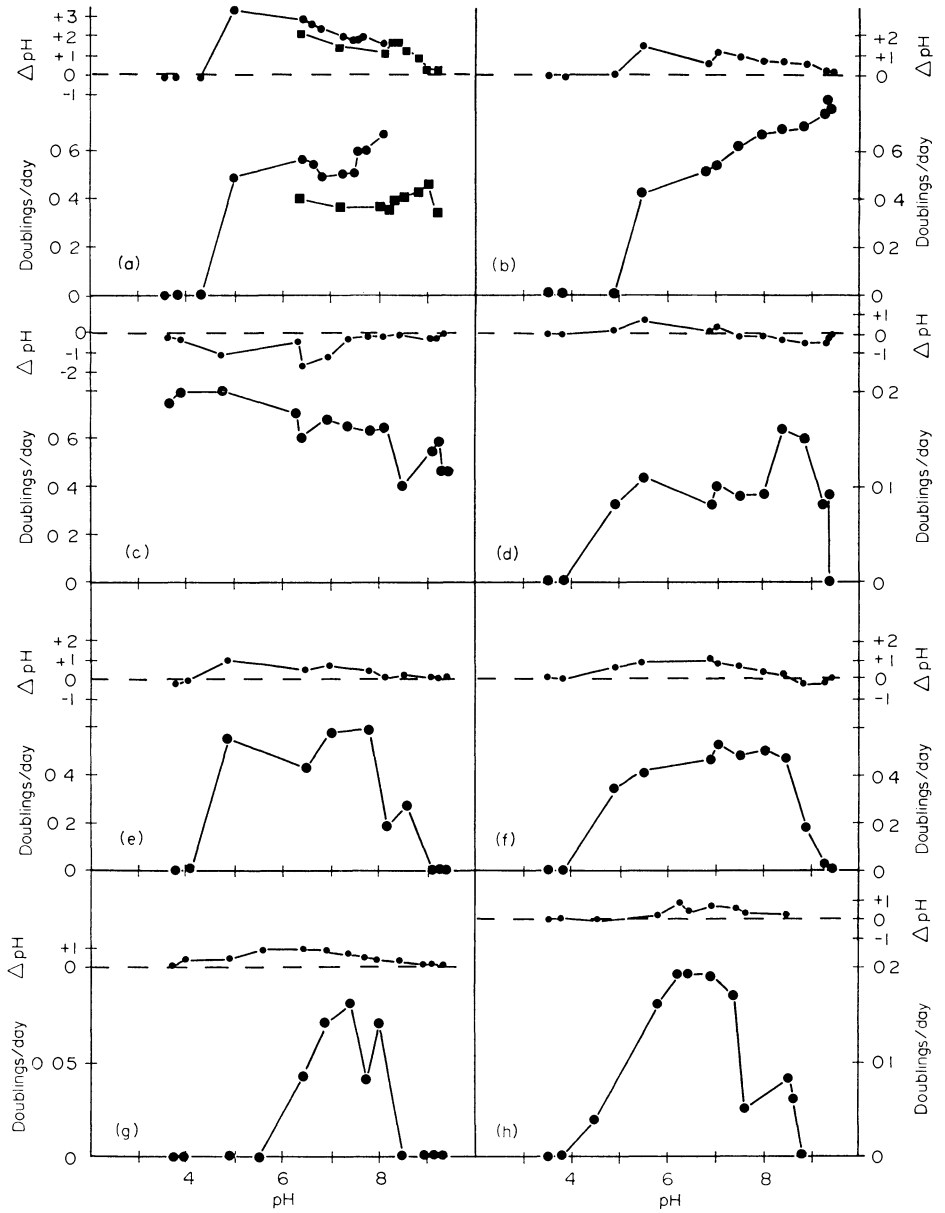

FIG. 3. Effects of pH on growth of representative species, and changes in pH in the media after 7–10 days. *Pandorina morum* (a), *Mesotaenium kramstai* (b), *Euglena gracilis* (c) and *Scenedesmus quadricauda* (d) are typically eutrophic species and *Desmidium swartzii* (g) and *Micrasterias americana* (h) are typically oligotrophic. *Cryptomonas ovata* var. *palustris* (eutrophic) (e) and *Gonatozygon monotaenium* (oligotrophic) (f) are atypical of their groups.

strength, additional iron (as $FeCl_3$) and additional chelating agent (NaEDTA) were variously added. Two typical oligotrophic species, *Micrasterias americana* and *Desmidium swartzii*, and the less typical (with respect to its behaviour at high pH) *Gonatozygon monotaenium* were grown. The typically eutrophic *Pandorina morum* and *Pediastrum tetras* were used for comparison. In no case did added trace elements, iron alone, chelating agent alone, or chelating agent plus iron increase growth at high pH. Addition of 12 mg/l NaEDTA (total in medium 15 mg/l) decreased growth of *P. tetras* at both high and low pH and prevented growth of *Desmidium swartzii* at low pH. No growth of the latter occurred in any treatment at high pH. The effect of the chelating agent may have been to remove ions from cell walls, thus resulting in disruption of the colonial *Pediastrum*

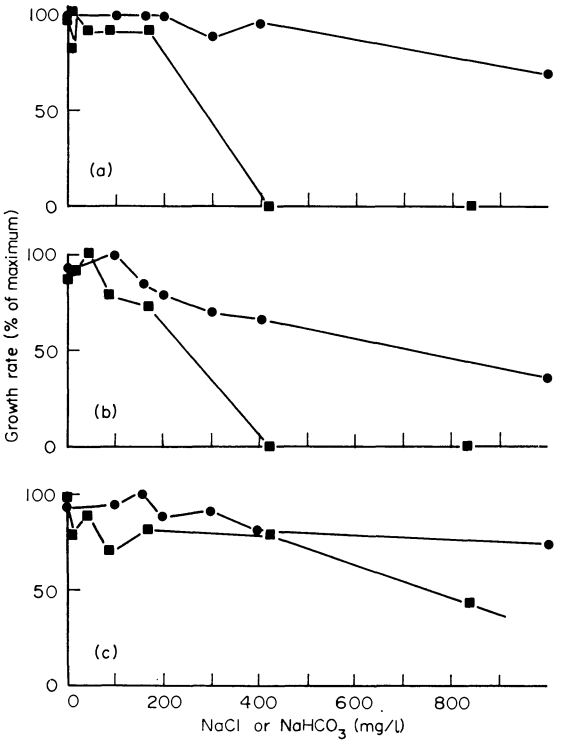

FIG. 4. Effects of sodium chloride (●) and sodium bicarbonate (■) on growth of three oligotrophic species: *Pleurotaenium trabecula* (a); *Micrasterias americana* (b); *Cosmarium* sp. (c). Growth conditions 21 ± 1° C, 2·6 klux, 15-h day.

and the filamentous *Desmidium*. The colonial *Pandorina* was not, however, similarly affected. A total of 9 mg/l NaEDTA in treatments where three times the normal trace element mixture was added did not diminish growth of *Pediastrum tetras* and *Desmidium swartzii* compared with controls.

Trace-element availability seems not to have been responsible for limiting growth at high pH in these experiments. It is possible, however, that in natural waters, where trace metals may be less well chelated, eutrophic species may be able to absorb these metals more easily from low concentrations than can oligotrophic species. Results of a limited experiment carried out with similar results on two independent occasions but using only

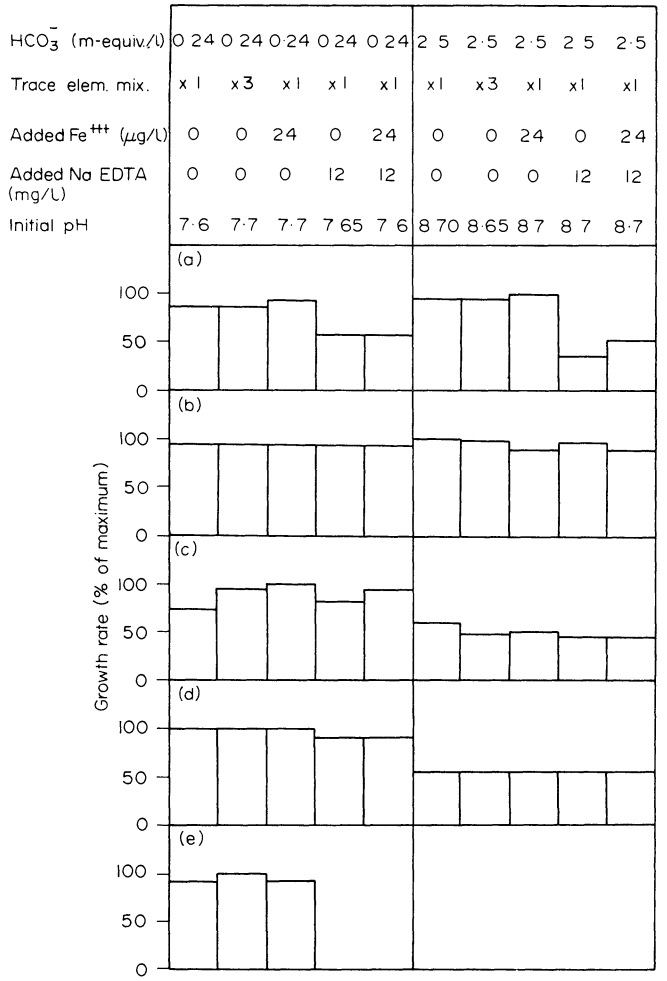

FIG. 5. Effects of increasing iron, trace element and EDTA levels on growth at low and high pH, of two eutrophic species, *Pediastrum tetras* (a) and *Pandorina morum* (b), and three oligotrophic species, *Gonatozygon monotaenium* (c), *Micrasterias americana* (d) and *Desmidium swartzii* (e). Growth conditions 21±1° C, 2·6 klux, 15-h day.

Table 4. *Effects of Fe^{+++} level on growth of three species (growth measured as doublings per day; growth conditions 22±1° C, 4·8 klux, 15-h day)*

	µg Fe^{+++}/l										
	0	0·1	0·5	1	2	5	10	20	30	40	50
Pandorina morum	0·62	0·51	0·60	0·57	0·59	0·62	0·60	0·56	0·61	0·61	0·64
Cosmarium botrytis	0·29	0·28	0·28	0·28	0·28	0·28	0·32	0·32	0·32	0·30	0·23
Micrasterias americana	0	0	0	0·09	0·14	0·11	0·11	0·14	0·13	0·14	0·11

three species (Table 4) seem to suggest this in the case of iron. Two eutrophic species, *Pandorina morum* and *Cosmarium botrytis*, were able to maintain optimal growth with only the iron transferred in the inoculum (about 20 ng Fe^{+++}/l of experimental medium). In contrast, for the oligotrophic *Micrasterias americana* between 0·5 and 1·0 μg/l Fe^{+++} had to be present for growth to begin.

DISCUSSION

Directly or indirectly pH seems likely to be an important factor in determining why oligotrophic species do not grow in hard-water lakes. Effects of pH on growth do not, however, explain why growth of eutrophic algae does not supersede that of oligotrophic algae in soft waters. This problem is discussed by Moss (1973).

There are several ways in which high pH might exclude oligotrophic algae from eutrophic waters: (1) an intrinsic effect of pH on enzymes, in the cell wall or membrane, responsible for uptake of one or more essential nutrients; (2) inability of oligotrophic species to absorb trace elements present in low concentration at high pH; (3) a toxic effect of relatively high total dissolved ion content associated with high pH; (4) co-precipitation of phosphate with calcium, magnesium, and carbonate at high carbonate levels; (5) a direct toxic effect of carbonate or of hydroxide ions, levels of which increase with increasing pH; (6) differential availability of different inorganic carbon compounds for photosynthesis.

The first possibility cannot be discarded without detailed studies on the isolated enzymes, and the second seems unlikely. Growth of oligotrophic species was not made possible at high pH by adding trace elements, or, more importantly, by adding extra chelating agent to maintain them in solution. The possibility of toxicity of high ionic content has also been experimentally eliminated.

Calcium and magnesium levels (usually 6·78 and 2·46 mg/l respectively) were too low for precipitation of these elements as carbonates and phosphates to have occurred. Even when as much as 16·78 mg Ca^{++}/l was present (*Chlamydomonas reinhardii* and euglenoid media) growth was ample at high pH in two cases out of three. Hutchinson (1957) notes that, in natural bicarbonate waters in equilibrium with air, at least 26 mg Ca^{++}/l may be held in solution.

Direct toxic effects of OH^- and CO_3^{--} are possible, but at pH 8·6, the highest pH at which typical oligotrophic species grew, CO_3^{--} levels are negligible (about 0·0026 m-equiv./l), as are those of OH^- (about 0·004 m-equiv./l). Both CO_3^{--} and OH^- are always present, though in lower concentrations at even lower pH values, and it seems unlikely that they should suddenly become toxic at or just less than pH 8·6. Decrease in growth of the oligotrophic species with increasing pH was very pronounced. Increasing toxicity of omnipresent ions, such as carbonate and hydroxide, would be expected to be reflected in a gradual decrease in growth with increasing pH, below the pH range (approaching 9) where carbonate level increases markedly. The sixth possibility, of different availabilities for photosynthesis of inorganic carbon compounds at different pH values, appears to explain most reasonably the experimental data and field distribution of the organisms studied.

Fig. 2 shows a progressive decline in the dissolved free CO_2 content of the medium with increasing pH, the curve levelling between pH 7·7 and 9·4. Increased bicarbonate content above pH 9 is not reflected in greatly increased pH so that the free CO_2 content rises a little above pH 8·7. Levelling of the free CO_2 curve at concentrations of about

0·011 mM is correlated with the decrease in growth rates of the oligotrophic organisms in this pH range. No growth was obtained for most oligotrophic organisms above about pH 8·6, which corresponds with the minimum of the free CO_2 concentration curve. Most ceased growth before the minimum was reached and growth of *Micrasterias denticulata* and *M. thomasiana* stopped when free CO_2 level was about 58% higher than the minimum. *Gonatozygon monotaenium* grew much less well at pH 8·85 compared with its growth at pH 8·4. Free CO_2 concentration differs by about 11% between these levels.

The simplest explanation of the results is that the oligotrophic algae were limited at high pH by lack of sufficient free carbon dioxide. The situation would be exacerbated by any rise in pH due to absorption of free CO_2 and nitrate (Fogg 1965). Rise in pH would further reduce free CO_2 concentration. Cessation of growth of two *Micrasterias* species at relatively high free CO_2 concentrations may result from the bulkiness of these species compared with *M. americana* and other oligotrophic species tested. High external free CO_2 levels might be necessary to provide adequately fast diffusion of CO_2 into the chloroplasts. Tolerance of very low free CO_2 level by *Gonatozygon monotaenium* is understandable since this species has long thin cells for which a less steep CO_2 gradient may suffice. Calculated free CO_2 level above pH 8·7 was greater than that at the calculated minimum. Theoretically some growth of oligotrophic species should have been observed as the CO_2 level rose at pH values above 8·7. However, this apparent rise may be less than that calculated because media containing salts additional to carbonates do not behave exactly as predicted from the behaviour of pure bicarbonate solutions. Rather less free CO_2 than predicted by calculation is found at high pH (Wood 1970). Also levels of potentially toxic carbonate (Österlind 1949) increase markedly as pH 9 is approached.

The lowest free CO_2 levels in the experimental media are about those expected in any aqueous solution in equilibrium with the air, regardless of pH. Theoretically, free CO_2 level attains a constant value, dependent on temperature and partial pressure of CO_2 in the atmosphere, and bicarbonate and carbonate levels adjust accordingly. Attainment of equilibrium with atmospheric carbon dioxide is, however, slow (Hutchinson 1957, p. 658), and the high calculated free CO_2 levels at low pH in these experiments imply a quasi-stable situation. When algal growth begins, and CO_2 is used up, bicarbonate decomposes to give free CO_2, and the pH rises. As the pH rises a smaller proportion of the total inorganic carbon can exist as free CO_2, but, if the initial pH was low, a moderate rise in pH could still permit relatively high absolute concentrations of free CO_2. At high pH values, e.g. those above 8 where growth of most of the oligotrophic species ceased or was greatly reduced, the initial free CO_2 concentration (calculated as about 0·011 mM/l, but probably less) was similar to that expected at air equilibrium. As the cells provided as inoculum withdraw this CO_2, the pH would begin to rise, permitting a smaller proportion of the total inorganic carbon to exist as free CO_2. At pH values above 8 the proportion of carbon able to be present as free CO_2 decreases very rapidly with increasing pH (2·5% at pH 8, 0·3% at pH 9, Hutchinson 1957, p. 657). Since diffusion of CO_2 into solution from the atmosphere is slow, the free CO_2 content of the medium would rapidly diminish, and absorption of CO_2 by cells of the inoculum might not be reflected in cell division, or a slight increase in numbers might occur. Organisms confined to free CO_2 as a carbon source for photosynthesis would be unable to grow very much under these conditions.

Growth of most species of the eutrophic group did not stop at low free CO_2 concentrations, but continued undiminished to pH values above 9·0 in most cases. The exceptions, *Synura petersenii*, *Volvox aureus* and *Cryptomonas ovata* var. *palustris*, behaved like members of the oligotrophic group and presumably their growth became limited at

high pH by lack of free CO_2. For the other eutrophic species growth could have been sustained by direct use of bicarbonate ions, though the possibility of their being able to absorb free CO_2 from extremely low concentrations cannot be eliminated. Rigorous proof of direct utilization of HCO_3^- ions is impossible (Raven 1970) since some free CO_2 is always present in solution. Carbonate toxicity may have limited growth of those species (*Closterium acerosum, Pediastrum duplex, P. tetras, Eudorina californica, Scenedesmus quadricauda, Peridinium cinctum* f. *oviplanum*) which would not grow at pH 9·2 or above. Substantial rise in pH in the least well-buffered media (Fig. 3) when experiments were sufficiently prolonged also suggests bicarbonate utilization (Österlind 1949). Decrease in pH in all treatments with *Euglena gracilis* (Fig. 3) probably reflects uptake of ammonium ions (Pratt & Fong 1940).

Potential ability to use both free carbon dioxide and bicarbonate ions or to use free CO_2 at very low levels by eutrophic species, and only carbon dioxide at relatively high concentrations by oligotrophic species, agrees reasonably with the observed natural distributions of the two groups of species (Moss 1972). Though oligotrophic species could grow at pH values typically above 8 in eutrophic waters if more than 0·011 mM free CO_2/l was present, their growth would be slow and they would probably be at serious competitive disadvantage compared with eutrophic species. Some oligotrophic species grow in certain habitats of eutrophic lakes under special circumstances. *Pleurotaenium trabecula* is found on highly organic sediments in Abbot's Pond, Somerset (Moss 1973). The alkalinity of the open water of the pond is up to 3 m-equiv./l and the pH is often above 8. However, bacterial decomposition in sediments may lower the pH of the interstitial water by up to 2 units and increases the free CO_2 level a hundred-fold (Yongue & Cairns 1971). Jackson (1963) found *Micrasterias sol* and *Pleurotaenium trabecula* in circumneutral water overlying organic mud in the *Typha latifolia* swamp surrounding a highly alkaline (pH 8 and above) hard water lake in Michigan.

Since the eutrophic species can grow well at pH values below 5, there is no reason why they should not grow in soft waters, in accordance with the field evidence (Moss 1972, Fig. 1).

Problems of the behaviour, exceptional to their group, of *Cosmarium* sp., *Cryptomonas ovata* var. *palustris*, *Synura petersenii* and *Volvox aureus* could be explained on the grounds that the clones used were atypical of the normal populations (see Part I, Moss 1972), but there are other possibilities. *Cosmarium* sp. was isolated in the standard medium, containing 0·238 m-equiv./l HCO_3^-. It is a small species not yet satisfactorily named and may be a species normally growing best in eutrophic lakes but present, like many eutrophic species, also in oligotrophic lakes. The three eutrophic flagellates unable to grow at high pH may be able to survive in eutrophic lakes because of their behaviour. Many flagellates stop moving and sink in the water column at night (Happey 1968; Happey & Moss 1967). Particularly at the thermocline and in the hypolimnion, free CO_2 levels increase, and pH decreases. *Cryptomonas* spp. are frequently found clustered at the thermocline (B. Moss, unpublished). Flagellates, sinking into deep water layers at night, could absorb sufficient free CO_2 for continued growth, whilst fixing light energy in the well-illuminated upper layers to which they move by day. Alternatively, since members of the genera *Synura*, *Cryptomonas*, and, in the absence of sexual stages, *Volvox* are difficult to identify correctly to species level, errors of identification in the literature may have given a misleading impression of the natural distribution of the species involved.

Distribution of oligotrophic species relative to the alkalinity of natural waters (Part I, Fig. 1, Moss 1972) suggests that a maximum of about 1·5 m-equiv./l weak acid salts is

tolerated. In these experiments most oligotrophic species tolerated up to 2·5 m-equiv./l and grew at pH values higher than they would be likely to do so in nature. It seems that neither the pH nor the bicarbonate level individually limits growth of oligotrophic species. Their influence is probably in their combined effects on free CO_2 level. Data for pH are not available for all instances where alkalinity data are given in Fig. 1, Part I (Moss 1972). Moreover individual pH readings can be misleading since photosynthetic withdrawal of free CO_2 can result in temporary pH increases of 1 or 2 units in soft, poorly buffered waters. For comparison of the experimental data with field observations, the extensive data, from eighty temperate lakes, of Sparling & Nalewajko (1970) and those collected together by Armstrong & Schindler (1971, Table 10) have been plotted (Fig. 6). For a given alkalinity pH values have been averaged and a curve fitted by eye. Calculated free CO_2 level decreases with increasing pH and alkalinity (assumed to be largely bicarbonate). The rate of decrease of free CO_2 falls markedly from 1 m-equiv.

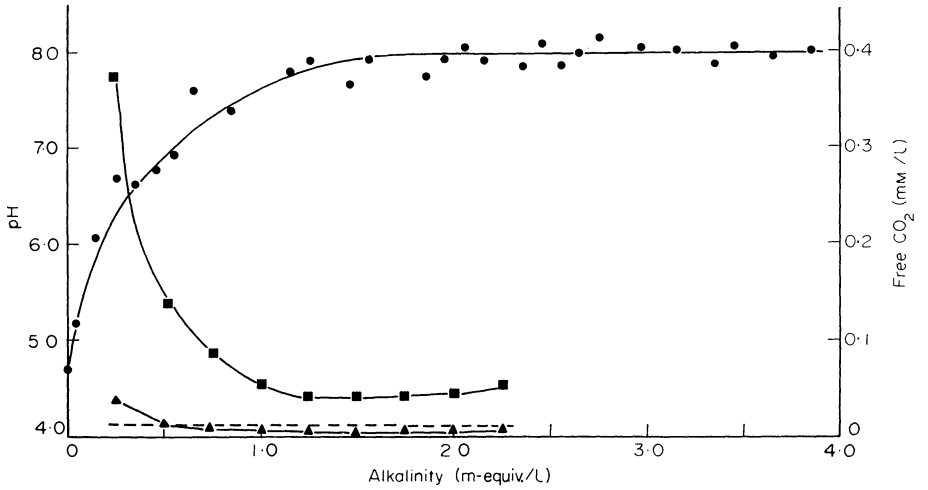

FIG. 6. The relationship of free CO_2 (■) and pH (●) to alkalinity in temperate lakes. Calculated from data of Sparling & Nalewajko (1970) and of various authors given by Armstrong & Schindler (1971). The pecked line indicates level of 0·011 mM free CO_2; and triangles indicate free CO_2 levels on increase in water pH of 1 unit.

HCO_3^-/l to 1·5 m-equiv. HCO_3^-/l where the minimum of 0·040 mM/l CO_2 occurs. Thereafter is a slight increase as weak acid salt level rises but pH rises less rapidly. Minimum free CO_2 levels occur between 1·25 and 1·5 m-equiv./l weak acid salts, in good agreement with the maximum weak acid salt levels at which oligotrophic species occur in nature (Fig. 1, Part I, Moss 1972). The minimum free CO_2 levels, however, are about four times higher than those in the present experiments, and, up to 1·75 m-equiv./l weak acid salts, a lower pH is associated with a given weak acid salt concentration than in the experimental media. Weak acid salts in the waters studied by Sparling & Nalewajko included organic acids, though these were not measured. Their presence causes overestimation, by calculation, of the bicarbonate and free CO_2 levels, particularly at low pH. Also most of the data are derived from November samples when phytoplankton growth was probably low, with little consequent depletion of carbon dioxide. During spring and summer, when pH tends to be higher, free CO_2 levels might be much lower, since a pH increase of 1 unit

would reduce the free CO_2 concentration to a tenth of its former value. At weak acid salt levels of 1·25–1·5 m-equiv./l, free CO_2 concentration would then be below the minima recorded in the experimental media. The shape of the curve (Fig. 6), however, would not be greatly altered.

Further comparison of field and experimental data can be made with the data of Brook (1964). For many Scottish lochs, measurements of pH and alkalinity were compared with the compound phytoplankton quotient (Nygaard 1949). This quotient relates the number of phytoplanktonic desmid species to the sum of those of certain other groups. A quotient of 0·8 or less is considered to indicate definite oligotrophy. In Fig. 7 the highest and lowest calculated free CO_2 concentrations for each of twenty-six lochs have been compared with the phytoplankton quotients of the lochs. A marked transition to low free CO_2 levels occurs associated with a quotient of just under 1·0. There is thus good agreement between relatively high free CO_2 levels and an oligotrophic flora, and low free CO_2 levels and a mesotrophic or eutrophic flora.

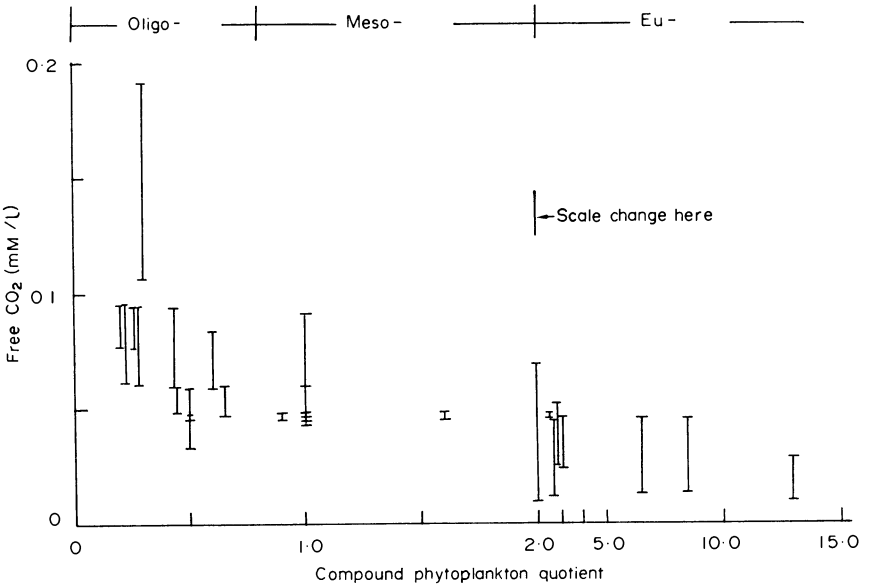

FIG. 7. The relationship of free CO_2 level to compound phytoplankton quotient in twenty-six Scottish lochs. Calculated from data of Brook (1964). Trophic designations are those suggested by Brook.

In African waters Talling & Talling (1965) noted that an 'appreciable desmid plankton' does not occur in waters with more than 2·5 m-equiv./l weak acid salts. In Lake Malawi (Lake Nyasa) (alkalinity 2·36–2·61 m-equiv./l) pH may be as low as 7·7, but sometimes reaches 8·5. Such conditions are similar to the upper extreme tolerated by oligotrophic desmids in the present experiments. A relatively high ratio of monovalent to divalent cations in African waters was regarded by Talling & Talling potentially to be responsible for the ability of the desmids to tolerate rather higher levels of alkalinity than they do in temperate waters. In view of the results reported in Part I (Moss 1972), this explanation is less likely than that sufficient free CO_2 is available in African waters at alkalinities up to 2·5 m-equiv./l to allow growth of species unable to use HCO^- or free CO_2 at very low levels for photosynthesis.

Hutchinson, Pickford & Schuurman (1932) found thirty to forty species of desmids in the Weltevreden West Pan (South Africa) at pH 9·0 and alkalinity 1·3 m-equiv./l. However, these conditions, and the accompanying very low free CO_2 level (0·0031 mM/l), were temporary owing to intense photosynthesis of macrophytes. Later the pH fell to 6·8 (0·495 mM free CO_2/l). The desmids were collected by plankton net and could have been dislodged, in a shallow pan, from populations growing on the bottom mud at much higher free CO_2 levels than were present in the open water. Many of the desmid genera mentioned characteristically live on sediment surfaces.

The potential ability of many eutrophic species of algae to use bicarbonate or free CO_2 at very low concentrations for photosynthesis means that their growth in nature is unlikely ever to be limited by lack of carbon, as suggested by Kuentzel (1969). *Pandorina morum* (Table 5) formed dense populations in axenic culture on standard inorganic (except for EDTA) medium enriched with 4 m-equiv./l bicarbonate, in completely filled, glass-stoppered flasks. Larger populations were found in a similar medium exposed to the air, but the sealed flask populations were much larger than those found in a eutrophic pond. The presence of organic substrates and bacteria seem, therefore, to be unnecessary for the production of large algal growths (Kuentzel 1969). In highly enriched sewage oxidation ponds where ample supplies of combined nitrogen and phosphate permit dense

Table 5. *Growth of* Pandorina morum *in sealed and unsealed flasks (standard medium with* 4·0 *m-equiv./l* $NaHCO_3$*; growth conditions* $22 \pm 1°$ *C,* 4·8 *klux,* 15*-h day; results as coenobia/ml)*

	Sealed flasks		Unsealed flasks		Abbot's Pond
Initial population	148	181	56	42	
Initial pH	8·83	8·83	8·80	8·80	
Largest population reached	14000	17350	57900	57800	4400*
Final pH	11·15	11·3	9·3	9·25	

* Surface water, July 1966 (B. Moss, unpublished).

growths of algae to increase pH so much that carbonate may be formed, carbon may become limiting to growth. However, sewage ponds constitute a special case (Goldman *et al.* 1971) and conditions in them are quite different from those in eutrophic lakes. Even in very soft water lakes with low pH and little bicarbonate present to provide a reserve of CO_2, sufficient CO_2 appears to be available for algal growth to be limited by phosphate levels rather than by lack of carbon (Schindler *et al.* 1972).

Through whatever mechanisms, it is clear that bicarbonate level does influence the distribution of freshwater algae, but may not necessarily be the only determinant. It is therefore appropriate to consider other ions which might be important.

Most ions in freshwaters are present in more than adequate quantities for the sizes of crops found (Lund 1965) and probably at insufficient levels to be toxic as judged from the relatively large quantities normally added to algal culture media (Starr 1964) and from previous culture experiments (Rodhe 1948; Österlind 1949). However, the possibility that some trace elements may be more toxic in the presence of naturally low levels of other substances such as iron and chelating agents than they are in well chelated culture media (Steemann Nielsen, Kamp-Nielsen & Wium-Andersen 1969; Steemann Nielsen & Kamp-Nielsen 1970) must be allowed, as must the 'principle of uncertainty',

that organisms may behave differently in single species culture under experimental conditions than they do as part of a diverse array of species under natural conditions.

Calcium, and to a lesser extent magnesium, sodium and potassium were discussed in Part I (Moss 1972). Kordé (1961) has deduced calcareous phases in post-glacial lake history from the presence of the calcareous loricae of *Phacotus lenticularis* Ehr. Experimental evidence that this species is confined to such waters is not available. A parallel case is that of the molluscs which do occur in calcium-poor waters, though in lesser abundance and variety than in calcium-rich waters (Boycott 1936).

Lund (1965, p. 254) refers to Russian work in which fish ponds were variously fertilized with potassium, phosphorus and nitrogen, and in which potassium alone appeared to stimulate production of a desmid flora rather than one of blue-green algae. However, the experiments appeared inadequately controlled to establish unequivocally the influence of potassium. Evidence has been given here that sodium chloride added at levels much greater than the sodium and chloride levels of eutrophic natural freshwaters did not decrease growth. Growth of one eutrophic species rather than another may be stimulated by differing cation ratios (Vollenweider 1950) and some blue-green algae in dense cultures require relatively high levels of monovalent ions (Allen & Arnon 1955; Provasoli 1969). Large natural crop sizes of blue-green algae, however, would be amply supplied by the concentrations found in even oligotrophic waters. Overall, no case can yet be made that major cation levels determine the different floras in oligotrophic and eutrophic lakes. The role of major cations in saline lakes and the sea, however, may be very significant (Lund 1965).

Levels of minor cations such as ammonium, iron, manganese and other trace elements have been less studied in freshwaters, perhaps owing to analytical problems. Many Euglenophyta so far tested in culture seem to require ammonium and be unable to use nitrate (L. Provasoli & I. J. Pintner, unpublished, cited by Provasoli 1958). Deoxygenated waters and organic muds, where ammonium levels are likely to be high, frequently have an abundance and variety of Euglenophytes. *Trachelomonas* spp. may sometimes require iron and manganese for their thecae and consequently grow best where levels of these elements are relatively high. Suitable habitats are found in both soft and hard waters. Iron and manganese are more soluble in aerobic acid waters than in aerobic alkaline ones. Possibly oligotrophic algae, growing in acid waters, may require more of these elements than eutrophic species, or may be less efficient at absorbing these metals from low ambient concentrations. Some evidence of this, though insufficient for generalization, has been given in this paper. Oligotrophic and eutrophic algae may be similarly differentiated in absorption of some anions, particularly nitrate and phosphate. Rate of supply of these substances to the algae is assumed to be smaller in oligotrophic lakes than it is in eutrophic lakes (Vollenweider 1969; Schindler 1971), and possible adaptations of algae to different rates of supply have been sought. Marine phytoplankters from 'oligotrophic' parts of the Pacific Ocean appeared to be more efficient at absorbing nitrogen compounds than did phytoplankters from 'eutrophic' upwelled waters (McIsaac & Dugdale 1969). Van der Ben (1970) found that a bog species, *Cosmarium impressulum*, reduced nitrate to nitrite *in vivo* maximally at pH 6·5 and hardly at all at pH 8, whereas a eutrophic species, *C. tetraophthalmum*, reduced nitrate better at pH 8 than at pH values down to 5·5. Differences between species in efficiency of uptake of nitrate and phosphate are undoubtedly validly studied only in continuous culture. In batch culture increasing phosphate and nitrate concentration increase final yield but may have little effect on growth rate (Österlind 1949). Golterman, Bakels & Jakobs-Mögelin (1969), however,

found increasing growth rate with increasing initial phosphate concentration for batch cultures of *Scenedesmus obliquus*.

In eutrophic lakes periods occur when the supply of phosphate and nitrate is low, e.g. in dry summers, and concentrations of these substances in the epilimnion are as low as they are in oligotrophic lakes. Oligotrophic desmid species do not then grow in eutrophic lakes, though Chrysophyta, particularly *Dinobryon* spp., which also occur in oligotrophic lakes, may do so. Marl lakes, with low phosphate availability (Wetzel 1969), are also not characterized by oligotrophic species (B. Moss, unpublished). Very high levels of phosphate may be toxic to *Chara* spp. (Forsberg 1964, 1965) and *Dinobryon* spp. (Rodhe 1948). At least one *Dinobryon* sp., however, will tolerate both very high and very low phosphate concentrations in culture (Lund 1965).

Sulphate and chloride levels in lakes are usually more than adequate and less than toxic, and there is neither evidence nor suggestion that they influence algal distribution in freshwaters.

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SUMMARY

Oligotrophic species of algae would not grow at pH values above 8·6–8·85, whilst eutrophic ones grew at pH values above 9, and at considerably higher bicarbonate levels than did the former. The results are interpreted in terms of the available free CO_2 at various combinations of pH and bicarbonate. Oligotrophic species are probably confined to free CO_2 as an inorganic carbon source for photosynthesis, and may be unable to absorb it below certain concentrations (at least 0·011 mM). Eutrophic species may either use bicarbonate directly or may be able to use free CO_2 at very low concentrations. Field observations support the hypothesis that the availability of free CO_2 prevents growth of oligotrophic species in hard waters.

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