PLANKTON PIGMENTS IN AUSTRALIAN WATERS

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The Concentration of Plankton Pigments in Australian Waters

By G. F. Humphrey

Division of Fisheries and Oceanography

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THE CONCENTRATION OF PLANKTON PIGMENTS IN AUSTRALIAN WATERS

By G. F. Humphrey*

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Summary

The method of Richards with Thompson for the determination of pigments in sea-water was modified to allow routine determination in the laboratory of samples filtered at sea. For each pigment, the S.D. of a series of replicates was about 10 per cent. of the mean. Weekly observations off Sydney in 1958 showed that from late winter to late summer there were bursts of pigment production. These were associated with intrusions of cold water on to the continental shelf. Chlorophyll *c* was a major pigment in the waters off Sydney, in the Coral and Tasman Seas, and in the Indian Ocean. There was no constant relation between chlorophylls *a*, *b*, and *c*. Calculating from the total chlorophyll content, the mean amount of organic matter under 1 m^2 of water surface was 3·0 and 3·8 g at the two stations off Sydney.

I. Introduction

This work was begun to provide a basis for the calculation of results of carbon fixation. Also, it was hoped to use the chlorophyll data as a measure of the amount of plant material in coastal and oceanic waters. The only previous measurements of pigment concentration in Australian waters were made by Davis who estimated chlorophyll *a* in Lake Macquarie (Davis 1959) and in coastal waters off Sydney (Davis, unpublished data for 1957).

II. Methods

Sampling was carried out as shown in Figure 1. Raw data were published as follows:

(1) 50 m station off Sydney (so called because the depth is 50–60 m) in latitude 34° 5′ S., longitude 151° 13′ E. at approximately weekly intervals from February 3 to December 30, 1958, at 0, 10, 20, 30, 40, and 50 m; data in C.S.I.R.O. Aust. (1959*b*); no observations between March 3 and April 8.

(2) 100 m station off Sydney (so called because the depth is 100 to 120 m) in latitude 34° 5′ 30″ S., longitude 151° 15′ 30″ E. at approximately weekly intervals from February 3 to December 31, 1958, at 0, 10, 20, 30, 40, 50, 75, and 100 m; data in C.S.I.R.O. Aust. (1959*b*); no observations between February 20 and April 9.

(3) Latitude 10° 43′ S., longitude 131° 58′ E. on one occasion off Darwin from H.M.A.S. *Warrego* at 0 and 20 m on April 26, 1958; data in C.S.I.R.O. Aust. (1960*a*).

* Division of Fisheries and Oceanography, C.S.I.R.O., Cronulla, N.S.W.

(4) During a cruise of H.M.A.S. *Quickmatch* from Brisbane to Sydney via New Caledonia and New Zealand from March 24 to April 26, 1958, at 0, 25, and 50 m; data in C.S.I.R.O. Aust. (1960*a*).

(5) During various cruises of F.R.V. *Derwent Hunter* in east Australian waters from April 23 to December 10, 1958, at 0, 25, 50, 100, 150, and 200 m; data in C.S.I.R.O. Aust. (1959*a*).

(6) During the Antarctic cruise of M.V. *Magga Dan* from January 6 to March 5, 1959, at 0 and 25 m; data in C.S.I.R.O. Aust. (1960*b*).

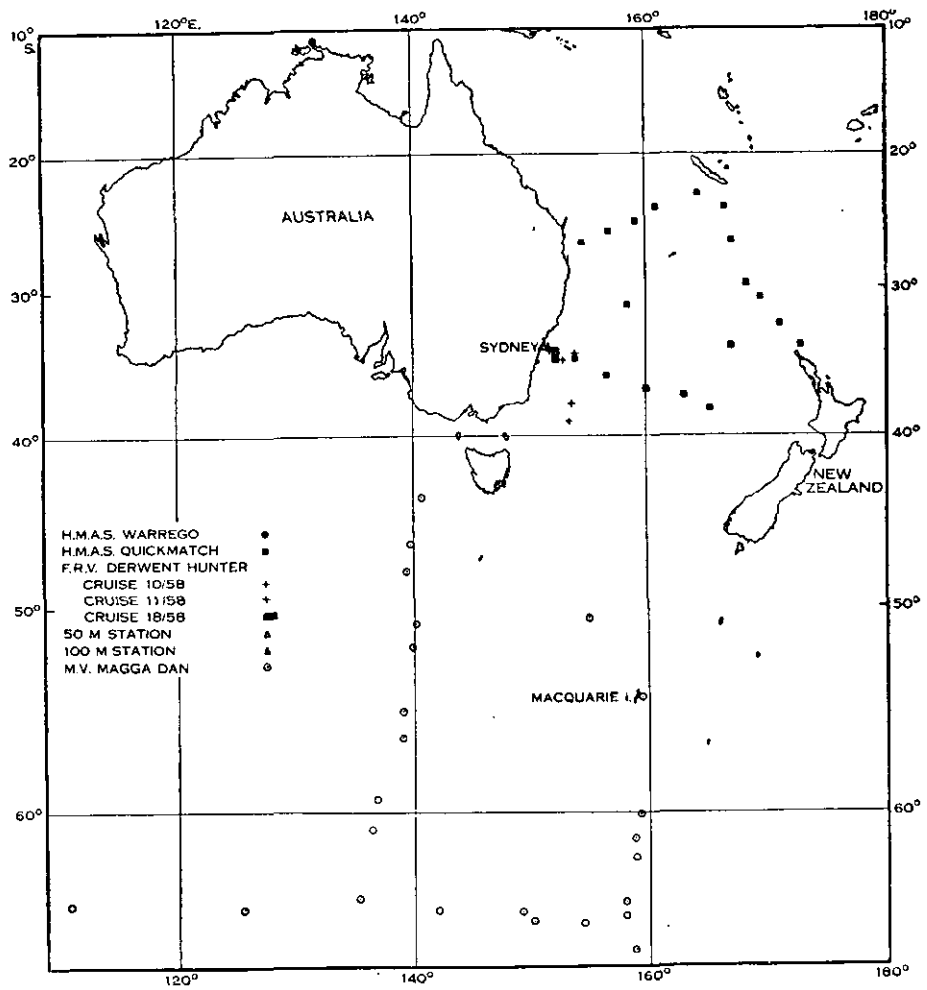


Fig. 1.—Positions of stations.

A plastic bucket was used to collect surface samples and the plastic sampler of Davis (1957) was used at depth. Samples were transferred to white translucent plastic bottles. Between 1 and 5 l. were filtered within 4 hours of collection (the only exception was from *Warrego* when 40 hours elapsed) and the analyses carried

out at once, or the filter papers placed in envelopes or nylon tubes and kept in a vacuum in a metal desiccator containing silica gel.

The analytical method used was based on those of Richards with Thompson (1952) and Davis (1957) but as it differs in many particulars the details are set out in full below.

The bottles containing the samples were inverted two or three times and a measured volume (usually 4·5 l.) from each placed in the jars on the stand (Fig. 2). Approximately 0·1 g of magnesium carbonate was then added to the millipore filter (HA, 47 mm, white, plain) in the plastic filter holder, the syphon tube connected and the vacuum pump switched on. Filtration was usually rapid (30 min) but if not less water was filtered. The filter was then placed in a nylon centrifuge

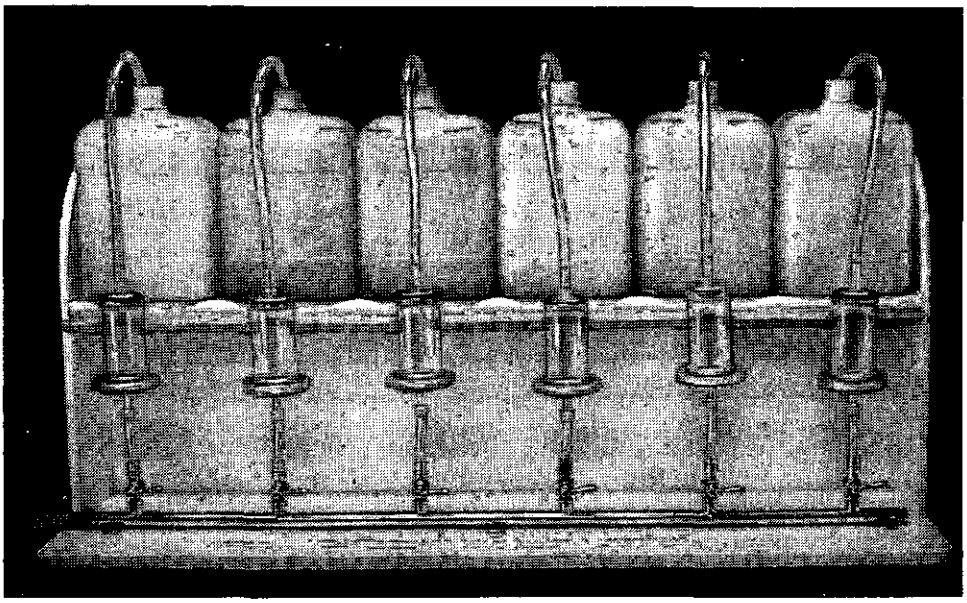


Fig. 2.—Filtration apparatus.

tube (15 by 70 mm) and 4 ml 90 per cent. acetone added. The mixture was stirred with a glass rod and kept overnight in the dark in a closed container. The mixture was again stirred and, if highly coloured, up to 5 ml 90 per cent. acetone added. After centrifuging for 10 min at 4300 *g* the supernatant was decanted into a 10-ml graduated tube. If the residue was still pigmented a further extraction was carried out for a few minutes with 4 ml 90 per cent. acetone. If the extract was turbid 90 per cent. acetone was added until a clear solution was obtained. The volume was then read and the optical density determined at 750, 665, 645, 630, 510, and 480 mμ in a 1-cm cell in a Unicam SP 500 spectrophotometer. The reading at 750 mμ served as a check on the clarity of the solutions; with clear solutions this density was always less than 0·01. The blank was 90 per cent. acetone.

The results were calculated as mg/m³ or MSPU/m³ (1 MSPU or milli-specific-plankton-unit is approx. 1 mg) according to the equations given by Richards with Thompson (1952).

TABLE 1

STABILITY OF ACETONE EXTRACTS

The solutions were kept at 21°C in cuvettes in the spectrophotometer; values are optical density readings

Time (min)	665 mμ	645 mμ	630 mμ	510 mμ	480 mμ
0	0·177	0·044	0·044	0·079	0·211
5	0·176	0·042	0·042	0·079	0·210
15	0·178	0·044	0·046	0·076	0·211
60	0·177	0·045	0·042	0·077	0·210
125	0·178	0·044	0·043	0·081	0·209
185	0·176	0·045	0·043	0·077	0·209

III. RESULTS

(a) *Method*

(i) *Stability of Acetone Extracts.*—The optical density of extracts was determined within 1 hour of centrifugation and there was no loss of colour in that time. In fact (Table 1) extracts were stable for several hours.

TABLE 2

EFFECT OF TIME ON PIGMENT EXTRACTION

Extraction Time (hours)	Chlorophyll *a* (mg/m³)	Chlorophyll *b* (mg/m³)	Chlorophyll *c* (MSPU/m³)	Astacin (MSPU/m³)	Non-Astacin (MSPU/m³)
1	1·34	−0·02	1·08	0·08	0·51
	1·45	0·01	1·14	0·06	0·62
3	1·58	−0·02	1·08	0·06	0·63
	1·72	−0·01	1·44	0·12	0·64
21	1·97	0·13	1·92	0·16	0·62
	2·09	0·05	1·58	0·14	0·75
45	2·26	0·08	1·66	0·14	0·76
	2·00	0·09	1·65	0·12	0·74

(ii) *Duration of Extraction.*—Eight surface samples were collected at the 50 m station and filtered 2 hours later. The filters were placed in acetone and centrifuged between 1 and 45 hours later. The results in Table 2 show that no further extraction occurred after standing overnight (21 hours).

(iii) *Effect of Standing before Filtration.*—On several occasions samples were allowed to stand before filtering. The samples were kept in the plastic bottles, out of direct sun and at ambient temperature. The results in Table 3 show that there was usually an increase in pigment concentration under these conditions, i.e. up to 6 hours after collection.

TABLE 3

CHANGES IN PIGMENTS DURING SAMPLE STORAGE

Filtration was carried out after the intervals shown. All collections were made in triplicate at about 10 a.m. at 0 m at the 50 or 100 m stations

Five values marked * are regarded as aberrant but have been included in calculating the means. Means are given in bold numbers

Date	Hours after Collection	Chlorophyll *a* (mg/m³)	Chlorophyll *b* (mg/m³)	Chlorophyll *c* (MSPU/m³)	Astacin (MSPU/m³)	Non-Astacin (MSPU/m³)
23. xi.58	1·5	1·65	0·22	0·40	0·11	0·75
		1·52	0·26	0·51	0·11	0·68
		1·54	0·14	1·26*	0·09	0·71
		1·57	**0·21**	**0·72**	**0·10**	**0·71**
	4·0	1·77	0·27	1·33	0·20	0·81
		1·88	0·23	1·26	0·16	0·80
		2·03	0·17	1·24	0·18	0·88
		1·89	**0·22**	**1·28**	**0·18**	**0·83**
22.xii.58	2·3	0·48	0·02	0·47	0·08	0·14
		0·45	0·01	0·54	0·07	0·11
		0·52	0·03	0·54	0·08	0·13
		0·48	**0·02**	**0·52**	**0·08**	**0·13**
	6·3	0·78	0·04	0·62	0·11	0·25
		0·75	0·06	0·57	0·13	0·26
		0·84	0·09	0·93	0·15	0·23
		0·79	**0·06**	**0·71**	**0·13**	**0·25**
23.xii.58	3·5	2·62	0·11	1·97	0·16	0·69
		1·93	0·07	1·61	0·17	0·57
		2·19	0·09	1·55	0·14	0·59
		2·25	**0·09**	**1·71**	**0·16**	**0·62**
	5·5	2·26	0·07	2·28	0·17	0·07*
		2·30	0·16	2·75	0·24	0·64
		1·77	0·14	2·57	0·21	0·48
		2·11	**0·12**	**2·53**	**0·21**	**0·40**
30.xii.58	2·0	1·14	0·13	0·96	0·13	0·29
		1·10	0·15	0·96	0·11	0·29
		1·05	0·14	0·92	0·14	0·24
		1·10	**0·14**	**0·95**	**0·13**	**0·27**
	5·0	1·33	0·26	1·80	0·16	0·35
		1·33	0·03	1·37	0·10	0·38
		1·10	0·00	1·55	0·08	0·34
		1·25	**0·10**	**1·57**	**0·11**	**0·36**
7. i.59	2·0	0·18	0·06	0·27	0·04	0·09
		0·13	0·04	−0·01	0·01	0·01
		0·19	0·08	0·01	0·02	0·11
		0·17	**0·06**	**0·09**	**0·02**	**0·07**
	4·0	0·27	0·03	0·39	0·07	0·11
		0·26	0·05	0·28	0·04	0·12
		0·29	0·00	0·41	0·03	0·15
		0·27	**0·03**	**0·36**	**0·05**	**0·13**
8. i.59	1·5	0·25	0·05	0·48	0·10	0·11
		0·30	0·08	0·48	0·09	0·09
		0·33	0·16	0·96*	0·14	0·06
		0·30	**0·10**	**0·64**	**0·11**	**0·09**
	3·5	0·39	0·07	0·67	0·29*	0·11
		0·25	0·07	0·37	0·05	0·10
		0·18*	0·02	0·24	0·03	0·09
		0·27	**0·05**	**0·43**	**0·12**	**0·10**

(iv) *Storage of Filters.*—Before filtration, about 0·1 g of magnesium carbonate was added to the filter, both to speed filtration and to prevent the filtered material becoming acid during any subsequent storage, thus giving pigment breakdown. Table 4 shows the results of an experiment designed to test these points and to discover if filters could be stored for several days. On July 9, 1958, 12 surface samples were taken at the 50 m station and 4·5 l. of each were filtered—six with magnesium carbonate, six without. Three filters from each treatment were analysed at once and three were kept in envelopes in the dark in a vacuum desiccator containing silica gel.

TABLE 4

STORAGE OF FILTERS

Means are given in bold numbers

Days	Magnesium Carbonate	Chlorophyll *a* (mg/m³)	Chlorophyll *b* (mg/m³)	Chlorophyll *c* (MSPU/m³)	Astacin (MSPU/m³)	Non-Astacin (MSPU/m³)
0	–	0·96	0·22	0·64	0·10	0·27
		1·06	0·18	0·42	0·05	0·41
		1·23	0·35	0·84	0·14	0·37
		1·08	**0·25**	**0·63**	**0·10**	**0·35**
	+	0·99	0·13	0·80	0·07	0·39
		1·26	0·22	0·83	0·10	0·43
		1·03	0·15	0·92	0·08	0·37
		1·09	**0·17**	**0·83**	**0·08**	**0·40**
27	–	1·21	0·21	0·85	0·11	0·24
		0·88	0·18	0·78	0·13	0·14
		1·26	0·26	1·20	0·15	0·21
		1·12	**0·22**	**0·94**	**0·13**	**0·20**
	+	1·20	0·24	1·01	0·12	0·27
		1·39	0·21	0·83	0·10	0·31
		1·26	0·24	0·93	0·14	0·24
		1·28	**0·23**	**0·92**	**0·12**	**0·27**

It can be seen that no loss occurred on storage and it is considered that the increases observed were not significant. There was no definite evidence that magnesium carbonate preserved the pigments, but as it hastened filtration by up to 25 per cent. it was routinely used.

(v) *Efficacy of Centrifugation Compared with Filtration.*—Samples taken close inshore, inside estuaries, or after bad weather were often slow to filter and sometimes the filter clogged completely before 4·5 l. had passed through. Under these conditions the centrifuge described by Davis (1957) was used. The rate of flow was about 4·5 l. in 20 min and the material was removed from the cup by washing thrice with a few ml of 90 per cent. acetone. This centrifuge was as effective as the millipore filter, in both poor and rich waters (Table 5).

(vi) *Precision of Determinations.*—Surface water was collected at the entrance to Port Hacking, and 6 gal were well mixed and distributed uniformly among six

1-gal plastic bottles so that each contained 4·4 l. These samples were filtered and pigments determined. The results in Table 6 show the order of precision obtained (S.D. about 10 per cent. of the mean).

TABLE 5

COMPARISON OF CENTRIFUGATION WITH FILTRATION

The value marked * was rejected as the solution was reddish-brown (all others were green) *indicating zooplankton. Means are given in bold numbers*

Method	Chlorophyll *a* (mg/m³)	Chlorophyll *b* (mg/m³)	Chlorophyll *c* (MSPU/m³)	Astacin (MSPU/m³)	Non-Astacin (MSPU/m³)
Filtration	0·66	0·04	0·72	0·08	**0·24**
	0·60	0·03	0·81	0·06	**0·23**
	0·69	0·02	0·60	0·06	**0·26**
	0·65	**0·03**	**0·71**	**0·07**	**0·24**
Centrifugation	0·78	0·01	0·75	0·10	0·23
	0·66	0·03	0·64	0·04	0·23
	0·48	0·03	0·65	0·92*	0·13
	0·64	**0·02**	**0·68**	**0·07**	**0·20**
Filtration	2·65	0·10	2·49	0·21	0·74
	3·20	0·09	3·49	0·21	0·95
	3·10	0·03	3·36	0·21	0·86
	2·98	***0·07***	***3·11***	***0·21***	***0·85***
Centrifugation	2·61	0·04	3·21	0·13	0·78
	4·02	−0·13	3·87	0·16	1·29
	3·83	0·11	3·54	0·12	1·11
	3·49	**0·01**	**3·54**	**0·14**	**1·06**

TABLE 6

PRECISION OF DETERMINATIONS

Expt. No.	Chlorophyll *a* (mg/m³)	Chlorophyll *b* (mg/m³)	Chlorophyll *c* (MSPU/m³)	Astacin (MSPU/m³)	Non-Astacin (MSPU/m³)
1	0·85	0·22	0·96	0·15	**0·21**
2	0·77	0·23	0·93	0·14	**0·20**
3	0·83	0·24	0·71	0·11	**0·24**
4	0·80	0·20	0·93	0·17	**0·19**
5	0·87	0·23	0·77	0·15	**0·22**
6	0·73	0·22	0·71	0·12	**0·21**
Mean	0·81	0·22	0·84	0·14	**0·21**
S.D.	0·053	0·011	0·108	0·020	**0·014**

(vii) *Variation between Samples.*—Several triplicate surface collections were made, particularly at the 50 and 100 m stations. As a measure of the variation of each constituent the average deviation from the mean was calculated for each set of triplicates. The means of these average deviations are shown as percentages in

Table 7 for six occasions chosen at random from the 23 for which figures were obtained. Triplicate samples at 30 and 50 m were obtained on four occasions and the results from these have been treated similarly.

Thus for these coastal waters it can be seen that, apart from astacin, the deep samples showed much more variation than those at the surface. This could have been caused by the boat drifting more during the deep sampling or by the deeper waters

TABLE 7

VARIATION BETWEEN SAMPLES

The numbers indicate the mean values of $100\Sigma(|X-\bar{X}|)/\Sigma X$, where X represents concentration of pigment

Depth (m)	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *c*	Astacin	Non-Astacin
0	8·0	23·0	18·9	18·0	26·3
30	34·5	36·3	32·1	17·8	14·3
50	24·6	71·3	37·0	14·2	41·5

being less uniform. On one occasion three samples were placed on the wire at 28·5, 30, and 31·5 m so that there was simultaneous collection but there was again no uniformity in the results (Table 8). This indicates that drifting is not the sole cause.

(*b*) *Concentration of Pigments*

(i) *Seasonal Changes.*—Figures 3 and 4 show the changes in pigment levels throughout 1958 for the 50 and 100 m stations. The following comments can be made.

TABLE 8

VARIATION BETWEEN SIMULTANEOUS SAMPLES

Depth (m)	Chlorophyll *a* (mg/m³)	Chlorophyll *b* (mg/m³)	Chlorophyll *c* (MSPU/m³)	Astacin (MSPU/m³)	Non-Astacin (MSPU/m³)
28·5	0·25	−0·05	0·31	0·10	0·09
30·0	0·50	−0·10	1·06	0·16	0·12
31·5	0·33	−0·08	0·47	0·14	0·09

At the 50 m station the peaks in chlorophyll *a* were observed throughout the water column, with the April, August, and September ones less marked at the bottom. Chlorophyll *b* gave much smaller peaks than *a* but they occurred at similar times. Chlorophyll *c* behaved very much like *a*. During blooms there was more *a* than *c* but between blooms *c* equalled or exceeded *a*. Astacin did not show seasonal variations and the fluctuations probably represent the fortuitous presence of zooplankton. The non-astacin fraction showed seasonal variations, with the April peak well pronounced at the surface and the August and September peaks missing at the bottom.

At the 100 m station the times of peaks were similar to, but not identical with, those at the 50 m station. The deficiencies that result from being unable to sample under all conditions are illustrated by the fact that a marked peak was observed on April 15 at the surface but the lack of samples at depth on that date resulted in no peaks being shown below this. At 0–20 m chlorophyll *a* showed many seasonal peaks which became progressively fewer to 50 m; at 75 and 100 m

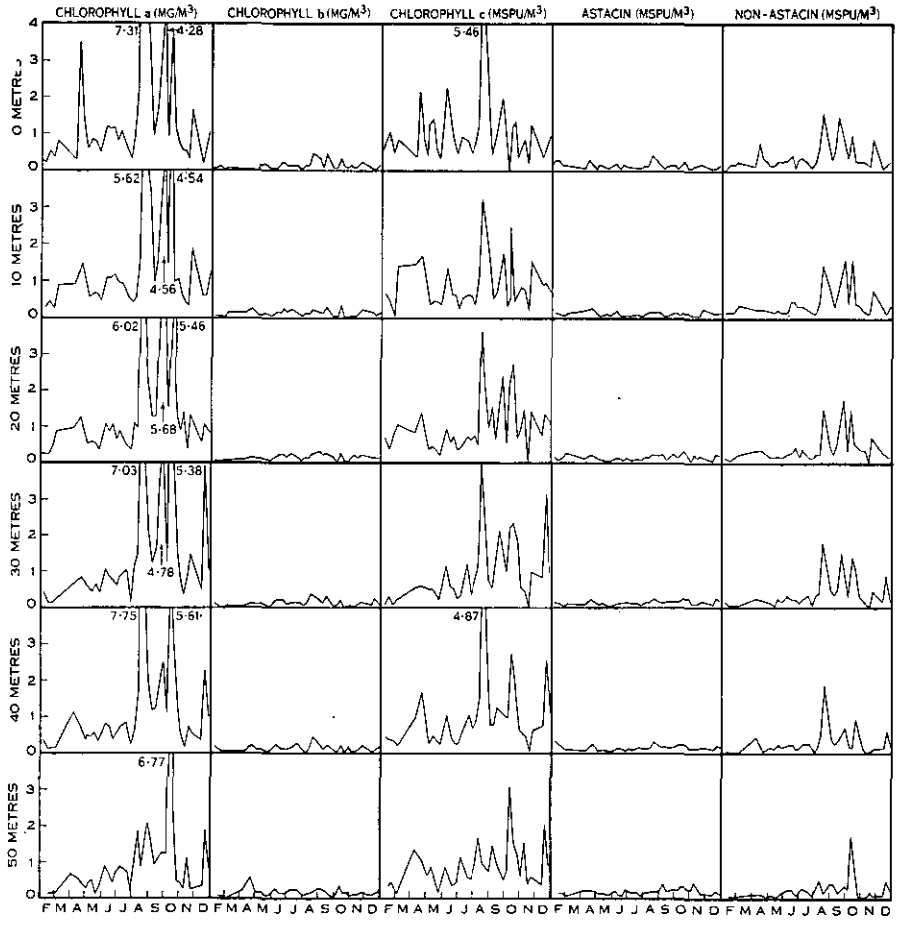


Fig. 3.—Pigment concentration at 50 m station, February to December.

the August peak was the only definite one. Chlorophyll *b* showed only an August peak which was most noticeable at 40 and 50 m. Chlorophyll *c* showed its most pronounced peaks at 0–20 m and there was considerable pigment at the bottom. The astacin results are very difficult to interpret but possibly some of the peaks do reflect a seasonal variation in zooplankton. The non-astacin values for 0–20 m showed seasonal changes similar to those in the chlorophylls. However, the November peak was missing from 30–100 m, the end-September peak from 40–100 m, and the August peak from 75 and 100 m.

(ii) *Geographical Distribution.*—The amounts of pigments in the coastal waters off Sydney showed a range of values which included those obtained for the Coral and Tasman Seas and the Indian Ocean during the cruises of H.M.A.S. *Warrego*

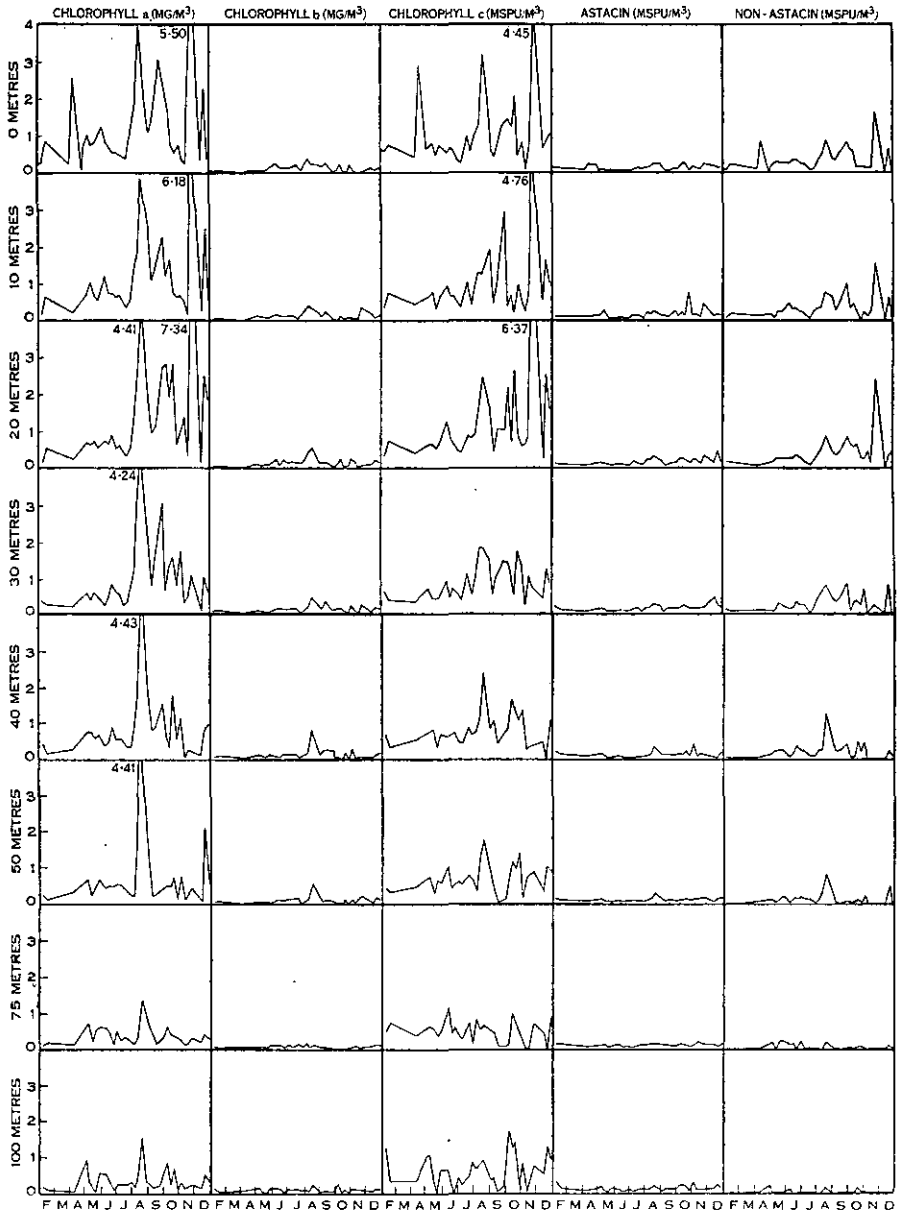


Fig. 4.—Pigment concentration at 100 m station, February to December.

and *Quickmatch* and M.V. *Magga Dan*. The results of these cruises emphasized the quantitative importance of chlorophyll *c* which was nearly always greater in amount than chlorophyll *a* or *b*.

IV. Discussion

(*a*) *Method*

The small increase in pigment shown by samples stored for a few hours in plastic jars (Table 3) may be due to reduced light intensity. Opposite changes (decreases) have been shown by Marshall (1956) and Yentsch and Ryther (1957) when samples were exposed to surface illumination. The latter authors mention photo-oxidation as an explanation. It is difficult to decide how to avoid changes after collection; if treatment cannot be carried out at once it seems best to adopt the procedure used in the present investigation, i.e. storage out of direct sun and at ambient temperature.

Once the sample has been filtered, the filter can be stored *in vacuo* either in the refrigerator (Creitz and Richards 1955) or at room temperature over silica gel (Table 4).

Creitz and Richards (1955), Hartman (1958), and Kutkuhn (1958) have shown that filtration with millipore filters is to be preferred to centrifugation with the Foerst centrifuge because many organisms (particularly blue-green algae) are lost with the supernatant. The analyses reported in Table 5 were done in January and February when ultra-plankton predominated.

No figures could be found in the literature giving the precision of the pigment methods but Littleford, Newcombe, and Shepherd (1940), investigating the precision of plankton counts made after concentration with the Foerst centrifuge, showed that counts of four 350-ml subsamples from a large container gave ± 10 per cent. deviation from the mean. The results in Table 6 show a precision of this order, which is smaller than the variation in the pigment content of replicate samples from the sea.

Laevastu (1957) has stated that ± 33 per cent. variation between replicate plankton catches can be considered reasonable. This is supported by the findings of Hasle (1954) who found big variations in the surface distribution of *Chaetoceros* in a fiord. Further, Littleford, Newcombe, and Shepherd (1940) found a mean count of 78,631 with a standard deviation of 12,261 for 14 samples collected at close time intervals over 4 hours. Much bigger variations in pigment concentration were found between duplicates during the 48-hour drift on *Derwent Hunter*, Cruise DH18/58 (C.S.I.R.O. Aust. 1959*a*) off Sydney. The results in Tables 7 and 8 show that big variations can occur, even with simultaneous sampling, particularly at depth.

The values obtained for non-astacin pigments were often negative (down to $-0{\cdot}15$ MSPU/m^3) and a few negative values were obtained for chlorophyll *b* and astacin. These may be experimental errors but it is more likely that the equations of Richards with Thompson (1952) do not reflect adequately the pigments occurring in Australian waters. This subject is being investigated separately.

(*b*) *Seasonal Variation*

Dakin and Colefax (1933) counted the diatoms in net tows off Sydney and found that there was a smooth annual variation, with peaks in February and late September. They concluded that there was "a similarity with the plankton rhythm of European Seas". The more comprehensive results in Figures 3 and 4 show that the changes cannot be represented properly by smooth curves and that many peaks

occur. At about the times of these peaks there were hydrological changes in the surrounding waters. Figure 5 summarizes these changes, which are given more fully in Figures 6–11 (data in C.S.I.R.O. Aust. 1959*b*). During late winter, spring, and summer there were intrusions of water of slope origin (Rochford 1958). These intrusions were arbitrarily defined by the occurrence of the following values: temperature, 14°C or less; phosphate, 0·7 μg-atoms P/l. or more; nitrate, 5·0 μg-atoms N/l. or more; pH, 8·15 or less.

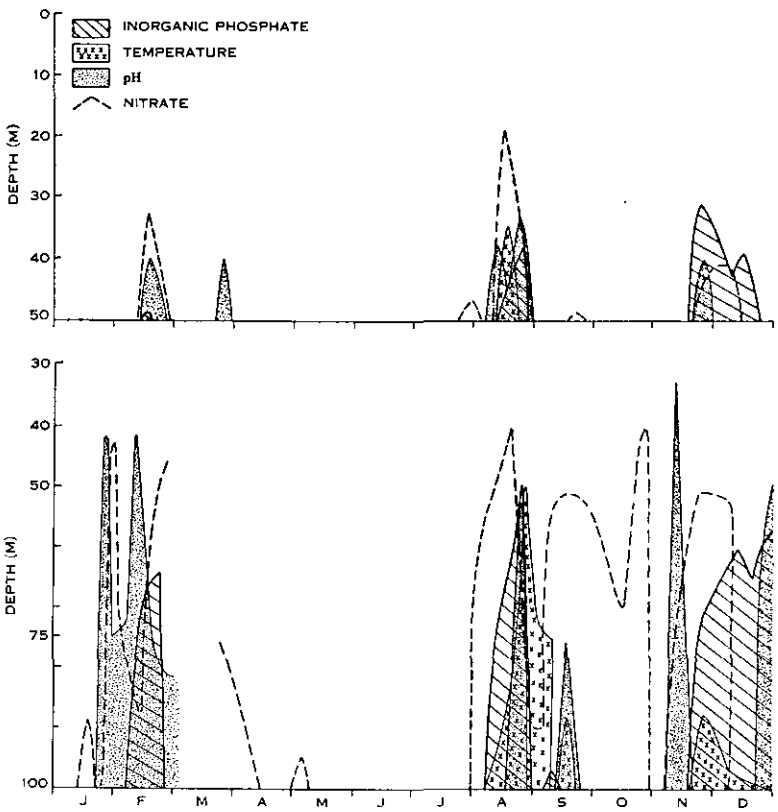


Fig. 5.—Summary of hydrological conditions at 50 m and 100 m stations.

It is not possible to conclude that this water of relatively high nutrient content caused the increases in pigment although it has often been shown that phytoplankton causes decreases in nutrient salts. For example Harvey (1934) showed a steady decrease in phosphate (at a station off Plymouth) during February to August, a period when there were several peaks in pigments. Again, Riley and Conover (1956) give results for Long Island Sound showing how phosphate and nitrate decrease rapidly at the onset of the early autumn chlorophyll peak. Without hydrological information for the whole of the water column and plots as in Figures 6–11 it is difficult to discover a relation between the properties of the water and pigment production; e.g. although the results of Ryther and Yentsch (1958) show definite early-winter and early-spring peaks in chlorophyll *a* in samples taken at 10 m from the continental shelf off New York, there was no correlation possible with the variations in nitrogen, phosphorus, and temperature.

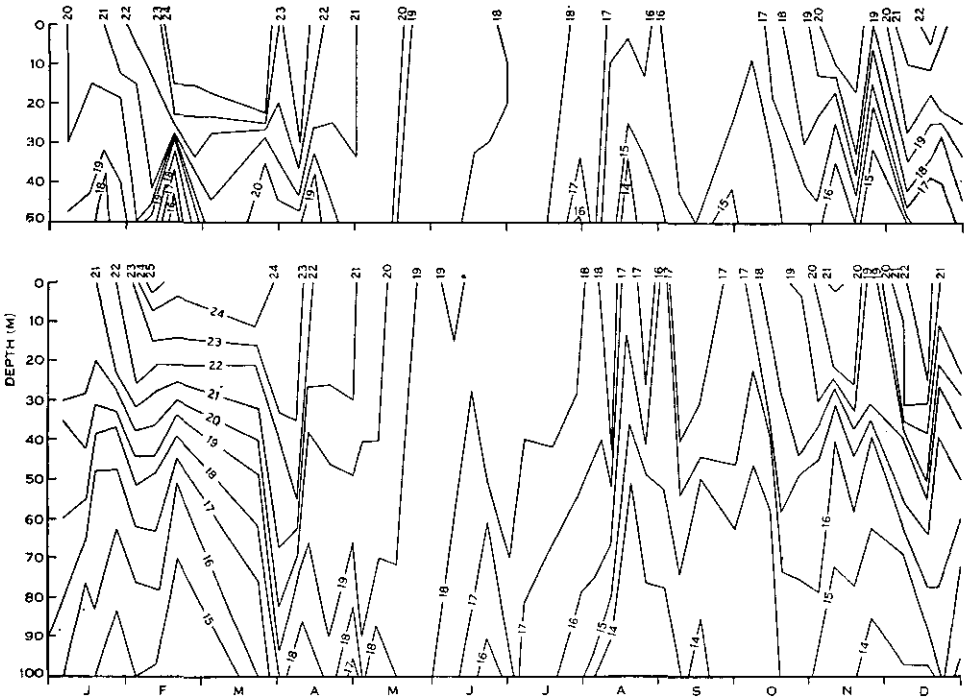


Fig. 6.—Temperature (°C) at 50 m and 100 m stations.

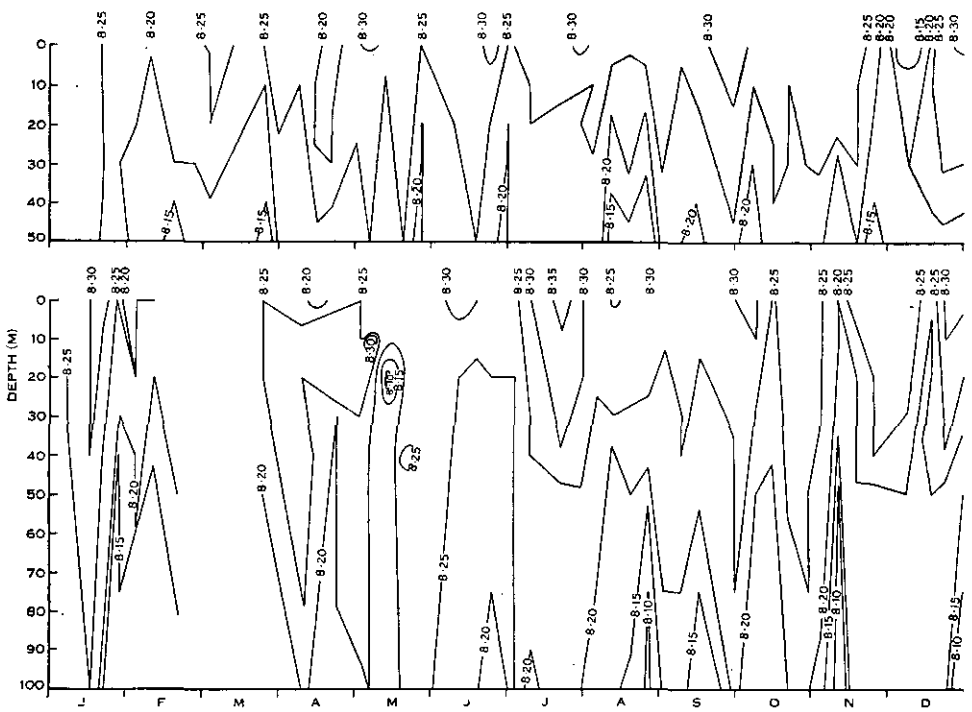


Fig. 7.—The pH at 50 m and 100 m stations.

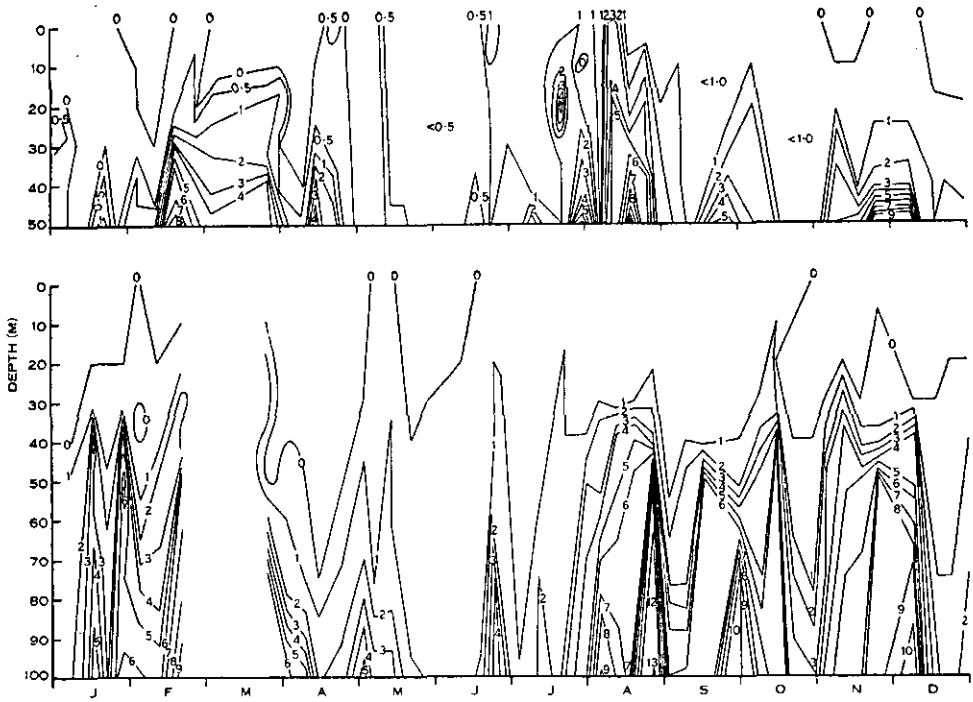


Fig. 8.—Nitrate (μg-atoms N/l.) at 50 m and 100 m stations.

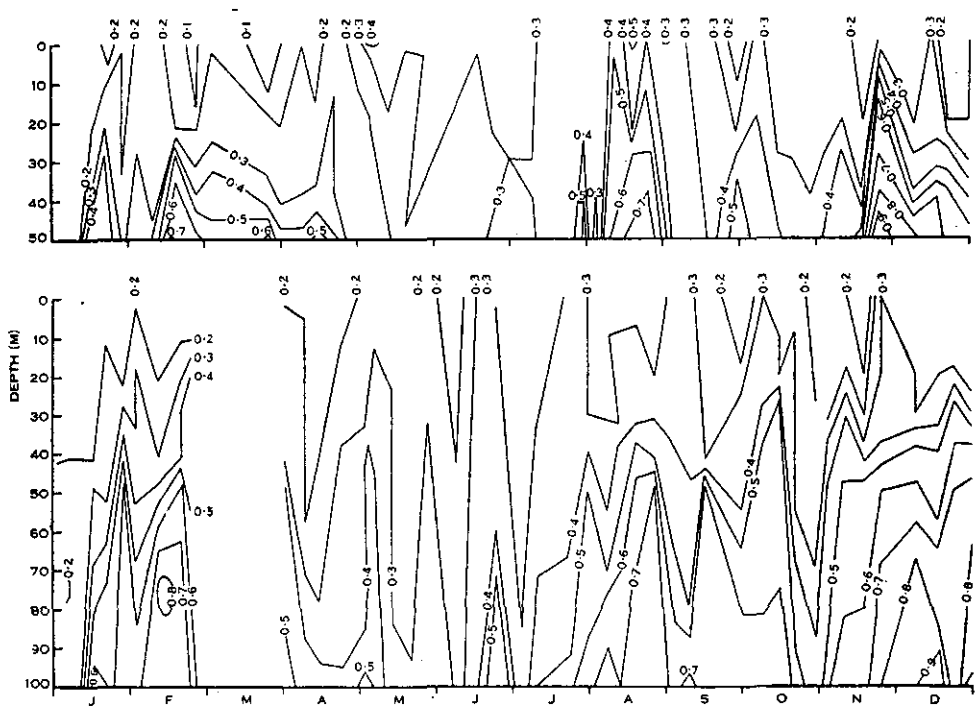


Fig. 9.—Inorganic phosphate (μg-atoms P/l.) at 50 m and 100 m stations.

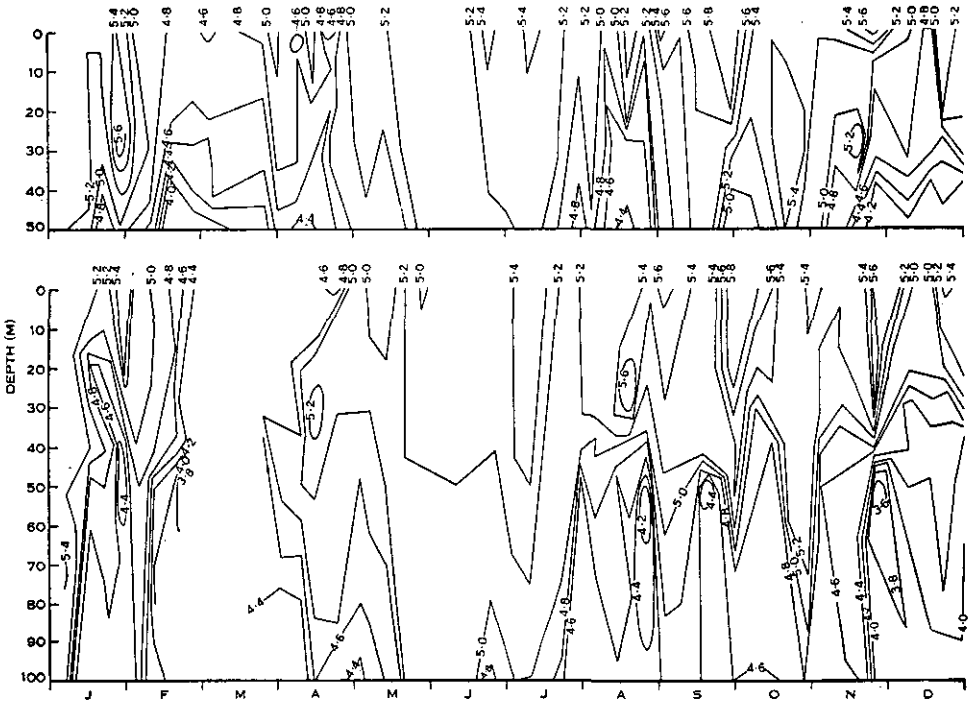


Fig. 10.—Oxygen (ml/l.) at 50 m and 100 m stations.

Fig. 11.—Chlorinity (‰) at 50 m and 100 m stations.

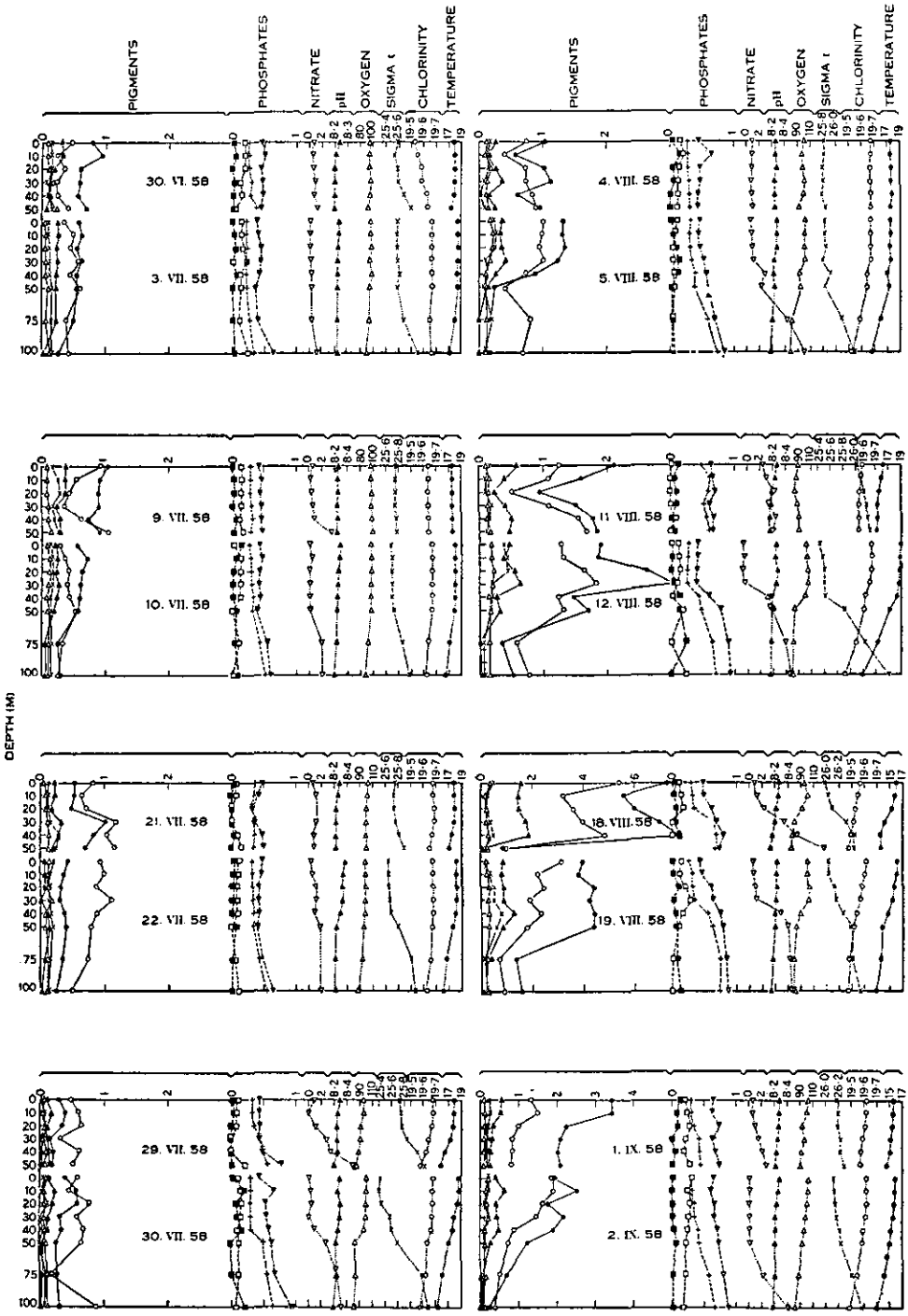
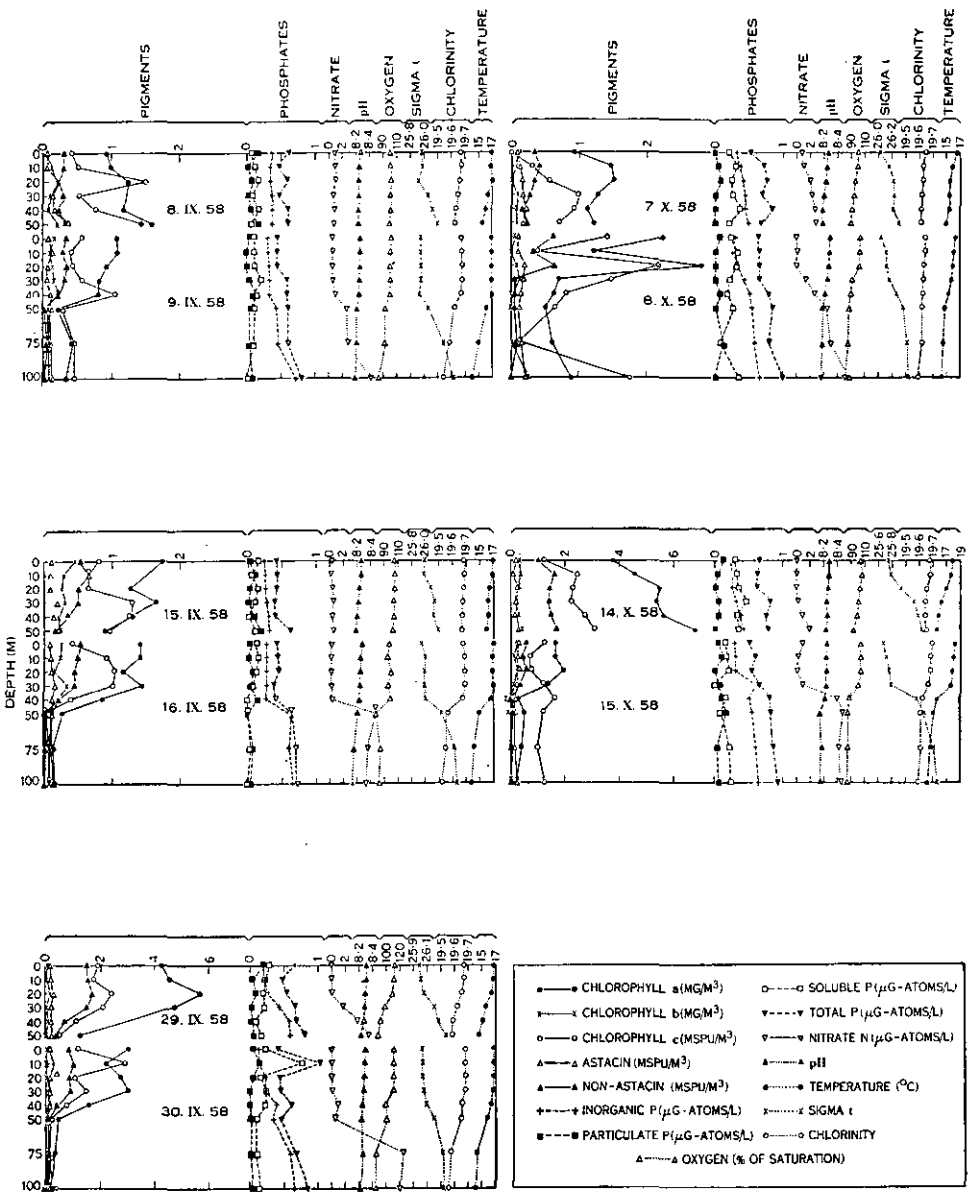


Fig. 12.—Depth profiles for pigment concentration and hydrological conditions at 50 m and 100 m stations from 30.vi.58 to 15.x.58.



UPPER GRAPH 50 M STATION

LOWER GRAPH 100 M STATION

Fig. 12 (*continued*).—Depth profiles for pigment concentration and hydrological conditions at 50 m and 100 m stations from 30.vi.58 to 15.x.58.

(c) *Depth Variation*

Figure 12 shows profiles for conditions at the 50 and 100 m stations during the period June 30–October 15. The following observations can be made on these results:

(1) During pigment blooms, chlorophylls *a* and *c* usually increased in proportion to each other but often *c* outstripped *a*, particularly below the surface.

(2) The concentration of non-astacin pigments ran moderately parallel to chlorophyll *a*—certainly better to *a* than *c*.

(3) From early August, astacin was greater than before. This may reflect a greater abundance of zooplankton in response to increased phytoplankton.

(4) On only one occasion (September 8) did the variation in particulate phosphate reflect the variation in chlorophyll.

(5) The results for soluble phosphate suggested proportionality with chlorophyll.

(6) Inorganic phosphate was inversely proportional to chlorophyll.

(7) Nitrate and total phosphate increased with depth, irrespective of changes in chlorophyll.

(8) pH tended to more acid values with depth. Exceptions to this were associated with increased pigments; thus these exceptions could be explained by postulating increased carbon dioxide uptake.

(9) Sharp changes in pigments occurred irrespective of a thermocline (cf. Holmes, Schaefer, and Shimada 1957). In fact thermoclines were never well developed.

(10) The relation between pigments and oxygen was sometimes direct, sometimes inverse. High chlorophyll concentrations at the bottom did not give high oxygen saturation.

Also it can be seen that there was often much chlorophyll (particularly *c*) in the bottom waters. There are no data available on the intensity of light at these depths but Jitts (1959) has shown that in 1955–57 the mean depth of penetration of 1 per cent. of the surface light was 57 m at the 50 m station (24 observations) and 68 m at the 100 m station (33 observations). Riley and Gorgy (1948) found that in the Sargasso Sea pigments were in greatest concentration at 100 m with significant amounts still present at 300 m. For the shallower waters of Long Island Sound, Riley and Conover (1956) stated that the presence of chlorophyll at the bottom was due to a proportion of the phytoplankton produced at the surface being removed to deeper water. It is possible that this happens at the stations off Sydney. Further, analyses made on oceanic waters off the southern New South Wales coast in June (*Derwent Hunter* Cruises DH10 and DH11/58) showed that chlorophyll *c* was present in significant quantities down to 200 m (Table 9).

(d) *Relation between Chlorophylls* a, b, *and* c

Krey (1957) has mentioned the desirability of determining the relation between the chlorophylls so that a determination of *a*, for example, would allow the cal-

culation of *b* and *c*. However, as Figure 13 shows, there is a variation throughout the year which in the case of *b/a* is quite marked. The values can be compared

TABLE 9
PIGMENTS IN THE TASMAN SEA

Position	Time	Depth (m)	Chlorophyll *a* (mg/m³)	Chlorophyll *b* (mg/m³)	Chlorophyll *c* (MSPU/m³)	Astacin (MSPU/m³)	Non-Astacin (MSPU/m³)
39° 02′ S.	0600	0	0·52	0·20	0·75	0·28	*
153° 14′ E.		25	0·52	0·26	0·83	0·23	*
		50	0·37	0·14	0·89	0·15	*
		100	0·61	0·22	1·01	0·16	0·03
		150	0·14	0·11	0·80	0·17	*
		200	0·07	0·08	0·47	0·11	*
37° 49′ S.	0600	0	0·62	0·30	0·66	0·17	0·05
153° 28′ E.		25	0·67	0·29	0·88	0·14	*
		50	0·55	0·26	0·66	0·25	*
		100	0·25	0·20	0·59	0·14	*
		150	0·22	0·18	0·54	0·21	*
		200	0·08	0·07	0·42	0·14	*
34° 12′ S.	0600	0	0·99	0·29	0·61	0·16	0·26
151° 31′ E.		25	0·67	0·20	0·61	0·13	0·15
		50	0·23	0·15	0·50	0·16	*
		100	0·07	0·17	0·29	0·17	*
		150	0·09	0·16	0·28	0·16	*
		200	0·11	0·16	0·44	0·19	*
	1345	0	1·05	0·42	0·70	0·17	0·25
		25	1·93	0·41	0·25	0·14	0·24
		50	1·92	0·47	0·45	0·18	*
		100	1·11	0·04	0·53	0·14	*
		150	1·11	0·04	0·62	0·17	*
		200	1·08	0·08	0·73	0·17	*
34° 51′ S.	0600	0	0·35	0·06	0·74	0·13	0·79
152° 48′ E.		25	0·45	0·10	0·99	0·12	0·09
		50	0·41	0·06	0·90	0·13	0·09
		100	0·45	0·07	0·83	0·13	0·08
		150	0·07	0·04	0·63	0·14	*
		200	0·03	0·06	0·23	0·10	*
	1300	0	0·43	0·12	0·40	0·10	0·12
		25	0·41	0·07	0·42	0·09	0·13
		50	0·35	0·11	0·46	0·13	0·09
		100	0·39	0·15	0·52	0·18	0·01
		150	0·06	0·08	0·33	0·18	*
		200	0·02	0·10	0·16	0·12	*
34° 30′ S.	0600	0	0·38	0·11	0·58	0·16	0·08
153° 57′ E.		25	0·36	0·03	0·42	0·11	0·11
		50	0·43	0·13	0·73	0·16	0·07
		100	0·39	0·15	0·86	0·16	0·04
		150	0·44	0·14	0·63	0·21	0·05
		200	0·33	0·18	0·93	0·29	*
	1300	0	0·46	0·16	0·62	0·17	0·06
		25	0·45	0·09	0·84	0·18	0·08
		50	0·56	0·12	0·87	0·14	0·12
		100	0·51	0·11	0·82	0·14	0·08
		150	0·14	0·06	0·56	0·18	*
		200	0·14	0·06	0·59	0·14	*

* Negative values.

with those given by Rabinowitch (1945) for *b/a* in green algae, i.e. from 0·3 in *Chlorella* to 0·8 in *Ulva*. The values plotted in Figure 8 are calculated from weighted

averages representing the whole water column, i.e. if X_r is the value at r m, A_{50} the weighted average for the 50 m station and A_{100} that for the 100 m station,

$$A_{50} = \frac{5X_0 + 10\sum_{10}^{40} X_r + 5X_{50}}{50}$$

and

$$A_{100} = \frac{5X_0 + 10\sum_{10}^{40} X_r + 17{\cdot}5X_{50} + 25X_{75} + 12{\cdot}5X_{100}}{100}.$$

It can be seen that b is always less than a and that a is bigger than c on a very slight majority of occasions. Although b can be regarded as a shade pigment

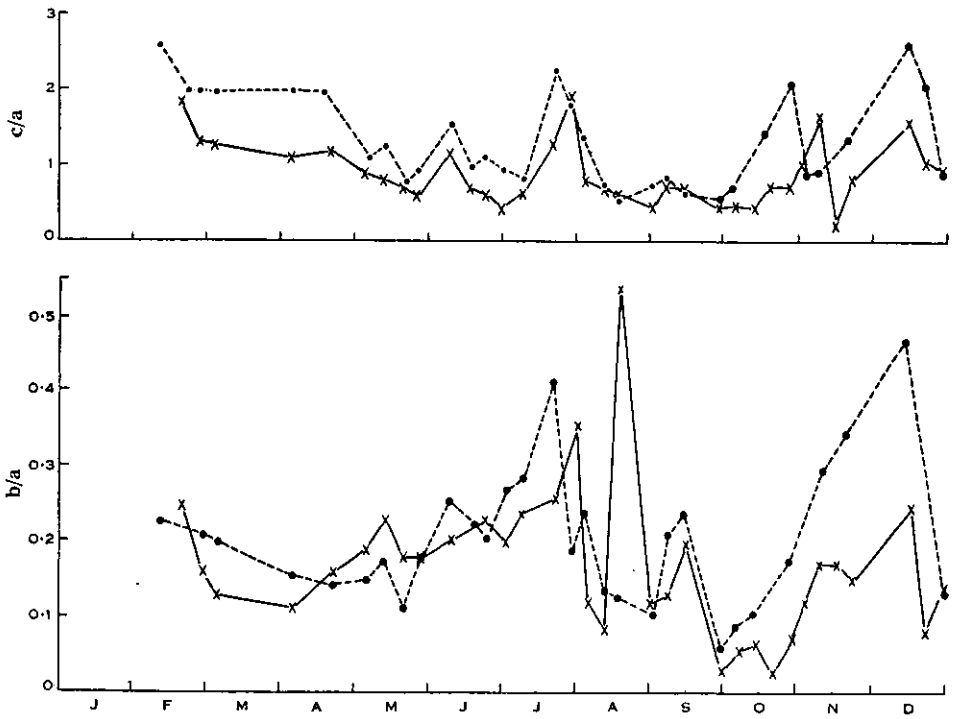


Fig. 13.—Ratios between chlorophyll concentrations (weighted averages) for water column at 50 m (×———×) and 100 m (●- - - -●) stations.

in some plants (Rabinowitch 1945), there was no suggestion in the results that b increased with depth. However, the highest value of b/a was during winter (Fig. 13). Shmaevskii (1955) has shown that in wheat the value of b/a varies during the day, independently of the night and day light-intensity, and he suggests that there is a daily periodicity of biochemical changes responsible for this variation.

(e) *Hourly Variation*

Ryther *et al.* (1958) observed a diatom bloom for several hours and found that there were changes in chlorophyll a which could not be explained by variations

in population density. They concluded that the chlorophyll content of diatoms increases gradually throughout the day, reaches its peak about sunset, and decreases rapidly throughout the night.

The means of the results for the 48-hour drift off Sydney have been plotted in Figure 14, and it can be seen that chlorophyll *a*, *b*, and astacin did not give

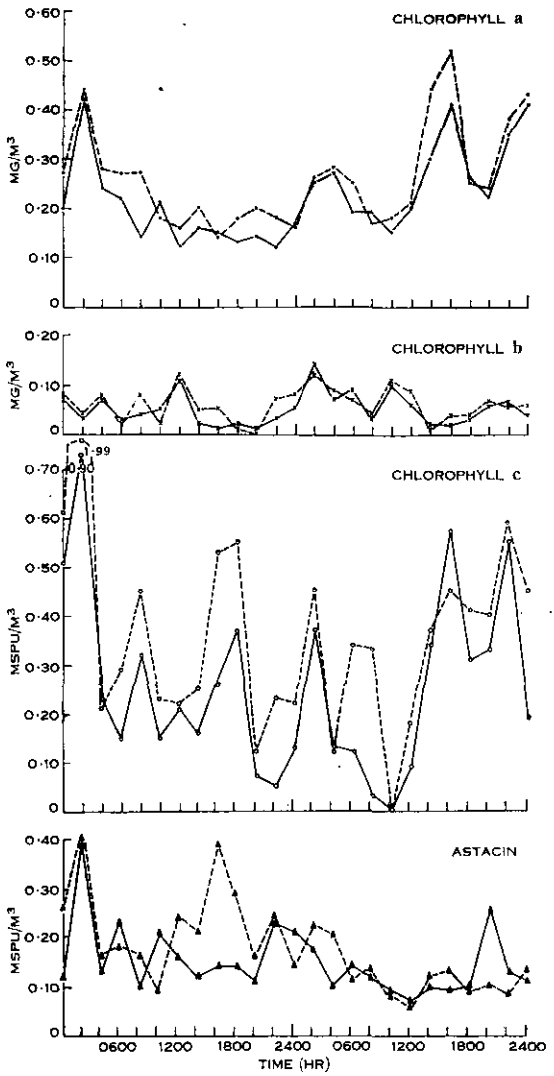


Fig. 14.—Variation in pigment concentrations during a 48-hour drift off Sydney: ———, 0 m; – – – –, 25 m.

uniform patterns over the two days. Chlorophyll *c* showed many peaks with the minima on each day at early morning, midday, and evening. The changes at 25 m usually resembled those at 0 m. In addition to patchiness in distribution while the boat drifted, the variation could be due to alterations in pigment content of individual cells.

(*f*) *Pigments as a Measure of Biomass*

Numerous methods have been used or suggested for giving the concentration of phytoplankton in the sea. To evaluate the importance of phytoplankton in food chains it is necessary to know the amount of each type of food which can be supplied to the next members of the chain. Thus, analyses for fat, protein, carbohydrate, vitamins, minerals, etc. are needed. All the methods used are approximations to this end and depend on conversion factors of doubtful validity.

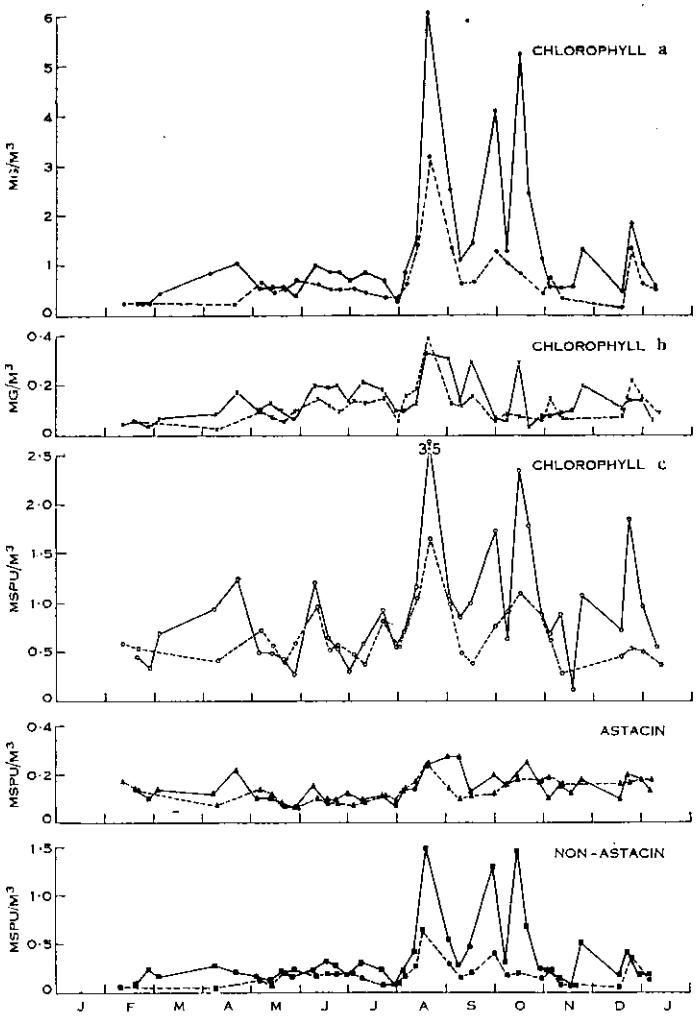


Fig. 15.—Weighted averages for pigment concentrations in water columns at 50 m (———) and 100 m (– – – –) stations.

The use of pigments has many disadvantages including the presence of colourless forms, the seasonal changes in pigments in individual cells (cf. Harris and Riley 1956), the lack of knowledge of the relation between pigments and the chemical composition of the organisms, and interference by non-cellular pigments. However,

pigments give more reliable results than cell counts or volume estimations because of the ease and precision of routine estimation and the greater uniformity of methods in different laboratories.

The weighted averages obtained at the time of maximum pigment concentration (Fig. 15; August 18 at 50 m station and August 19 at 100 m station) gave the following estimates of biomass (Table 10) after calculation using the factors 1 mg chlorophyll = 12 mg C (Laevastu 1957) and 1 mg C = 2·24 mg organic matter (Harvey 1950). Thus, using the total chlorophyll value, the maximum amount of phytoplankton found at the two stations during 1958 corresponded to 13·9 and

TABLE 10

PHYTOPLANKTON BIOMASS OFF SYDNEY ON AUGUST 18 AND 19

The calculated values refer to the column under 1 m² of water surface

Position	Pigment	Carbon (g)	Organic Matter (g)
50 m station	6·1 mg/m³ chlorophyll *a*	3·7	8·2
	0·3 mg/m³ chlorophyll *b*	0·2	0·5
	3·8 MSPU/m³ chlorophyll *c*	2·3	5·2
	Total	6·2	13·9
100 m station	3·2 mg/m³ chlorophyll *a*	3·8	8·5
	0·4 mg/m³ chlorophyll *b*	0·5	1·1
	1·7 MSPU/m³ chlorophyll *c*	2·0	4·5
	Total	6·3	14·1

14·1 g of organic matter. The mean values for organic matter in 1958 were 3·0 and 3·8 g. These means are lower than those found in similar depths by Harvey (1950) (4 g off Plymouth in 1949) and Riley (1955) (16 g in Long Island Sound in 1953).

V. CONCLUSIONS

The following conclusions can be made:

(1) Overnight extraction with 90 per cent. acetone was needed to remove pigments from phytoplankton.

(2) Such extracts were stable for several hours after centrifugation.

(3) Unless water samples were filtered or centrifuged at once there was usually a slight increase in pigments during the next few hours.

(4) Filters could be stored for at least a month without appreciable change in pigments.

(5) Magnesium carbonate hastened filtration.

(6) Centrifugation could replace filtration.

(7) The precision of estimations was such that the S.D. was about 10 per cent. of the mean.

(8) Satisfactory replicate samples could be collected from surface waters but not at depth.

(9) Chlorophyll *c* was a major pigment in both surface and deeper waters (200 m).

(10) In vertical profiles, chlorophylls *a* and *c* changed roughly in proportion. Particulate phosphate did not reflect the chlorophyll changes. Soluble phosphate was directly, and inorganic phosphate indirectly, related to chlorophyll. There were sharp changes in pigments irrespective of thermoclines.

(11) The ratios between the chlorophylls varied during the year.

(12) The mean amount organic matter (calculated from pigments) under 1 m^2 of water surface during 1958 was 3·0 g at the 50 m station and 3·8 g at the 100 m station.

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