CORRELATION OF SOME OF THE PHYSICAL AND CHEMICAL PROPERTIES OF THE SEA WITH THE CHEMICAL CONSTITUTION OF THE ALGAE

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INTRODUCTION

Although reliable data are available for the seasonal variation of the nutrient salts of surface off-shore waters in various parts of the world (Harvey, 1945, pp. 75–8, 85–6), information on inshore coastal waters is scanty. In North

America, however, extensive investigations have been carried out within the last 20 years on the coastal waters bordering on the states of Washington and British Columbia. These waters are cut off from the Pacific Ocean by Vancouver Island, and thereby possess certain characteristic properties. Hutchinson, Lucas & McPhail (1929) have described the seasonal variations in the waters of the Strait of Georgia. The phosphate figures are much higher than in the open sea, and these authors attribute the high nutrient content to the inflow of water from the Fraser River.

The waters of Puget Sound are also particularly rich in phosphates, nitrates and silicates. Thompson & Robinson (1933) believe this to be due to upwelling off the entrance to Juan de Fuca Strait and to vigorous mixing and agitation of the waters. Phifer & Thompson (1937) give the results of 5 years' study of the inshore conditions at Friday Harbour, on the San Juan Channel. In general, the nitrate and phosphate figures are about four times higher than those reported in our waters, and they are not subject to the same marked seasonal variation, while dissolved oxygen saturations rarely exceed 80%. In this channel strong tidal currents throughout the year create turbulent conditions, which are responsible for the high nutrient and low oxygen contents of these waters.

Newcombe, Horne & Shepherd (1939) have studied the waters of Chesapeake Bay, in the region of Solomons Island, Maryland, U.S.A., during the period 1936–38. Conditions in Chesapeake Bay, however, are typically 'estuarine', surface chlorinities being low (5·8–10·2%) and summer temperatures high (25°C.). The phosphate content of the Bay waters in summer commonly ranges about 0.23×10^{-3} mg. atom P/l. at the surface, while the river waters may contain 1.0×10^{-3} mg. atom P/l. Minimum concentrations are obtained during the winter period, when the abundance of phytoplankton organisms is maximum. Estuarine waters are rich in ammonium and silicate.

Marshall & Orr (1927) obtained a close relation between the growth of diatoms and chemical changes in the Clyde Sea Area in 1924, 1925 and 1926 and again in 1927 and 1928 (1930). In 1926 the seasonal variation in temperature, density, pH, salinity, dissolved oxygen and phosphate were determined for two points in Loch Striven. The two localities visited were Clapochlar (depth c. 60 m.) and Loch Striven head (depth c. 28 m.), and samples were taken at 0, 10, 20, 40 and 60 m. at weekly intervals from the end of January till the end of November. Loch Striven is not polluted to any extent by the Clyde, although the surface water is often affected by drainage from the hills, which give rise to erratic salinity values.

No other work, at least to the writers' knowledge, appears to have been carried out on the seasonal variation in the nutrient content of the Scottish inshore waters, and it seemed possible that lack of nutrients during the summer months might influence the growth of algae.

This investigation was undertaken, therefore, in order to determine the

seasonal variation in the nutrient content of our sea water and to ascertain if any correlation existed between the composition of the sea water, in particular, nitrate, and that of the algae growing in it.

LOCATION AND METHODS OF SAMPLING

The three positions selected were Eilean Coltair (at the entrance to Loch Melfort), Rudh-an-Aoil (Shuna Island) and Cullipool (Luing Island). Eilean Coltair is the most sheltered of the three positions, being situated in a loch with the water cut off to a certain extent. Shuna Island is more exposed, with strong tidal currents running, but it is protected from the open sea by the Island of Luing. Cullipool, situated on the north-west coast of Luing Island, is open to the Atlantic and is the most exposed position.

The upper waters pass in general direction from the Irish Sea, through the North Channel and up the west coast of Scotland, through the Minch and round the north coast to the Orkney region. The water at the three positions selected can therefore be regarded as typical coastal inshore water, practically free from oceanic water.

Samples from the three localities were taken monthly for 13 months from March 1948 to March 1949 by means of a Nansen-Pettersson water bottle at a depth of 4–5 m. (low water), just above the weed bed. Temperatures were recorded with a reversing thermometer and the transparency of the waters measured with a standard Secchi disk.

The samples of water, carefully protected from light, were transported to Inveresk Gate and the chemical analysis carried out within 24 hr.

EXPERIMENTS AND RESULTS

The usual methods for the various determinations were utilized, and the results are summarized in Tables I and II and Figs. 1–6.

The pH was measured with the Marconi pH meter, and the observed readings corrected for the rise in temperature since sampling. The pH values for the three localities are given in Table I but, because of the relative constancy for any given month during the period, only the Shuna Island figures are reproduced in Fig. 5. Dissolved oxygen was estimated only in the Shuna Island waters and the results are given in Table II and Fig. 3.

Nitrate was determined by the diphenylbenzidine method of Atkins (1932), which was modified slightly, as outlined below, to allow the colour intensities to be measured by the Spekker Photoelectric Absorptiometer. The intensity of the blue colour which develops does not bear a linear relationship to the nitrate content, but the graph of colour intensity plotted against nitrate concentration gives a smooth curve. The slope of this curve increases with increased nitrate content, so that the higher nitrate figures are more accurate than the lower figures. The accuracy of the method, however, increases from

TABLE I. SEASONAL VARIATION IN TEMPERATURE, pH AND TRANSPARENCY

Month	Temperature (°C.)			pH *in situ*			Secchi disk (m.)		
	Eilean Coltair	Shuna Island	Culli-pool	Eilean Coltair	Shuna Island	Culli-pool	Eilean Coltair	Shuna Island	Culli-pool
1948									
March	7·5	7·5	—	8·03	7·96	—	—	—	—
April	8·2	8·1	7·8	8·09	8·11	8·11	9	9	5
May	9·8	9·8	9·0	8·17	8·13	8·10	7	7	9
June	12·4	11·1	10·6	8·13	8·13	8·10	7	8	9
July	12·2	12·3	11·7	8·11	8·12	8·11	9	7	5
August	13·0	13·0	12·7	7·91	7·95	7·96	9	9	7
September	12·5	11·9	12·2	7·86	7·91	7·90	7	9	7
October	12·2	11·9	12·1	8·01	8·06	8·06	7	6	5
November	10·5	10·9	11·0	8·01	8·04	8·06	5	7	7
December	9·7	10·0	10·2	7·96	8·02	8·05	8	7	7
1949									
January	6·5	7·0	8·1	8·04	8·07	8·07	7	7	7
February	7·8	7·7	8·0	8·01	8·03	8·05	7	7	5
March	6·1	6·9	6·9	8·04	8·05	8·07	7	7	7

TABLE II. SEASONAL VARIATION IN SALINITY, OXYGEN, PHOSPHATE AND NITRATE

Month	Salinity (g./kg.)			Oxygen. Shuna Island		Phosphate (mg. atoms P/m.³)			Nitrate (mg. atoms N/m.³)		
	Eilean Coltair	Shuna Island	Culli-pool	ml./l. at N.T.P.	% satura-tion	Eilean Coltair	Shuna Island	Culli-pool	Eilean Coltair	Shuna Island	Culli-pool
1948											
March	34·05	34·12	—	—	—	0·55	0·55	—	6·4	6·1	—
April	33·56	33·81	33·94	6·24	93·5	0·35	0·52	0·41	0·7	3·1	5·0
May	33·78	33·86	34·02	6·14	95·4	0·19	0·26	0·33	1·0	2·6	4·6
June	33·90	33·93	33·93	5·94	94·9	0·16	0·20	0·18	1·4	2·4	1·6
July	33·83	33·72	33·92	6·02	98·2	0·16	0·16	0·20	0	0	0·9
August	34·01	34·02	34·05	5·73	94·9	0·19	0·19	0·20	0·5	1·0	1·5
September	33·61	33·06	33·76	5·83	93·9	0·30	0·23	0·30	3·0	3·1	4·9
October	33·63	33·57	33·65	5·71	92·4	0·37	0·31	0·31	5·0	5·4	4·8
November	33·34	33·67	33·83	5·86	93·0	0·40	0·50	0·42	4·9	5·6	5·7
December	33·16	33·59	33·83	5·85	90·4	0·75	0·65	0·50	5·5	6·1	5·7
1949											
January	32·92	33·26	33·44	6·41	92·8	0·57	0·53	0·53	6·3	6·5	6·9
February	33·45	33·62	33·56	6·40	94·3	0·51	0·53	0·53	6·5	6·7	6·5
March	33·62	33·83	34·03	7·00	101·5	0·48	0·53	0·52	5·5	5·8	5·9

± 10 mg. at 0–30 mg. nitrate – N/m.³ to ± 4 mg. at 90 mg. N/m.³. This method cannot compete with Harvey's strychnidine method at low nitrate concentrations, for the latter gives a linear relation between colour intensity and nitrate content. It was not used, however, in these investigations, because of the difficulty of preparing a satisfactory reagent.
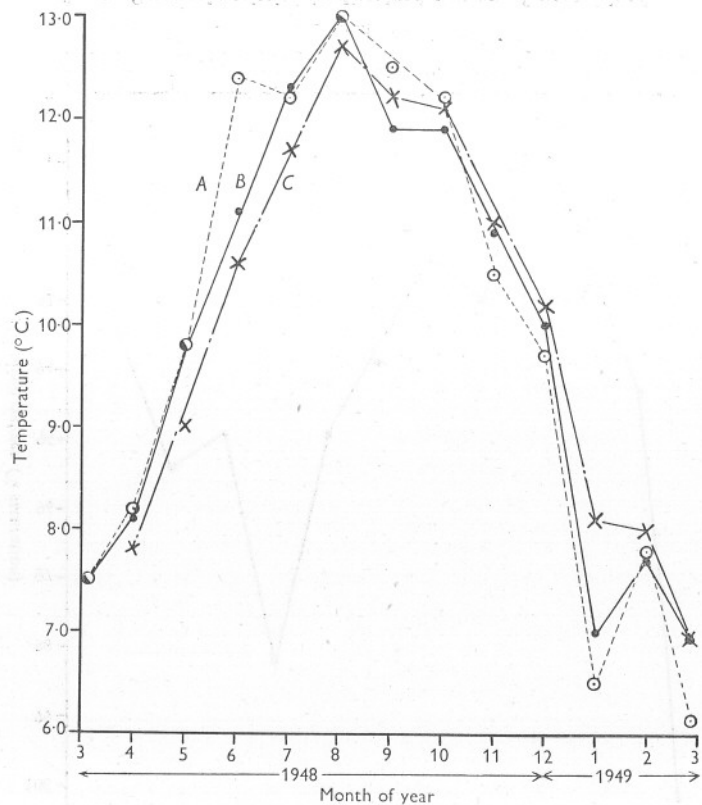
Fig. 1. Seasonal variation in temperature. (A) L. Melfort; (B) Shuna Island; (C) Cullipool.
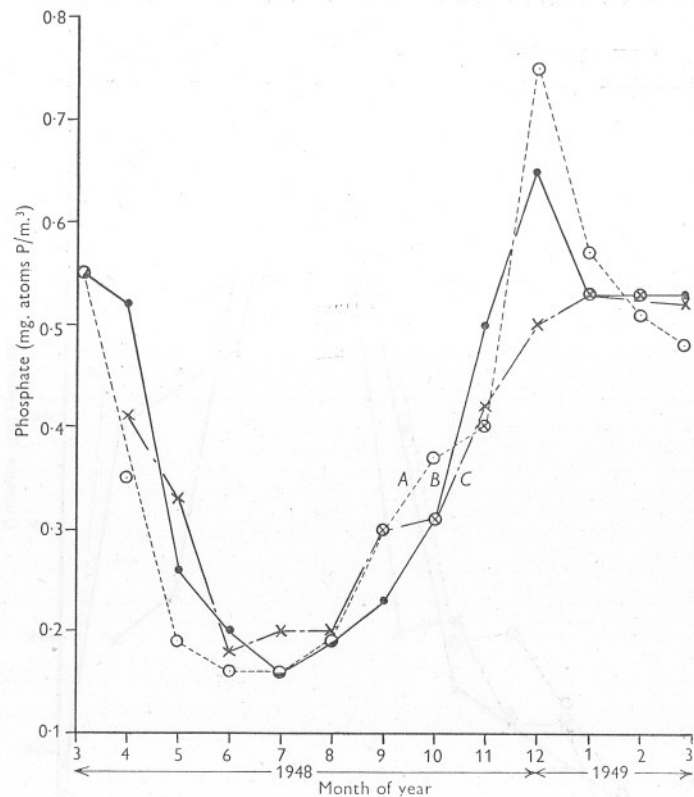

Fig. 2. Seasonal variation in phosphate. (A) L. Melfort; (B) Shuna Island; (C) Cullipool.
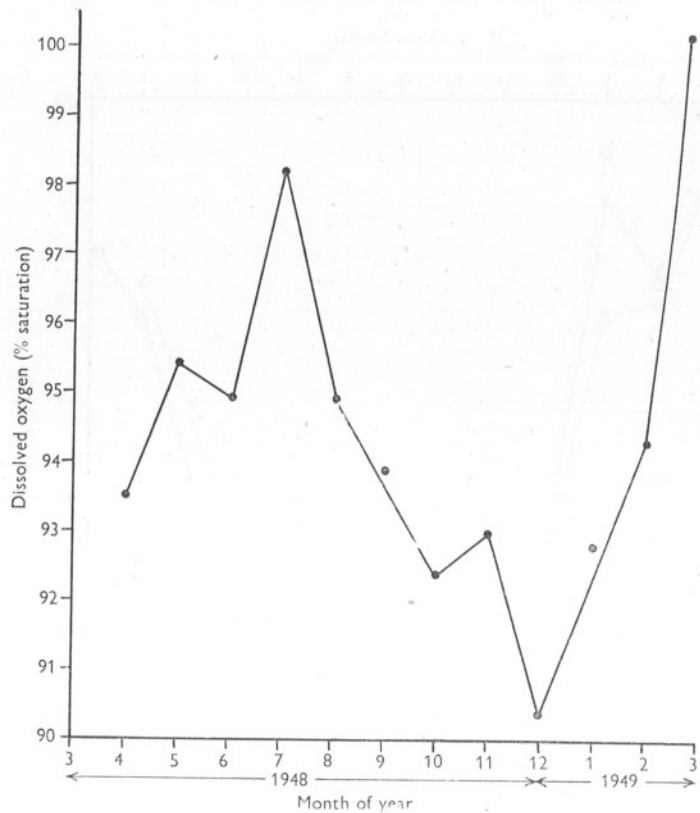
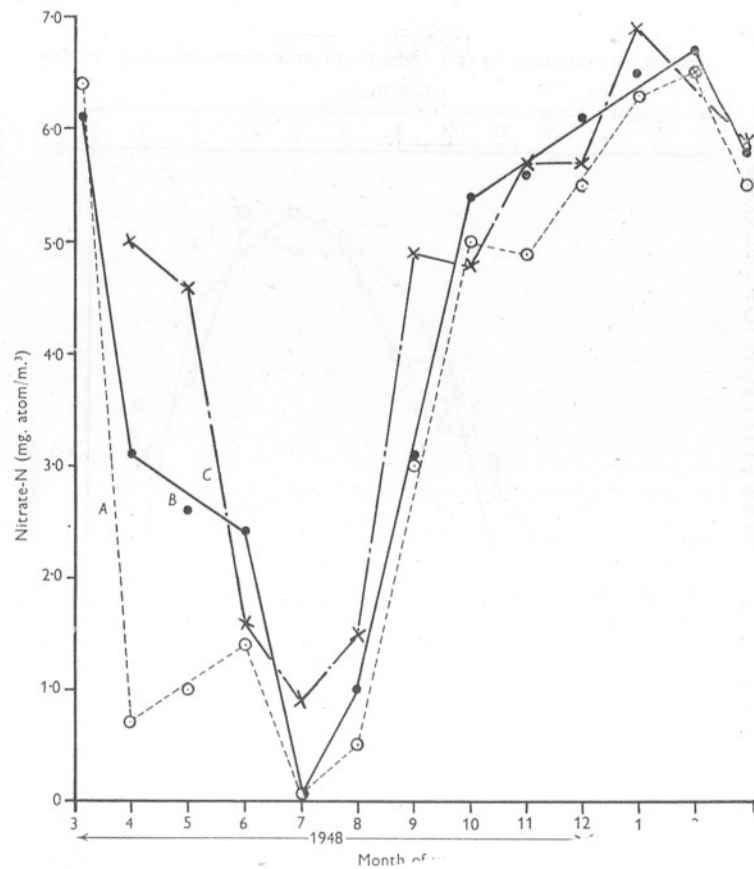Fig. 3. Seasonal variation in dissolved oxygen. Shuna Island.
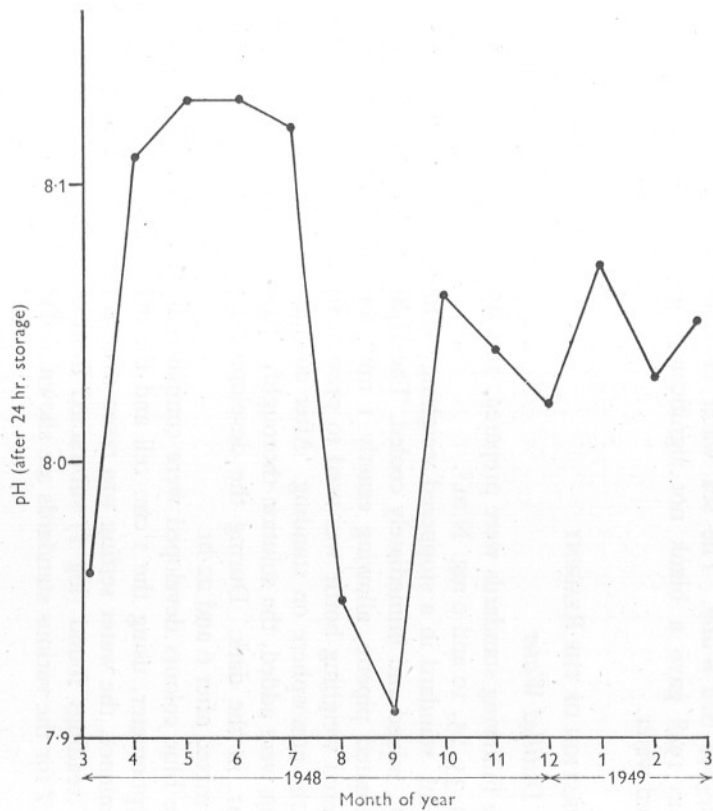
Fig. 4. Seasonal

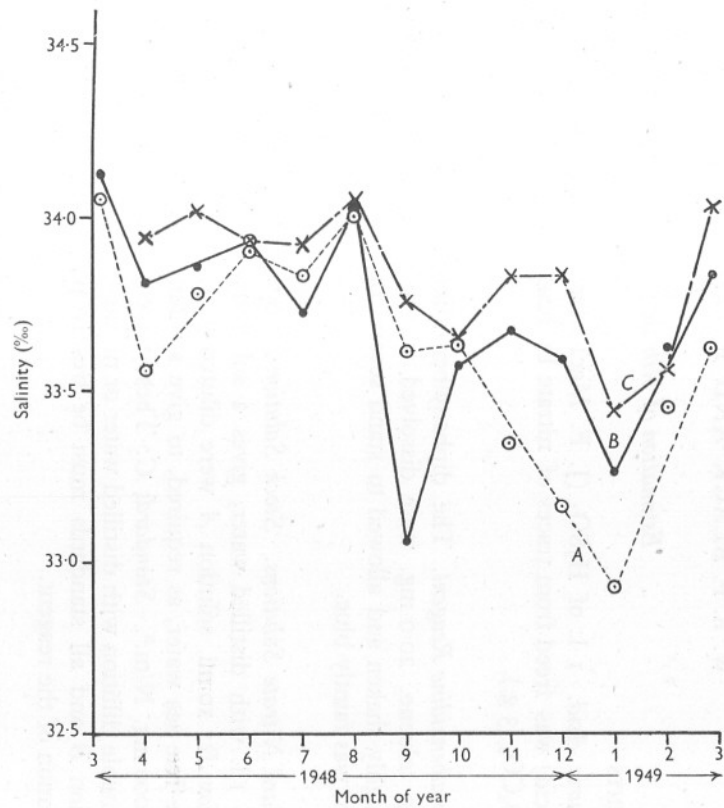Fig. 5. Seasonal variation in pH. Shuna Island.

Fig. 6. Seasonal variation in salinity. (A) L. Melfort; (B) Shuna Island; (C) Cullipool.

Estimation of Nitrate

REAGENTS

Sulphuric Acid. 1 l. of H_2SO_4 (J. F. MacFarlan and Co's special nitrate free acid) was freed from traces of nitrate by heating at 300° for 2 hr. with $(NH_4)_2CO_3$ (3 g.).

Diphenylbenzidine Reagent. The diphenylbenzidine was recrystallized from boiling toluene. 20·0 mg. were dissolved in 100 ml. H_2SO_4, the solution thoroughly shaken and allowed to stand several days before use. The final reagent was faintly blue.

Standard Nitrate Solutions. Stock Solution A: 0·7218 g. A.R. KNO_3, made up to 1 l. with distilled water, gives a solution containing 0·1 g. N/litre. *Solution B:* 10 ml. solution *A* were diluted to 1 l. with distilled water or nitrate-free sea water, as required, to give a solution containing 1 mg. N/l, i.e. 1000 mg. $N/m.^3$. *Standards C:* These were prepared from solution *B* by suitable dilution with distilled water or nitrate-free sea water as required. Solution *B* and all standards must be freshly prepared each day during calibration of the reagent.

Nitrate-free Sea Water. The sea water collected from Eilean Coltair on 11 May 1948 gave a blank not significantly greater than that given by distilled water.

CALIBRATION OF THE REAGENT

(1) *In Distilled Water*

The following standards were prepared: 100, 95, 90, 80, 75, 70, 60, 55, 50, 45, 40, 35, 25, 10 and 0 mg. $N/m.^3$.

2·5 ml. standard in a stoppered weighing bottle were treated with 6·0 ml. H_2SO_4, mixed and immediately cooled. The H_2SO_4 was added by means of a graduated pipette, allowing exactly 1 min. for delivery in each case. A stoppered weighing bottle was used to prevent the solution taking up water from the atmosphere on standing. After cooling, 1·5 ml. diphenylbenzidine reagent were added, the solution thoroughly mixed, and allowed to stand for 22·5 hr. in the dark. During the development period, the solutions were again mixed after 6 and 22 hr.

The blue colours developed were compared in the Spekker Photoelectric Absorptiometer, using the 1 cm. cell and the orange Ilford 607 filter. For convenience, the water setting was taken as 0·70 on the drum scale, and the drum readings found (log I) subtracted from 0·70 (log I_0). The values of log I_0/I for the various standards are shown in Table III.

TABLE III

Distilled water		Distilled water		Sea water		50% (v/v) sea water	
Standard	$\log I_0/I$	Standard	$\log I_0/I$	Standard	$\log I_0/I$	Standard	$\log I_0/I$
100	0·523	50	0·203	100	0·331	100	0·468
95	0·485	45	0·177	75	0·211	75	0·295
90	0·456	40	0·162	50	0·136	50	0·161
80	0·413	35	0·141	25	0·080	25	0·088
75	0·329	25	0·080	10	0·050	10	0·048
70	0·324	10	0·042	0	0·035	0	0·032
60	0·238	0	0·030				
55	0·239						

There is not a linear relationship between colour intensity ($\log I_0/I$) and nitrate concentration, but the graph gives a smooth curve.

(2) *In Sea Water*

In order to determine the salt error, a calibration curve was carried out using nitrate-free sea water in the preparation of the standards. The results, shown in Table III, indicate that the salts in sea water decrease the colour formation, when the colour is measured after 22·5 hr.

(3) *In 50°/₀ (v/v) Sea Water*

Solution B was made up in distilled water. The standards were prepared by taking the appropriate amount of B, adding 50 ml. nitrate-free sea water and making up to 100 ml. with distilled water. The results in Table III show that the colour intensities lie, for the most part, between the distilled water and sea-water values.

ESTIMATION OF NITRATE IN SEA-WATER SAMPLES

2·5 ml. of the unknown sample were treated with H_2SO_4 and diphenyl-benzidine exactly as described in the calibration of the reagent, and the value of $\log I_0/I$ determined after 22·5 hr. The nitrate content of the sample in mg. nitrate-N/m.³ was read off from the sea-water calibration curve.

ACCURACY OF THE METHOD

It was found that the value of $\log I_0/I$ for any given standard could, in general, be reproduced within ± 0·02. From the sea-water calibration curve, the accuracy at various concentrations has been estimated as follows:

Nitrate concentration	Maximum error
30 mg. N/m.³	± 10 mg. N/m.³
40	± 8·5
50	± 7·5
60	± 7·0
70	± 5·5
80	± 4·5
90	± 4·0

Estimation of Phosphate

Phosphate was estimated by the Denigés method, as described by Wattenberg (1937).

<div style="text-align:center">DISCUSSION OF RESULTS</div>

Temperature

In the spring, temperatures are at a minimum, 6–7° C. (Table I and Fig. 1). The loch water at Eilean Coltair gets warmer more rapidly than the open sea, reaching a temperature of over 12°C. by June. At Cullipool the surface waters take considerably longer to heat up, 12°C. only being reached in August. For Shuna Island, in general, the graph lies between the other two.

The opposite state of affairs prevails during the autumn and winter months. Cullipool maintains a temperature of 8°C. or more until February, while the loch water has dropped to 6·5°C. by January. One would expect loch water, because of its proximity to, and isolation by, land, to warm up and cool down more rapidly than the open sea. It is interesting to note that the maximum and minimum temperatures for the three positions do not vary appreciably, although the times required to reach these values do.

Marshall & Orr (1927) found the temperature of the surface waters (0–10 m.) at Clapochlar to be at a minimum (6°C.) in January/February, rising to a maximum of 14°C. in August.

pH

The pH was measured 24 hr. after sampling. The effect of storage is to lower the pH for the following reasons: (1) On standing, the water warms up to that of its surroundings, and the pH falls with rising temperature. The pH *in situ*, however, can be calculated from the pH at temperature of observation by allowing for the temperature coefficient of sea water. In most cases, the decrease in pH per 1°C. rise can be taken as 0·01 pH unit. (2) Bacteria liberate carbon dioxide in respiration, and this lowers the pH. This effect cannot be corrected for, and may be appreciable during warm weather. Although care was taken to keep the temperature of the samples as low as possible during transport, the results may be slightly low.

In April a rapid increase in pH occurs, indicating that plant growth is beginning to utilize carbon dioxide at a greater rate. From April to July the pH values for all positions are 8·10 or greater, but in August and September figures of 7·86–7·96 indicate a slowing up in the rate of photosynthesis which coincides with a low nutrient content of the water. An increase in pH occurs in October, and from October to March fairly constant values of 8·00 ± 0·07 are obtained.

The pH of the surface water at Clapochlar (Marshall & Orr, 1927) was close to 8·0 during the early part of the year. This was followed by a sudden increase at the beginning of April following the spring diatom outburst, and figures of 8·3–8·4 are recorded during this period. Another peak in the pH graph was obtained in September due to the autumn phytoplankton growth.

Transparency

It was hoped, by means of a Secchi disk, to detect the phytoplankton out-burst in the spring, but in practice very little variation was observed through-out the year. Maxima of 7–9 m. and minima of 5–6 m. are recorded, but these do not occur at any specific time and appear to have no significance.

Salinity

In ocean water away from the influence of land, variations in surface salinity are not great. Böhnecke (1938) has shown for an area of the North Atlantic Ocean, extending between latitudes 18° and 42° N., that the highest average surface salinity, 36·70‰, occurs in March and the lowest, 36·59‰, in November. The variations from one month to another are irregular, but on the whole the salinity is somewhat higher in spring than it is in autumn. In the open ocean variations in surface salinity depend mainly upon variations in the difference between evaporation and precipitation, in association also with currents. In inshore waters salinity values are complicated by the influence of land, which gives rise to coastal currents and dilution due to rivers and land drainage. For example, when heavy rain falls during the winter the increased volume of waters discharged by the rivers of the region will tend to decrease the salinities during the winter months. If, however, the precipitation is in the form of snow, the effect upon the salinity of the coastal waters may not be experienced until the summer.

The high salinity values obtained in this investigation, particularly for Cullipool, indicate that these waters have not been diluted by fresh water to any great extent, and the figures bear some relation to the rainfall for the 13 months. For all positions, highest values were obtained in March and August, and lowest values in January. In September an abnormally low figure, 33·06‰, was reported for Shuna Island. In general, the salinity of Cullipool water is higher than the loch water, and this is to be expected because land drainage after heavy rain will affect loch more than open sea water. Again, the graph for Shuna Island lies between the other two for most months of the year, although there are some exceptions.

Marshall & Orr (1927) found the salinity of the surface layer at Clapochlar lay between 33 and 34‰, although some values for the immediate surface layer were much lower, for example, some very low figures are recorded during March and October/November.

Oxygen

With the exception of March 1949, the samples were treated with the reagents, manganous chloride and sodium hydroxide-potassium iodide, about 24 hr. after sampling. In March 1949, the reagents were added immediately after sampling, and the iodine liberated and titrated the following day. This water was found to be slightly supersaturated (% saturation, 101·5). Thus it may be

assumed that all the results, except the last, are low to the extent of 5–10%. Nevertheless, the relative values are of interest in detecting the period of photosynthesis in the spring. The explanation for these low results is twofold: (1) on standing, the water warms up to that of its surroundings, and a certain amount of oxygen tends to be expelled from solution at the higher temperature; and (2) when sea water is stored, bacteria rapidly consume oxygen in respiration, the amount utilized being quite appreciable even after 24 hr. Moreover, bacteria are particularly abundant in inshore water.

The highest saturations are obtained during the spring and summer months, the maximum (98·2%) being reached in July, which is further evidence of photosynthesis during these months. Saturations then decrease steadily to a minimum of 90·4% in December. Because of the uncertainty of these results, further discussion would be unwise, but it appears that this water is saturated, or very nearly saturated, for the greater part of the year, and probably slightly supersaturated during the period of photosynthesis.

Marshall & Orr (1927) found the dissolved oxygen saturations for the surface layers were close to 100% throughout the year, except during the diatom outbursts in the spring and autumn when values up to 138% were occasionally recorded.

Phosphate

The phosphate estimations were carried out one day after sampling, the water being centrifuged before analysis if any turbidity was noticed. It is advisable, however, to estimate phosphate immediately after sampling, or to saturate the water with chloroform if estimations are to be carried out at a later date. On standing, the inorganic phosphate may increase slightly if much organic phosphorus is present, or it may decrease due to utilization by bacteria, but this error will not be great after only 24 hr., provided the temperature of the water is prevented from rising appreciably.

Fig. 2 indicates that the phosphate content begins to decrease after March, the decrease being most marked in the loch water, although the open sea at Cullipool has a phosphate content in April not much greater than the loch. Minimum values of 0·16–0·20 mg. atom phosphate-$P/m.^3$ are obtained in all localities by June, and remain at this low level until September, when there is an increase again to the winter maximum in December, January and February. Abnormally high values were obtained in December for Eilean Coltair (0·75 mg. atom $P/m.^3$) and Shuna Island (0·65 mg. atom $P/m.^3$), which can only be attributed to contamination by land drainage, as the value for Cullipool was normal.

The winter maximum for 1949 of 0·52 mg. atom $P/m.^3$ is in good agreement with the values quoted for the English Channel, although the summer minimum tends to be higher than that found in the Channel.

The seasonal variation in phosphate at a position in the English Channel

has been followed at monthly intervals for a number of years. From winter maximum values varying between 0·69 and 0·47 mg. atom $P/m.^3$ it has fallen in some years to less than 0·02 in the surface layers, in other years 0·06 or 0·10 mg. atom remain (Atkins, 1923–30; Cooper, 1933 a, b, 1938). The winter maximum values of phosphate in the water at the beginning of the year are not constant but fluctuate from year to year. Cooper (1938) has found lower winter maxima during the 1930's as compared with the 1920's, and these have been reflected in decreased plankton and fish populations (Russell, 1935, 1936 a, b).

During February at Clapochlar (Marshall & Orr, 1927), phosphate in the surface layer was about 0·65 mg. atom $P/m.^3$. Within a few weeks, at the end of March and beginning of April, phosphate was reduced to 0·16 mg. atom $P/m.^3$, while by the end of May no trace of phosphate existed in the surface layer. Small quantities appeared in the waters at odd periods throughout the summer, but no full-scale regeneration of phosphate became apparent until the end of September. Maximum winter values were obtained by November.

Nitrate

The estimations were carried out 1 day after sampling. The effect on the nitrate content of storing samples for 24 hr. is negligible, provided the samples are shielded from light. Fig. 4 shows that the seasonal variation of nitrate follows closely that of phosphate. After March nitrate begins to decrease, and again this decrease is most marked in the loch. Between March and April 1948 nitrate-N in Loch Melfort dropped from 6·4 mg. atoms/m.³ to almost zero, and remained at this level until September. Complete exhaustion of nitrate was not observed at Shuna Island and Cullipool until July. Nitrate is almost certainly absent in all localities during July and August. As in the case of phosphate, nitrate is regenerated during September and increases gradually during the autumn and winter, reaching a maximum of about 6·8 mg. atoms $N/m.^3$ in January. Little or no evidence was obtained of the plankton out-burst in the autumn, either from the phosphate or nitrate records.

The seasonal variation in nitrate has been followed during the course of several years in the English Channel (Harvey, 1926, 1928; Cooper, 1933 a). Exhaustion of nitrate due to utilization by phytoplankton is frequently found in the surface water during the summer, while winter maxima may exceed 7 mg. atoms nitrate-$N/m.^3$. The absence of nitrate, however, does not neces-sarily mean that plant production in the sea is reduced to a low level, as nitrogen may still be available as ammonium, the concentration of which may reach 2 mg. atoms ammonium-$N/m.^3$ during the autumn and winter months. Harvey (1940) has shown that plankton diatoms utilize ammonium in pre-ference to nitrate. This may explain why the autumn plankton outburst was not detected by following the seasonal variation in nitrate (Fig. 4), although the flattening of the graph from September to December might be taken as an

indication of its utilization by plankton. In the spring, nearly all the available nitrogen is present as nitrate.

The Nitrogen/Phosphorus Ratio at the Winter Maximum

If 0·52 mg. atom P/m.^{3} and 6·8 mg. atoms N/m.^{3} are taken as the winter maximum values for phosphate and nitrate respectively in 1949, the N/P ratio is 13·2.

The significance of these results on the growth of the adjacent seaweeds is discussed in Part II.

SUMMARY

Monthly samples of sea water, taken from three localities on the Argyllshire coast, from March 1948 to March 1949, have been analysed for pH, dissolved oxygen, salinity, nitrate and phosphate, while temperatures and transparencies have been recorded.

Surface temperatures range from 6–7°C. in January/March to 13° in August. Transparencies remain fairly constant at 7–9 m. throughout the whole period, with occasional minimum values of 5–6 m.

pH values close to 8·0 are obtained from October to March, followed by an increase to 8·1 or over during the period of photosynthesis (April to July). Low pH values (7·86–7·96) are recorded during August and September.

Salinities vary over the narrow range, 33–34°/$_{oo}$, with maxima in March and August and minima in January. These high salinities indicate that the inflow of fresh water from streams in the neighbourhood is negligible, even for Loch Melfort.

Dissolved oxygen saturations are highest in the spring and summer months and lowest in December.

Phosphate and nitrate begin to decrease after March, the reduction being most marked in the loch, and remain at a low level until September, when these nutrients are again regenerated. Winter maximum values of 0·52 mg. atom P/m.^{3} and 6·8 mg. atoms nitrate-N/m.^{3} are obtained by January.

Atkins's diphenylbenzidine method has been used to estimate nitrate, and a description of the method is given.

PART II. CORRELATION OF THE COMPOSITION OF SEA WATER WITH THE CHEMICAL CONSTITUTION OF THE ALGAE

INTRODUCTION

The larger algae, like most other forms of marine plant life are, apart from the influence of light, wholly dependent for their growth and development on the nutrient content of the surrounding sea water.

Although there is no experimental evidence to show that the growth rate of sea-weeds in the sea is ever brought to a standstill through lack of nutrients, Harvey's work (1926) indicated that lack of nitrate can limit plant growth. He also showed (1933) that a marked reduction in the rate of photosynthesis for the diatom *Nitzschia closterium* occurred with phosphate concentrations below 0·32 mg. atom P/m.^{3}, being most marked below 0·16 mg. atom P/m.^{3}. There appears to be no doubt that nitrate and phosphate are most important factors in the productivity of the sea, and plankton, at least, is richer where vertical currents aid in increasing their concentration. Whereas Harvey (1926, 1928) has recorded marked seasonal variations in the nitrate-content in the English Channel with non-detectable amounts in August, Dakin (1934) showed that at Sydney there was a much more uniform distribution throughout the year. It would appear, therefore, that marked seasonal variations in the nutrient content, with depletion of nitrate in the summer, occur mainly in inshore waters where a thermocline is set up during the summer preventing regeneration of the nutrients in the photosynthetic layer.

Marshall & Orr (1948), in carrying out experiments in Loch Craiglin on the effect of different plant nutrients on the phytoplankton, found that after adding a large excess of fertilizer a good increase in plankton was obtained, after an initial lag. At that time attached algae were abundant and these investigators believed that the needs of the algae had to be satisfied before any nutrients could be used by phytoplankton.

With marine diatoms, therefore, scientific investigations have established a direct correlation between their efflorescence and abundance, and the concentrations of nutrient salts, especially nitrates and phosphates, in sea water, and scientific literature pertaining to European, American, Antarctic and other waters furnishes numerous examples.

Parke (1948), in her studies on the British Laminariaceae, found for *Laminaria saccharina* that there were two periods of growth, a period of rapid growth between January and June/July with the most rapid growth between March and June, and a period of slow growth from July to December, but unfortunately no attempt was made to correlate this with the nutrient content of the water.

Tikhovskaya (1940) investigated the seasonal variations in the productivity and photosynthesis of *L. saccharina* in the Barents Sea. He reported maximum

growth in April with a sharp drop in June and a slight increase in October, and stated, although no figures are given, that in summer the N and P content was the smallest for the whole year.

In view of the fact that in Norway, it has been possible to correlate the presence of herring with water of a particular temperature and salinity, it was thought that, since correlation appeared to exist between the chemical composition of the algae and the physico-chemical properties of the water, it might be possible from an analysis of the water to forecast the optimum annual harvesting periods. No previous investigator, however, appears even to have attempted to correlate the seasonal changes in the chemical composition of the fixed algae with changes in the composition of the sea water.

PREPARATION AND ANALYSIS OF SAMPLES

Monthly samples comprising six plants of the following species of Laminariaceae were taken, two were used for dry weight determinations, and two were analysed by the methods previously employed (Black, 1948).

The inorganic nitrogen was obtained by difference, i.e. by subtracting the organic nitrogen from the total nitrogen. The total nitrogen was determined by modifying the standard Kjeldahl method using a reduction mixture containing sodium thiosulphate as a preliminary, to the ordinary digestion (Kelley, Hunter & Sterges, 1946). Any ammonium salts, however, present in the plant will be estimated as organic nitrogen (Kjeldahl).

(1) *L. saccharina* from 4 m. (low water) at Eilean Coltair, Loch Melfort.

(2) *L. saccharina* from 4 m. at Rudh-an-Aoil, Shuna Island.

(3) *L. cloustoni* from 4 m. at Cullipool, Luing Island.

The results for crude proteins, inorganic nitrogen, mannitol, laminarin, dry-weight content, fresh weight, total ash, and alginic acid are summarized in Figs. 7–14.

As the marked seasonal variations in composition occur in the fronds the results for the stipes, with the exception of the fresh weights for *L. saccharina*, have been omitted.

DISCUSSION OF RESULTS

In comparing the variations in chemical constitution it should be borne in mind that *L. cloustoni* differs in many respects from *L. saccharina*, and it is unfortunate that the latter species was unobtainable at Cullipool.

On the west coast of Scotland, with *L. saccharina*, growth of the new frond begins much earlier than with *L. cloustoni*, and from August losses due to the shedding of the old frond of the current year may exceed the weight increment. In the autumn the rapid drop in laminarin may be due to this or it may be due to sporogenesis. On the other hand, *L. cloustoni* does not usually cast its old frond until April/May, although considerable wear has no doubt occurred
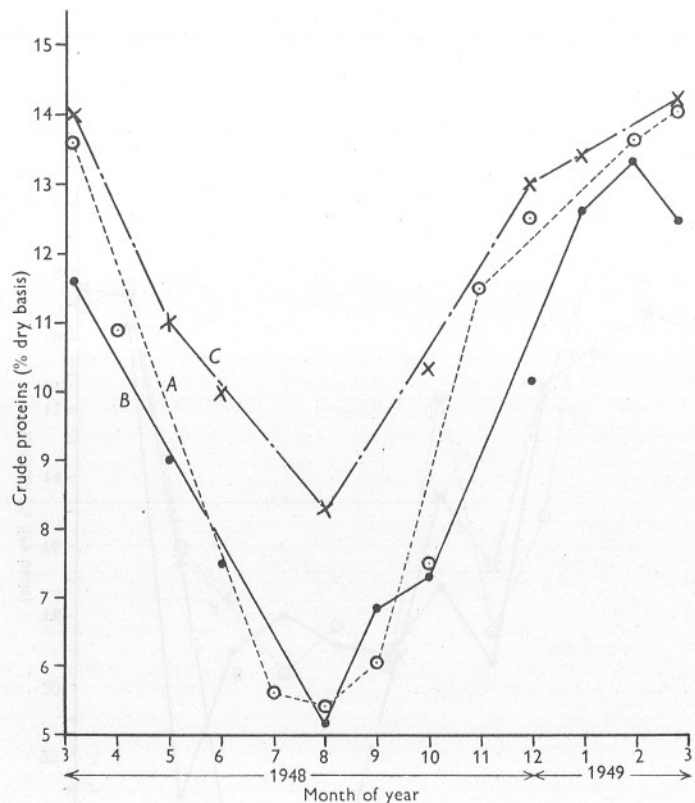
Fig. 7. Seasonal variation in crude proteins. (*A*) *L. saccharina* frond, L. Melfort; (*B*) *L. saccharina* frond, Shuna Island; (*C*) *L. cloustoni* frond, Cullipool.
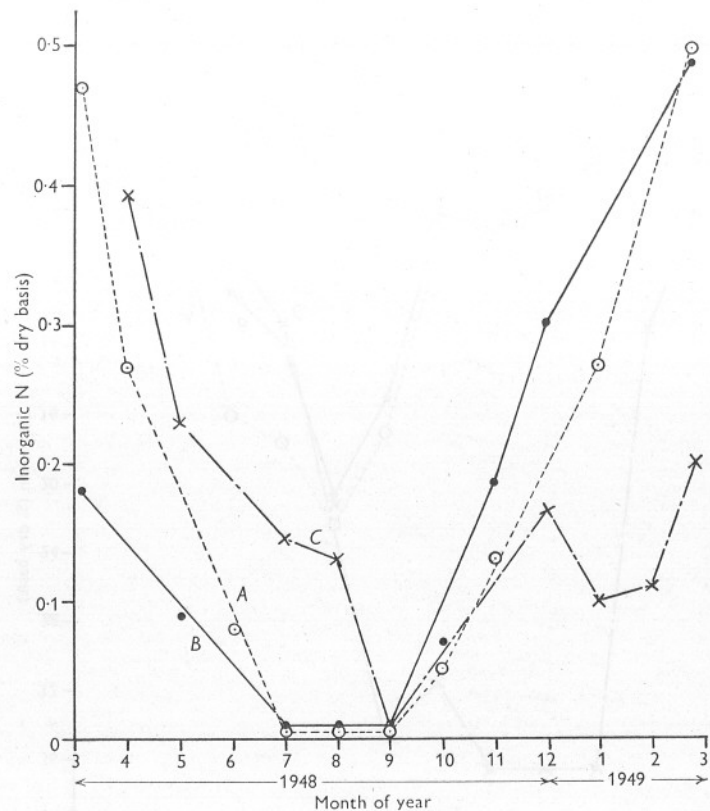
Fig. 8. Seasonal variation in inorganic nitrogen. (*A*) *L. saccharina* frond, L. Melfort; (*B*) *L. saccharina* frond, Shuna Island; (*C*) *L. cloustoni* frond, Cullipool.
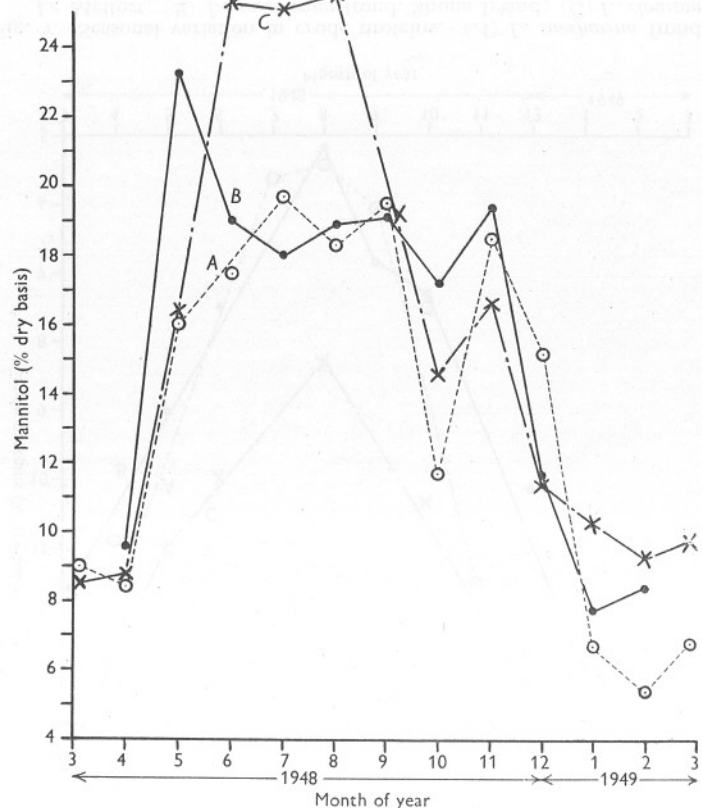
Fig. 9. Seasonal variation in mannitol. (A) L. saccharina frond, L. Melfort; (B) L. saccharina frond, Shuna Island; (C) L. cloustoni frond, Cullipool.
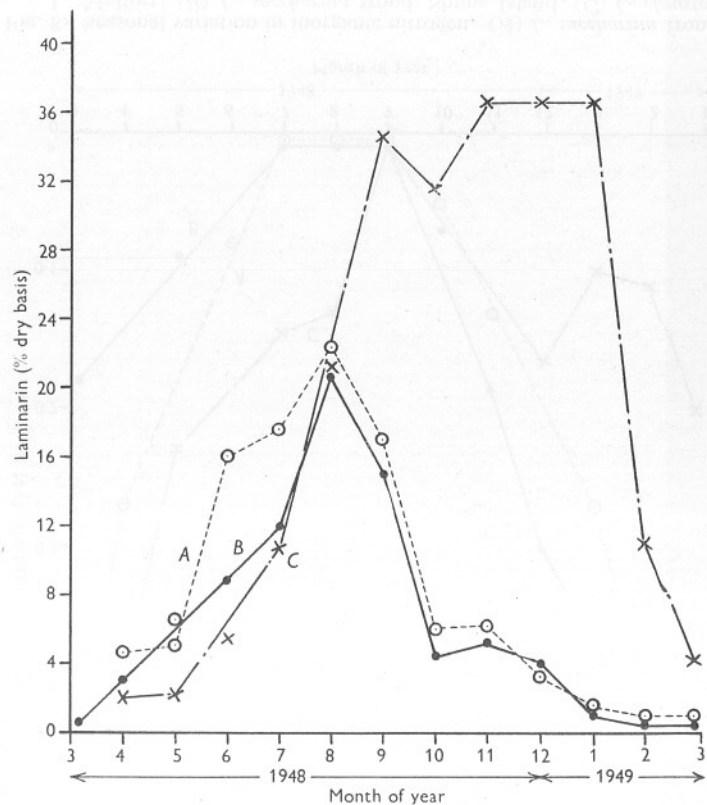
Fig. 10. Seasonal variation in laminarin. (A) L. saccharina frond, L. Melfort; (B) L. saccharina, frond, Shuna Island; (C) L. cloustoni frond, Cullipool.
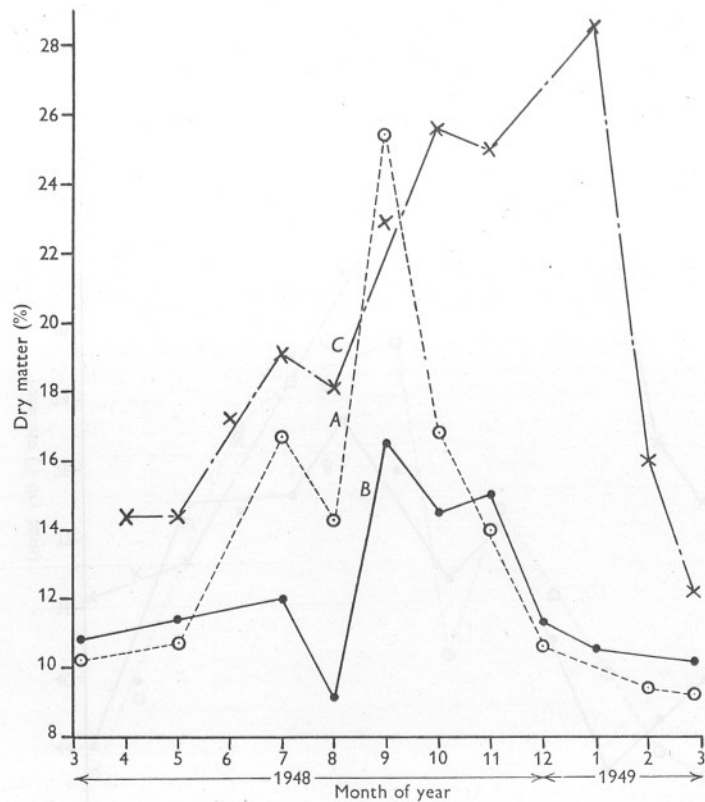
Fig. 11. Seasonal variation in dry matter. (A) L. saccharina frond, L. Melfort; (B) L. saccharina frond, Shuna Island; (C) L. cloustoni frond, Cullipool.
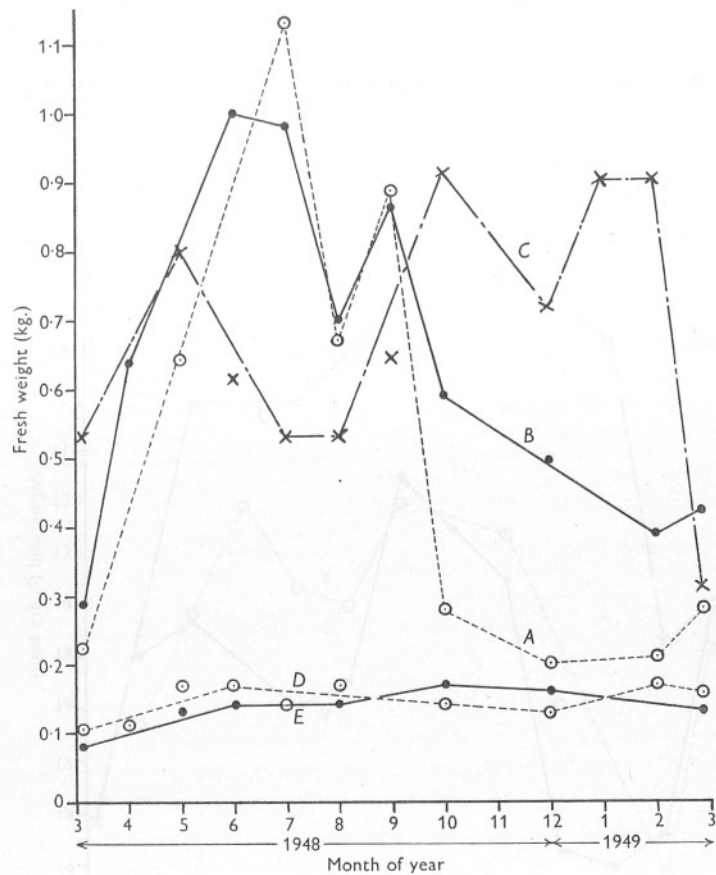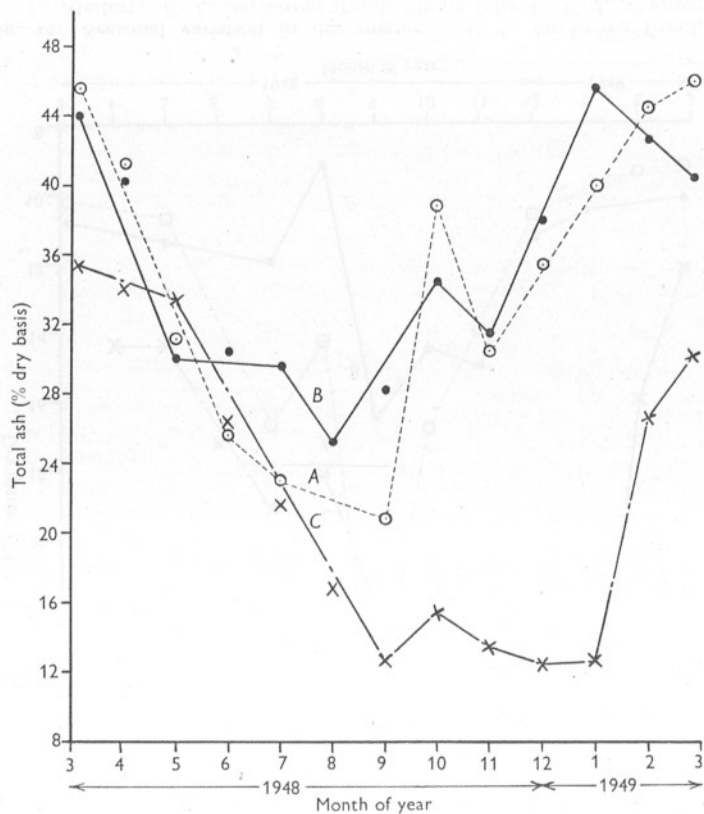
Fig. 12. Seasonal variation in fresh weight. (A) L. saccharina frond, L. Melfort; (B) L. saccharina frond, Shuna Island; (C) L. cloustoni frond, Cullipool; (D) L. saccharina stipe, L. Melfort; (E) L. saccharina stipe, Shuna Island.

Fig. 13. Seasonal variation in total ash. (A) L. saccharina frond, L. Mel-
fort; (B) L. saccharina frond, Shuna Island; (C) L. cloustoni frond,
Cullipool.
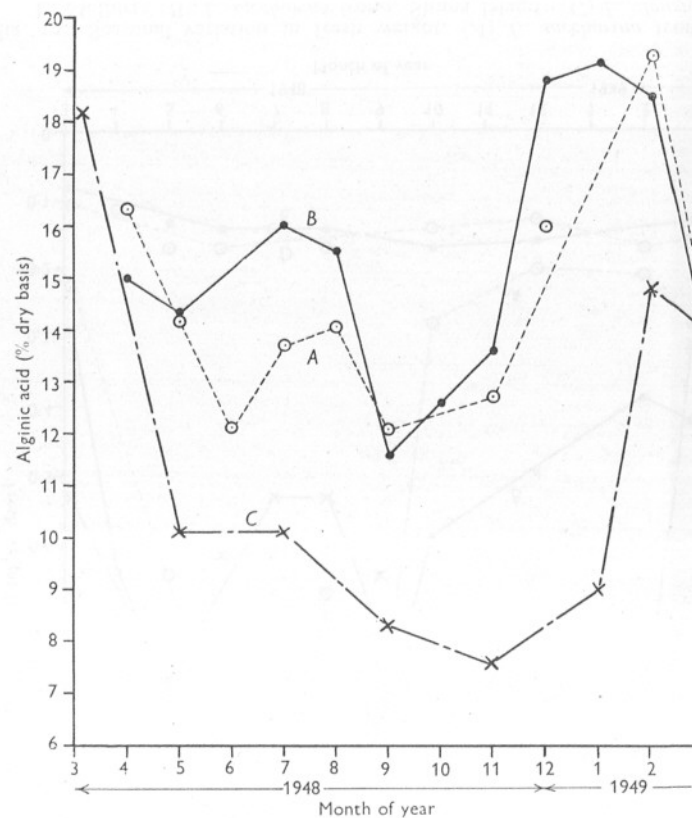
Fig. 14. Seasonal variation in alginic acid. (A) L. saccharina frond,
L. Melfort; (B) L. saccharina frond, Shuna Island; (C) L. cloustoni
frond, Cullipool.

before then. As distinct from *L. saccharina* laminarin is found at a high percentage in January when sporing of this species usually occurs. With *L. cloustoni*, therefore, the new frond may begin growth in water already depleted of nutrients, and this may account to some extent for the results obtained, and particularly the high laminarin content which the writers believe to be an indication of 'restricted growth'. It may be significant, however, that *L. saccharina* is often regarded as an annual, while *L. cloustoni* is a perennial.

In this investigation the plants have been weighed, but these weights cannot be taken as a measure of growth. First, differences may be due to variations in the weights of the individual plants; and secondly, the weight is determined by the rate of growth minus the rate of wear. Sporogenesis may also influence the weight, as a sporogenous frond has been found to be approximately twice as heavy as a sterile one (Report by State Oceanographical Institute, 1933).

It might be argued that the results expressed on the anhydrous basis do not give a true picture of the living plant and that a decrease in a particular constituent, for example, is due to growth and its redistribution in a larger plant. When the results are calculated on the wet basis, however, the graphs are, in general, parallel to those for the anhydrous basis.

The results, on the other hand, could be expressed per unit plant. If we consider the crude protein content, the maximum in the fronds, on the anhydrous basis, occurs in March 1948, when it is 50 g. per fresh frond. In August when it is at a minimum, on the anhydrous basis, it is 140 g. per fresh frond. In March the proteins will consist of reserve proteins and protoplasmic proteins. During rapid growth, however, the reserve proteins will no doubt be utilized to produce protoplasm; and synthesis of proteins, and consequently growth, will continue as long as inorganic nitrogen is present in the sea water or in the plant. By August the reserve proteins may all have been converted, and absence of inorganic nitrogen both in the water and the plant will consequently retard growth.

Although many of the changes in the composition of the sea water, such as the rapid utilization of nitrate in the spring, may in part be due to phytoplankton, during the summer, when the light is strong, plankton probably keep well below the surface and out of the sea-weed zone.

If carbohydrate assimilation with a corresponding decrease in crude proteins and ash can be taken as a measure of photosynthesis, the results indicate that a period of rapid photosynthesis occurs from March to June/July. During this period, with the exception of *L. cloustoni*, an increase in the fresh weight of the plants occurs indicating that this is also a period of rapid growth. A marked increase in the pH and oxygen saturation is further evidence of rapid photosynthesis. Growth and/or photosynthesis during this period utilizes all the nutrients in the water, so that by August nitrate is undetectable and phosphate is as low as 0·16–0·19 mg. atom/m.^{3}. The warming of the waters may have resulted in the setting up of a thermocline, thereby restricting vertical mixing

so that regenerated nutrients do not reach the photosynthetic layer. In September cooling of the waters results in the autumn mixing and the replenishment of the photosynthetic layer with nutrients, so that in October/November a second burst of photosynthesis but on a reduced scale occurs, resulting again in increased carbohydrate assimilation. With light, no doubt, now the limiting factor, photosynthesis slows up and the nutrients accumulate again to reach their winter maxima in January/February.

In foreign waters such as the San Juan Channel, Washington, U.S.A., where *Nereocystis luetkeana* predominates, nitrate and phosphate have been determined for a period of 5 years (Phifer & Thompson, 1937). The great velocity of the tidal currents there creates very turbulent conditions and no thermocline is set up as in our inshore waters. At all times of the year the phosphate concentration was always sufficient for plant growth and could never be considered as a limiting factor. From a maximum of about 3 mg. atoms/m.^{3} in December it decreased to 0·8 mg. atom/m.^{3} in August, and this minimum value is considerably greater than our winter maximum. Nitrate, at a maximum of about 35 mg. atoms/m.^{3} in December/January, dropped to 0·5 mg. atom/m.^{3} in August, a minimum value of the order of magnitude of our winter maximum.

Although it appears that some correlation exists between the seasonal variation in the composition of the British Laminariaceae and variations in the sea water, it does not appear evident to the authors how, by taking and analysing a sample of sea water, it is possible to predict the appropriate time of harvesting, when a desired constituent will be at a maximum. A 4-year investigation (Black, 1948, and in the press) of the seasonal variation of some of the common British Laminariaceae has provided that information. To establish the correlations between the physico-chemical properties of the water and the fixed algae would require quite extensive work over a period of years, and once established it would still be necessary to keep sampling the sea water at intervals to ensure that the prognostication was being followed as expected.

In March the fronds of the Laminaria are low in mannitol and laminarin (Figs. 9 and 10) and high in proteins and inorganic nitrogen (Figs. 7 and 8), after the carbohydrates have been used up during the winter in respiration and probably in the synthesis of proteins, which are believed to be the product of a 'dark' synthesis. In March also, the alginic acid content is at a maximum (Fig. 14), but the frond is exceedingly small (Fig. 12) and high in water content (Fig. 11), while the cell sap contains much mineral matter (Fig. 13). During the winter months the rate of photosynthesis has been very low, if any photosynthesis occurs at all, and consequently the surrounding sea water is high in nitrates and phosphates.

In March/April a marked increase in photosynthesis occurs, accompanied by an increase in the fresh weight of the plant and an increase in the mannitol, while a decrease occurs in the crude proteins and inorganic nitrogen, all

indicating that vigorous growth has commenced. Simultaneously, in the surrounding sea water a drop occurs in the nitrate and phosphate content as a result of their utilization by algae and other forms of marine life. At the same time an increase in the pH and oxygen saturation content occurs, which is further evidence of a marked increase in photosynthesis.

While a drop of only 0·03 mg. atom/m.^{3} occurs in the phosphate content of the water at Shuna Island, the phosphate content at Loch Melfort falls 0·20 mg. atom/m.^{3} during March/April. The change in the nitrate content is more marked during this period; at Shuna Island it falls from 6·1 to 3·1 mg. atoms/m.^{3}, while at Loch Melfort it decreases from 6·4 to 0·7 mg. atom/m.^{3}.

During May photosynthesis continues. *Laminaria saccharina* increases in weight, but a decrease in the weight of *L. cloustoni* occurs, no doubt due to the shedding of the old frond at this time. Mannitol shows an increase, and a decrease occurs in the crude proteins, inorganic nitrogen, alginic acid and ash. In the sea, the temperature of which is gradually increasing, the pH and oxygen saturation content show an increase, and a marked drop in the nitrate content at Cullipool and Shuna Island occurs, while phosphate decreases to 0·33, 0·26 and 0·19 mg. atom/m.^{3} at Cullipool, Shuna Island and Loch Melfort respectively.

In June there is a suggestion of a 'falling off' in the rate of photosynthesis. The fronds of *L. saccharina* from Shuna Island show a decrease in mannitol, but an increase in fresh weight, however, occurs in both samples of *L. saccharina*, while a decrease in the weight of *L. cloustoni* is recorded. The crude proteins, inorganic nitrogen, ash and alginic acid contents show a further decrease with an appreciably higher protein figure for *L. cloustoni* at Cullipool. While the pH and oxygen saturation of the water remain constant, a further drop in the nitrate content occurs, while phosphate is between 0·16 and 0·20 mg. atom/m.^{3}.

In July/August there is evidence of a marked change in the rate of growth and/or the rate of photosynthesis. Laminarin shows an increase but this is characteristic of 'restricted' growth, mannitol remains relatively constant, but a drop occurs in the dry matter and fresh weight of *L. saccharina*. The water analysis shows the temperature rising to a maximum of 13°C. at the three localities. In July, nitrate and phosphate are at a minimum, the nitrate being 0·9 mg. atom/m.^{3} at Cullipool, while at Shuna Island and Loch Melfort it is undetectable in the water, and phosphate is between 0·16 and 0·20 mg. atom/m.^{3}.

The pH and dissolved oxygen saturation, which reach maxima in July, fall rapidly in August, indicating reduced photosynthesis in the water. The ash content shows a slight decrease, but a marked increase occurs in the alginic acid content.

The months of July and August appear to be critical ones, and in all our work, and in the work of other investigators, comment has been passed on the change

in composition and rate of growth which occurs at this time. There may be other influencing factors, but the water analysis shows that lack of nutrients must be a limiting factor. Although low concentrations of nitrate, for example, exist in the water at Loch Melfort in April the weed still contains a reserve of inorganic nitrogen, so that it is not until July, with the exception of *L. cloustoni* which always exhibits a lag, that nitrate is undetectable in the plant and the water.

Temperature may also be an influencing factor, as it reaches a maximum of 13°C. at this time of the year. The intensity of respiration and assimilation increases with temperature, but the effect on respiration is considerably greater than on assimilation. Consequently with increasing temperature, the surplus will eventually disappear and growth will cease.

In September/October a rise occurs in the protein content of all the plants, coinciding with an increase in the nitrate and phosphate contents of the water. This is the result of the autumn cooling of the water bringing about the breakdown of the thermocline, giving vertical mixing and regeneration of nutrients in the photosynthetic zone. A marked increase in the pH of the water signifies a second 'burst' of photosynthesis, less intense however, than the spring outburst. An increase in the fresh weight and dry-matter content occurs, but a considerable drop in the laminarin occurs which might be the result of further growth.

In November/December, although a drop in the dry matter and fresh weight of the plants occurs, there is a rise in the mannitol, laminarin and crude protein contents and a decrease in the ash, indicating that the rate of assimilation still exceeds the rate of respiration. The rapid increase since August in the nitrate concentration is checked, indicating its utilization.

From December to March photosynthesis proceeds at a low rate as light may now be the limiting factor. Little change occurs in the pH, while the carbohydrates are now utilized in growth and respiration at a greater rate than they are assimilated.

Nitrate and phosphate increase to a maximum in January/February and then begin to decrease again, the exception being the high phosphate figures for Shuna Island and Loch Melfort in December.

The large store of inorganic nitrogen present in the fronds during the winter and early spring is rather surprising and is worthy of further investigation.

These seasonal variations in composition appear to be characteristic of most forms of marine life. For example, Daniel (1922) investigated the seasonal variation in chemical composition of the mussel and found from May onwards a decrease in the proteins, on the dry ash free basis, reaching a minimum in September/October and then rising to a maximum the following March. The carbohydrates showed a steady increase from March, reaching a maximum in September/October, with a tendency to form a second maximum in December, and then a rapid decrease until March.

SUMMARY

Monthly samples of the Laminariaceae, *L. saccharina* and *L. cloustoni*, from three localities on the Argyllshire coast have been taken from March 1948 to March 1949, and analysed for dry matter, ash, mannitol, laminarin, crude proteins, inorganic nitrogen and alginic acid, and the seasonal variation in these constituents correlated with the changes in composition of the sea water.

The results show that a correlation does exist and that a period of rapid photosynthesis occurs from March to June/July, but is restricted in July/August when nitrate is undetectable in the water and phosphate is as low as 0·16–0·20 mg. atom/m.³. The replenishment of the photosynthetic layer with nutrients is retarded in July/August, apparently due to the warming of the inshore waters, which may set up a thermocline restricting vertical mixing.

The autumn cooling of the uppermost waters facilitates vertical mixing, regenerating the nutrients in the photosynthetic region, and a second burst of photosynthesis at a reduced rate from the spring 'outburst' occurs in October/November.

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