THE SEASONAL VARIATION IN WEIGHT AND CHEMICAL COMPOSITION OF THE COMMON BRITISH LAMINARIACEAE

By W. A. P. Black, B.Sc., Ph.D., F.R.I.C.

Scottish Seaweed Research Association, Musselburgh

(Text-figs. 1–20)

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Introduction

In previous publications the author (Black, 1948) has reported the seasonal variation in the total ash, iodine, crude protein, mannitol, laminarin and alginic acid contents of the Laminariaceae, *Laminaria cloustoni*, *L. digitata* and *L. saccharina*, for the 2-year period from November 1944 to October 1946.

The work has been continued for a further 2 years and this paper summarizes the seasonal variation in the above constituents, with the exception of iodine, for the period November 1946 to October 1948 inclusive. In addition, the seasonal variation in the fresh weight and dry weight contents has also been recorded.

The algae, in general, undergo such marked variations in chemical composition depending on the season of the year, the habitat, and the depth at which they grow, that any analysis of a sample for which the complete history is not given is of little value.

Cast weed, which usually has all the water-soluble constituents leached out either by the buffeting action of the waves before it is finally deposited on the beach, or by rain while exposed on the shore, may also have undergone appreciable bacterial decomposition, giving a false picture of the composition of the living plant. It is quite possible, too, that the time which elapses between the collection and drying may influence the composition, so that this time, together with the conditions of drying, should also be recorded.

The present investigation has shown that the method of sampling adopted is relatively sound, and that at any one time surprisingly small differences occur in the composition of individual plants taken from the same habitat, at the same depth, and dried under identical conditions.

The investigation is unique in that the method of sampling prevented the weed from coming into contact with rain, etc., which would affect its composition, and the plants were immediately dried under controlled conditions to reduce any chemical and biological changes to a minimum. Previous workers, on the other hand, do not state how their samples were collected, whether the weed was cut, or whether the samples were those of drift weed, and in what manner their samples were dried.

Although Stanford (1883, 1884*a*, *b*, 1886) in the second half of the nineteenth century carried out the pioneer work and laid the foundations for an industry based on the organic constituents of seaweed, it was not until 1919 that Lapicque carried out the first systematic investigation into the seasonal variation in the chemical composition of the marine algae. During the first world war, however, the demand for potash led to an intensive investigation being carried out by Hendrick (1916), but he was chiefly concerned with the analysis of the mineral matter of the algae.

From 1927 to 1929, Colin & Ricard (1929, 1930) collected monthly samples of *L. flexicaulis* and *L. saccharina* and examined them for dry weight, ash, mannitol, laminarin, algin and cellulose. Lunde (1937), investigating the possibilities of the seaweed off the coast of Norway as a source of raw material, collected monthly samples of *L. digitata* from 1935 to 1937 and analysed them for ash, mannitol, alginic acid, laminarin, fucoidin and nitrogen.

Similar investigations have been carried out in Japan by Atsuki & Tomoda (1926*a*, *b*), in Russia by Kizevetter (1938) and Vedrinskii (1938), and in Eire by Dillon (1943). Our knowledge, however, of the organic constituents of the marine algae is still incomplete. Accurate methods of analysis have been devised for the estimation of mannitol, laminarin, alginic acid and cellulose, while work is continuing on a method for the estimation of fucoidin. The fats and pigments require further investigation. Their nature and relative proportions in the algae are in many cases indeterminate.

No work appears to have been carried out on the algal proteins.

Minor constituents such as fucosterol and the neutral oils are at present being investigated, and it may well be that other valuable constituents have yet to be identified.

The work described in this paper forms part of the programme of research and development on seaweed undertaken by the Scottish Seaweed Research Association.

The author wishes to thank Miss B. Graham and Mr W. Cornhill for assistance with the analytical work and the Association for permission to publish.

Investigations on separate constituents

In a brief review it is impossible to summarize all the work which has been carried out on the known constituents of the brown algae. The inorganic and several of the organic constituents have attracted the attention of numerous investigators, but it is unfortunate that often the results are somewhat conflicting.

Total ash

The total ash consists of inorganic salts, presumably in solution in the cell sap, and the cations combined with the organic constituents such as alginic acid and fucoidin. In addition, the ash figure contains salts from the sea water retained on the surface of the plant. The plants were only allowed to drip before drying, since it was not considered advisable to wash off this surface water with distilled water which might affect the composition. Until about 1850 the ash of seaweeds, chiefly of the fucoid types, was the sole source of alkali for the soap and glass industries. This kelp industry was then threatened by the Le Blanc process, but as the use of iodine in medicine developed, a new kelp industry arose based on the ash of the laminarias; but this industry is also obsolete. Any future industry, however, based on seaweed will most likely depend on its organic constituents, which are mainly polysaccharide in character.

Trace elements

The accumulation of trace elements in marine algae, which can be explained by considering alginic acid as an ion-exchange material, has been studied by Cornec (1919), Vernadskii (1930), Jones (1922), Öy (1940), and Wilson & Fieldes (1941).

A study of the trace elements present in the Laminariaceae and Fucaceae common to Scotland is at present being carried out and the results will be published in a future communication.

Mannitol

Mannitol, a hexahydric alcohol common to all brown seaweeds, was first detected in *L. saccharina* by Stenhouse (1844). It appears to be the primary product of photosynthesis, previous workers having found only traces, or the complete absence, of free-reducing sugars. Like the mineral matter, the mannitol is probably all in solution in the cell sap, for if the living plant is put into distilled water the salts and mannitol rapidly diffuse through the cell wall into the surrounding water.

Although there is a recent patent by Berk (1940) on the extraction of mannitol from seaweed, the mannitol of commerce is obtained from manna, or is prepared synthetically by the catalytic reduction of fructose.

Laminarin

Laminarin has been the subject of investigation by various workers. It was first described by Schmiedeberg (1885), who isolated it from the Laminariaceae. It has since been studied by Krefting & Torup (1909), Kylin (1913), Gruzewska (1923), Colin & Ricard (1929), Lunde (1937), Nisizawa (1940), Le Gloahec & Herter (1940), and Barry (1938, 1939, 1941, 1942), and their work has been reviewed by Hassid (1944). Barry (*loc. cit.*) showed that laminarin consisted exclusively of glucose units, and that after methylation and hydrolysis it gave 2, 4, 6-trimethyl glucopyranose. He concluded that laminarin consisted of a chain of β-glucopyranose units bent into a spiral form.

Laminarin, therefore, differs fundamentally from starch and cellulose in that the glucose residues are combined by 1, 3-glycosidic linkages and not through carbon atoms 1 and 4.

The extent of oxidation of laminarin with periodic acid was used by Barry (1942) as an end group assay for this polysaccharide, and the results indicated a chain of sixteen glucose units. This chain length was not in agreement, however, with that obtained by the Haworth-Hirst method which indicated a chain length of about seventy-four glucose units.

The present investigation has shown that laminarin, present only in the frond, can be isolated in two forms, an almost water-insoluble form from *L. cloustoni*, which separates from cold water, and a more soluble form from *L. digitata*, which can only be obtained from aqueous solution on the addition of alcohol. The two forms are at present being studied.

It is not yet known what part laminarin plays in the metabolism of the algae.

Alginic acid

Alginic acid, found in all brown seaweeds, where it is believed to play an important part in the cell wall, was first isolated by Stanford (1883, 1884*a*, *b*, 1886) and has since been studied by the following workers: Hoagland & Lieb (1915), Nelson & Cretcher (1929), Bird & Haas (1931), Dillon & McGuinness (1931), Gomez (1933), Barry & Dillon (1935, 1936), Hirst, Jones & Jones (1939), Stewart & Lucas (1940), Speakman & Chamberlain (1944), Astbury (1945), and Wasserman (1949). It has been shown to be a polyuronide composed entirely of D-mannuronic acid.

Dillon & McGuinness (1931) believed that the alginic acid in the growing plant was combined with calcium and iron, and that desiccation destroyed the colloidal character of these compounds and rendered them insoluble. Bird & Haas (1931), on the other hand, believed that the alginic acid in the cell wall was in two forms: (*a*) a water soluble form, and (*b*) the acid in the free state. Recent adsorption experiments carried out by Wasserman (1949), however, have shown that the alginic acid occurs in the cell tissue of brown algae in the form of various metal salts, and is not present as the free acid.

Crude proteins

Except for the work of Haas and co-workers (1929, 1931, 1933, 1938), who succeeded in isolating an octapeptide of glutamic acid, no other work has been carried out on the nitrogen metabolism of the brown algae.

Cellulose

Although Kylin (1913, 1915, 1918, 1944) had shown that the cell-wall constituents of various seaweeds gave, with iodine and sulphuric acid, the characteristic blue colour of cellulose, considerable doubt existed for some time as to the occurrence of normal cellulose in marine algae. Thus Atsuki & Tomoda (1926*a*, *b*) stated that the greater part of the crude fibre of the laminarias consisted of the hemicelluloses, and that there was no evidence of the normal cellulose, while Ricard (1931), in the algae examined by him, did not obtain the characteristic reaction of cellulose, as found by Kylin. On the other hand, Naylor & Russel-Wells (1934) and Dillon & O'Tuama (1935) demonstrated the existence of normal cellulose in marine algae. Viel's (1939) survey of the literature reveals numerous contradictions, but recent work by Percival & Ross (1948*a*) has shown conclusively that the brown algae do contain cellulose which is fundamentally similar to the cellulose of the land plants.

Fats

Except for the work of Russel-Wells (1932) and Takahashi and his co-workers (1933, 1935, 1939), no other work appears to have been carried out on the fats of the Laminariaceae, and there is no information on the seasonal variation. Russel-Wells (1932) showed that a correlation existed between the fatty constituents and the depth of immersion of the algae. Takahashi *et al.* have, over a number of years, studied the fats of the indigenous algae of Japan and identified the various fatty acids.

Pigments

Although an appreciable amount of work has been carried out on the pigments of the Fucaceae, those of the Laminariaceae have received very little attention, the only recent work of a systematic nature being that of Manning & Hardin (1944).

Preparation and Analysis of Samples

Each month, the samples of *L. cloustoni* were collected on the reef off Cullipool, Luing Island, in approximately 4 m. of water (D.L.W.O.S.T.), every effort being made to take them from the same spot and at the same depth. The open sea samples of *L. saccharina* were taken at Rudh-an-Aoil, Shuna Island and the sea-loch samples at Eilean Coltair, Loch Melfort, where the plants were growing in 3–4 m. of water (L.W.). As with *L. cloustoni*, the samples were obtained by trawling a multi-pronged grapnel for 2 min., hoisting the grapnel and lifting the weed into the boat.

The open-sea samples of *L. digitata* were taken at Atlantic Bridge and the

sea-loch samples at Eilean Coltair, Loch Melfort, where the plants were growing in 1 m. of water (L.W.). The samples were taken by hand at low water, when the plants were partially exposed. With each species, twenty plants were taken, measured and weighed. Two plants were selected for dry-weight determinations, and two were chosen at random for chemical analysis. The plants, separated into stipes and fronds, were draped over racks in a heated shed and dried at a temperature of 25–35° C. for approximately 48 hr., after which they were ground in a Christy and Norris No. 8 Laboratory Mill, fitted with a $\frac{1}{64}$ in. perforated plate screen, giving a powder which practically all passed through a sieve of 90 meshes to the inch. As the analytical methods were evolved it was found that this fine state of division was essential, especially for the method of estimating alginic acid.

The methods of analysis used were those previously employed by the writer (1948).

In the preparation of the samples for analysis, changes in composition due to respiration, etc., were negligible provided drying was carried out immediately after sampling; samples dried rapidly at 80–90° C. confirmed the results obtained at the temperatures employed in this investigation. On the other hand, plants killed in boiling absolute alcohol before drying, as is the normal procedure in plant analysis, showed considerable loss of mannitol and mineral matter, but it was found possible to kill sections of the laminarias in boiling chloroform without materially affecting the chemical composition.

Results

The results, calculated on the anhydrous basis for the frond, stipe and whole plant, are given in Figs. 1–19 (pp. 53–67).

Before drying, the plants were divided into stipes and fronds which were weighed separately. From these results each constituent determined in stipe and frond was calculated for the whole plant.

Figs. 2, 8 and 12, giving the seasonal variation in the dry-matter contents of the fronds, the stipes and the whole plants, can be used to recalculate any of the results (expressed on the dry basis) on the fresh-weight basis.

In Fig. 20 (p. 68) the seasonal variation in the fresh weight of *L. saccharina* from Loch Melfort and Shuna Island is given, while the average figures for the three species are given in Table I.

Discussion

General

In general, the results agree reasonably well with those of the first 2 years investigated (Black, 1948), despite the fact that the summer of 1947 was exceptionally good with considerable sunshine, while 1948 was a very poor summer with considerable cloud and rain. Slight differences are occasionally found,

however, in the spring (March–April). At this period of the year, when a marked increase in the rate of photosynthesis occurs, the plant is actively producing a new frond, while the old frond is wearing away. The composition of the old frond (as shown in Table II) differs somewhat from that of the new frond, to which it is still attached, so that conditions such as rough weather, which influence the shedding of the old frond, will have an effect on the composition of the whole plant.

It would appear, therefore, that when the older part of the frond detaches itself it still contains mannitol and laminarin while the new growth contains no laminarin. This, no doubt, accounts for the sudden drop in laminarin in the spring. The new frond then undergoes a period of rapid growth and laminarin

TABLE I. MAXIMUM, MINIMUM AND AVERAGE WEIGHT OF PLANTS IN GRAMS

		Maximum	Minimum	Average of 450 plants
L. cloustoni	Frond	936	510	681
	Stipe	1787	681	1192
L. saccharina (open sea)	Frond	1022	198	595
	Stipe	227	85	198
L. saccharina (loch)	Frond	936	198	595
	Stipe	227	85	170
L. digitata (open sea)	Frond	1901	340	965
	Stipe	426	142	255
L. digitata (loch)	Frond	823	198	426
	Stipe	227	85	142

TABLE II. COMPOSITION OF THE OLD AND NEW FROND OF *LAMINARIA CLOUSTONI* COLLECTED AT CULLIPOOL ON 14 APRIL 1947 (DRY BASIS)

	Total ash	Mannitol	Laminarin
Old frond	38·8	4·0	2·7
New frond	36·7	8·0	Trace

is absent. In general, this polysaccharide is almost completely absent when there is rapid growth, i.e. in all the laminarias in the spring, in *L. digitata* at Atlantic Bridge for the greater part of the year, in the annual *Saccorhiza bulbosa* already reported, and in all the samples of *Macrocystis pyrifera*, *Nereocystis luetkeana* and *Lessonia flavicans* so far examined.

While the percentage of laminarin in *Laminaria cloustoni* fronds is over 30 from August to December (dry basis), in *L. saccharina* and *L. digitata* it falls rapidly in September, reaching 4·7% in *L. saccharina* (open sea), 5·9% in *L. saccharina* (loch) and 7·5% in *L. digitata* (open sea). Parke (1948, and private communication) has found that growth in *L. saccharina* and *L. digitata* continues at a greater rate during summer and autumn than in *L. cloustoni*. With *L. cloustoni* fronds there was practically no growth from July to December in the sublittoral fringe zone, which confirms the author's suggestion that laminarin is generally found when there is 'restricted growth'.

In March, therefore, we find the algae high in proteins and alginic acid with

the cell sap high in mineral matter and low in carbohydrates, which have been used up during the winter in respiration and probably in the synthesis of amino-acids. In the spring a rapid increase in the rate of photosynthesis occurs accompanied by an increase in the mannitol content and a decrease in the ash content, while rapid growth of the plant results in a decrease in the crude protein content. A decrease in alginic acid occurs as a result of this increase in mannitol. As summarized in Fig. 19, when the results are calculated on the anhydrous basis, ash, proteins and alginic acid are at a maximum and mannitol and laminarin at a minimum at the beginning of the spring, while in the autumn the reverse is true.

In the first 2 years investigated, with the exception of *L. digitata* in 1948, a break or flattening out of the mannitol graph for the fronds occurs in July–August of each year. This coincides with (*a*) a slowing up in the rate of growth, (*b*) the absence of nutrients in the waters, and (*c*) the probable period of sporogenesis in the case of *L. digitata* and *L. saccharina*, so that these factors together with light which is at its maximum intensity at this time are all contributory factors influencing the chemical composition of the algae.

Fronds *Laminaria cloustoni*

In May 1947 the dry-weight content is at a minimum of 13·3% (Fig. 2), laminarin is at a minimum of 1·0% (Fig. 1), while the total ash is at a maximum 37·6% (Fig. 1). At this period the new frond has probably taken over photosynthesis and the old frond has been cast. The new frond contains 14·2% mannitol (Fig. 3) and 11·8% crude proteins (Fig. 4), the mannitol having been at a minimum in March (6·4%) and the proteins at a maximum (15%), while the alginic acid was also at a maximum (19·3%).

As the ash falls to a minimum of 13% in September-October the dry-weight content reaches a maximum of 32% in September, the alginic acid a minimum of 8%, and the laminarin a maximum of 32·4%, the ash graph (Fig. 1) being the inverse of the laminarin graph.

In 1948, maxima and minima of the same order of magnitude occur at approximately the same periods, with the exception of mannitol which is 25% from June to August compared with a maximum of 22·9% in August 1947. In general, the laminarin, dry weight and mannitol are parallel, showing minima in the spring and maxima in the autumn, while the ash, crude proteins and alginic acid are the reverse, showing maxima in the spring and minima in the autumn.

In Fig. 6 the ash graphs for the 4 years investigated are given and show that the results are reproducible within a month, e.g. an ash content of 30% occurs in June–July in 1945, 1947 and 1948, and in July–August in 1946.

The only striking difference in the composition of the fronds during the 4 years now investigated is in the laminarin content which progressively increases each year from 29% in 1945 to 34% in 1948, while later work to be

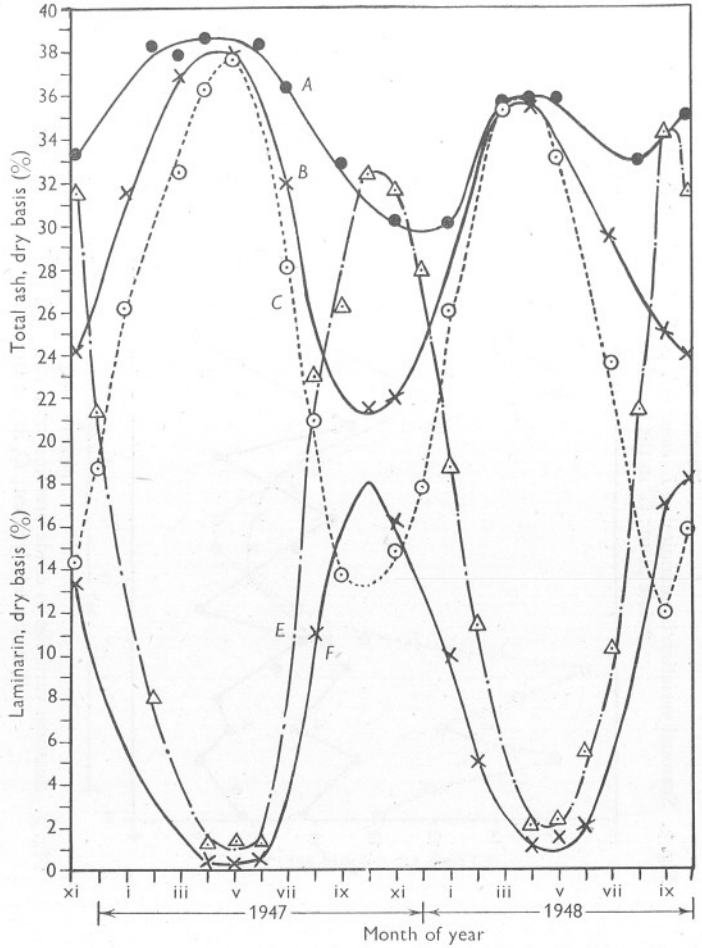

Fig. 1. Seasonal variation in total ash and laminarin in *L. cloustoni*. *A*, total ash in the stipes; *B*, total ash in whole plant; *C*, total ash in the fronds; *E*, laminarin in the fronds; *F*, laminarin in the whole plant.

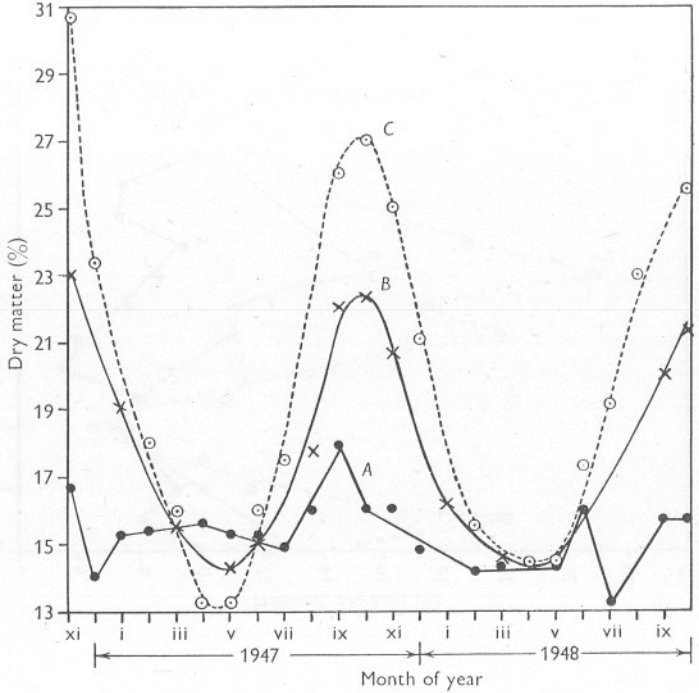

Fig. 2. Seasonal variation in dry matter in *L. cloustoni*. *A*, in the stipes; *B*, in the whole plants; *C*, in the fronds.

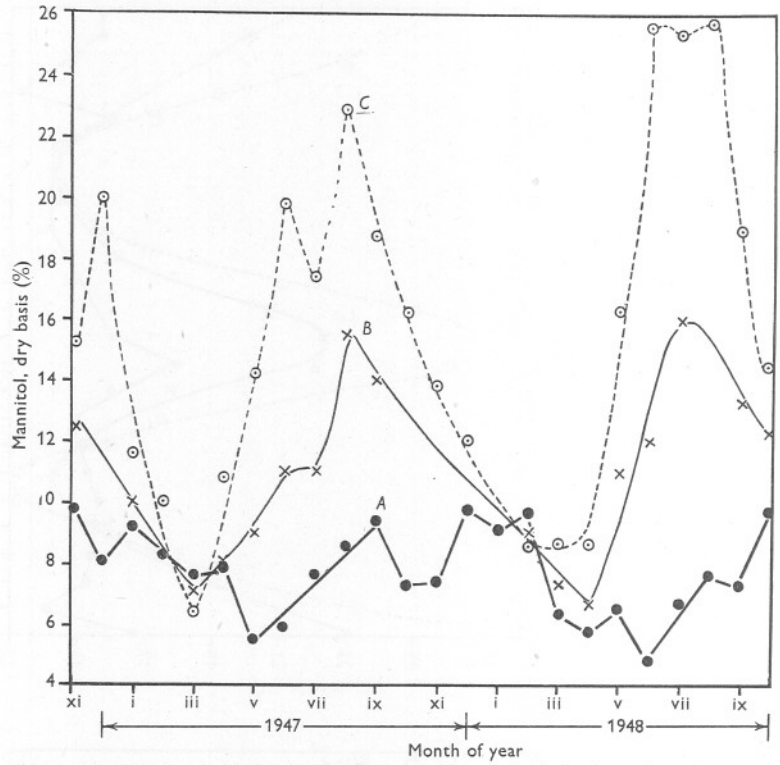

Fig. 3. Seasonal variation in mannitol in *L. cloustoni*. *A*, in the stipes; *B*, in the whole plant; *C*, in the fronds.

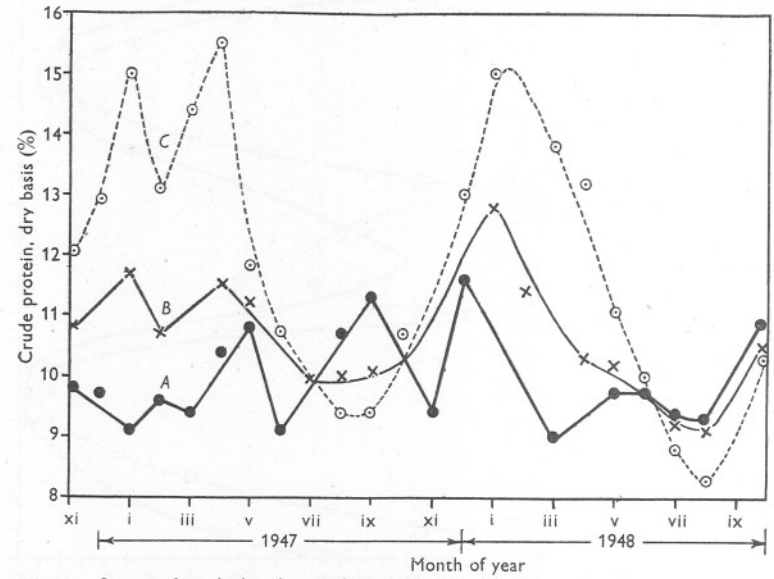

Fig. 4. Seasonal variation in crude proteins in *L. cloustoni*. A, in the stipes; *B*, in the whole plant; *C*, in the fronds.

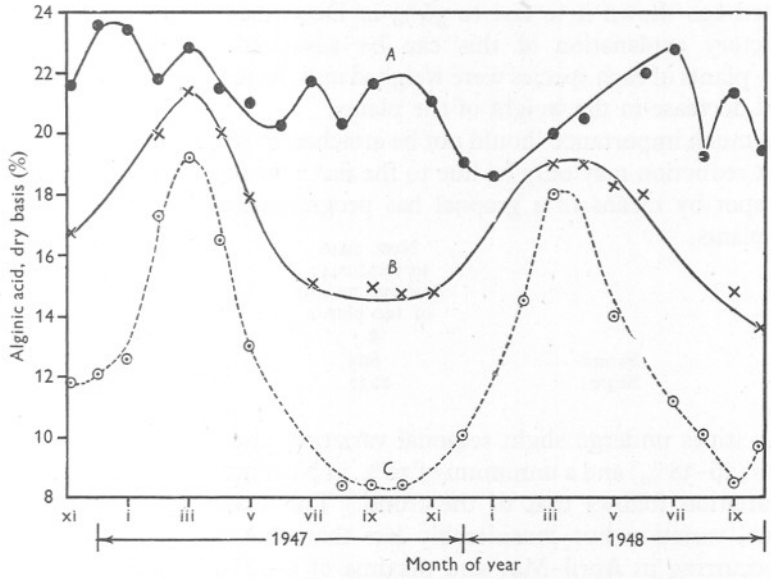

Fig. 5. Seasonal variation in alginic acid in *L. cloustoni*. *A*, in the stipes; *B*, in the whole plant; *C*, in the fronds.

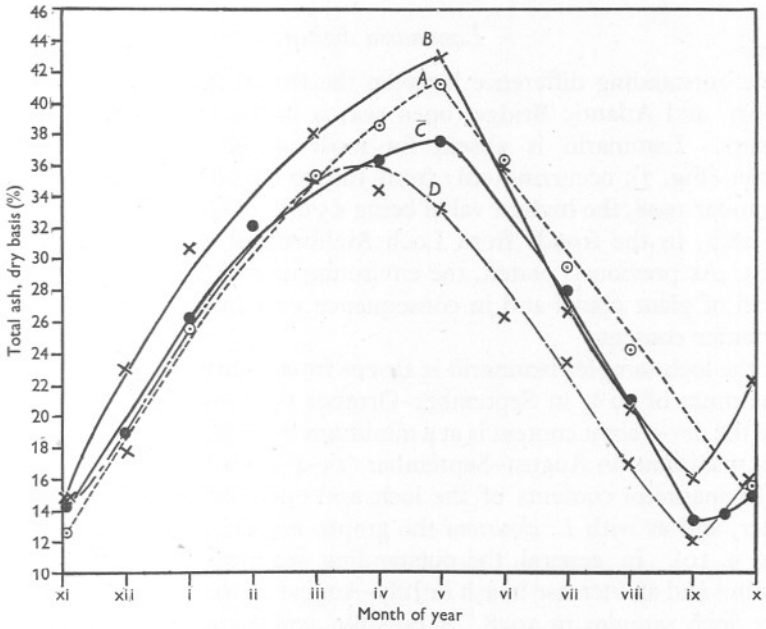

Fig. 6. Seasonal variation in total ash in *L. cloustoni* fronds. *A*, 1944–1945; *B*, 1945–1946; *C*, 1946–1947; *D*, 1947–1948.

reported has shown it to rise to 36% in December 1948–January 1949. No satisfactory explanation of this can be advanced. However, each month twenty plants of each species were weighed and the results indicate a progressive annual decrease in the weight of the plants.

Too much importance should not be attached to such figures, as the apparent weight reduction may only be due to the fact that repeated sampling from the same spot by means of a grapnel has progressively removed the larger and older plants.

	Nov. 1946 to Oct. 1947 Average weight of 240 plants (g.)	Nov. 1947 to Oct. 1948 Average weight of 240 plants (g.)
Frond	693	656
Stipe	1231	1141

Stipes

The stipes undergo slight seasonal variation, the ash being at a maximum in May (36–38%) and a minimum of 30% in November–December; in general, the variation follows that of the frond. The mannitol graph also shows seasonal variation but considerably less than that in the fronds, minima of 5% occurring in April–May and maxima of 9–10% in September–October. The dry weight content, however, shows very little variation (13–18%; Fig. 2).

The alginic acid shows no regular variation and fluctuates between 19 and 24%.

Laminaria digitata

Fronds

The outstanding difference between the fronds from Eilean Coltair (Loch Melfort) and Atlantic Bridge (open sea) is in the laminarin and dry-weight contents. Laminarin is absent for most of the year from the open-sea samples (Fig. 7), occurring only from July to October 1947 and from August to October 1948, the highest value being 15·6% in October 1948, as compared with 28% in the fronds from Loch Melfort and over 30% in *L. cloustoni* fronds. As previously stated, the environment at Atlantic Bridge favours the growth of giant plants and in consequence very little variation occurs in the dry matter content.

In the loch samples laminarin is absent from February to April, and reaches a maximum of 20% in September–October 1947 and 28% in October 1948, while the dry-weight content is at a minimum from January to March (11–12%) and a maximum in August–September (23–4%) in both 1947 and 1948.

The mannitol contents of the loch and open-sea samples are remarkably similar, and as with *L. cloustoni* the graphs are the inverse of the ash graphs (Figs. 9, 10). In general, the outstanding features are a temporary drop in mannitol and an increase in ash in July–August of 1947, and at the same period in the loch samples in 1948. A possible explanation of this is advanced in a recent publication (Black & Dewar, 1949).

The crude-protein graphs (Fig. 15) are very similar for loch and open-sea samples, exhibiting maxima of 12–14% between February and April and

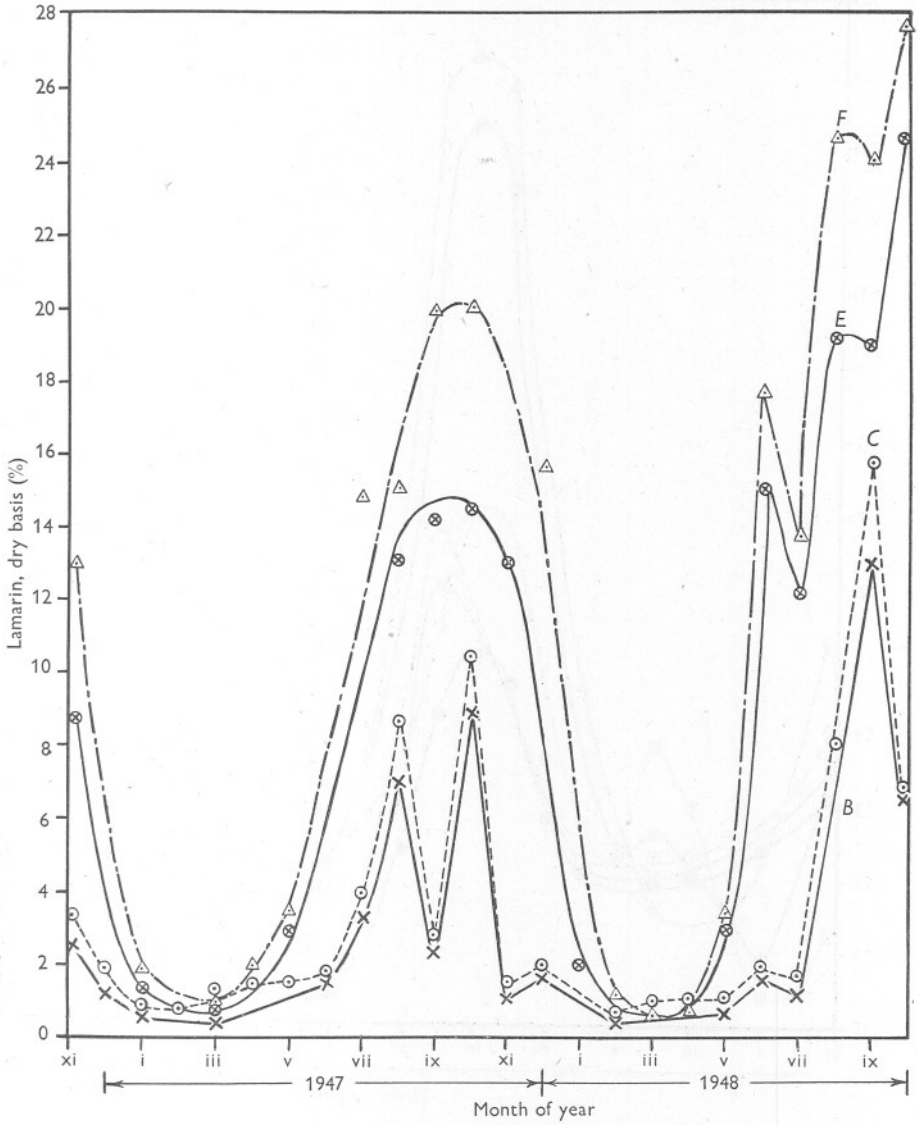

Fig. 7. Seasonal variation in laminarin in *L. digitata*. *B*, open-sea whole plant; *C*, open-sea fronds; *E*, loch whole plant; *F*, loch fronds.

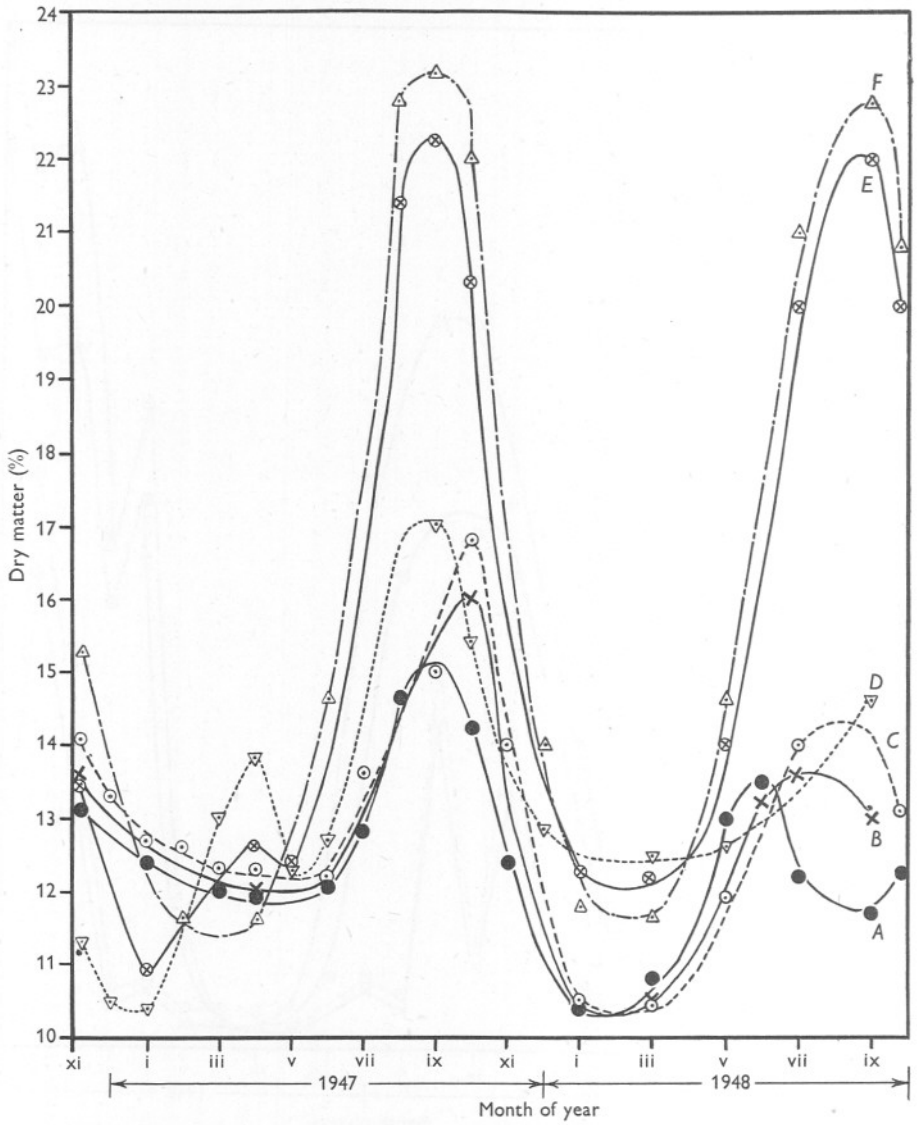

Fig. 8. Seasonal variation in dry-matter content in *L. digitata*. *A*, open-sea stipes; *B*, open-sea whole plant; *C*, open-sea fronds; *D*, loch stipes; *E*, loch whole plant; *F*, loch fronds.

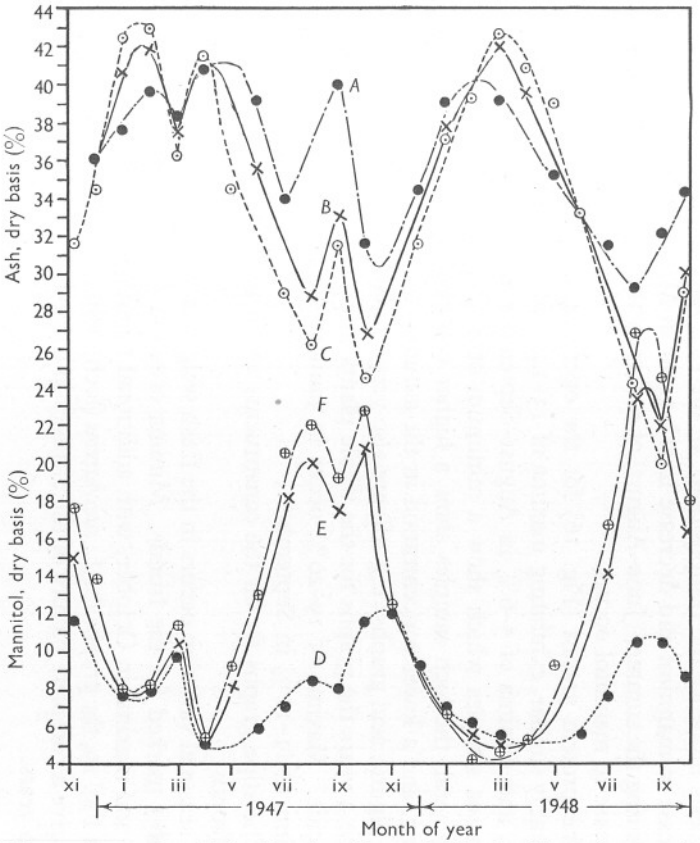

Fig. 9. Seasonal variation in ash and mannitol in *L. digitata* (open sea). *A*, ash in the stipes; *B*, ash in the whole plant; *C*, ash in the fronds; *D*, mannitol in the stipes; *E*, mannitol in whole plant; *F*, mannitol in the fronds.

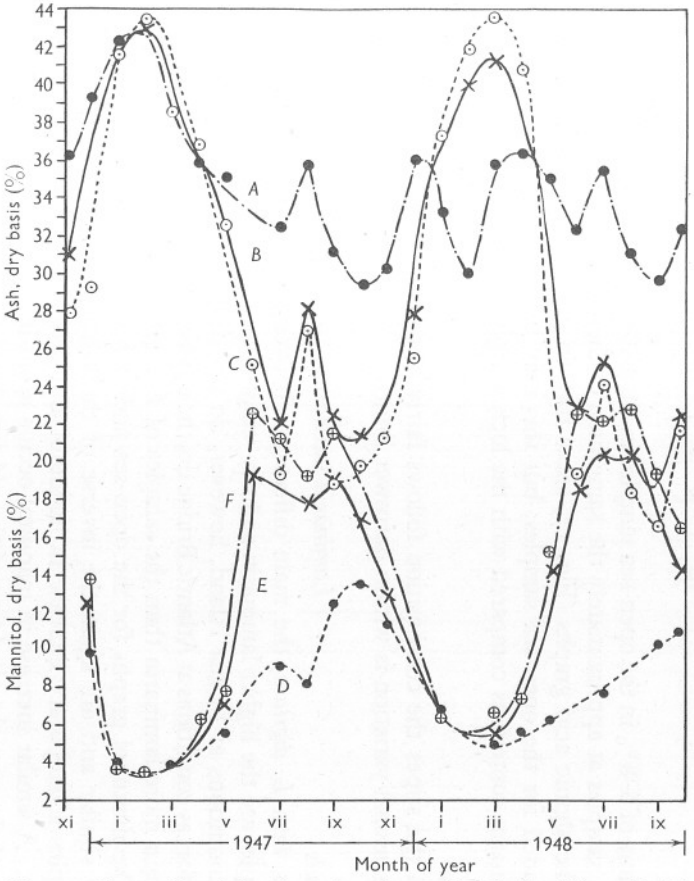

Fig. 10. Seasonal variation in ash and mannitol in *L. digitata* (loch). *A*, ash in the stipes; *B*, ash in whole plant; *C*, ash in the fronds; *D*, mannitol in the stipes; *E*, mannitol in the whole plant; *F*, mannitol in the fronds.

minima of 6–8% in the open-sea samples in August–October, and 4–6% in the loch samples at approximately the same time of the year.

The alginic acid graphs (Fig. 17) show that, in general, higher values are obtained for the open-sea samples, but this may be the result of the lower laminarin content as compared with the loch samples.

Stipes

In the stipes the composition follows fairly closely that of the fronds but the seasonal variation is within narrower limits.

Laminaria saccharina

Fronds

As with *L. digitata*, the main differences between the open-sea and loch samples are the higher laminarin and dry-weight contents of the loch samples. As conditions at Shuna Island, however, do not differ so much from Loch Melfort as conditions at Atlantic Bridge do, the open-sea samples of *L. saccharina* contain more laminarin than the samples of *L. digitata* from Atlantic Bridge.

The mannitol graphs for the open-sea and loch samples (Figs. 13, 14) are very similar and are again the inverse of the ash graphs. In the open-sea samples a temporary increase in mannitol and drop in ash occur in March 1947. A similar increase in mannitol occurs again in March 1948, but at this time of the year the shedding of the old frond and a rapid increase in photosynthesis influence the composition. In the loch samples a similar temporary increase in mannitol and decrease in ash occur in March 1947.

During the summer (June–August) of each year the characteristic temporary decrease in mannitol occurs.

The protein graphs (Fig. 16) for the open-sea and loch samples are remarkably similar, exhibiting maxima of 13–14% in February–March of each year, and minima of 5–6% in August–September with the exception of the open-sea samples which show a minimum of 8% in July–September 1947. In general, the loch samples show a higher concentration of proteins in the spring, and a lower concentration in the autumn, than the open-sea samples. The alginic acid graphs (Fig. 18) are also very similar, and for the greater part of the 2 years the graphs for the whole plants from the two localities actually coincide. Maxima of 19–20% occur in January–February of each year and minima of 11–12% in September.

The stipe graphs for all the constituents are in general parallel to those of the fronds.

A seasonal variation occurs in the fresh weight of the plants (Fig. 20) which is most marked for the fronds. Minima occur in January–February of each year and maxima in October, with additional maxima in July 1948.

In Fig. 19, the graphs for *L. saccharina* (loch), whole plant, are superimposed and give a complete picture of the changes in composition which occur during the 2 years.

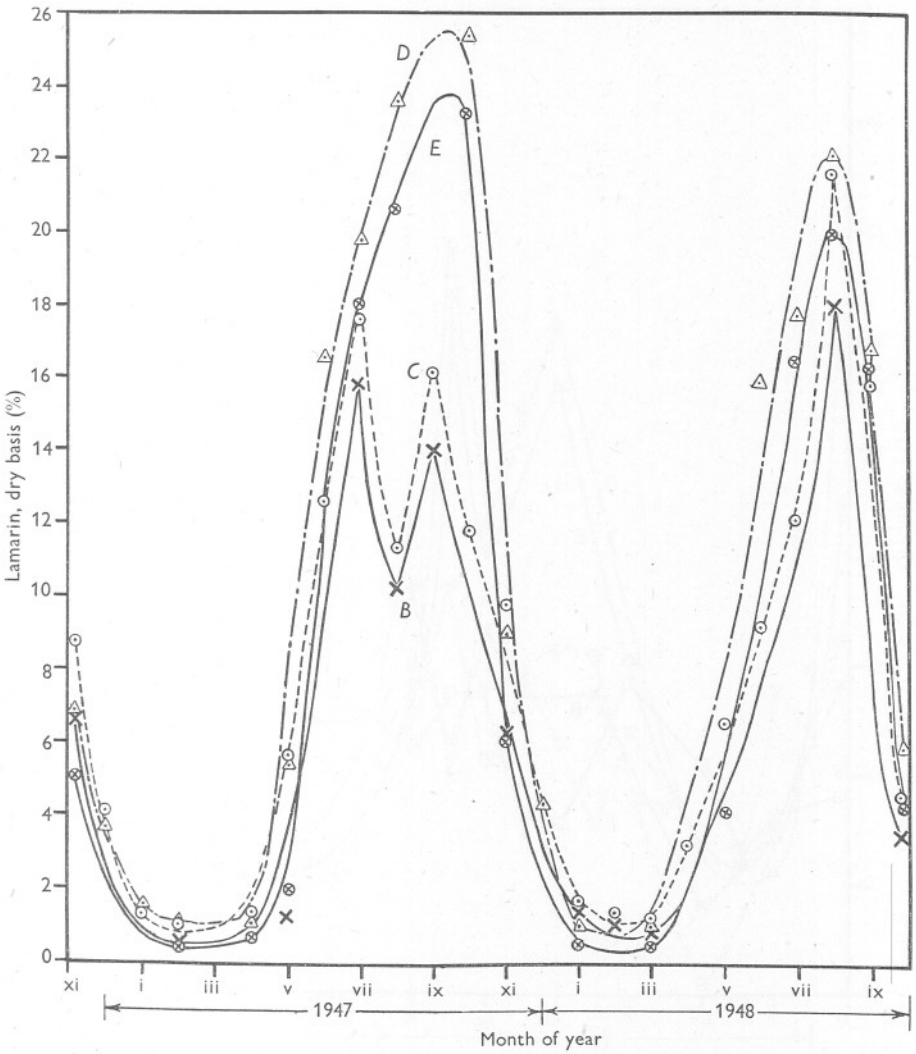

Fig. 11. Seasonal variation in laminarin in *L. saccharina*. *B*, in the open-sea whole plants; *C*, in the open-sea fronds; *E*, in the loch whole plants; *D*, in the loch fronds.

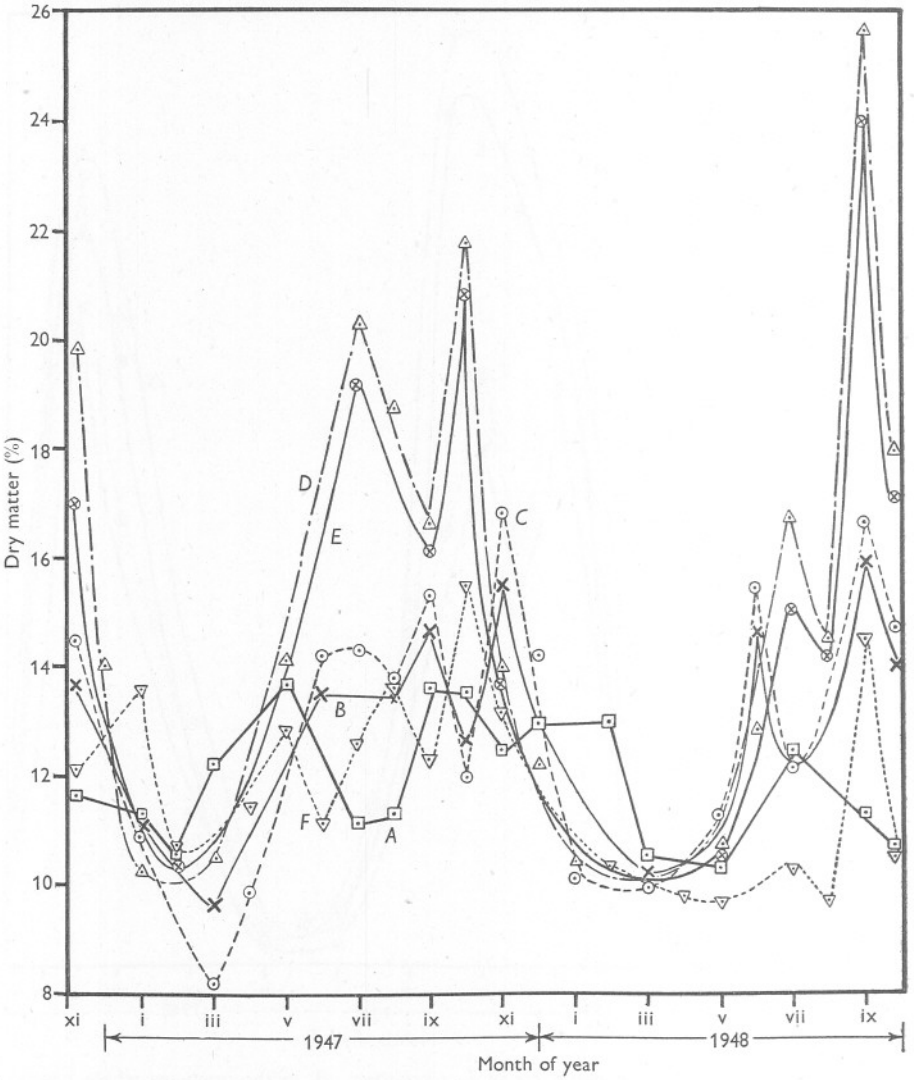

Fig. 12. Seasonal variation in dry matter in *L. saccharina*. *A*, in the open-sea stipes; *B*, in the open-sea whole plants; *C*, in the open-sea fronds; *D*, in the loch fronds; *E*, in the loch whole plants; *F*, in the loch stipes.

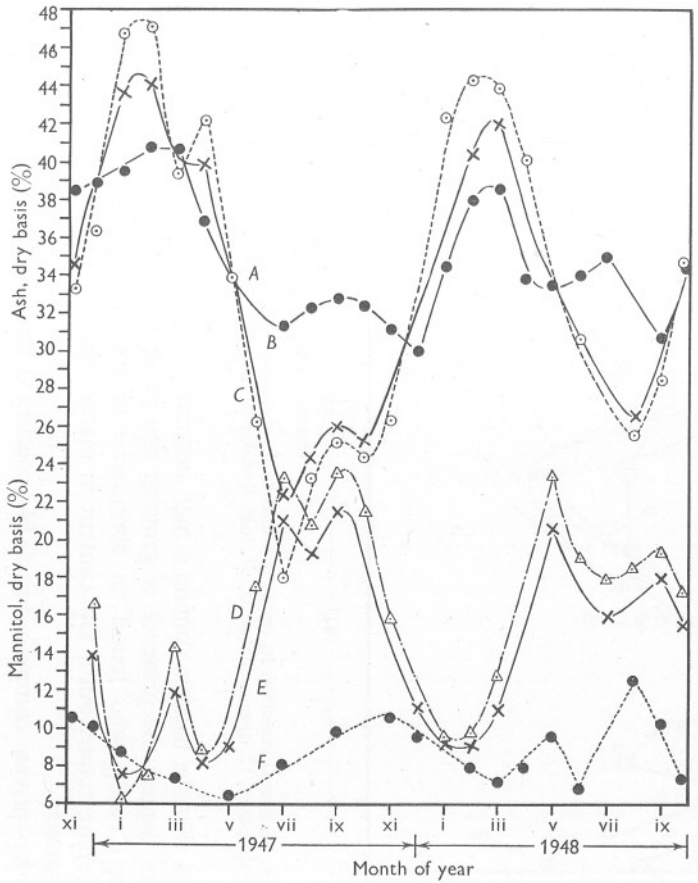

Fig. 13. Seasonal variation in ash and mannitol in *L. saccharina* (open sea). *A*, ash in the stipes; *B*, ash in the whole plant; *C*, ash in the fronds; *D*, mannitol in the fronds; *E*, mannitol in the whole plant; *F*, mannitol in the stipes.

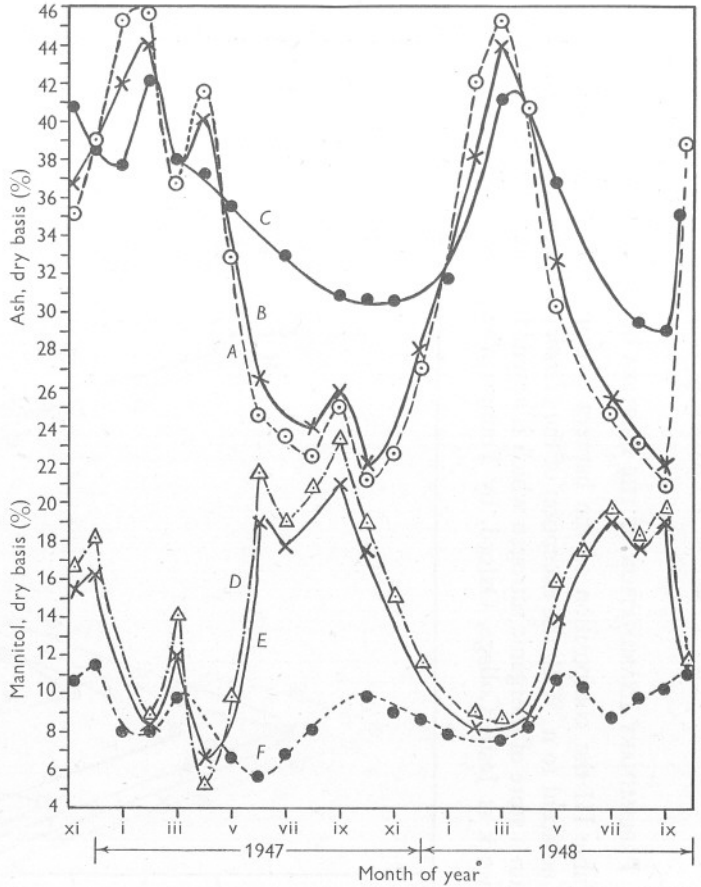

Fig. 14. Seasonal variation in ash and mannitol in *L. saccharina* (loch). *A*, ash in the fronds; *B*, ash in the whole plant; *C*, ash in the stipes; *D*, mannitol in the fronds; *E*, mannitol in the whole plant; *F*, mannitol in the stipes.

Preliminary Examination of the Haptera of the Laminarias

In Table III the composition of the haptera is compared with that of the stipe adjacent to it. With the exception of the *Nereocystis* stipe the haptera all contain a store of inorganic nitrogen which is absent from the adjacent stipes, and work at Jesus College, Oxford, by Young (private communication) has

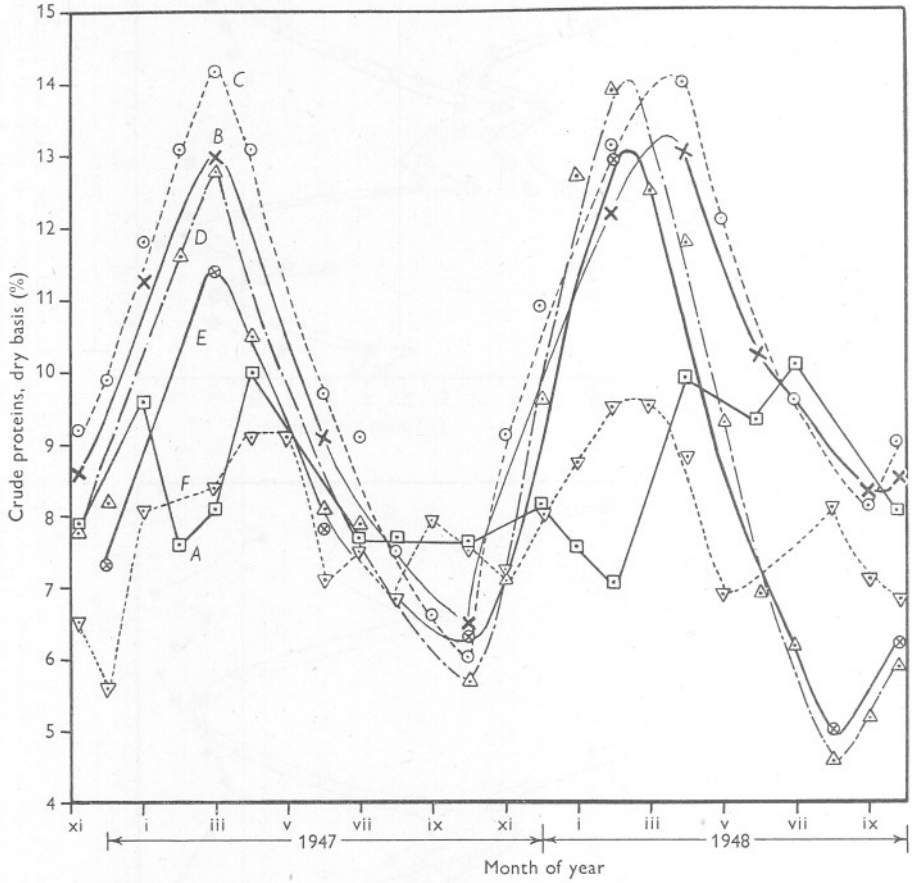

Fig. 15. Seasonal variation in crude proteins in *L. digitata*. *A*, open-sea stipes; *B*, open-sea whole plant; *C*, open-sea fronds; *D*, loch fronds; *E*, loch whole plants; *F*, loch stipes.

shown that the haptera contain a high percentage of free amino-acids. These results suggest the presence of growing tips in agreement with the result of Allsop (1948), who found an abundance of free amino-acids in actively developing tissue, but a low content in mature tissues not specially adapted for storage.

Parke (private communication) has confirmed that the haptera develop as

TABLE III. COMPOSITION OF THE HAPTERA OF THE LAMINARIACEAE *LAMINARIA CLOUSTONI*, *L. DIGITATA* AND *L. SACCHARINA*, AND *NEREOCYSTIS LUETKEANA* (DRY BASIS).

Sample	Date and place of collection	Total ash	Mannitol	Laminarin	Alginic acid	Crude proteins	In-organic nitrogen
L. cloustoni, holdfast	July 1948, Cullipool	34·1	6·0	< 1·0	6·6	14·0	0·17
L. cloustoni, stipe adjacent	July 1948, Cullipool	35·9	6·7	< 1·0	22·9	9·4	Nil
L. digitata, holdfast	Oct. 1948, Atlantic Bridge	32·4	8·4	< 1·0	18·8	15·3	0·20
L. digitata, stipe adjacent	Oct. 1948, Atlantic Bridge	34·2	8·6	< 1·0	30·6	8·1	Nil
L. saccharina, holdfast	Oct. 1948, Shuna Island	37·3	7·4	< 1·0	7·5	15·9	0·18
L. saccharina, stipe adjacent	Oct. 1948 Shuna Island	34·6	7·3	< 1·0	24·9	9·1	Nil
N. luetkeana, holdfast	Oct. 1948, San Juan, Wash., U.S.A.	39·0	1·2	< 1·0	6·9	16·3	0·41
N. luetkeana, stipe adjacent	Oct. 1948, San Juan, Wash., U.S.A.	40·8	1·2	< 1·0	13·8	5·8	0·29

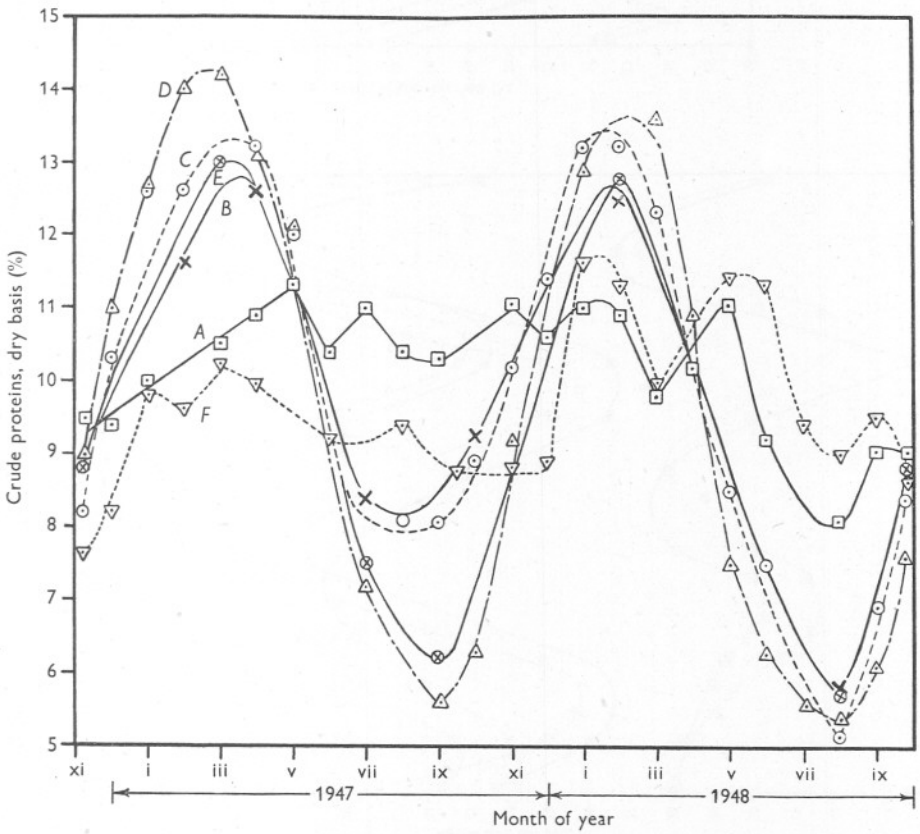

Fig. 16. Seasonal variation in crude proteins in *L. saccharina*. *A*, open-sea stipes; *B*, open-sea whole plants; *C*, open-sea fronds; *D*, loch fronds; *E*, loch whole plants; *F*, loch stipes.

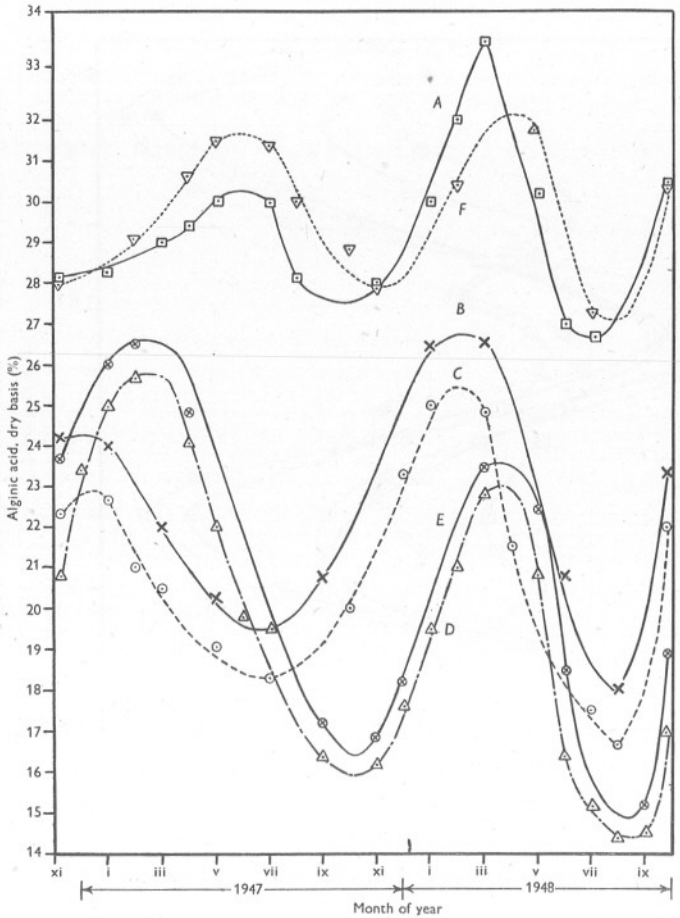

Fig. 17. Seasonal variation in alginic acid in *L. digitata*. *A*, open-sea stipes; *B*, open-sea whole plant; *C*, open-sea fronds; *D*, loch fronds; *E*, loch whole plants; *F*, loch stipes.

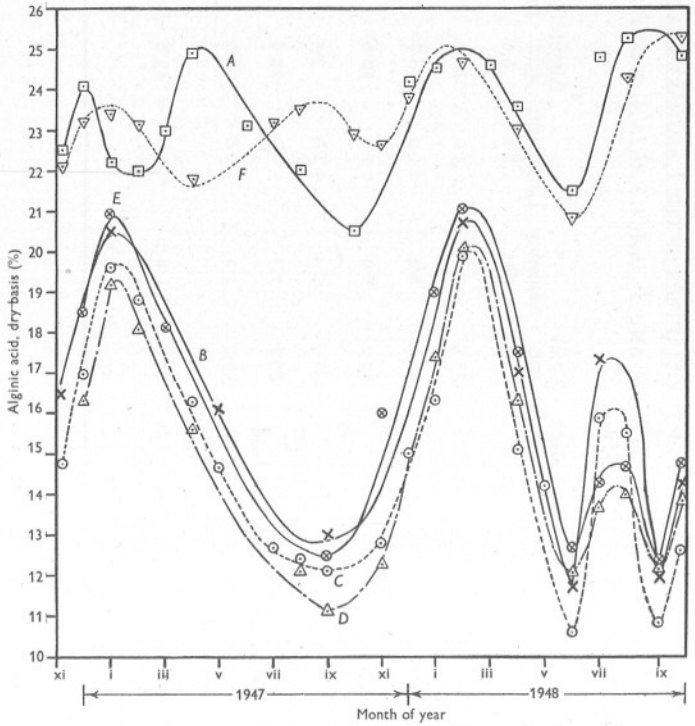

Fig. 18. Seasonal variation in alginic acid in *L. saccharina*. *A*, open-sea stipes; *B*, open-sea whole plant; *C*, open-sea fronds; *D*, loch fronds; *E*, loch whole plants; *F*, loch stipes.

outgrowths from a meristematic layer on the outside of the stipe, and once they start to form the tips can be regarded as growing tips.

It is interesting to note the low percentage of alginic acid, when determined by the standard method (Black, 1948). When the method was modified so that after soaking in acid the sodium carbonate was added without filtering off the

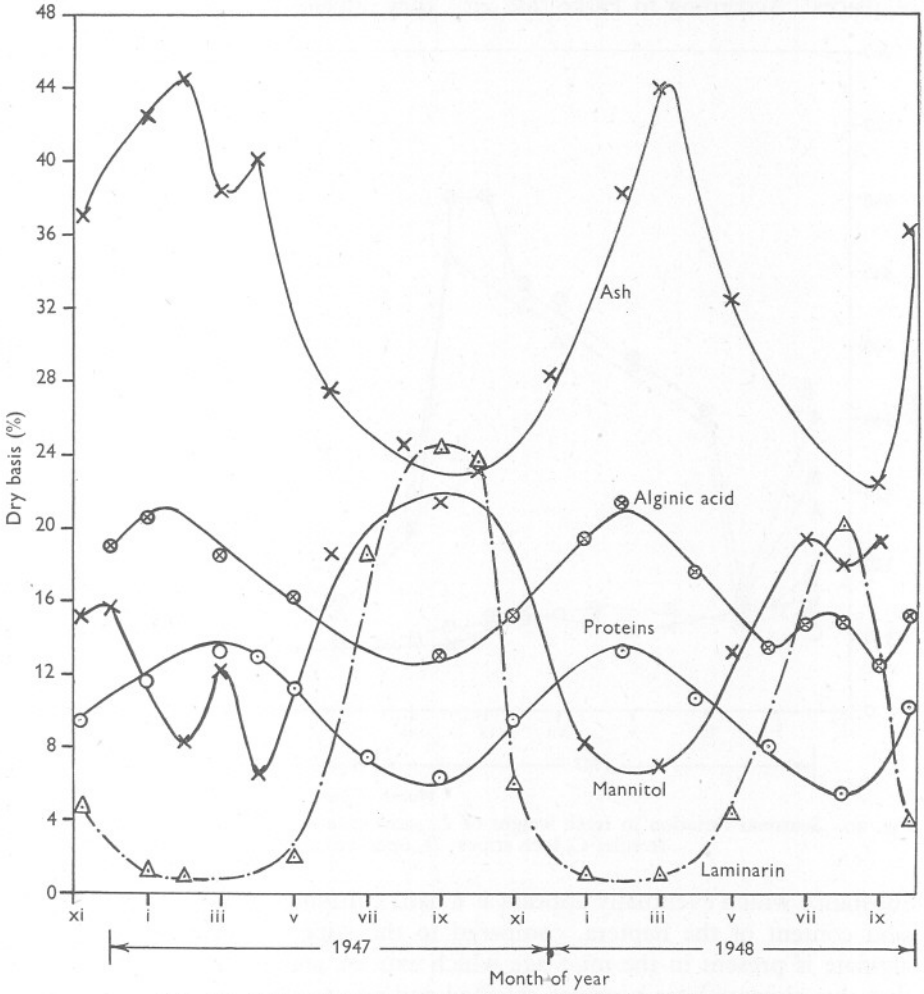

Fig. 19. Seasonal variation in *L. saccharina* (loch) whole plant.

weak acid solution, a result of 14·8% was obtained for the haptera of *Laminaria cloustoni*. When the alginic acid was determined by the carbazole method of Percival & Ross (1948*b*) a figure of 20·4% was obtained. The results indicate that in the haptera there is present either a water-soluble or a very low-grade alginate which requires further investigation.

The results show, however, that the haptera of *L. digitata* are considerably higher in alginic acid than those of *L. cloustoni* and *L. saccharina*, which may account for the very firm attachment which the holdfast of *L. digitata* has for the substratum.

The haptera as they touch the substratum become attached in a number of places. According to Parke (*loc. cit.*) they appear to secrete some sticky

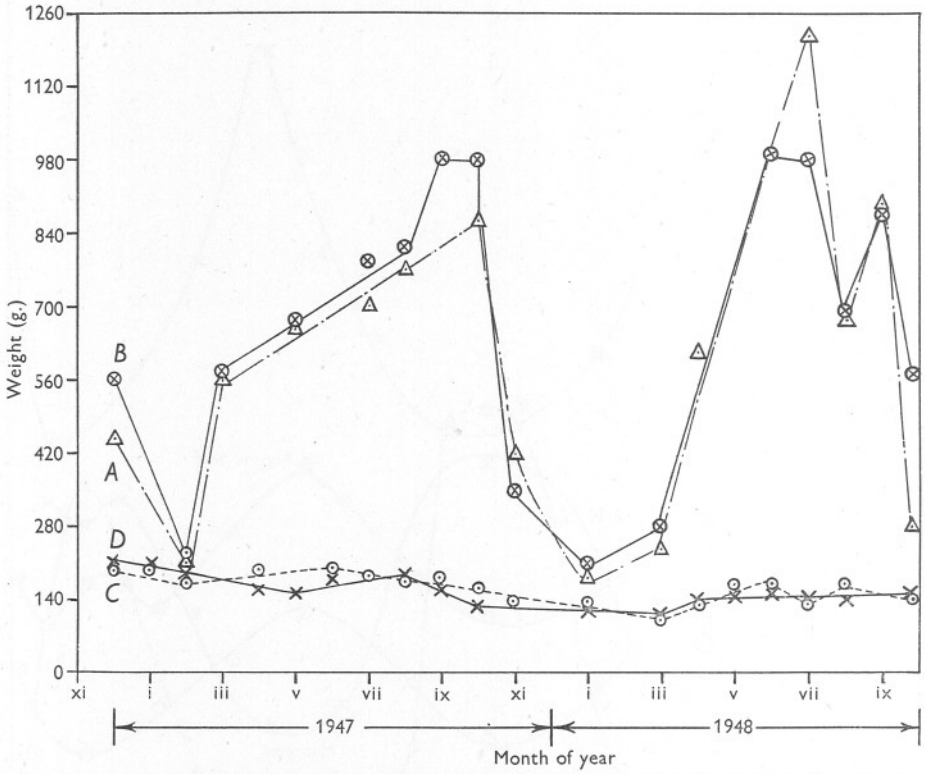

Fig. 20. Seasonal variation in fresh weight of *L. saccharina* in *A*, loch fronds; *B*, open-sea fronds; *C*, loch stipes; *D*, open-sea stipes.

substance which eventually appears as a hard chitinous layer. The low alginic acid content of the haptera, compared to the stipe, might indicate that an alginate is present in the mucilage which exudes, and there is the possibility that this alginate later becomes calcified and assists in cementing the haptera to the substratum.

Summary

The seasonal variations in the total ash, crude proteins, mannitol, laminarin and alginic acid contents are given for monthly samples of the Laminariaceae, *L. cloustoni*, *L. digitata* and *L. saccharina* from November 1946 to October

1948, samples of *L. digitata* and *L. saccharina* having been taken at different localities to determine the effect, if any, of the degree of exposure on the chemical composition.

The results agree favourably with those of the first 2 years examined and indicate that, with only a few exceptions, results might be reproducible in the corresponding season of any year, and it should be possible, therefore, to predict the approximate composition in subsequent years.

As before, the marked seasonal variations in chemical constitution occur in the fronds, where the bulk, if not all, of the photosynthesis occurs. The stipes undergo some variation parallel to that in the fronds, but within narrower limits, while laminarin is absent throughout the year.

In the fronds in the spring, mannitol is at a minimum and laminarin is absent, while the crude proteins, ash and alginic acid are at a maximum. In the autumn the reverse is true. The dry-matter content shows a corresponding variation, being at a minimum in the spring and a maximum in the autumn, but the variation is greatest in the loch samples and in *L. cloustoni* at Cullipool.

In the case of *L. digitata* and *L. saccharina*, the main effect of different degrees of exposure is that in the plants from the more sheltered localities (lochs) the laminarin and consequently the dry-weight contents are higher than in the more exposed samples, which might indicate 'restricted' growth in the lochs.

The fresh weight of the plants undergoes a similar seasonal variation, being at a minimum in the spring and a maximum in late summer.

A preliminary examination of the haptera has been carried out, and indicates a high inorganic nitrogen and free amino-acid content, while there is evidence of the presence of a lower grade alginate than is present in the adjacent stipe.

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