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CONCENTRATION GRADIENTS AND THEIR SIGNIFICANCE IN *LAMINARIA SACCHARINA* (L.) LAMOUR.

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(Text-figs. 1–5)

In previous publications the author (Black, 1948*a–c*, 1949, 1950*a*) has reported the seasonal variations in chemical composition that occur in certain of the common Phaeophyceae. In the sublittoral and littoral zones it has been found that a correlation exists between the chemical composition and the depth of immersion of the plant (Black, 1950*b*, and unpublished work). Differences in composition, particularly during the summer months, also occur in the same species taken at the same time and depth but from different habitats (Black, *loc. cit.*), and there is also evidence that in the laminarians the stage of development of the plant has a profound influence on the composition. In all the above-mentioned analyses the entire plants have been separated into stipe and frond, dried under controlled conditions, milled, and a well-mixed representative sample of each taken for analysis.

Work on the distribution of iodine and potassium in *Laminaria flexicaulis* (= *digitata*) has been carried out by Spindler (1948), Rinck (1948), Rinck & Brouardel (1949*a*, *b*), and Brouardel & Rinck (1950). Rinck & Brouardel (1949*a*) determined the iodine content of 325 samples of the fresh and dried alga, and found it to vary regularly with the distance along the frond, being at a minimum at the base of the frond where the youngest cells are and then increasing with the length of the frond. A similar variation was also obtained in the water content of different parts of the frond. As with iodine, they found a regular variation in the potassium content, the results indicating a direct relation between the morphological portions of the alga—holdfast, stipe, stipe-frondal zone and frond—and the concentration of the chemical elements. Moss (1948, 1950), in her studies on the structure and chemical composition of *Fucus vesiculosus*, has shown that a concentration gradient exists in the thallus and that the receptacles and sterile tips differ markedly in composition. As anatomical differentiation proceeded from the apex, the region of growth, to the base of the thallus, so did the chemical composition vary: the percentage dry weight and organic nitrogen (expressed as percentage fresh weight) increased while alginic acid decreased.

Recent work by Gerdes (1951) on the distribution of aneurin in the algae has shown a constant decrease in the aneurin content with distance from the growing point. Sections of *Laminaria saccharina* were analysed from the transition zone, the upper part of the frond, the stipe and the holdfast. The transition zone contained 7·25 p.p.m. and the upper part of the frond 1·31 p.p.m. (dry basis).

Observations on the rate of growth of *L. digitata* and *L. saccharina* in the Dalne-Zelenetzky Gulf were made by Kuznetzov (1946), and Parke (1948) has shown that in *L. saccharina*, from the coasts of Devon and Argyll, there are two periods of growth, (i) a period of rapid growth from January to June/July with the most rapid growth between March/June, and (ii) a period of slow growth from July to December with the slowest period between September and December. Throughout a yearly cycle regular changes in growth rate affected the length, width, and thickness of the frond, while loss of frond tissue from the distal end started when a plant was a few months old and continued throughout its life. According to Parke (1948) there can be present in a *L. saccharina* frond in October a portion of newly formed tissue and tissue at least 7 months old. It is logical, therefore, to expect variations in composition with age. As the stipe of this species is relatively small compared to the frond, and as no variation existed in the dry-matter content along the stipe, only the frond was in each case analysed.

No work appears to have been carried out to show the presence of a concentration gradient in *Laminaria*. Moss (1948, 1950) has studied *Fucus vesiculosus*, but, whereas in *Fucus* the growing region is near the tips of the branches, in *Laminaria* it is at the base of the frond.

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Preparation of Samples and Methods of Analysis

For the investigation now to be reported samples were collected and treated as follows.

The initial sample of *L. saccharina* (L.) Lamour. was collected on 19 August 1949 at Shandon in the Gareloch at 4 m. (low water). This was plant 1, which had the dimensions shown in Fig. 1 A. The frond was divided transversely into five equal sections 30·5 cm. long and dried at 25–30° C. for 24 hr.

Plants 2–4 were collected in Kerrera Sound, Oban, Argyllshire, at approximately 4 m. (low water), on the 17 October 1950, 26 April 1951 and 30 May 1951 respectively, and sectioned as shown in Fig. 1. The sections were dried at 100° C. for 16 hr. Each of the dried sections was then milled in a Christy and Norris no. 8 Laboratory Mill fitted with a 64-mesh screen, and analysed as previously described (Black, 1948*a*; Black & Cornhill, 1951).

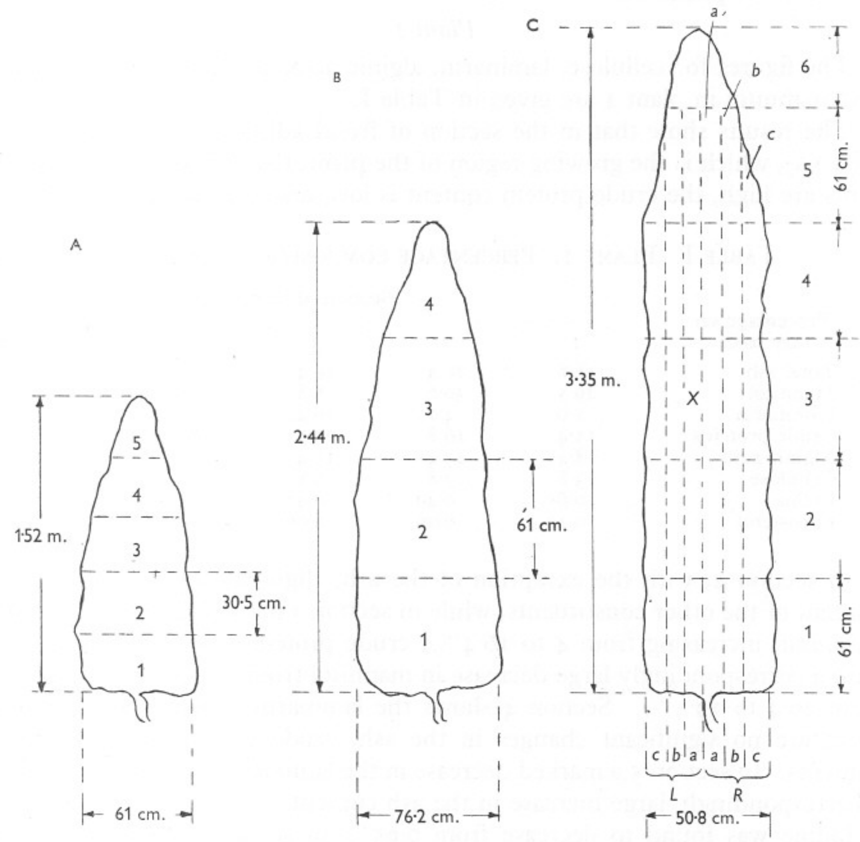

Fig. 1. Method of dividing the fronds investigated. A, plant 1, Gareloch, 19 August 1949. B, plants 3 and 4, Kerrera Sound, 26 April 1951 and 30 May 1951. C, plant 2, Kerrera Sound, 17 October 1950. All were divided into transverse 'sections' numbered from the base. In A there were five sections of 30·5 cm.; in B, four of 61 cm.; in C, six sections of which the first five were 61 cm. long and the terminal 30·5 cm. In C there were, in addition, longitudinal divisions. The frond was first divided into right and left halves (R and L). Each half was divided further into three strips, *a*, *b* and *c*, the first the innermost. $a=b=7{\cdot}6$ cm., $c=10{\cdot}2$ cm. (Any of the 30-odd subdivisions of the frond can be specified by a combination of three symbols: thus the unit marked *X* would be called L 3*a*.)

Results

The results obtained are expressed as fresh-weight and dry-weight percentages. When calculated as percentage concentrations in the water present the graphs were parallel to those for the fresh weight and have been omitted.

The calculation of the results on the 'residual dry weight' was also considered, but, as this underwent considerable variation, calculation on the fresh weight, which approximately gives the results in terms of concentration in the water present, was preferred.

Plant 1

The figures for cellulose, laminarin, alginic acid, crude proteins, total ash and mannitol in plant 1 are given in Table I.

The results show that in the section of frond adjacent to the stipe, (1 in Fig. 1A), which is the growing region of the plant, the mannitol and ash contents are high, the crude protein content is low, and laminarin is absent.

TABLE I. PLANT 1. PERCENTAGE COMPOSITION (DRY BASIS)

Percentage on dry basis	Section of frond				
	1	2	3	4	5
Total ash	28·8	26·4	19·4	20·0	26·0
Mannitol	29·5	30·6	8·8	9·6	10·6
Laminarin	1·0	4·0	16·4	22·0	12·4
Crude proteins	15·4	16·8	20·5	19·6	18·2
Alginic acid	8·4	13·4	13·4	18·3	17·7
Cellulose	3·8	3·8	3·8	3·8	3·8
Iodine	0·65	0·49	0·47	0·42	0·40
Fucosterol	0·10	0·06	0·06	—	—

In section 2, with the exception of the ash, slight increases occur in the content of the other constituents, while in section 3 marked differences occur, laminarin increasing from 4 to 16·4%, crude proteins from 16·8 to 20·5% with a correspondingly large decrease in mannitol from 20·5 to 8·8% and ash from 26·4 to 19·4%. Section 4 shows the laminarin still increasing, while there are no significant changes in the ash, crude proteins and mannitol contents. In section 5 a marked decrease in the laminarin content occurs with a correspondingly large increase in the ash content.

Iodine was found to decrease from 0·65% in section 1 to 0·49% in 2, 0·47% in 3, 0·42% in 4 and 0·40% in 5 (dry basis), while fucosterol decreased from 0·10% in section 1 to 0·06% in section 2, quite a significant difference for this sterol. The alginic acid content increased from 8% in section 1 to 18% in sections 4 and 5.

Plant 2

These results having shown that marked differences in composition occurred along the frond, a more detailed investigation was carried out with plant 2 which was divided into thirty-one areas (Fig. 1C), one at the tip, with the five transverse sections subdivided each into six longitudinally. The frond being thickened medially the innermost sections were about twice as heavy per unit area as the outer with the percentage water only slightly higher in the central strip.

Dry Matter

As one passes up the axis of the frond there is a marked increase in dry matter (Fig. 2). This reaches a maximum two-thirds of the way up and then decreases towards the eroded tip. It is interesting that although the dry-matter content increases, e.g. from 12·6% in L 1*a* to 31·1% in L 4*a* the fresh weights of the sections remain practically constant (45·5 and 47·0 g respectively). Also sections L 1*a* and R 1*a* contain double the amount of dry matter of the adjoining sections L 1*b* and R 1*b*, but the same percentage dry weight, indicating that they have approximately the same percentage composition but differ in thickness. No significant difference in concentration occurs across the frond.

Mineral Matter

The figures for the mineral matter (total ash) expressed on the dry basis, and on the fresh-weight basis, are given in Figs. 2 and 3. When expressed on the dry basis (Fig. 2) the mineral matter is high in the sections adjoining the stipe (25–35%), then decreases half-way up the frond (19–23%), and then increases slightly. This is also true if the ash is calculated on the 'residual dry-weight' basis. On the fresh basis (Fig. 3) almost the reverse occurs, the mineral matter increasing two-thirds of the way up the frond and then decreasing towards the tip. The mineral matter, therefore, increases as the dry-matter content increases and as the mannitol decreases. Possibly mannitol is being utilized in respiration or for further synthesis and mineral matter is diffusing into the cells to control the osmotic pressure. The concentration of mineral matter across the frond is somewhat irregular.

Mannitol

Although mannitol is unlikely to be the primary product of photosynthesis the value found is usually regarded as indicative of the amount of photosynthesis. In the sections adjacent to the stipe (Fig. 3) the mannitol content is high but increases to a maximum one-third of the way up the frond then decreases towards the tip, falling from 35% to 11·5% of the dry matter.

Laminarin

Laminarin is regarded as a storage foodstuff. It is absent from the stipe of the laminarians and from the frond during the period of rapid growth in the spring. It appears to be formed during the period of slow growth when the nitrate and phosphate content of our inshore waters is very low. Laminarin is almost entirely absent from plants growing in habitats where the above nutrients are available all the year as a result of pollution or upwelling. The results for this carbohydrate are given in Fig. 4. Laminarin is entirely absent from the active growing sections adjacent to the stipe, and increases up the frond to 30·4% in L 4*c* and 34·5% in R 3*b*; in general it increases with the dry-matter content and then decreases to 5% at the tip.

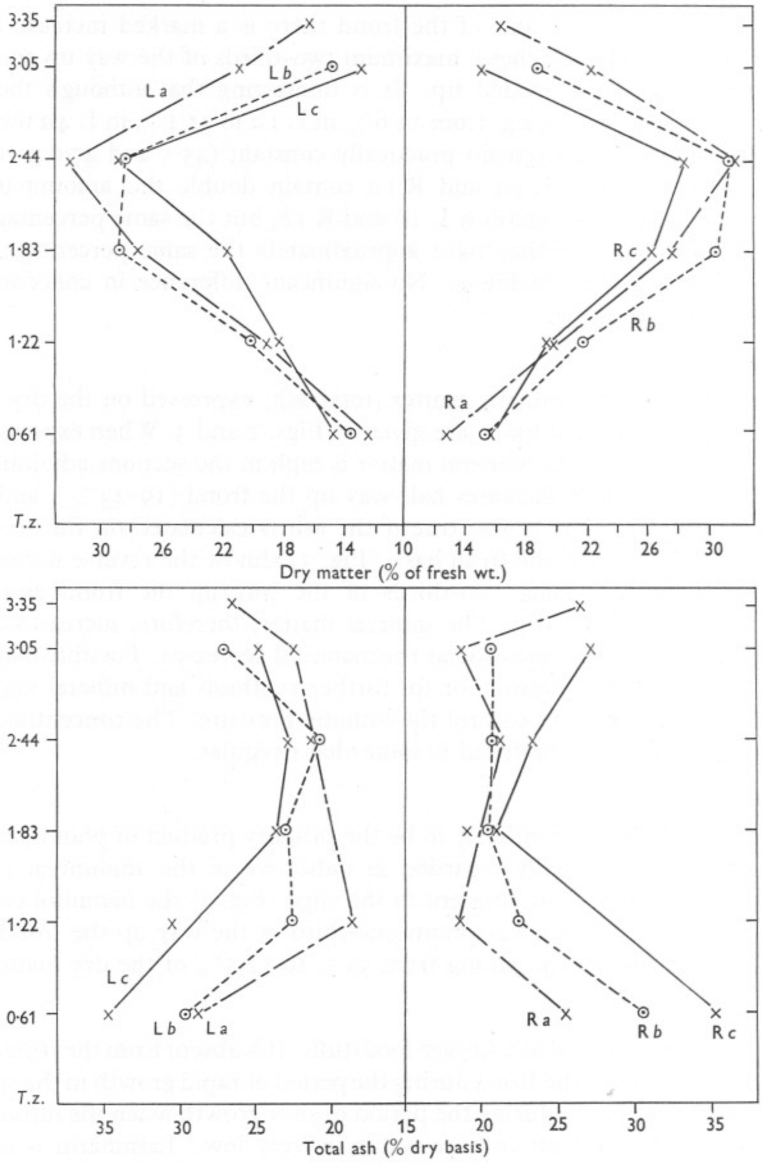

Fig. 2. Plant 2. Variation in (above) *dry-matter content* as percentage of fresh weight, and (below) *total ash* as percentage of dry matter. Each graph line represents one strip of the frond, with one value entered for each section. Left and right sides are graphed separately. *T.z.*, transitional zone at base of frond. Vertical scale *d* represents distance along the frond, from the base, in metres.

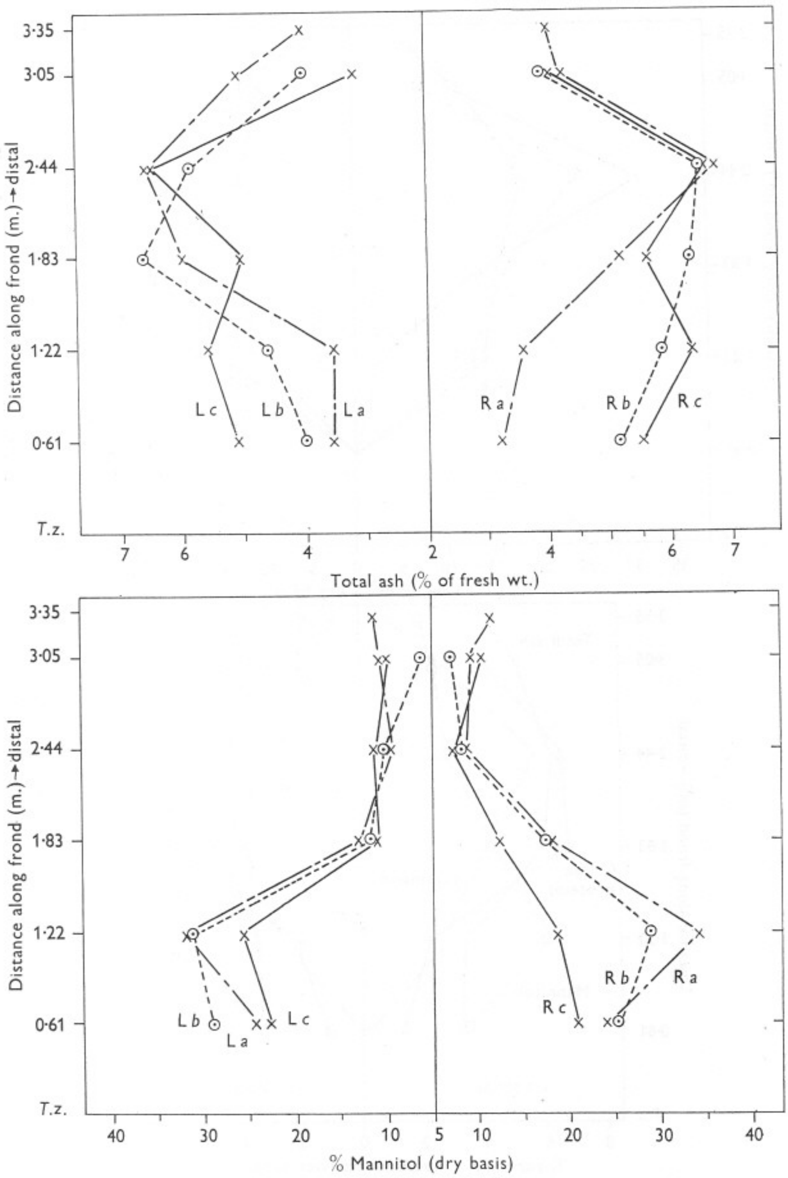

Fig. 3. Plant 2. Variation in (above) *total ash* as percentage of fresh weight, and (below) *mannitol* as percentage of dry matter. Particulars as in Fig. 2.

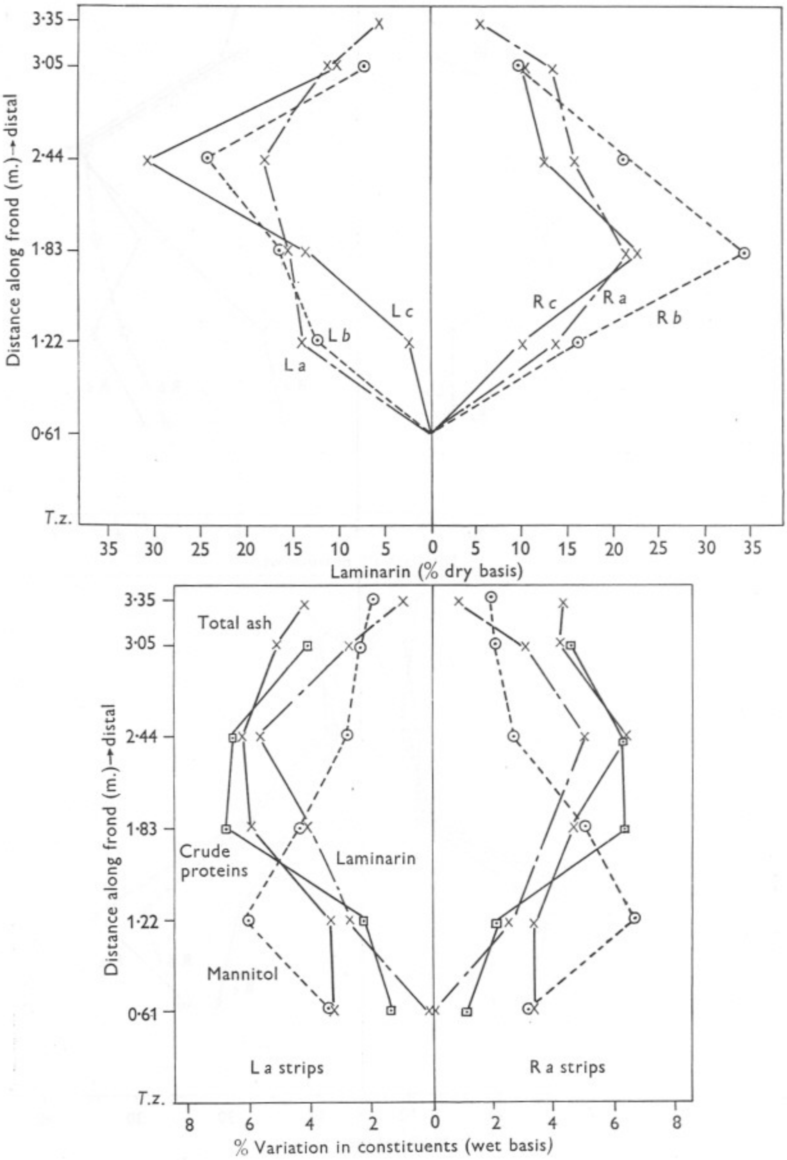

Fig. 4. Plant 2. Above: variation in *laminarin* as percentage of dry matter. Below: percentage variation in four constituents compared, on a wet basis, only the *a* strips considered.

Alginic Acid

The alginic acid content is exceedingly low in the sections L 1*a* and L 2*a* and is of such a low grade (i.e. of low molecular weight) that it is indeterminable by the standard method of analysis. In L 3*a* and L 4*a* values of 13·6 and 13·1 % were obtained, indicating that a higher polymer of alginic acid is no doubt present.

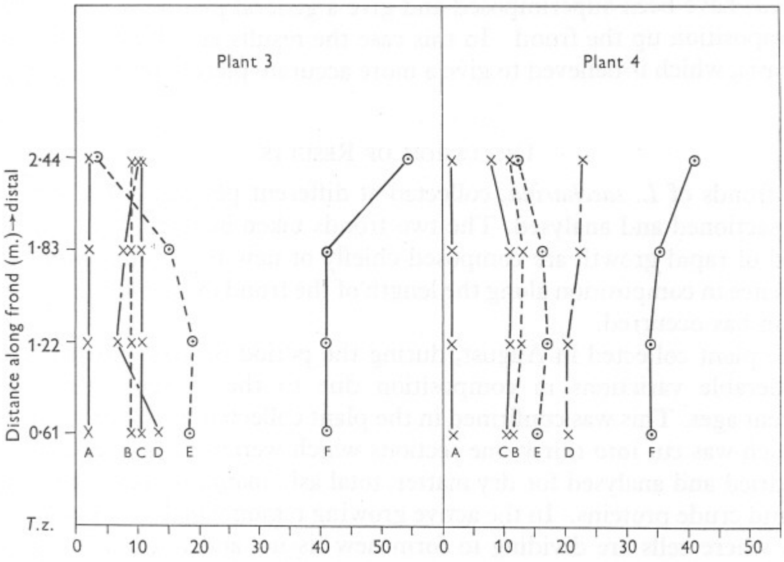

Fig. 5. Plants 3 and 4, illustrating the period of rapid growth. Variation in (A) *laminarin*, (C) *crude protein*, (D) *alginic acid*, (E) *mannitol*, (F) *total ash* (on dry basis); and (B) *dry matter* (on wet basis)

Plants 3 and 4

In order to prove that variations such as those recorded above were improbable during the period of rapid growth in the spring, plants 3 and 4 were taken and sectioned as in Fig. 1 B, and the analytical results are given in Fig. 5. The graphs show clearly that at this time of the year the composition of the frond is constant throughout its length, only at the tip where erosion takes place is there any noticeable change.

In previous publications (Black, 1948*b*, 1950*a*) the composition of the frond of *L. saccharina* is given for a period of 4 years, and the results agree with those of the present investigation. Plants 3 and 4, taken in April and May during the period of rapid growth, are composed mainly of tissue laid down during the preceding 3 months with perhaps only the distal part retained from the previous year, whereas plants 1 and 2 are composed of new tissue adjacent to the stipe and tissue up to 7–8 months old.

Analysis of a frond of *L. cloustoni* in May, when it was possible to differentiate between the new and old growth, showed that the old frond was low in mannitol but still contained laminarin, while the new frond was appreciably higher in mannitol but contained no laminarin. In April/May the composition of an *L. saccharina* frond will, therefore, depend on whether old growth from the previous year has been retained or cast. From May onwards loss of frond is then due to erosion at the tip.

In Fig. 4 the graphs for the sections representing the central portion (L*a* and R*a*) have been superimposed and give a general picture of the variations in composition up the frond. In this case the results are all expressed on the wet basis, which is believed to give a more accurate picture of the living plant.

Discussion of Results

Four fronds of *L. saccharina*, collected at different periods of the year, have been sectioned and analysed. The two fronds taken in April/May during the period of rapid growth are composed chiefly of new tissue and there is little difference in composition along the length of the frond except at the tips where erosion has occurred.

The plant collected in August, during the period of slow growth, showed considerable variations in composition due to the presence of tissue of different ages. This was confirmed in the plant collected in October, the frond of which was cut into thirty-one sections which were weighed separately and later dried and analysed for dry matter, total ash, mannitol, laminarin, alginic acid and crude proteins. In the active growing region which is proximal to the stipe, where cells are dividing to form new tissue, and practically along the whole length of the frond during the period of rapid elongation, laminarin is absent and the dry-matter content is very low (10–12 %) and is composed chiefly of mineral matter (36–40 %) and mannitol (20–30 %). In the active growing region the proteins are at a minimum, and the alginic acid is of such a low grade as to be indeterminable by the standard method. The results are in good agreement with those of Moss (1948) who found the dry matter and inorganic nitrogen, for example, lowest, and the mineral matter highest in the distal samples (tips) of *Fucus vesiculosus* where the growth is apical.

During the period of rapid growth there is extensive elongation due to cell enlargement with vacuolation. Then the tissue is low in dry matter composed mainly of mannitol and mineral matter and laminarin is absent. In July (Parke, 1948) a period of slow growth commences with the slowest growth between September and December when plants 1 and 2 were collected. June to September is also the period (Black & Dewar, 1949) when the nitrate and phosphate content of our inshore waters is at a minimum. During the period of slow growth, therefore, the stipo-frondal zone would alone represent new cells while the remainder of the frond, although able to photosynthesize, ceases active cell division and accumulates carbohydrates fated to become the food reserve for the winter or for sporogenesis. What Parke described as mature tissue is, therefore, the storage portion of the frond two-thirds of the way up and containing a reserve of laminarin with proteins; the distal portions represent tissue which has spored or is badly eroded. Iodine and fucosterol are highest in the stipo-frondal (or transition) zone where they are in all probability required for new growth.

The results show that at certain times of the year concentration gradients of carbohydrates, proteins and mineral matter exist up the frond; they may be the result of restricted growth and may not be present in plants from habitats where nutrients are available all the year round and where laminarin has already been shown to be absent (Black, 1950*a*).

There is no evidence of a concentration gradient across the frond although the thickness shows considerable variation.

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