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Cultivation of *Laminaria hyperborea* in situ and in continuous darkness under laboratory conditions

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KURZFASSUNG: **Kultivierung von *Laminaria hyperborea* in situ und im Dauer-Dunkel unter Laboratoriumsbedingungen.** Zwei- und dreijährige Exemplare der Braunalge *Laminaria hyperborea* wurden bei Helgoland auf untermeerische Zuchtstationen in 2 und 6 m Wassertiefe gebracht. In 2 m Tiefe wurde die maximale Wachstumsrate in der ersten Maihälfte erreicht, in 6 m Tiefe während der ersten Junihälfte. Verglichen mit der im Juli erreichten Thallusgröße der Pflanzen in 2 m Tiefe, erreichten die Pflanzen in 6 m Tiefe mindestens 75 % der Phylloidfläche, aber nur 1/3 der Stiellänge. Dunkelgehaltene Exemplare von *Laminaria hyperborea* zeigten von Februar bis Mai nur dann ein beträchtliches Wachstum des neuen Phylloids, wenn das alte Phylloid an der Pflanze belassen wurde.

INTRODUCTION

The technical difficulties involved in control of the environmental factors in an algal culture increase with the size of the cultivated specimens. Taking *Laminaria hyperborea* as an example, we are well informed about light and temperature requirements of the microscopic gametophytes as well as of early sporophytes up to a length of a few centimeters (KAIN 1964, 1965). These experiments were conducted in Petri dishes. Specimens of larger size, however, can be successfully cultivated only in running sea water (NEUSHUL & HAXO 1963, NEUSHUL & DAHL 1967). Control of physical and chemical properties of flowing sea water is achievable only through great technical efforts. Many experiments, however, conducted in the laboratory in case of microscopic algae, may be performed in the field in case of large algae due to advancements of SCUBA diving techniques (NORTH 1961, NEUSHUL & HAXO 1963). A technique for transplantation of *Laminaria hyperborea* specimens from natural rocks onto PVC plates mounted at sub-tidal growth stations has been described by LÜNING (1969a). The first objective of this investigation concerned the growth of individuals of *L. hyperborea* of known age at different water depths. The second objective was to study the growth of partly amputated specimens of *L. hyperborea* cultivated in darkness from February until May in the laboratory.

MATERIAL AND METHODS

The laboratory studies were performed in "Eternit"-tanks (125 × 80 × 20 cm) supplied with flowing sea water obtained directly from the sea. Specimens of *Laminaria hyperborea* of nearly equal size (stipe length 18–20 cm; area of old frond about

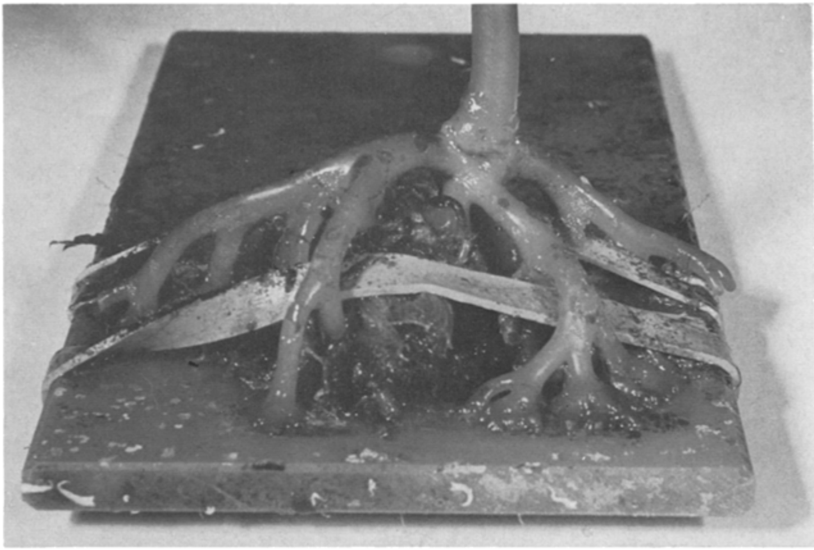

Fig. 1: Lower part of stipe and holdfast of a specimen of *Laminaria hyperborea* at end of the second period of fast growth. The alga had originially settled on a piece of rope which was later on mounted onto a numbered PVC plate

Fig. 2: Underwater growth station (iron frame, 2 × 1 m) with mounted PVC plates bearing two year old specimens of *Laminaria hyperborea*. The station has been hoisted on board a boat for photographic recording

2 dm²; age presumably 3–4 years) were sampled at 2–3 m water depth in February. At the time of sampling the algae had a small new frond of 0.21 to 0.26 dm² in area. The haptera were cut off the stipe and the stipe lengths adjusted to 19 cm (± 1 cm). Of 4 experimental groups, each contained 5 specimens: (a) new frond with stipe and old frond (cut to a frond area of 1.7 to 2.0 dm²; Fig. 7a); (b) new frond with old frond (old frond cut as in group a), but without stipe (Fig. 7b), (c) new frond with stipe, but without old frond (Fig. 7c); (d) isolated new frond (Fig. 7d). The specimens were cultivated in darkness from February until May by placing them into light-tight sea water tanks.

Field studies were conducted in the following manner: Polypropylene ropes (8 mm in diameter) were fastened to iron frames (2 × 1 m) which were submerged at 3 m water depth in January. Sporophytes of *Laminaria hyperborea*, settling on the ropes during the following months, were transplanted to sub-tidal growth stations by cutting the rope into pieces and fastening these onto numbered PVC plates (8 × 15 cm, Fig. 1). The PVC plates were mounted on iron frames (2 × 1 m, Fig. 2) which were submerged at 2 or 6 m water depth. The algae were brought to the surface every month and photographed on board a boat.

RESULTS AND DISCUSSION

Figure 3 shows the increase in frond area and stipe length of *Laminaria hyperborea* specimens, growing at 2 and 6 m water depth, during the second year of life.

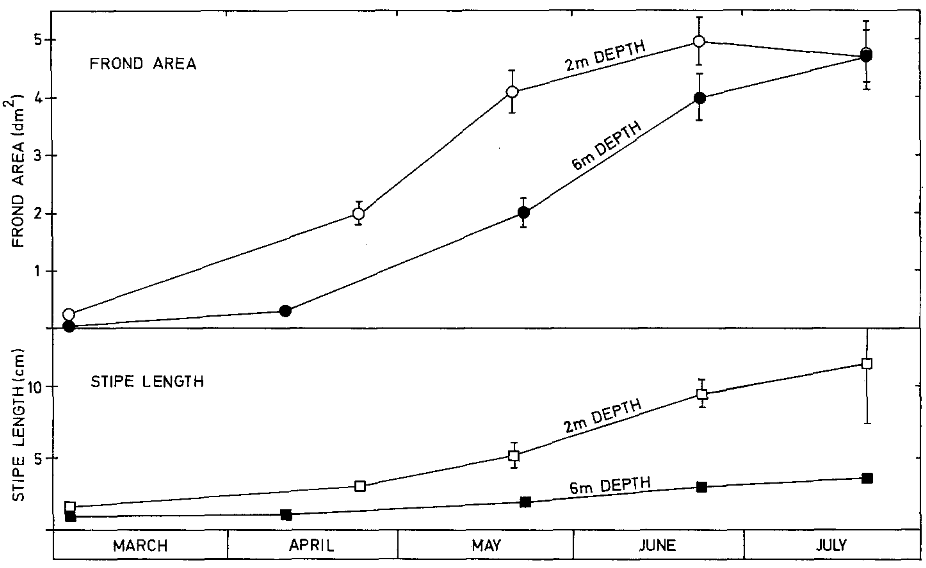

Fig. 3: Increase in frond area and stipe length in *Laminaria hyperborea* specimens during second year of life. Open symbols: specimens growing at 2 m water depth. Solid black symbols: specimens growing at 6 m water depth. Vertical ranges indicate fiducial limits ($P = 0.05$)

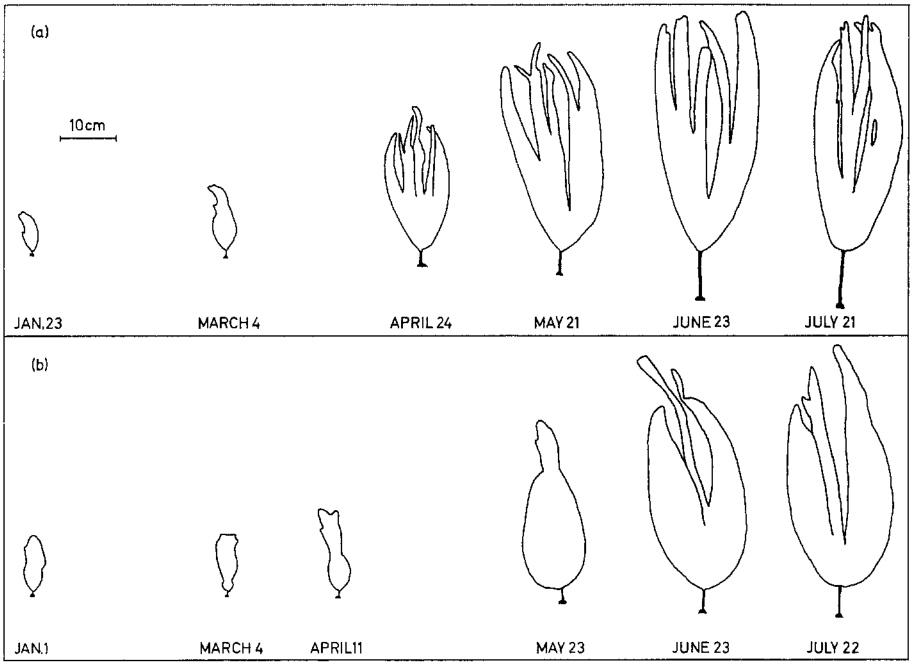

Fig. 4: Records of growth of two specimens of *Laminaria hyperborea* during second year of life. Upper: plant growing at 2 m water depth; lower: plant growing at 6 m water depth

Plants at 2 m depth show maximum increase in frond area in early May, specimens at 6 m depth in late May and early June. In July, growth of the frond ceases in plants at 2 m depth, whereas a small increase in frond area is noticed in specimens at 6 m depth. At the end of July, frond areas attained by plants at 2 and 6 m depth, respectively, are of similar size. The difference in stipe length, however, is considerable. Mean stipe length in July is 12 cm in plants growing at 2 m depth, and 4 cm in plants at 6 m depth.

The growth records of two specimens of *Laminaria hyperborea* obtained from January to July during the second year of life are illustrated in Figure 4. It can be seen that part of the old phylloid of the specimen grown at 6 m water depth (Fig. 4b) is still present in June. The same situation is noticed in 27 % of the specimens growing at 6 m depth. The majority of individuals growing at 2 m water depth, however, have already shed the old frond by May (Tab. 1).

Shedding of the old frond is also delayed in three year old specimens growing in 6 m water depth, if compared to individuals of the same age group growing in 2 m depth (Fig. 5, Tab. 1). At 6 m depth, all plants were still in possession of their old fronds in May, whereas 66 % of the three year old specimens growing at 2 m depth had lost their old fronds by this time.

The stipes of the three year old individuals growing at 6 m water depth are very short, if compared to specimens of the same age group at 2 m depth. Mean stipe length in July is 18 cm at 2 m depth, and 6 cm at 6 m depth (Fig. 6). As was

Table 1

Percentage of *Laminaria hyperborea* specimens possessing the old phylloid in successive months

Age (years)	Depth (m)	March	April	May	June	July
2	2	100	100	3	0	0 %
2	6	100	100	89	27	0 %
3	2	100	100	44	0	0 %
3	6	100	100	100	75	0 %

the case with the two year old plants, reduction of the frond growth rate occurs earlier in plants at 2 m depth than at 6 m depth (Fig. 6). At the end of the period of rapid growth (July) the specimens at 6 m depth have a frond area of about 75 % of that attained by individuals at 2 m depth.

The reason for the maximum frond growth rate occurring about 1 month later in specimens exposed to 6 m depth, if compared to plants at 2 m depth, is possibly to be seen in the fact that local underwater irradiance increases until June/July (LÜNING 1969b). It seems reasonable to suggest that underwater irradiance necessary for maximum growth rate of the frond is reached only in June at 6 m water depth.

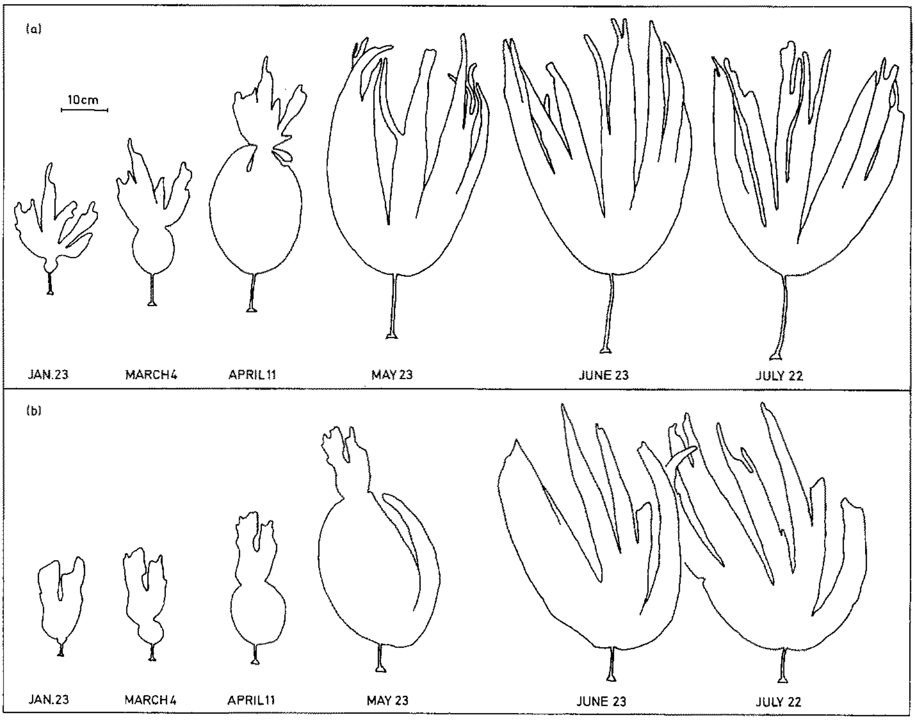

Fig. 5: Records of the growth of two specimens of *Laminaria hyperborea* during third year of life. Upper: plant growing at 2 m water depth; lower: plant growing at 6 m water depth

The question, however, as to which factors stop the growth of *Laminaria hyperborea* in summer, seems to allow no simple answer. Near Helgoland this species has its maximum assimilation surplus during summer, since underwater irradiance is

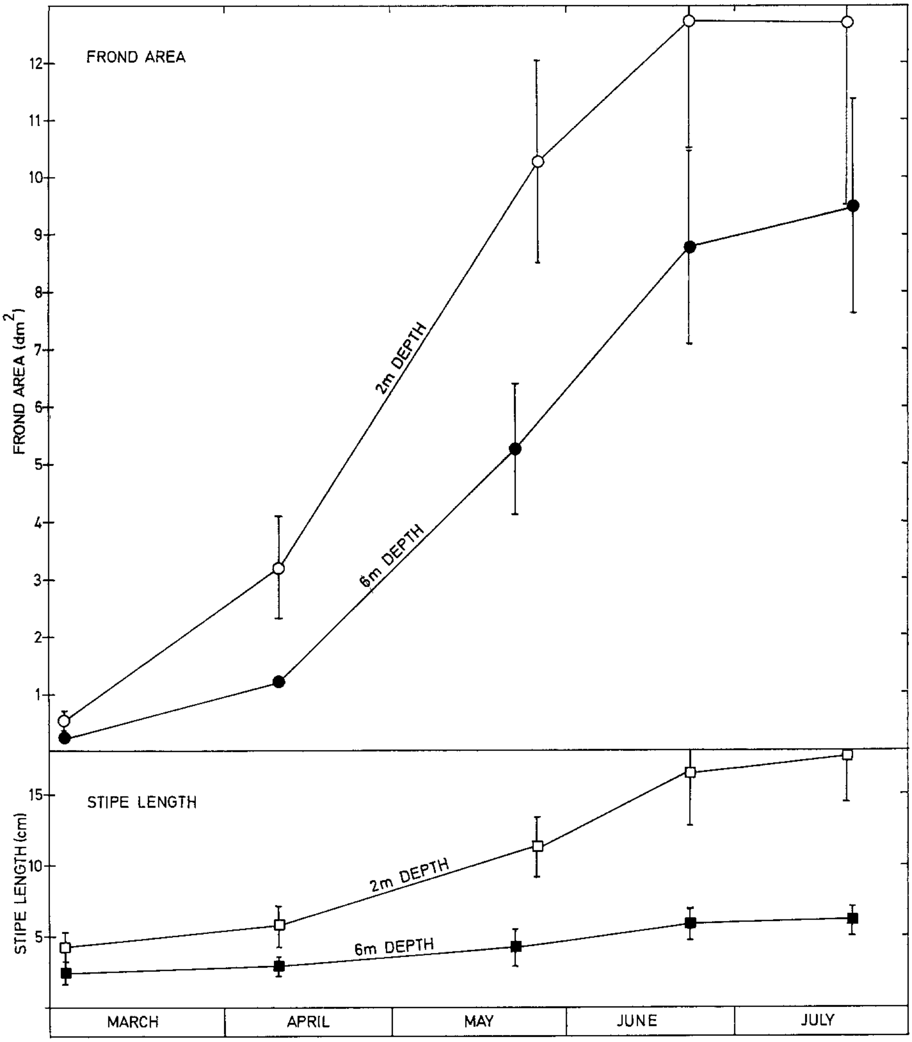

Fig. 6: Increase in frond area and stipe length in *Laminaria hyperborea* specimens during third year of life. Open symbols: specimens growing at 2 m water depth. Solid black symbols: specimens growing at 6 m water depth. Vertical ranges indicate fiducial limits (P = 0.05)

strongest during summer and respiration is by no means consuming the greatest part of gross photosynthetic products at this time (Lüning 1969b). The assimilation surplus occurring in summer is layed down as reserve materials (Black 1948). It has been argued, on the other hand, that lack of nutrients in the sea water during the summer months may probably be the cause for reduction of growth rate occurring in *Laminaria* species at this time (BLACK & DEWAR 1949, GESSNER 1955). Unfortunately, this hypothesis has not yet been verified by experimental evidence. The discovery that growth rate of *Laminaria hyperborea* fronds is reduced about 1 month later at 2 m depth, if compared to 6 m depth, probably cannot be explained in terms of

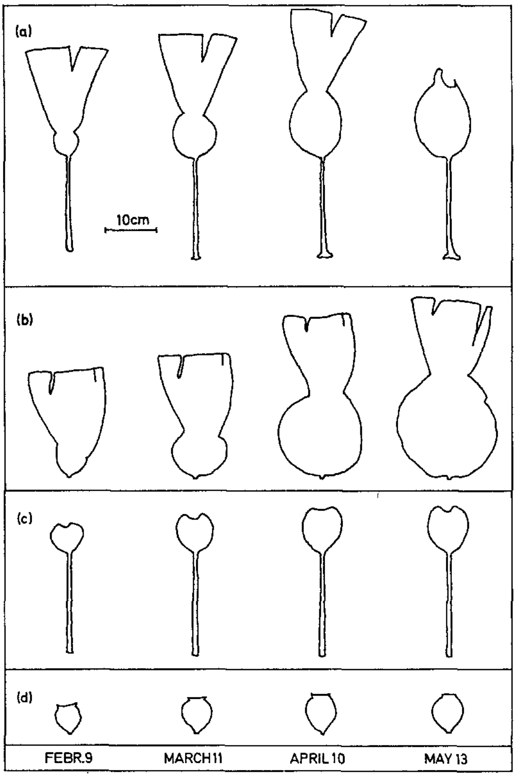

Fig. 7: Records of growth of 4 specimens of *Laminaria hyperborea* grown in darkness in a tank (running sea water) from February until May. Specimen *a*: new phylloid with stipe and old phylloid; *b*: new phylloid with old phylloid; *c*: new phylloid with stipe; *d*: isolated new phylloid

nutrient content of the surrounding sea water. It seems unlikely that the nutrient content is different at 2 and 6 m water depth, since a strong tidal current occurs at the stations where the experimental algae were growing.

During the early months of the year the sea water near Helgoland is generally very turbid. Underwater irradiance is reduced to levels below the compensation intensity of *Laminaria hyperborea* phylloids during this time even at moderate water depths (LÜNING 1969b). In spite of this fact the growth of the new frond begins in December/January. *L. hyperborea* produces a small new phylloid during the first half year even in complete darkness (LÜNING 1969a). It has been suggested that the organic material needed for production of the phylloid in darkness is due to reserve materials, presumably laminaran, built up by the old frond during the preceding season of slow growth (summer and autumn), when light penetrates deep into the water (Lüning 1969b). Until now the question has remained open, however, whether the majority of reserve materials responsible for frond growth in darkness are localized in the old frond or in the stipe. Chemical analyses performed by Black (1948) indicated that the wide seasonal variations in composition of *L. hyperborea* specimens

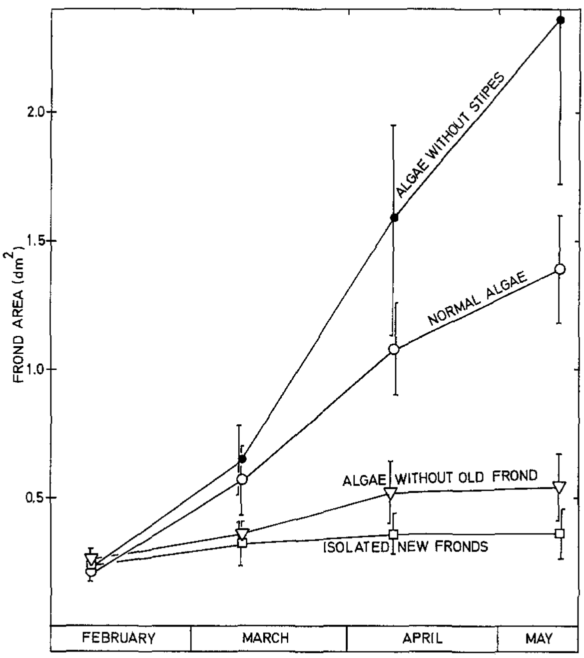

Fig. 8: Increase in frond area in partly amputated specimens of *Laminaria hyperborea* (Fig. 7). Vertical bars indicate standard deviations

are due almost entirely to variations in the composition of the fronds. Cultivation experiments on partly amputated specimens of *L. hyperborea* conducted in the laboratory revealed a further answer to this problem.

In darkness, considerable increase occurs in the area of new fronds connected to their old fronds (Fig. 7a, b). New fronds without stipes, but connected to their old fronds (Fig. 7b) attain a new frond area in May which may be even larger than the area of the old frond. Small increases in stipe length (1–2 cm) occur in groups (a) and (c), as well as the formation of new haptera in some cases.

Figure 8 shows mean values and standard deviations of new frond areas attained by all specimens. A small increase in new frond area is also recorded in isolated new phylloids during the early weeks of darkness. Fouling was noticed in the isolated new phylloids in April and May. New fronds with stipe, but without an old phylloid, increase their area from February until May by a mean factor of 2.2, new fronds with stipe and old frond 7.9, new fronds with old frond, but without stipe 10.3. The mean ratio of new frond area (attained in May) to old frond area is 1.3 in plants possessing the old phylloid, but no stipe, and 0.8 in plants possessing their old phylloid and stipe. It is concluded from this experiment that the bulk of reserve materials supporting the growth of the new frond are stored in the old frond. Moreover, it seems that in darkness the stipe consumes part of the reserve materials deposited in the old frond, particularly from April onwards. This is inferred from the better growth of new fronds without stipes (but with old fronds) if compared to new fronds with stipes and old fronds (Fig. 7, Fig. 8).

It does not seem likely, however, that this "parasitic behaviour" of the stipe occurs in young *Laminaria hyperborea* specimens growing in the light. Some preliminary experiments on photosynthesis and respiration of *L. hyperborea* stipes give the impression that brown stipes of young plants make up for their needs of respiration by their own photosynthesis. In old specimens, however, stipes are often covered with epizoans (*Membranipora* spec.) for most of their length. Hence, "parasitic behaviour" is to be expected in stipes of old specimens. This may partly explain the fact that the normal rhythm in the growth of young plants, of producing each year a larger frond, finally ceases.

Comparison has been made of the new frond area produced in darkness in plants with old phylloid, but without stipe (2.2 dm^2 = 2.4 dm^2 attained in May minus 0.2 dm^2 already present at the beginning of darkness) with the new frond area attained by specimens of similar size growing under natural conditions at 2.5 m water depth (new frond area in May about 8 dm^2). It seems reasonable to suggest that about 30 % of the new frond area produced during the period of rapid growth, i. e. the first half year, is due to reserve materials stored mainly in the old frond.

It should be mentioned that some preliminary experiments on frond growth in darkness were also performed with larger specimens of *Laminaria hyperborea* (stipe length about 80 cm, area of old frond in February about 18 dm^2). Growth of the new phylloids was very slow in these specimens. The majority of experimental algae showed effects of fouling some weeks after the beginning of the dark regime. This finding seems to indicate that the capacity of *L. hyperborea* to produce part of the new phylloid even in complete darkness decreases with increasing age.

SUMMARY

1. Two and three year old specimens of *Laminaria hyperborea* were transplanted to sub-tidal growth stations at 2 and 6 m water depth, respectively.
2. Maximum growth rate occurred in early May in specimens growing at 2 m depth, and in early June at 2 m depth.
3. Frond area size, attained by the specimens growing at 6 m depth in July, was at least 75 % of frond area size attained at 2 m depth. Stipe length of plants at 6 m depth, as recorded in July, was only about $1/3$ of the stipe length attained at 2 m depth.

4. Growth rates of new phylloids of *Laminaria hyperborea* specimens kept in darkness from February until May are considerably reduced, if the old frond is amputated.

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