GROWTH IN SOME LAMINARIALES

20 August 1982

Annette M. Olson

Marine Algology
Botany 545a

Friday Harbor Laboratories
Friday Harbor, Washington, U.S.A.

The intercalary growth pattern of kelps (Phaeophyta, Laminariales) is well-documented (e.g., Bold and Wynne 1978). However, the degree to which the region of elongation is restricted to the base of the frond, varies interspecifically and seasonally (e.g., Calvin and Ellis 1981, Fallis 1915 1916, Kain 1976b, Sundene 1964). The relative rates and timing of elongation and broadening in various regions of a thallus signal the developmental events (cell division and cell growth) which ultimately result in the gross morphology of the adult plant. Consequently, accurate measures are needed of the rate, location, and duration of growth within the thallus in order to understand the developmental origins of kelp forms.

Techniques for studying growth in kelps vary in their accuracy in measuring growth and their specificity in locating growth regions. In field studies, when the length of the blade is measured to determine total elongation (e.g., Burrows 1964, Chapman and Craigie 1977, Fallis 1915 1916, Kawashini 1972, Parke 1948), erosion of the distal portions of the blade can be a major source of error. To overcome this difficulty, investigators have been successful in using natural features of the thallus (e.g., the "collar" produced in the slow growth season of some Laminariaceae, John 1970, Kain 1976a, Setchell 1905; or the internodes of <u>Macrocystis</u>, Clendenning 1964, Neushul and Haxo 1963, North 1971 1972) as reference points against which to measure subsequent growth. However, this method does not permit identification of regions of maximum growth in the thallus. Additionally, in most kelps, such points of reference are present only seasonally, if at all. Consequently, researchers have developed a variety of artificial markers which permit both accurate measurement of growth rates (corrected for erosion) and specific identification of growth regions. These methods include (1) cutting the thalli to different initial lengths, protecting them from erosion, and measuring overall elongation of each thallus after growth (Fallis 1915 1916, Parke 1948), and (2) marking the thallus with one or a series of scratches (Fallis 1916), notches Sheldon 1915) or holes (Calvin and Ellis 1981, Chapman and Craigie 1977, Fallis 1915, Kain 1976a 1976b, John 1970, Mann 1972, Parke 1948, Sundene 1964, Widdowson 1971) and measuring elongation between pairs of adjacent marks.

An additional technique, initially developed for microscope studies of cell wall growth (e.g., Walland and Waaland 1975), has recently been modified for study of macroscopic growth of kelps by Louis Druehl and his colleagues at Bamfield Marine Station, B.C. (Druehl, pers. comm.). By staining the thallus with Calcofluor White--a vital cell wall stain which fluoresces under U.V. light--the investigator may directly visualize regions of thallus growth. Cell walls synthesized after staining (both wall growth and newly divided cells) do not incorporate the stain. Hence, when viewed under U.V. light, areas of the thallus which grow after the plant is removed from the stain bath appear dark, while areas where cell wall material existed prior to staining fluoresce.

In the present study, I had two main objectives: First, to determine the rates, regions, and duration of elongation in a variety of kelps and, second, to compare the results obtained from use of the hole-punch and fluorescent labelling techniques. After reporting these results, I will briefly compare them with the results of previous studies of kelp growth. In conclusion, I will discuss the advantages and disadvantages of the various techniques, concentrating on those used in this study.

MATERIALS AND METHODS

Organisms

The algae used in this study are listed in Table 1. Specimens of *Laminaria longipes*, *L. groenlandica*, and *Pterygophora californica* (hereafter referred to as *Pterygophora*) were dredged from subtidal banks in 12-15 m of water. The specimens of *L. longipes* were from a geographically isolated population at the southernmost extent of the species' range in North America (Markham 1972). The remainder of the plants were collected intertidally on San Juan Island, Washington, or from the floating breakwater at Friday Harbor Laboratories, Washington, U.S.A. After collection, the plants were held for seven to ten days until the initiation of the study in an indoor tank of running seawater, lighted by room lights and daylight from windows. Prior to use, the distal portions of some blades were cut off with a razor blade if they appeared to be deteriorating.

Fluorescent Labelling

Whole plants were stained by immersing them in 0.01% Calcofluor White ST (disodium salt of 4,4'-bis[4-anilino-6-bis-(2-hydroxyethyl)amino-*s*-triazin-2-yl amino]-2-2'-stilbenedisulfonic acid) (American Cyanimid Co., Bound Brook, N.J., U.S.A.) in filtered seawater for 12-24 h. The plants were then rinsed briefly in seawater (without stain) and immediately examined under U.V. light to record any irregularities in the initial staining.

Hole-punch Labelling

Immediately after staining, 2-15 1 mm-diameter holes were punched at a 2 cm spacing down the center of each frond, distally from the stipe-blade transition zone (see Figure 1). On one large *Alaria marginata* plant (hereafter referred to as *Alaria*), the distal sporophyll, rather than the transition zone, was used as the basal reference point. The following initial measurements were made:

a. distance from the first hole in the blade to the first hapteron arising from the the stipe.

b. total length of the blade.

c. total length of selected haptera and sporophylls.

Growth

During the growth period, plants were held in one of two ways: Most plants were attached (by a short nylon cord tied to the stipe) to one of three 1 m nylon lines. These lines, in turn, were attached to a 1 m square PVC frame suspended horizontally at 1 m depth from a floating frame (design, J. Robert Waaland, University of Washington, Department of Botany), moored to the breakwater at Friday Harbor Laboratories. Laminaria groenlandica and L. saccharina specimens were placed under fluorescent lights in a shallow (10-15 cm) seatable in running seawater.

Plants were allowed to grow for 11-16 d. Periodically during the growth period, the distances between pairs of adjacent holes and the distances a. to c., above, were measured. In addition, the plants were regularly examined under U.V. light, the thalli were traced onto paper, and unstained areas representing new growth were sketched onto the tracing. Notes were also made on the general appearance of each plant.

RESULTS

Hole-punch Technique

Rates and Regions of Elongation

Table 2 summarizes the mean daily blade elongation for each species studied. The mean daily growth of each 2 cm blade segment is summarized for the Laminariaceae and Alariaceae in Figures 2 and 3, respectively. In Figure 2, data for two Costaria costata plants (hereafter referred to as Costaria) are omitted because the plants had seriously deteriorated by the end of the study. Error bars on the L. longipes graph represent one standard deviation. In Figure 3, data for a second individual of Alaria (#2) are not included, because the plant was of considerably smaller size than the other plant studied. (For more detailed growth data on selected plants, see Appendix I, Figures I-1 to I-13.)

In the Laminariaceae, the greatest overall rate of blade elongation was observed in Costaria, which increased by 0.819 $cm \cdot d^{-1}$. Laminaria longipes had the slowest rate--0.052 $cm \cdot d^{-1}$. For most species, the region of maximum elongation was the proximal 2 cm blade segment. Costaria increased the most in this segment--30%$\cdot d^{-1}$ (0.600 $cm \cdot d^{-1}$)--while L. longipes increased only 2.1%$\cdot d^{-1}$ (0.042 $cm \cdot d^{-1}$). An exception was Cymathere triplicata (hereafter referred to as Cymathere), where the maximum rate--3.6%$\cdot d^{-1}$ (0.073 $cm \cdot d^{-1}$)--occurred in the second blade segment.

The degree to which the region of elongation was restricted to the basal region of the blade varied among the species observed (see Figure 2 and Table 2). For example, in L. longipes and L. saccharina, 100% and 97%, respectively, of the overall elongation occurred in the proximal 4 cm of the blade, with over 80% occurring in the proximal 2 cm. In Costaria and L. groenlandica, approximately 90% of the total elongation was recorded in the proximal 4 cm, with approximately 70% occurring in the proximal 2 cm. In some L. groenlandica plants, however, measurable elongation was recorded up to the seventh blade segment. Finally, elongation in Cymathere was more diffusely located, with only 52% of the total elongation recorded in the proximal 4 cm. However, measurable elongation was not observed distal to the fourth blade segment.

In the Alariaceae, the larger Alaria specimen (#1) elongated at an overall rate of 2.229 $cm \cdot d^{-1}$, while the smaller plant (#2) grew at 0.091 $cm \cdot d^{-1}$ and Pterygophora specimens grew at a mean rate of 0.150 $cm \cdot d^{-1}$ (Table 2).

The region of maximum elongation was the second blade segment (distal to the distal sporophylls) in Alaria #1, with 25%$\cdot$d^{-1} (0.055 cm$\cdot$d^{-1}) growth; the first segment in Alaria #2, with 3.7%$\cdot$d^{-1} (0.073 cm$\cdot$d^{-1}); and the first segment in Pterygophora, with a mean elongation of 5.9%$\cdot$d^{-1} (0.118 cm$\cdot$d^{-1}).

The region of elongation was quite diffuse in the large Alaria specimen (#1). Only 32% of the total elongation occurred in the proximal two blade segments (Table 2) and daily elongation equal to, or in excess of, 10% was observed in the first through fifth blade segments (Figure 3), accounting for 78% of the total elongation. In Alaria #2 and Pterygophora specimens (all of which lacked sporophylls), elongation was largely restricted to the basal 2 cm, where 100% and 79%, respectively, of the total elongation occurred. Measurable elongation was not observed distal to the first blade segment in Alaria #2 or the fourth blade segment in Pterygophora specimens.

Duration of Elongation

In some plants, a blade segment which was elongating at the beginning of the study ceased its growth during the period of observation. Thus, the total amount of time during which the cells in that blade segment were dividing or growing to produce elongation of the thallus could be estimated (Table 3, Column 5). The average period of growth of a blade segment was approximately 26 d (range 16 to 50 d). For species represented by two or more specimens--L. groenlandica (n=3) and Pterygophora (n=4)--the average estimated duration of growth in a blade segment was 24 d (range 21 to 26 d) and 28 d (range 22 to 40 d), respectively.

Fluorescent-labelling Technique

The results of the fluorescent staining procedure are shown in Figures 4 to 10. Laminaria saccharina (Figure 7) displayed the "typical" intercalary growth pattern of the Laminariaceae, with a dark band of unstained new tissue at the base of the lamina. The intermediately stained zone distal to the dark band represents a region where cell wall material existed at the time of staining, but where new cell walls were synthesized throughout the region after staining. The stipe and distal portion of the lamina remained brightly stained, indicating that, after staining, little or no new cell wall material was synthesized in these areas.

The length of the first blade segment on the tracing in Figure 7 (6.8 cm) is 13% shorter than that measured on the living plant (7.8 cm) (see Appendix I, Figure I-11), because the somewhat bullate blade could be stretched flat when measuring with a centimeter scale. (This underestimation of elongation was found to varying degrees in all tracings made.) Nevertheless, the width of the dark basal band of tissue (5.3 cm) was consistent with the measured growth of the basal blade segment (5.8 cm), indicating that nearly all of the elongation of this segment could be attributed to the production of new tissue at the base of the blade. The tracing also suggests that all of the 0.9 cm of elongation observed in the second blade segment was produced by diffuse growth. Note that the 0.2 cm of measured elongation in the third blade segment was not visible as a darkening of the lamina.

Laminaria longipes, L. groenlandica and Costaria represent modifications of the pattern seen in L. saccharina. Beginning with the simplest case, L. longipes (Figure 6), zones of unstained and intermediately stained tissue were observed on the stipe (proximal to the unstained band at the base of the blade), suggesting elongation of the stipe during the period of observation. This pattern was observed in only two specimens of L. longipes examined. Due to problems encountered in measuring stipe length, however, it was not possible to confirm that the stipe elongation suggested by these results had indeed occurred.

Laminaria groenlandica (Figure 5) presented a more complex pattern of growth. The non-bullate surface of the lamina showed the typical dark basal region, with intermediately and brightly stained zones distally. However, in the intermediately stained zone, the bullae were unstained and, in the brightly stained zone, the bullae were intermediately stained. Apparently, the bullae continue to grow in both length and breadth after growth has ceased in the surrounding areas, thus forcing the bullae out of the plane of the blade as they grow. This is a case where non-elongate growth appears to result in a dark pattern on the blade. Consequently, when using the fluorescent labelling technique to monitor growth, elongation cannot always be inferred from observations of unstained areas. This limitation becomes particularly apparent when considering the most complex pattern of growth observed in this study--that of Costaria costata.

In Costaria (Figure 4), brightly stained ribs extend through the intermediately stained and unstained portions of the blade. Superficially, this result suggests differential rates of elongation of rib and adjacent blade, an interpretation which requires that the blade must somehow slide past the ribs as it grows. A more feasible interpretation, however, is that while the ribs and blade elongate at the same rate, lateral growth of the blade between the ribs is much greater, resulting in relatively more "dilution" of the stain on the blade than on the ribs. In addition, lateral growth of the blade may continue after elongation has ceased. Consequently, the portion of the rib which elongated slowly or not at all after staining would appear bright, while the adjacent blade which grew laterally after staining would appear dark. Thus, differential rates of elongation need not be invoked to account for the observed pattern.

This interpretation was supported by the measurements reported in Figure I-1, where 4.2 cm of elongation was recorded in the first blade segment 11 d after staining. The unstained and intermediately stained portions of the ribs in this segment accounted for 80% (3.4 cm) of the recorded elongation. Thus, elongation of the ribs was about the same as that of the blade (in which the hole was punched), taking into account the under-estimation of growth in the tracing. Note, however, that the elongation in the second through fourth blade segments (2.3 cm, 0.7 cm, and 0.4 cm, respectively) was not visible as darkening of the ribs in these segments. This may be the result of relatively brighter initial staining of the ribs.

In the Alariaceae, a different pattern of growth was observed. In a small specimen of Alaria (#2) lacking sporophylls, 75% of the elongation of the first blade segment (0.8 cm) (Table 2) was accounted for by a basal dark band (0.6 cm) on the mid-rib (Figure 9). Distal to this band, the mid-rib was brightly stained, but the adjacent portions of the lamina were dark or intermediately stained. Measurable elongation was not observed distal to the basal blade segment. These results suggest that elongation occurred mainly in the most basal portion of the mid-rib and that subsequent lateral growth of the blade was not accompanied by elongation. In a larger specimen (#1), however, this pattern was not observed. Rather, elongation occurred up to 59 cm distal to the distal sporophyll. Both longitudinal and lateral growth in the lamina were so rapid that specific boundaries of the fluorescent stain were not visible.

In the specimens of Pterygophora (all of which lacked sporophylls), a growth pattern similar to that observed in the small Alaria was observed. Approximately 50% of the measured elongation (e.g., Figure I-13) in the basal blade segment (3.0 cm) can be attributed to a 1.5 cm dark band at the base of the blade (Figure 10). A brightly stained medial area (distal to this basal band) is flanked by dark lateral "wings." It appears that elongation is restricted to the basal portion of the lamina, but that, after longitudinal growth slows or ceases medially, lateral growth continues on the basal edges of the lamina. Note that the 0.6 cm elongation of the second segment was not visible as a darkening of the lamina in this segment.

Additional Observations

Haptera

Hapteron elongation was monitored in L. saccharina (n=1) and Pterygophora (n=3), but measurable growth was observed only in L. saccharina. Mean daily growth of 5% and 2% occurred in two haptera (Figure I-10). Localized dark areas were not observed on these haptera when examined under U.V. light.

Sporophylls

In the larger specimen of Alaria (#1) (Figure 8), the petioles of the two proximal pairs of sporophylls were unstained at the end of the period of observation (16 d). During the final 11 d of observation, the petioles of the proximal pair of sporophylls did not elongate, but those of the second pair increased from 1.5 to 3.0 mm. The lengths of the third pair of sporophylls increased 132% and 283% over the final 11 d of observation. Their blades were unstained and their petioles intermediately stained at the end of the period of observation. The distal pair of sporophylls became measurable between the third and sixth day of observation. By the sixteenth day, their lengths had increased 75% and 100% and their petioles and blades were entirely unstained. The stipe in the area of sporophyll attachment was intermediately stained and between the distal sporophyll and the base of the blade it was unstained at the end of the period of observation. These results suggest that in Alaria sporophylls arise distally, elongate rapidly throughout their length, and upon approaching maturity grow only in the petiole.

DISCUSSION

Growth

Rates of elongation reported for plants in the Laminariales vary considerably among species (Appendix II), from 0.03 cm·d^{-1} for Laminaria groenlandica growing at 7-12 m depth (Calvin and Ellis 1981) to ≤45 cm·d^{-1} for Macrocystis pyrifera (North in Clendenning 1964). Additionally, rates vary seasonally, with peak growth for Laminaria species occurring from January to July, depending upon species, depth, and site (Calvin and Ellis 1981, Chapman and Craigie 1977, Kain 1976a, Mann 1972, Parke 1948, Sundene 1964). Mann (1972) reports that Agarum cribrosum reaches its maximum growth in March to June, depending upon the site. In Alaria species, peak growth occurs in April to June (Widdowson 1971).

Similarly, reported regions of maximum elongation in the thallus vary from the basal 1 cm in Alaria costata (=?) to a region 5-15 cm distal to the base of the blade in L. hyperborea (Kain 1976b). In addition, Kain (1976b) and Sundene (1964) report that the region of maximum growth varies seasonally in Laminaria species. Consequently, when evaluating the results of the present study, midsummer growth data must be compared for each species.

Costaria grew in the present study (Table 2) at 40% to 60% of the rates observed by Fallis (1916) (see Appendix II). While I found the growing region restricted to the basal 8 cm in Costaria (Figure 2), Fallis observed growth throughout the basal 30 cm of the blade. Factors which could account for these discrepancies include (1) the small sample sized (n=1 in the present study; n presumably =1 in Fallis 1916), (2) the relatively small initial size of the specimen in the present study (16.5 cm) compared to that in Fallis' study (35 cm). Kain (1976b), finding that the region of elongation was more restricted in smaller plants, defined the growing region in terms of percent of total blade length, rather than in absolute values. However, corrected for blade length, the growth region of Fallis' plant is still considerably more extensive than in the plant I examined. Perhaps factors such as light, water quality, or age or health of the plants influence the duration of growth activity in a specific region of the thallus. Thus, if a portion of the blade could be induced by such factors to grow for a longer duration, then more of the blade would be involved in elongation and a greater growth rate would result.

Cymathere (Table 2) grew at only 21% the rate observed by Fallis (1916)

(Appendix II). In the present study, the growing region extended through the basal 8 cm of the blade, while Fallis observed that most of the growth occurred within 100 cm of the base of the lamina. Again, the small sample sizes and the relatively small specimen in the present study (12.9 cm) compared to that studied by Fallis (190 cm) may account to some degree for the differences. In addition, most Cymathere specimens used in this study did not do well. They rotted and grew poorly before they were lost from the floating frame. The one remaining plant was only just recovering a "healthy" appearance at the end of the study.

Calvin and Ellis (1981) found Laminaria groenlandica to be very slow growing in July (Appendix II). It grew approximately 0.05-0.07 $cm \cdot d^{-1}$ in shallow water and approximately 0.03 $cm \cdot d^{-1}$ in deeper water. They report that the region of maximum growth varied between individuals, from the basal 10 cm in some to throughout the blade in others. In contrast, I found that L. groenlandica grew much faster (0.284 $cm \cdot d^{-1}$) (Table 2), with virtually all of the measurable growth concentrated in the basal 8 cm (Figure 2). The plants and the growing conditions differed considerably between the two studies, however. Calvin and Ellis marked and grew established plants in situ at 3-12 m depths. I selected several small individuals (5.1-15.0 cm) and, during the period of observation, allowed them to grow in 10-15 cm running seawater in a seatable lighted by fluorescent lights approximately 15 cm above the water's surface. Consequently, higher growth rates might be expected under these conditions.

Published growth data for Laminaria longipes were not available. However, the extremely slow observed growth rates (0.05 $cm \cdot d^{-1}$) are comparable to those reported by Calvin and Ellis (1981) Kain,(1976b), and Sundene (1964) for subtidal Laminaria species during their slow growth periods. Year-round growth data for L. longipes could test the hypothesis that the observed midsummer growth rates represent the slow-season growth for this species.

Parke (1948) recorded midsummer elongation of 1.0 cm·d^{-1} for *Laminaria saccharina* (Appendix II). Of the total elongation, 65% occurred within the basal 2.5 cm of the blade and the remaining 35% within the next 7.5 cm. In the present study, I found growth in *L. saccharina* to be comparable. It grew at 0.627 cm·d^{-1}, with 73% of the total elongation occurring within 2 cm of the transition zone (Table 2 and Figure 2).

In the present study, the large *Alaria marginata* plant grew at a rate (2.229 cm·d^{-1}) comparable to that reported for the species (≈2.0-2.5 cm·d^{-1}) by Widdowson (1971) (Appendix II). However, while Widdowson found growth restricted to the transition zone and basal portions of the midrib, I observed elongation throughout most of the blade. In a smaller *Alaria* specimen, growth was restricted to the basal 2 cm of the blade, but the growth rate (0.074 cm·d^{-1}) was not comparable to that reported by Widdowson. I cannot speculate on the reasons for these discrepancies without additional information on the relative sizes and growing conditions of the plants in Widdowson's study.

Published growth data were not available for *Pterygophora californica*. The relatively slow growth rate (0.150 cm·d^{-1}) and the relatively restricted region of elongation are, however, similar to the results for the small *Alaria* specimen. Perhaps this pattern is typical of Alariaceae lacking sporophylls.

Although the results of fluorescent-labelling studies of kelp growth have not yet been published, some published and unpublished studies suggest patterns which might be expected for various species. For instance, Parke (1948) describes a region of new tissue formation ("primary growth") within 2.5 cm of the base of the lamina in *L. saccharina*, with a zone of cell division and cell elongation ("secondary growth") 2.5-7.5 cm from the base. These regions may correspond to the unstained and intermediately stained bands observed in the Laminariaceae in the present study. If cytological studies support this notion, fluorescent labelling would prove a very simple and graphic method of identifying the regions of primary and secondary growth in a thallus.

In a second related study, Druehl (pers. comm.) has observed a "wing-like" lateral growth region at the base of the lamina in *Alaria* in unpublished studies using fluorescent labelling methods. That a similar pattern was observed in immature *Pterygophora* (as well as in *Alaria*) in the present study, suggests that such a growth pattern may be typical of the Alariaceae. In contrast to *Laminaria* species, where lateral growth seems to accompany elongation, increase in breadth seems to occur subsequent to the cessation of elongation in young *Pterygophora* and *Alaria*. Interestingly, a similar "wing-like" pattern of growth was observed in *Cymathere* in the present study (pers. obs.). (A tracing and sketch of the fluorescent labelling results were inadvertently not recorded for this species.)

Techniques

A measurement technique for the study of kelp growth must meet three criteria: The technique must produce the desired information accurately, it must be relatively simple to use, and it must result in a minimum of trauma to the plants under study. The simplest, least traumatic techniques--measurement of overall thallus length and use of natural features of the thallus as points of reference--unfortunately provide little information on the relative rates of growth in different portions of the thallus. Early attempts to more specifically locate growth regions by cutting the blade to various initial lengths involve slightly more work and may seriously traumatize the plant. For instance, Fallis (1915) found that the basal 3 feet of *Nereocystis* luetkeana fronds cut into 3-inch segments grew a total of 38% less than control fronds of the same initial length which were left intact. Methods in which the plant is marked with notches, scratches, or punched holes represent a compromise in the amount of trauma inflicted on the study organisms while permitting very specific localization of growth. The precision of hole-punch or other such marking techniques may be increased by minimizing the distance between markers. By marking the thallus with a grid, two-dimensional patterns of growth may also be observed.

Such methods have several limitations, however. First, the marks may provide an entry-way into the thallus for disease. In the present study, Cymathere plants began rotting around the punched holes (pers. obs.). Second, in some species, the marks may themselves alter growth rates or patterns. Widdowson (1971), for example, reported that elongation ceased in Alaria when a hole was made in the transition zone and that sorus development was inhibited by holes punched in the sporophyll. Finally, the marks may structurally weaken the thallus.

These negative effects may be partially offset by minimizing the size of the marker with respect to the size of the thallus. However, in small plants, or when a closely-spaced grid would be required to determine relative rates of growth in differen regions of the thallus, the limit of utility of the hole-punch technique is reached. Here, the fluorescent-labelling method may be the most appropriate technique. Plants may be labelled at any size and, after growth patterns have been recorded, may be re-labelled.

The most striking advantage of the fluorescent-labelling method is its ability to demark visible boundaries between non-synchronous regions of growth in a thallus. This capacity may seem trivial in the case of L. saccharina, where it may be easily demonstrated by the hole-punch technique that the distal portion of the blade has ceased elongation, while the proximal region continues to grow. By the fluorescent-labelling technique, however, the bullae of L. groenlandica are shown to arise not simply by faster growth than the surrounding flat surfaces, but by more prolonged growth. Similarly, in Costaria, Alaria, and Pterygophora, lateral growth is seen by this method to begin or continue after elongation of the region has ceased. These more complex patterns of growth would be quite difficult, if not impossible, to analyze by the hole-punch technique.

Fluorescent-labelling nevertheless has several limitations. First, the staining procedure seems to traumatize some plants. In the present study, several plants which appeared healthy prior to staining seemed harmed by the prolonged staining (12-24 h) and either died or did not grow after staining. Improved circulation and aeration in the staining bath may correct this problem.

Second, the direction of growth in the thallus cannot necessarily be inferred from fluorescent-labelling results, alone. For instance, lateral growth between the ribs in Costaria initially was confused with elongation. Only by comparing the hole-punch results could the total amount of elongation be determined and a feasible interpretation made of the fluorescent-staining results.

Finally, comparison with hole-punch results in the present study indicates that the technique is not as accurate in defining rates of growth. Where discrete boundaries between growing and non-growing regions are not visible--regions of diffuse growth or of extreme fast or slow growth--accurate determination of growth rates is not possible. Brighter U.V. illumination than that used in the present study would improve the contrast visible between stained and unstained areas of the thallus (Druehl, pers. comm.). Where more accuracy is needed, the punched-hole method could be used in conjunction with fluorescent-labelling. To further define the uses and limitations of this technique, future studies should incorporate these suggested improvements and compare two-dimensional growth results for both the punched-hole grid and fluorescent-labelling methods.

ACKNOWLEDGEMENTS

L. Druehl and J.R. Waaland suggested the topic for this study, which was conducted as a term project for the Marine Algology (Bot 545a) class at Friday Harbor Laboratories, Washington, U.S.A. in the summer of 1982.
I wish to thank them and A. Sylvester for their support, both logistal and moral, during the project.

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Table 1. Algae Studied

Family/Species	No. Individuals Initial	Final	Mean (Range) Initial Length (cm) Blade+	Stipe
Laminariaceae				
Costaria costata (C. Ag.) Saunders	4	3	8.6 (4.1-16.5)	4.3 (1.7-5.9)
Cymathere triplicata (Post. & Rupr.) J. Ag.	4	1	12.9	3.9
Laminaria groenlandica Rosenvinge	5	5	10.5 (5.1-15.0)	1.2 (0.8-1.6)
L. longipes Bory	19	12	9.7 (5.2-19.8)	9.7 (2.0-19.1)
L. saccharina (L.) Lamouroux	1	1	6.8	2.0
Alariaceae				
Alaria marginata Post. & Rupr.	2	2	15.4, 43.5	12.4, 7.4
Pterygophora californica Rupr.	6	4	11.0 (9.0-15.0)	14.7 (8.1-21.4)

+Most plants were pruned to remove rotting tissue. Pruned length given.

Table 2. Contribution of Five Basal Blade Segments to Overall Elongation of Laminae

Species	Total Elongation ($cm \cdot d^{-1}$)	Percent Total Elongation in Blade Segment 1st	2nd	3rd	4th	5th
Costaria (n=1)	0.819	73	18	5	3	-
Cymathere (n=1)	0.173	10	42	37	10	-
Laminaria groenlandica (n=5)	0.284	70	18	5	1	1
L. longipes (n=12)	0.052	81	19	-	-	-
L. saccharina (n=1)	0.627	84	13	3	-	-
Alaria (#1)	2.229	10	22	21	15	9
Alaria (#2)	0.073	100	-	-	-	-
Pterygophora (n=4)	0.150	79	13	4	4	-

Table 3. Duration of Growth in Selected Blade Segments

	(1) Distance From Base of Blade		(2)	(3)	(4)	(5)
Species (Specimen #)	Initial (a)	Final (a)	Daily Growth	Prior Growth	Observed Growth	Total Growth
<u>Costaria</u> (#1)	4.0 - 6.0 cm	10.1 - 12.7 cm	0.55 cm·d^{-1}	7.3 d	11 d	18.3 d
	6.0 - 8.0	7.7 - 10.0	0.61	9.8	6	15.8
<u>Cymathere</u> (#3)	4.0 - 6.0	4.7 - 7.3	0.09	44.4	6	50.4
<u>Laminaria groenlandica</u> (#1)	2.0 - 4.0	3.0 - 5.3	0.13	15.4	8	23.4
" " (#2)	2.0 - 4.0	3.2 - 5.6	0.15	13.3	8	21.3
" " (#7)	2.0 - 4.0	2.9 - 5.0	0.11	18.2	8	26.2
<u>L. longipes</u> (#7)	2.0 - 4.0	2.6 - 4.8	0.10	20.0	6	26.0
<u>L. saccharina</u> (#1)	4.0 - 6.0	7.9 - 10.1	0.49	8.2	8	16.2
<u>Alaria</u> (#1)	24.0 -26.0	45.8 - 48.0	1.98	12.1	11	23.1
	26.0 -28.0	48.0 - 50.2	2.00	13.0	11	24.0
<u>Pterygophora</u> (#3)	2.0 - 4.0	3.2 - 5.4	0.11	18.2	11	29.2
" (#4)	2.0 - 4.0	4.0 - 6.6	0.18	11.1	11	22.1
" (#5)	2.0 - 4.0	2.7 - 5.1	0.12	16.7	6	22.7
" (#6)	2.0 - 4.0	2.8 - 5.0	0.07	28.6	11	39.6

(1) Distance of segment from base of blade (a) initial, (b) final.

(2) Mean daily elongation of blade proximal to the segment during period of observation.

(3) Time to reach initial position (Column 2 × Column 1a(lower #)).

(4) Days of observation from initial position until growth ceased.

(5) Estimated total days of growth (Column 3 + Column 4).

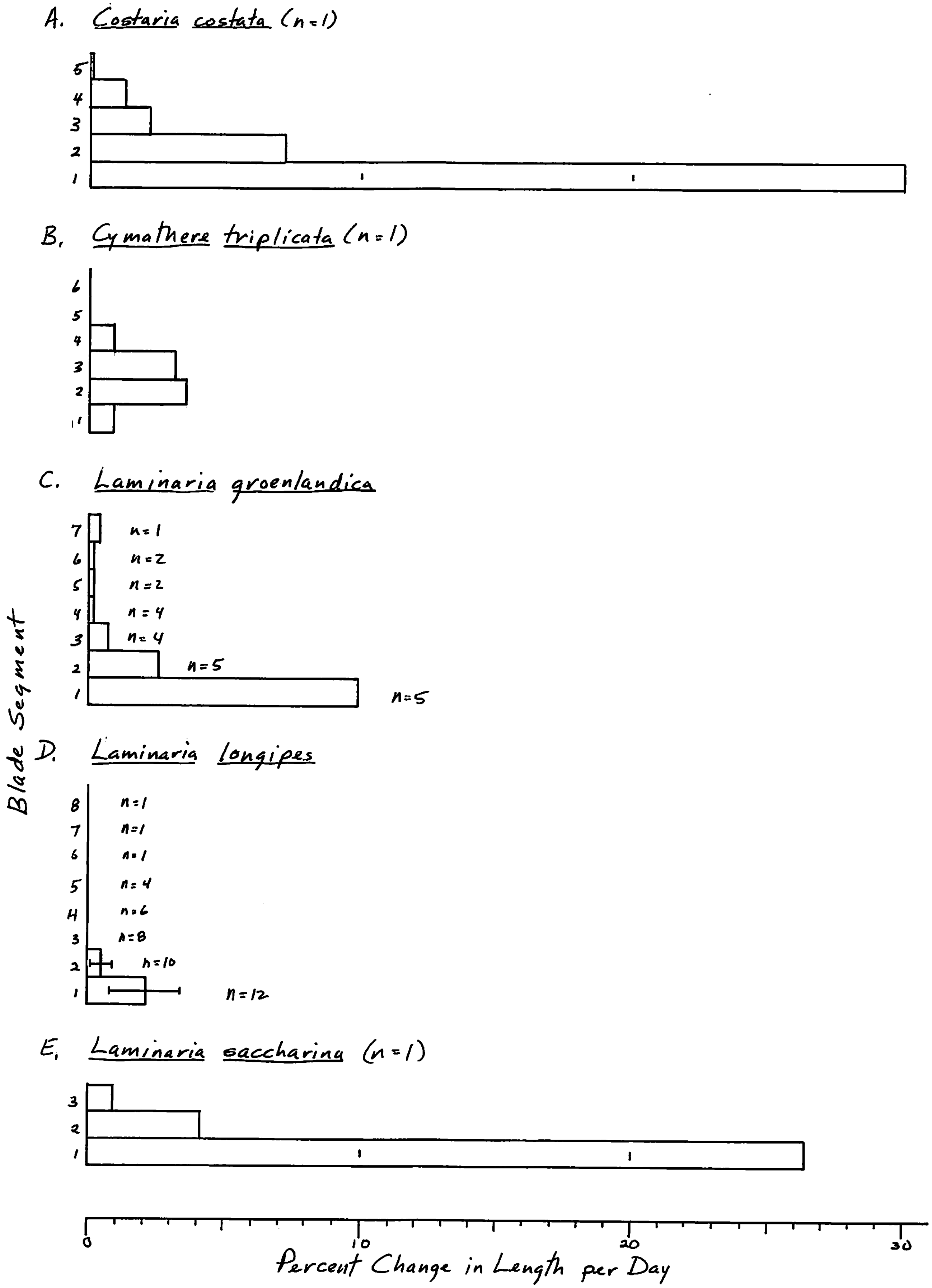


Figure 2. Mean Daily Growth: Laminariaceae

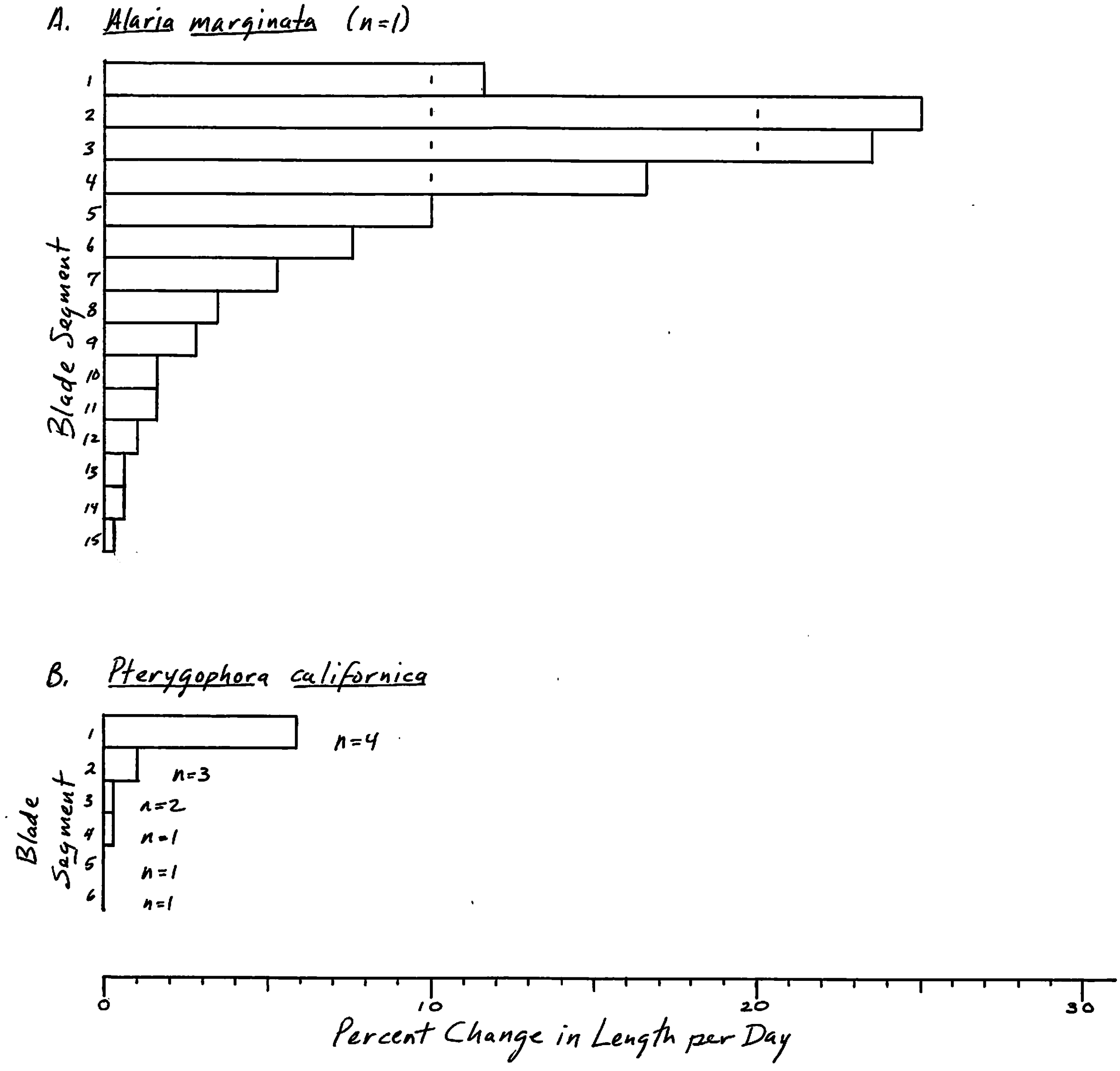


Figure 3. Mean Daily Growth: Alariaceae

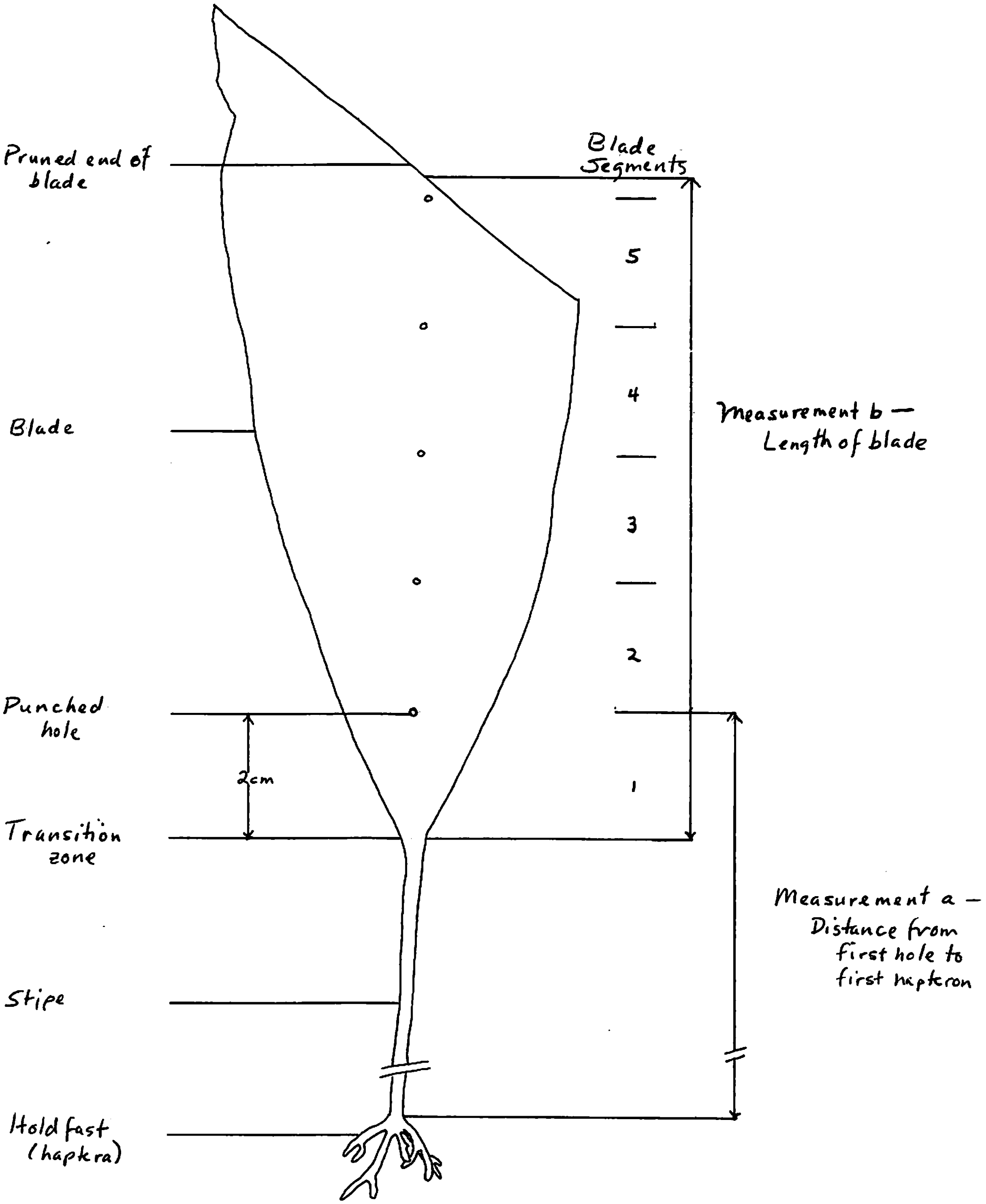
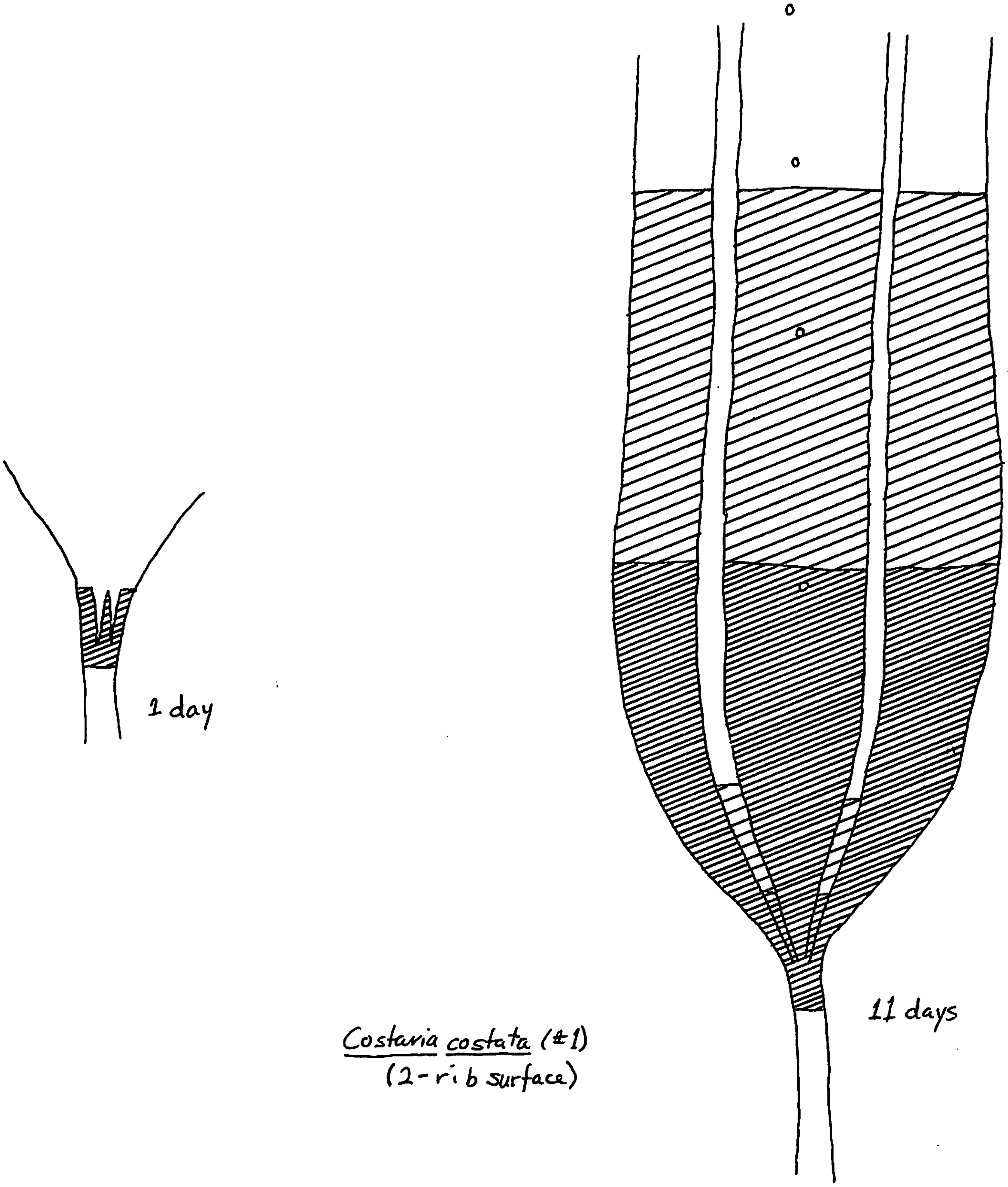


Figure 1. Hole-punching Technique — Terminology

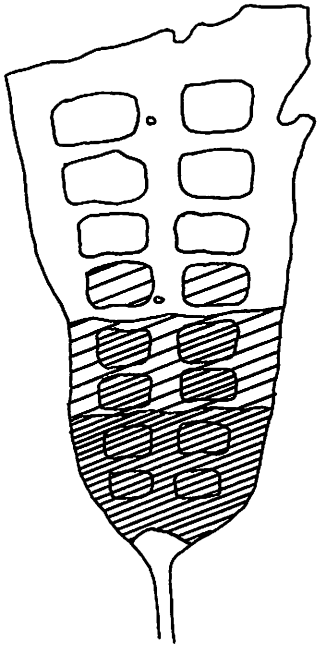
Figures 4 to 10. Key

□	Stained portion of thallus. Bright white fluorescence under U.V. light.
▨	Stained portion of thallus Dim (intermediate) white fluorescence under U.V. light.
▩	Unstained portion of thallus Appears dark under U.V. light.



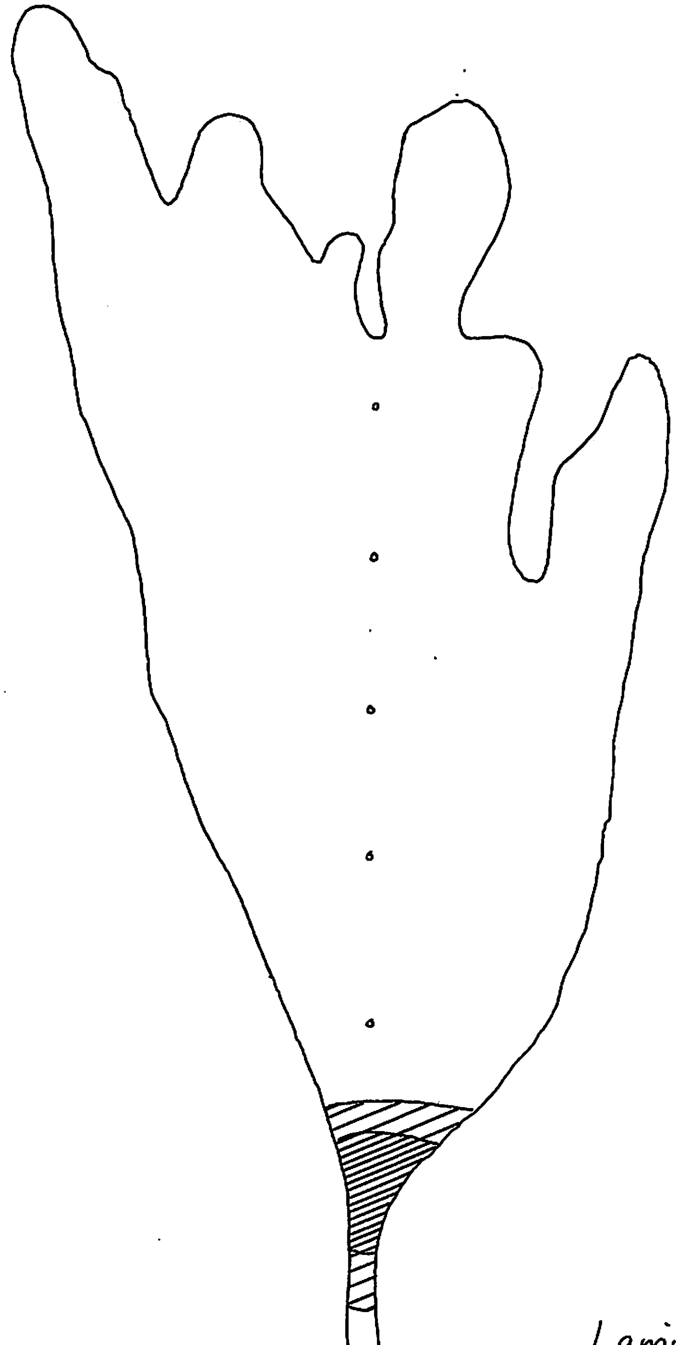
Costaria costata (#1)
(2-rib surface)

Figure 4. Calcofluor Stain After Growth



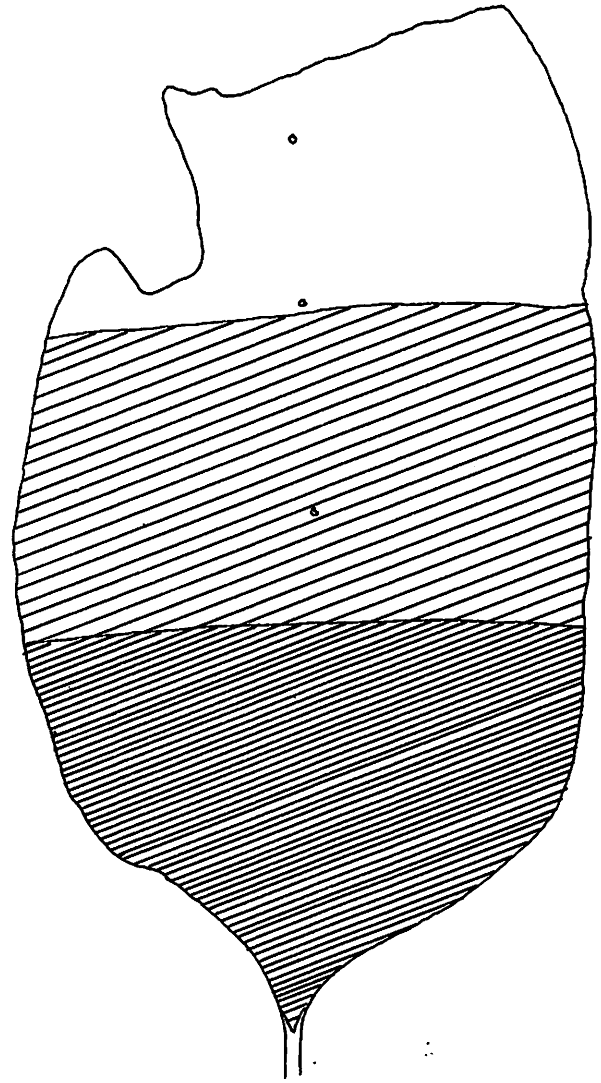
Laminaria groenlandica (#1)
11 days

Figure 5. Calcofluor Stain After Growth



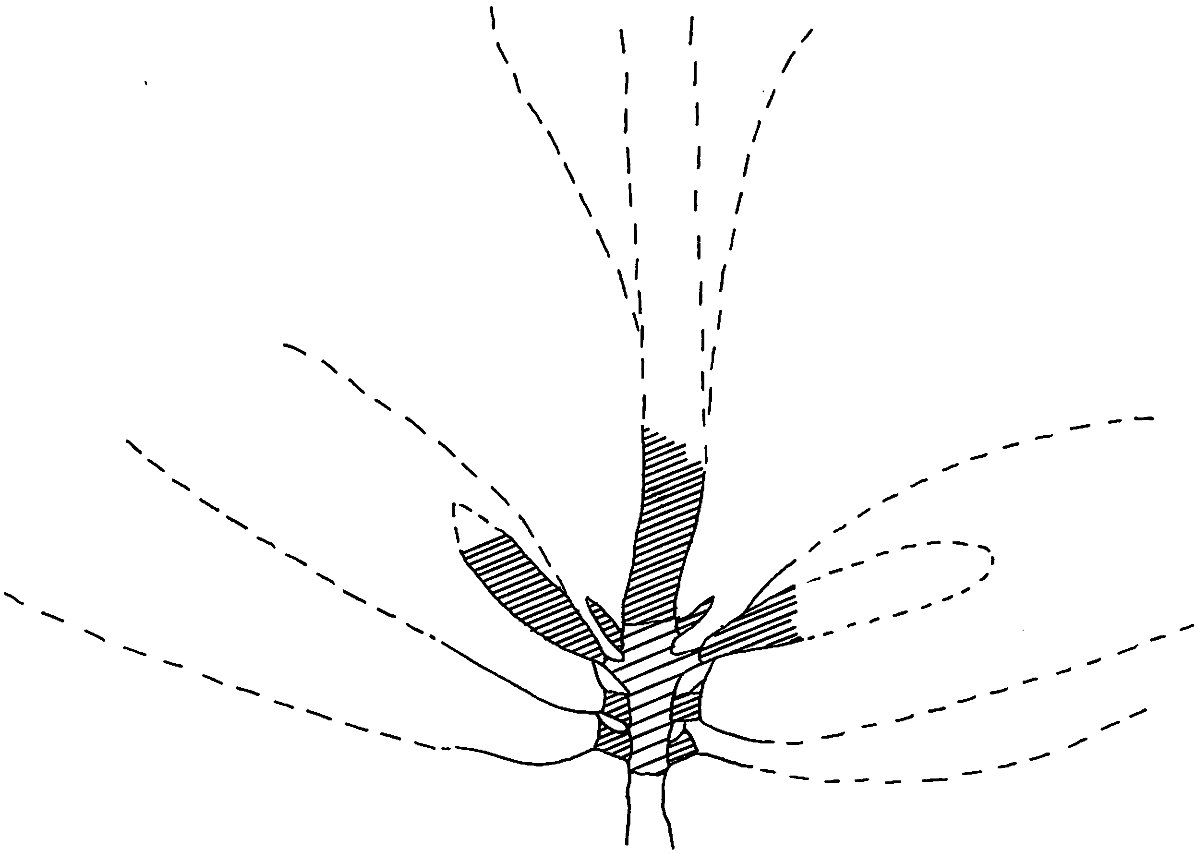
<u>Laminaria</u> <u>longipes</u> (#7)
11 days

Figure 6. Calcofluor Stain after Growth



Laminaria saccharina
11 days

Figure 7. Calcofluor Stain After Growth



<u>Alaria</u> <u>marginata</u> (#1)
11 days

Figure 8. Calcofluor Stain After Growth

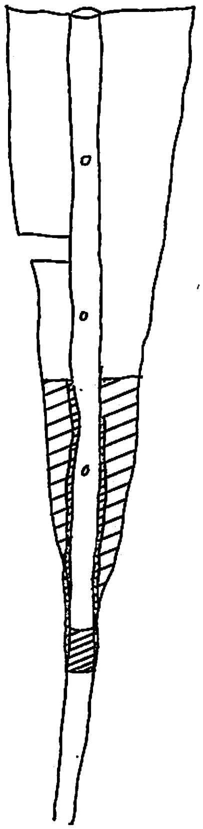


Figure 9. Calcofluor Stain After Growth

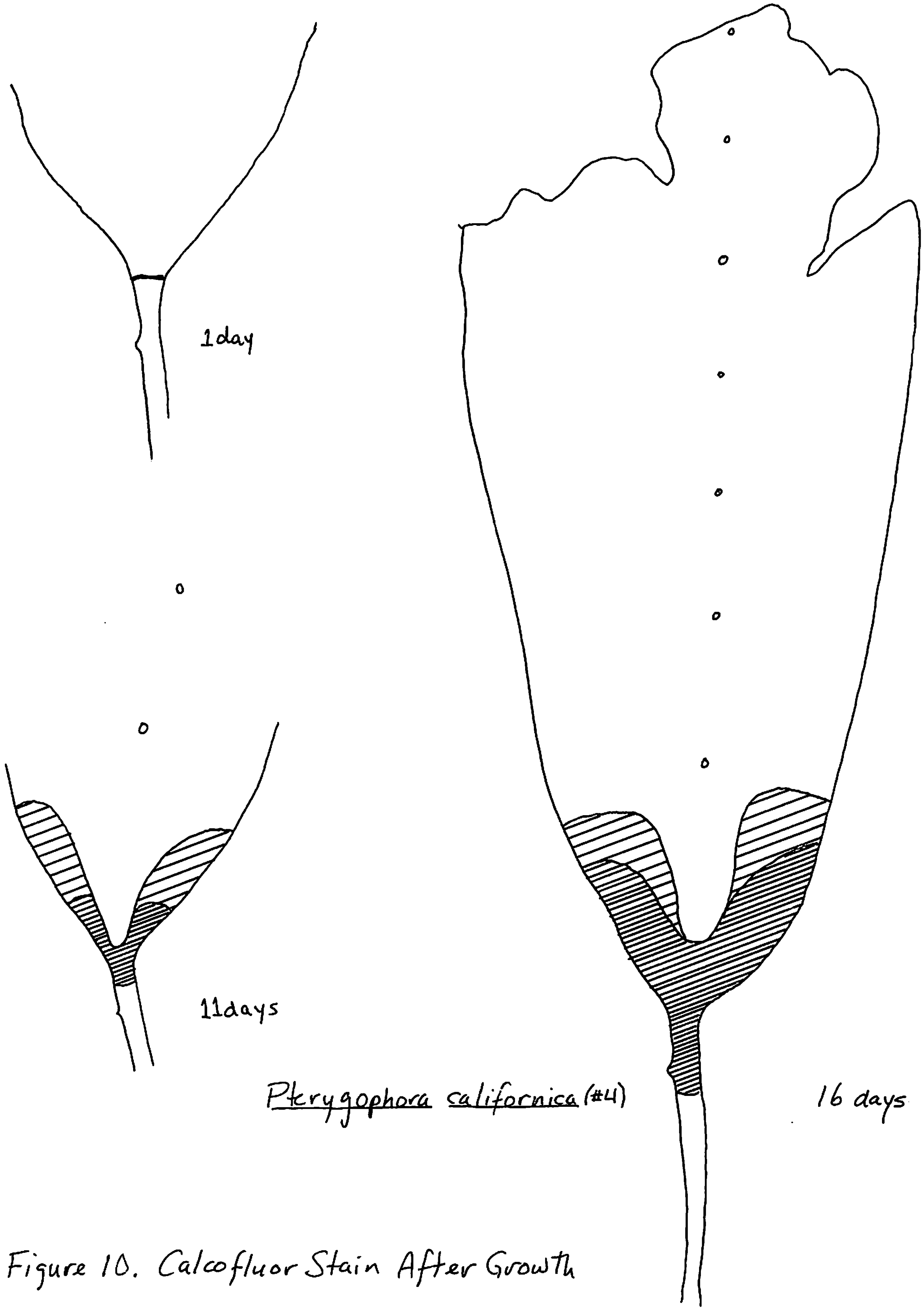


Figure 10. Calcofluor Stain After Growth

APPENDIX I. Key

▨ Measurable growth (≥ 0.2 cm) in blade segment during period indicated

☐ No measurable growth (<0.2 cm) in blade segment during period indicated

Figure I-1. Length of Blade Segments: Costaria costata (#1)

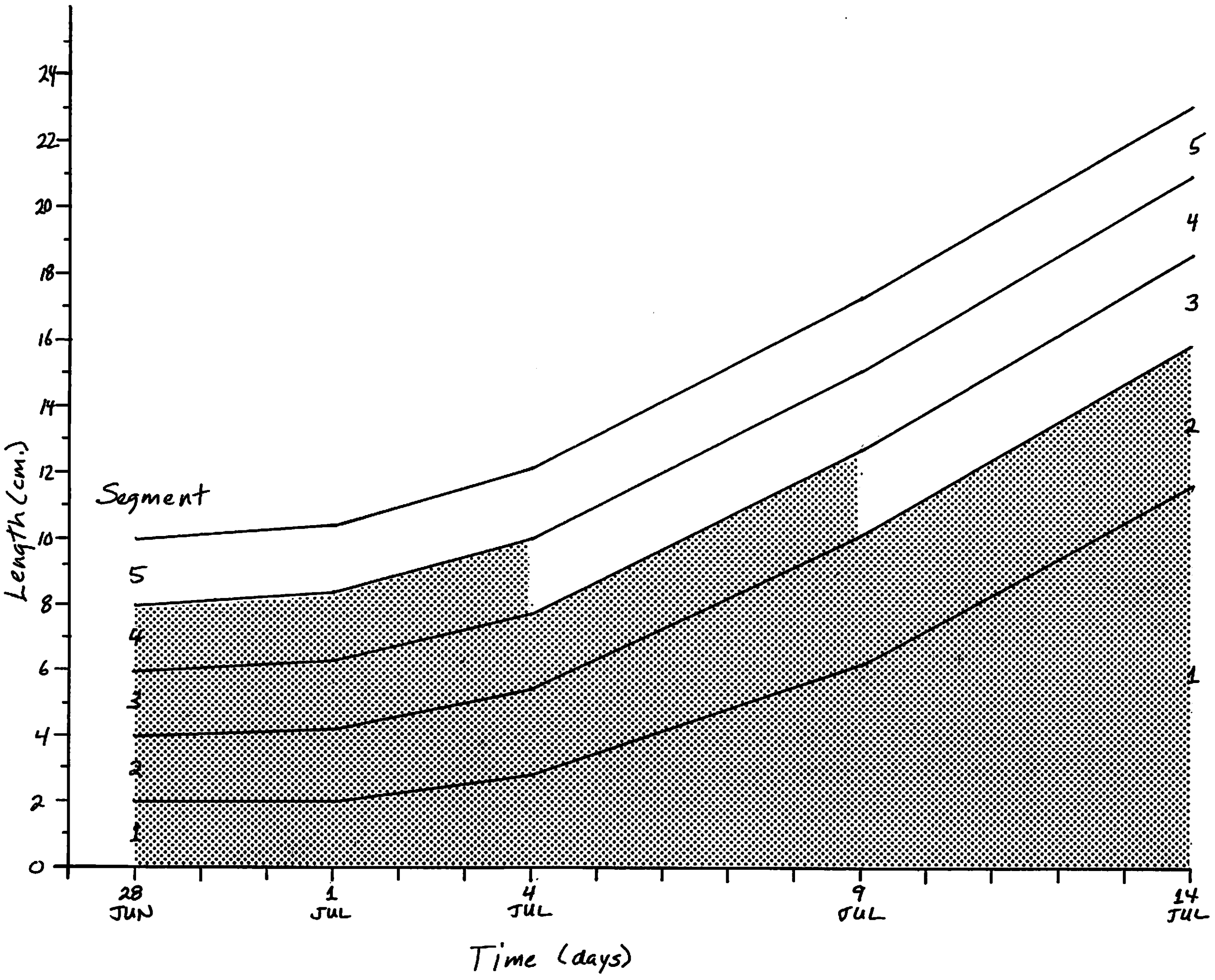


Figure I-2. Length of Blade Segments: <u>Costaria</u> <u>costata</u> (#4)

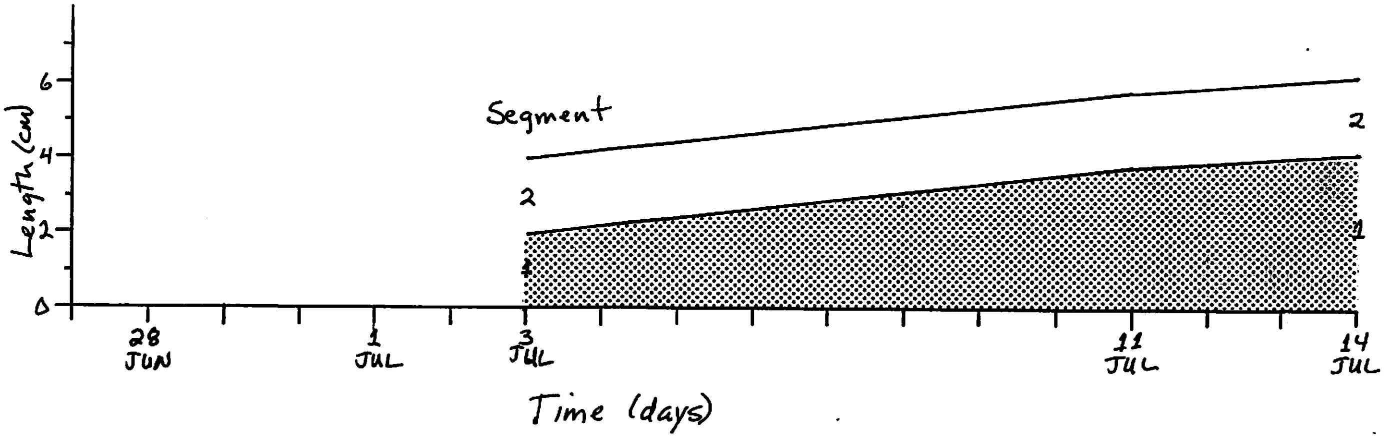


Figure I-3. Length of Blade Segments: <u>Cymathere</u> <u>triplicata</u> (#3)

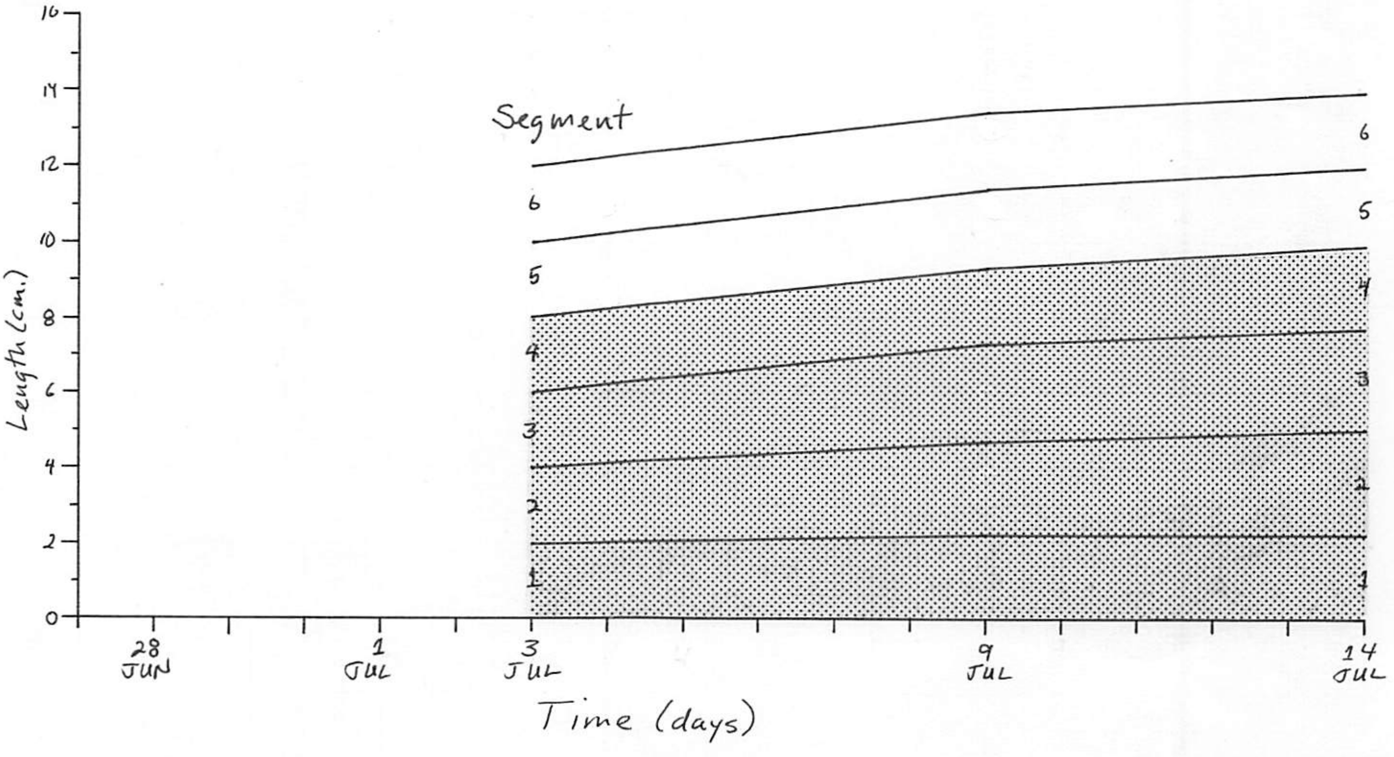


Figure I-4. Length of Blade Segments: _Laminaria_ _groenlandica_ (#1)

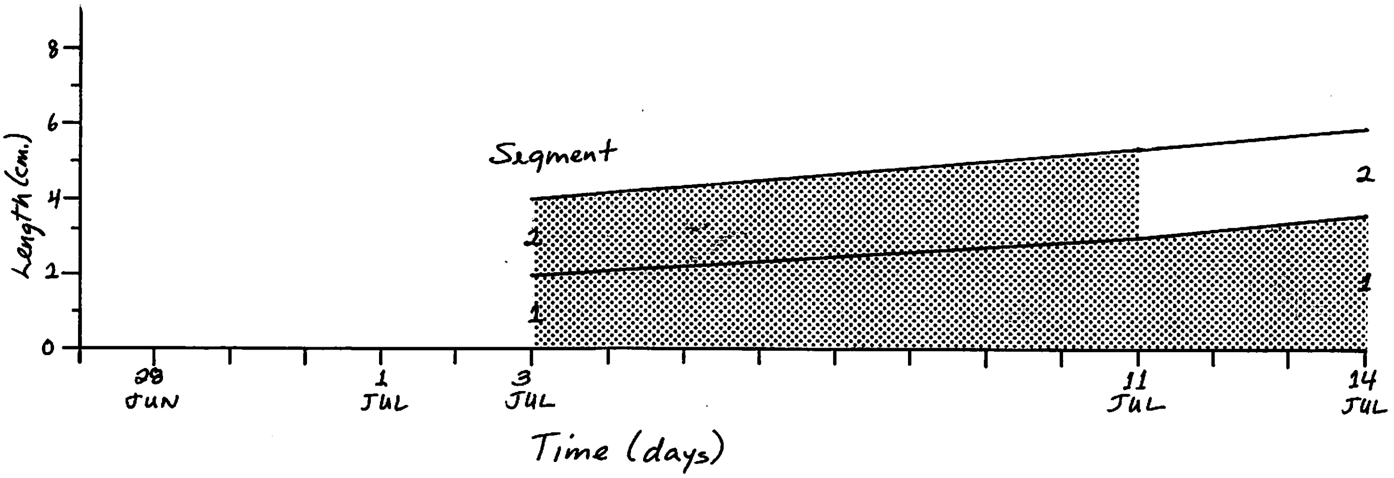


Figure I-5. Length of Blade Segments: *Laminaria groenlandica* (#6)

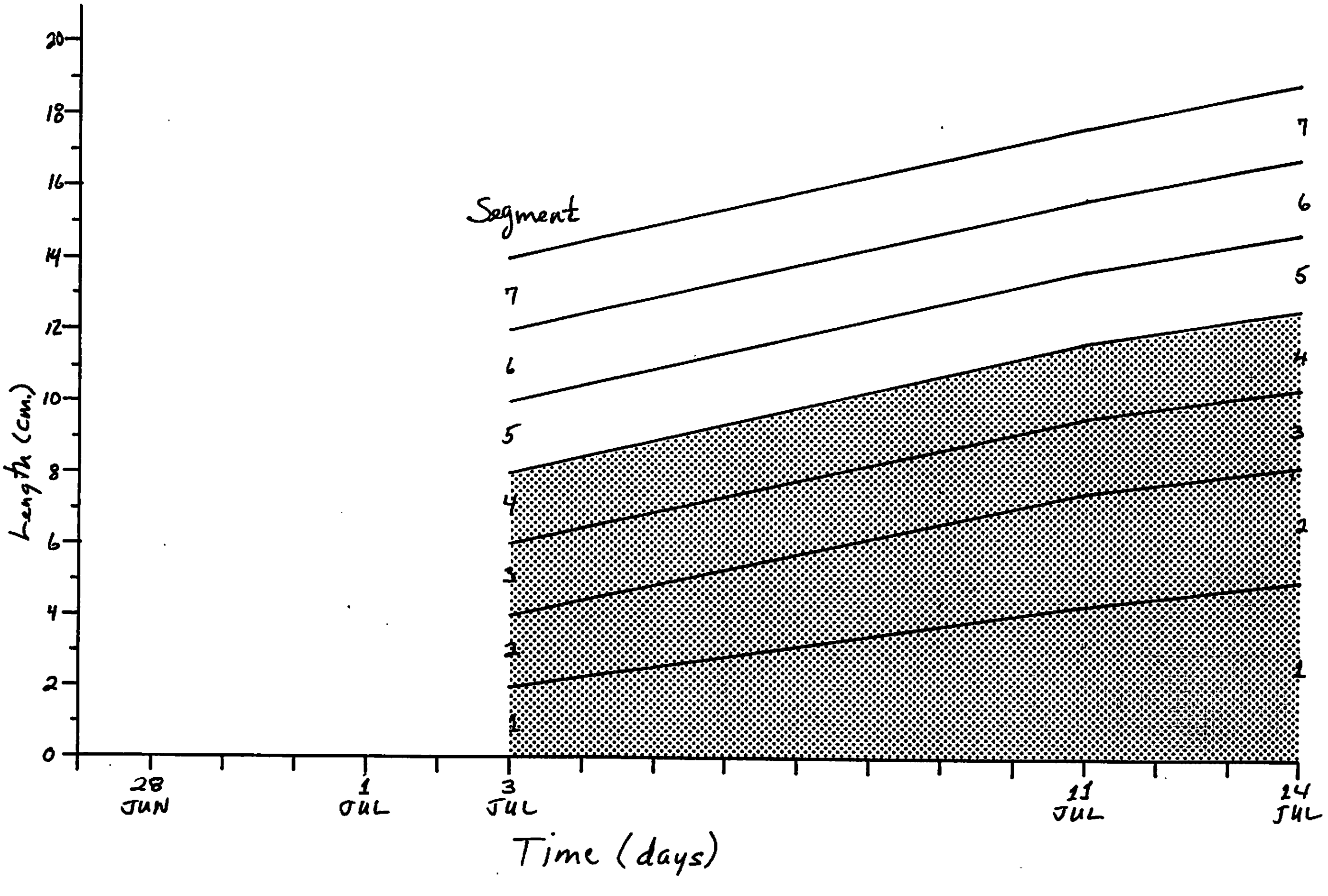


Figure I-6. Length of Blade Segments: *Laminaria longipes* (#7)

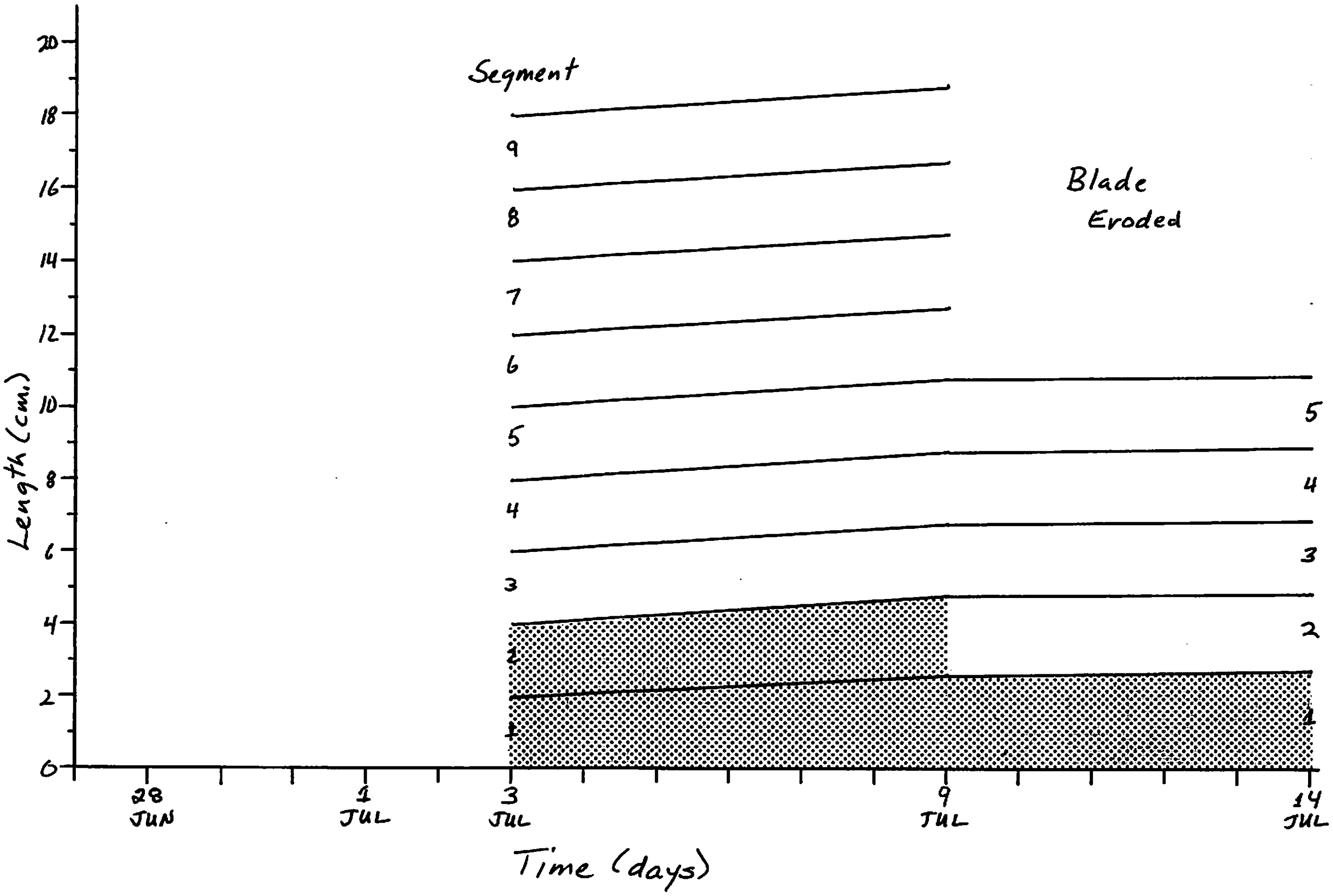


Figure I-7. Length of Blade Segments: <u>Laminaria</u> <u>longipes</u> (#13)

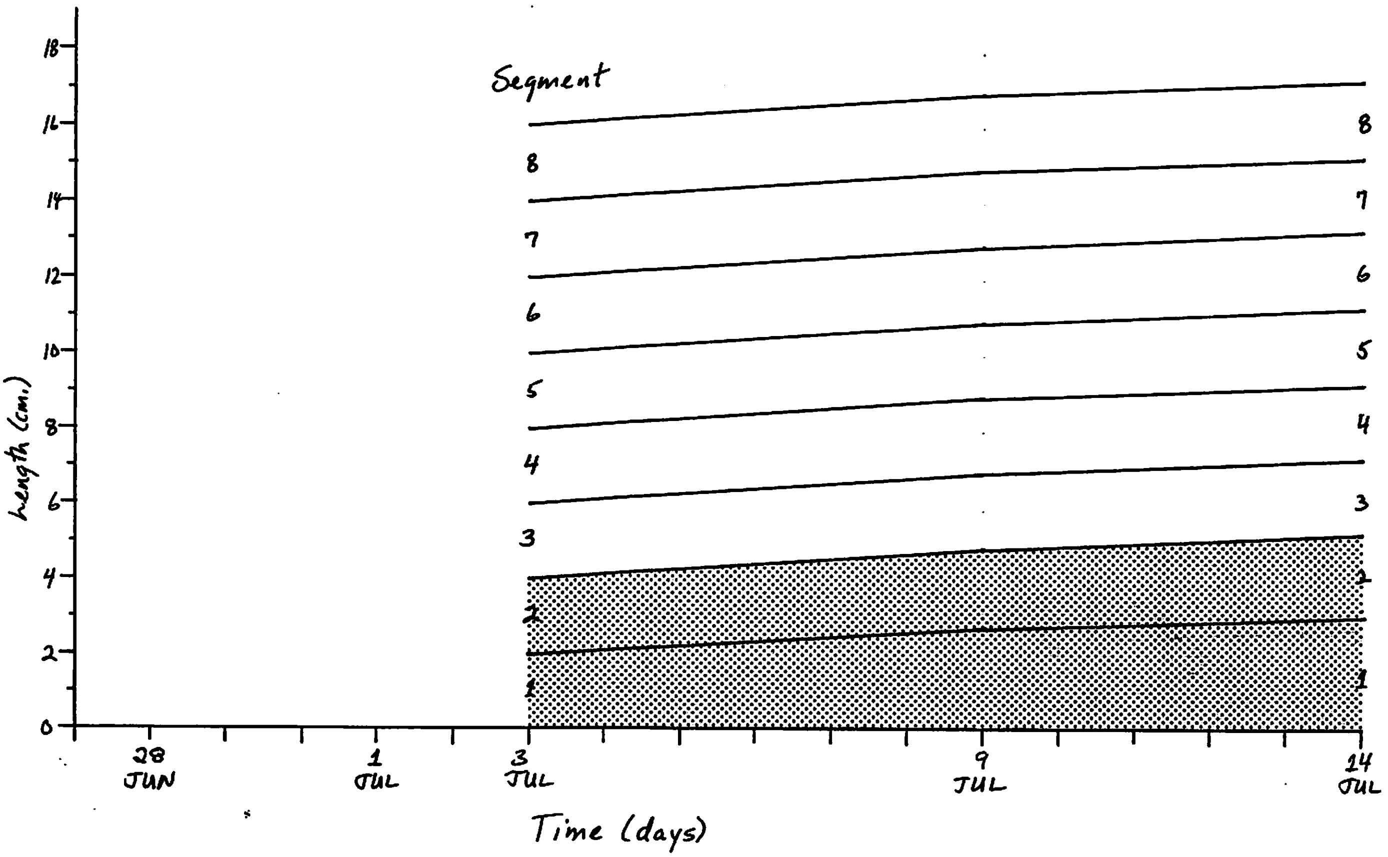


Figure I-8. Length of Blade Segments: <u>Laminaria</u> <u>longipes</u> (#20)

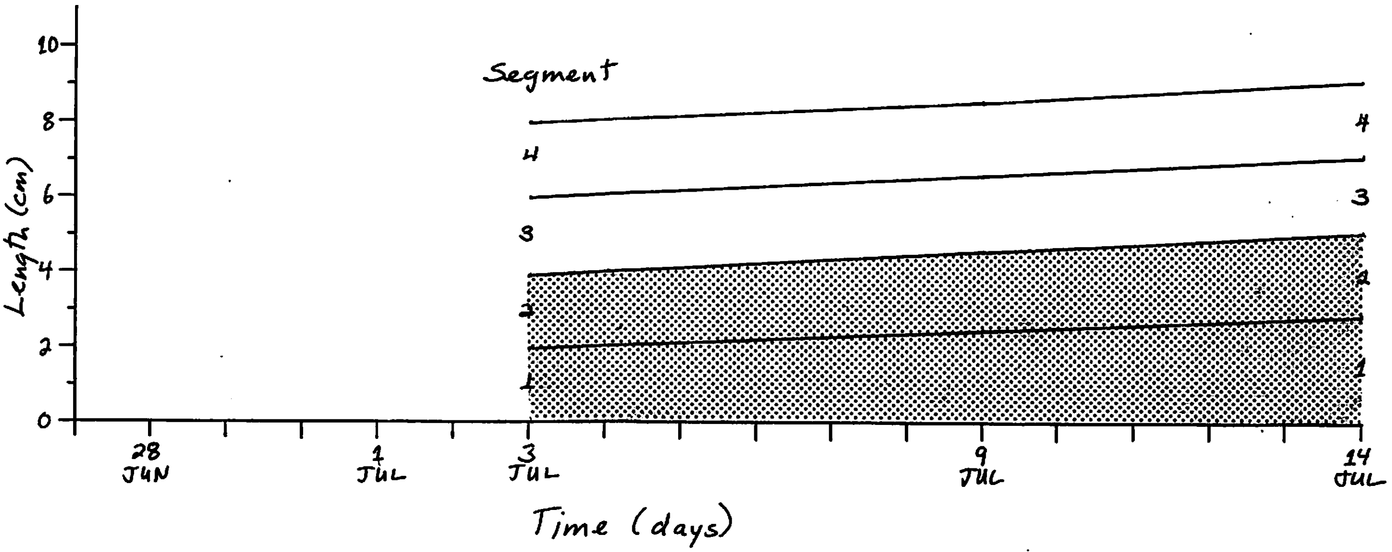


Figure I-9. Length of Blade Segment: *Laminaria longipes* (#22)

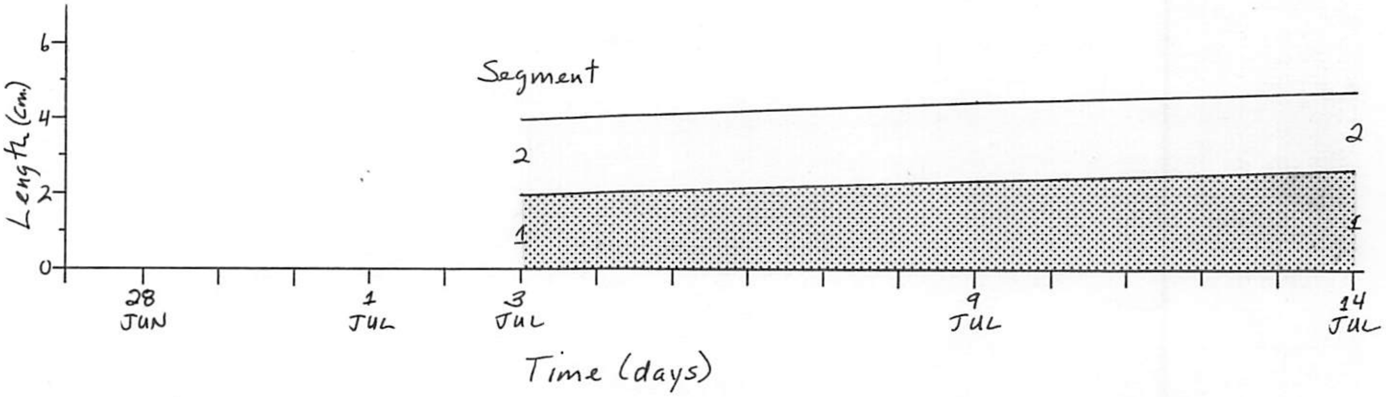


Figure I-10. Length of Haptera: *Laminaria saccharina* (#1)

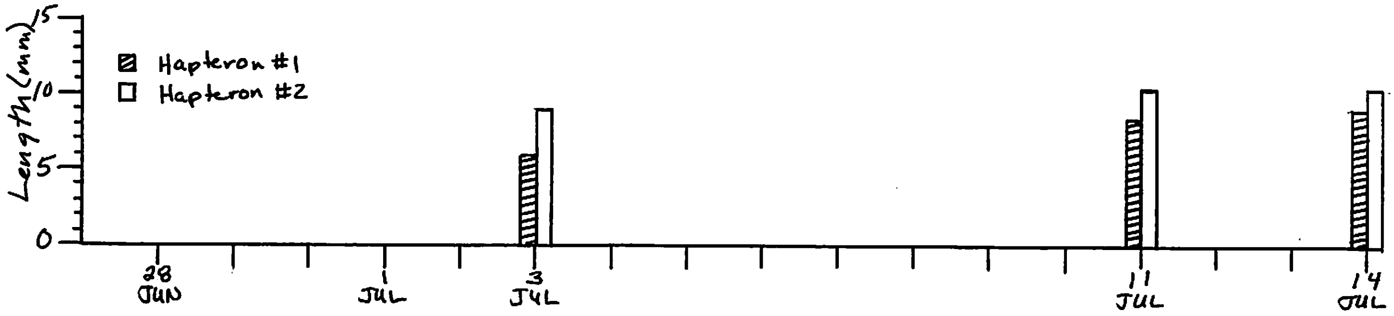


Figure I-11. Length of Blade Segments: *Laminaria saccharina* (#1)

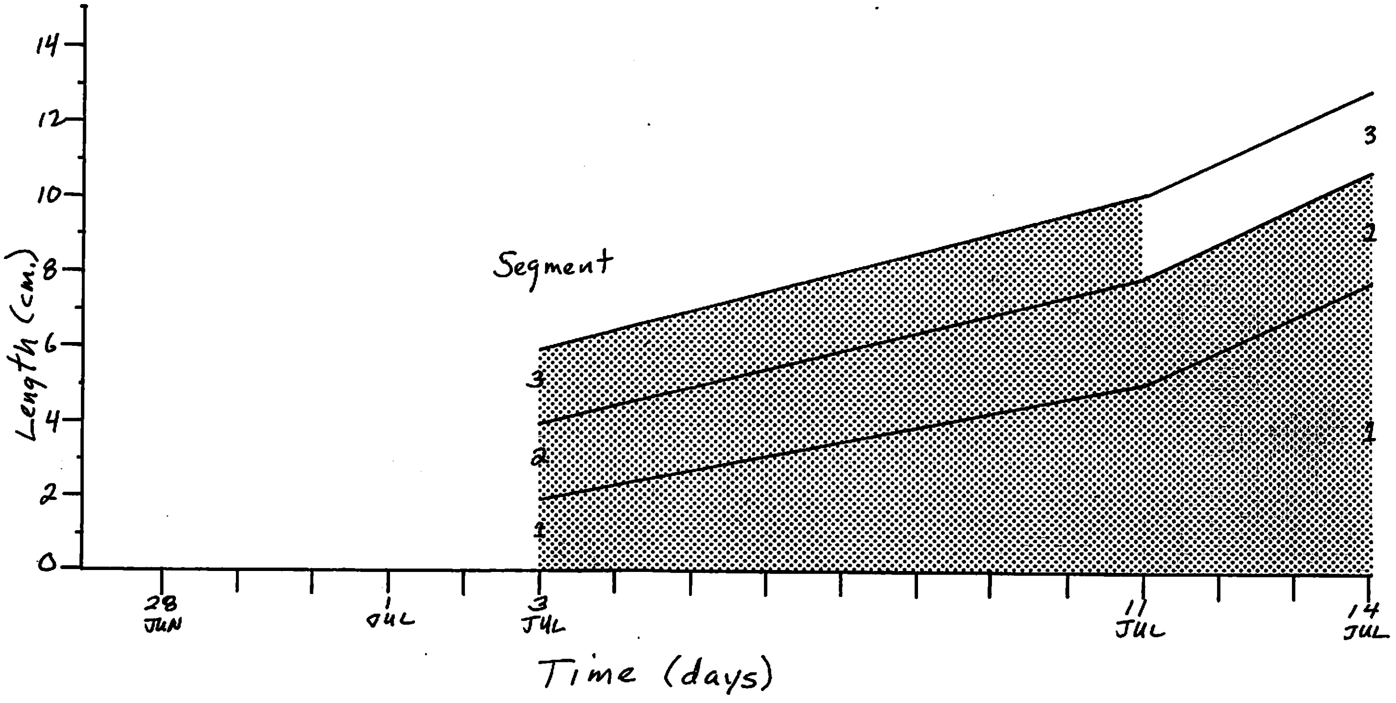


Figure I-10. Length of Haptera: Laminaria saccharina (#1)

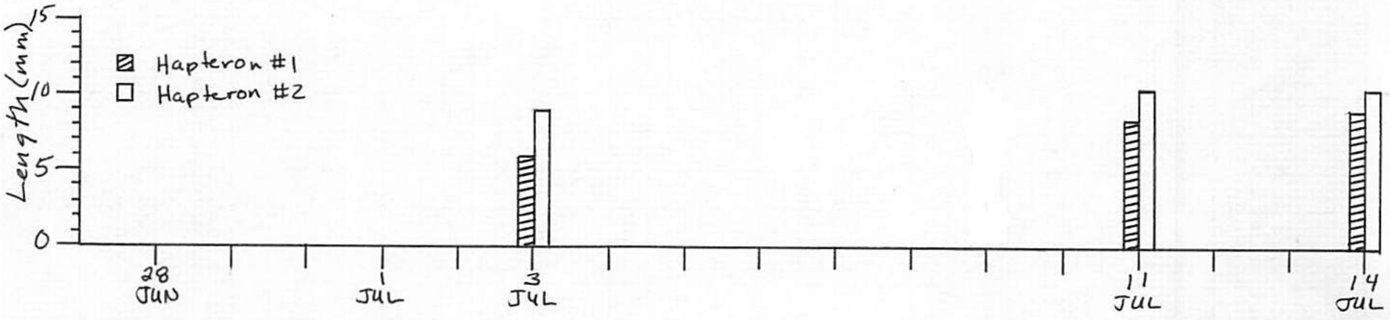


Figure I-11. Length of Blade Segments: Laminaria saccharina (#1)

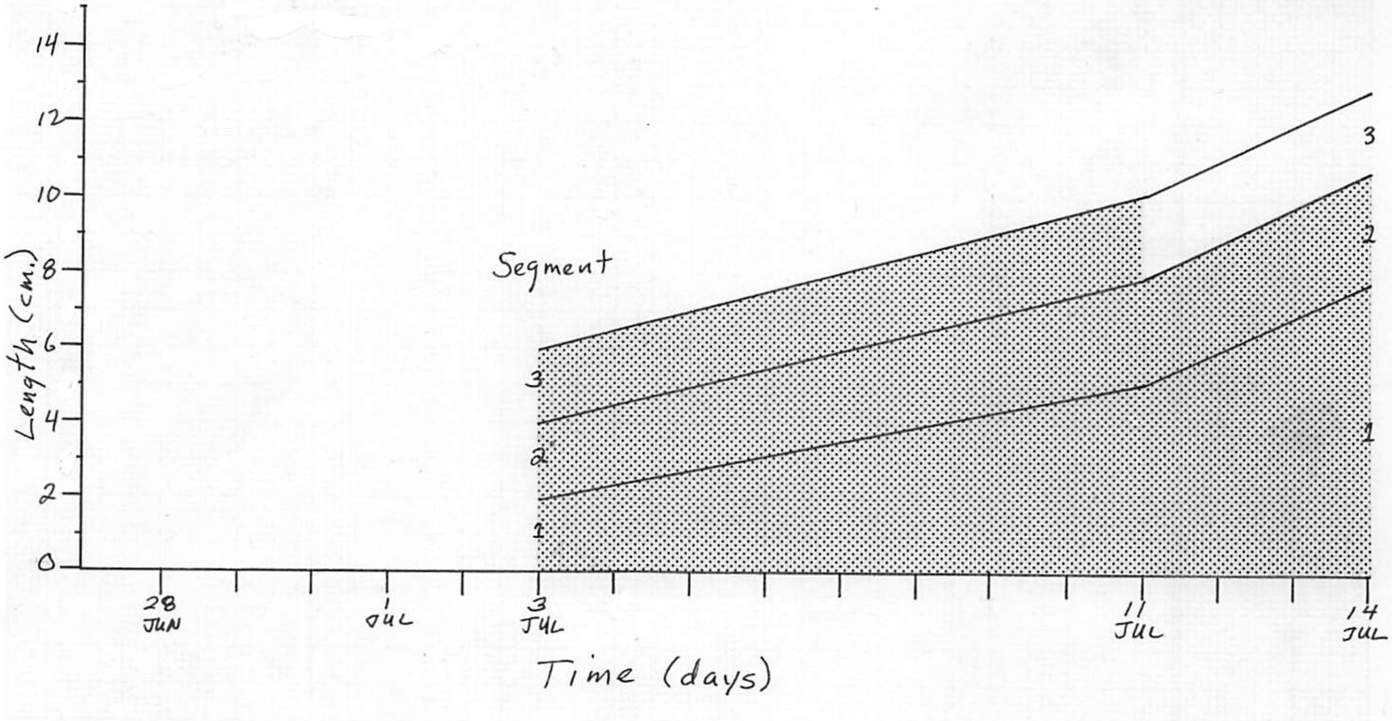


Figure I-12. Length of Blade Segments : *Alaria marginata* (#1)

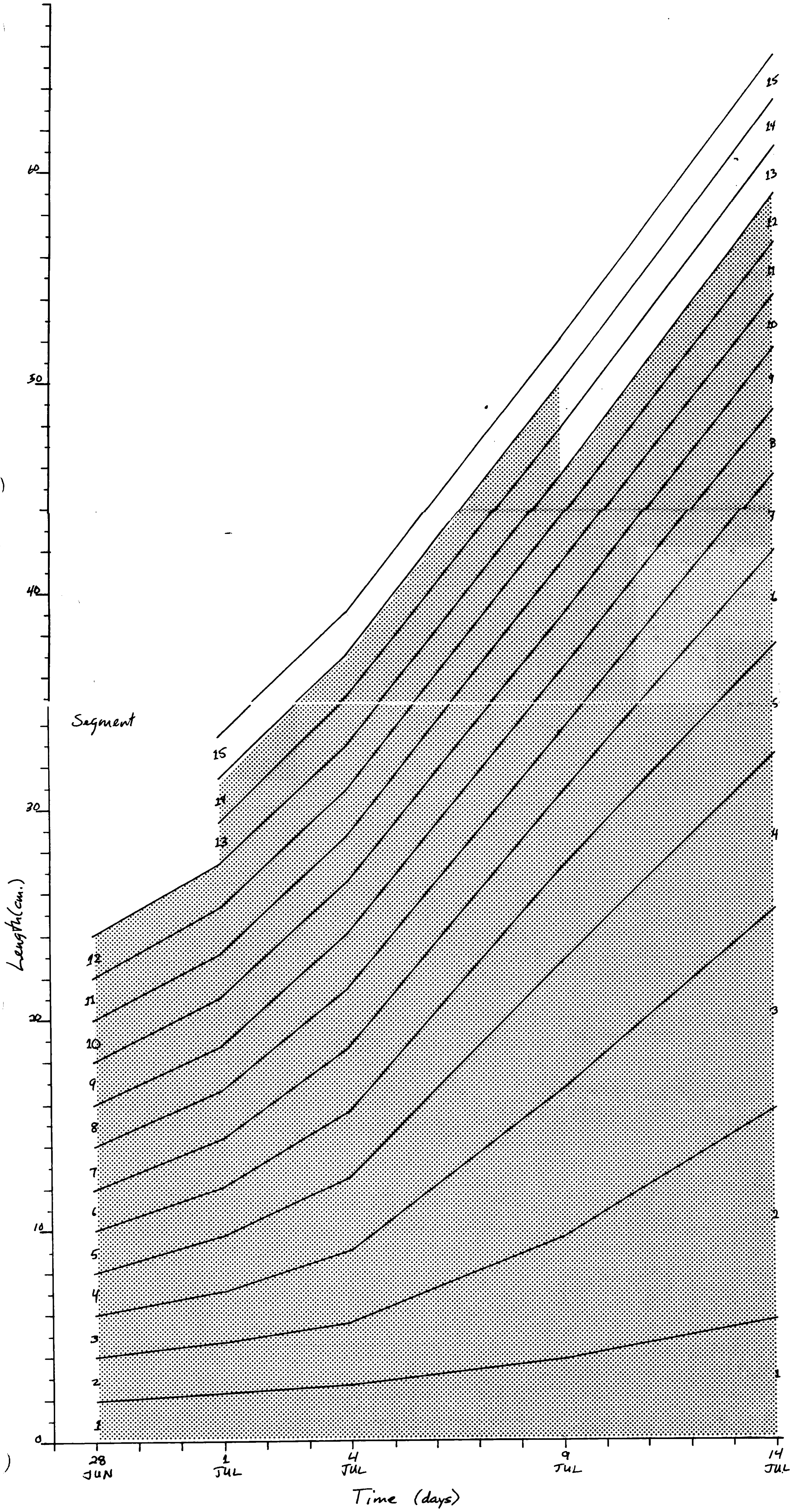
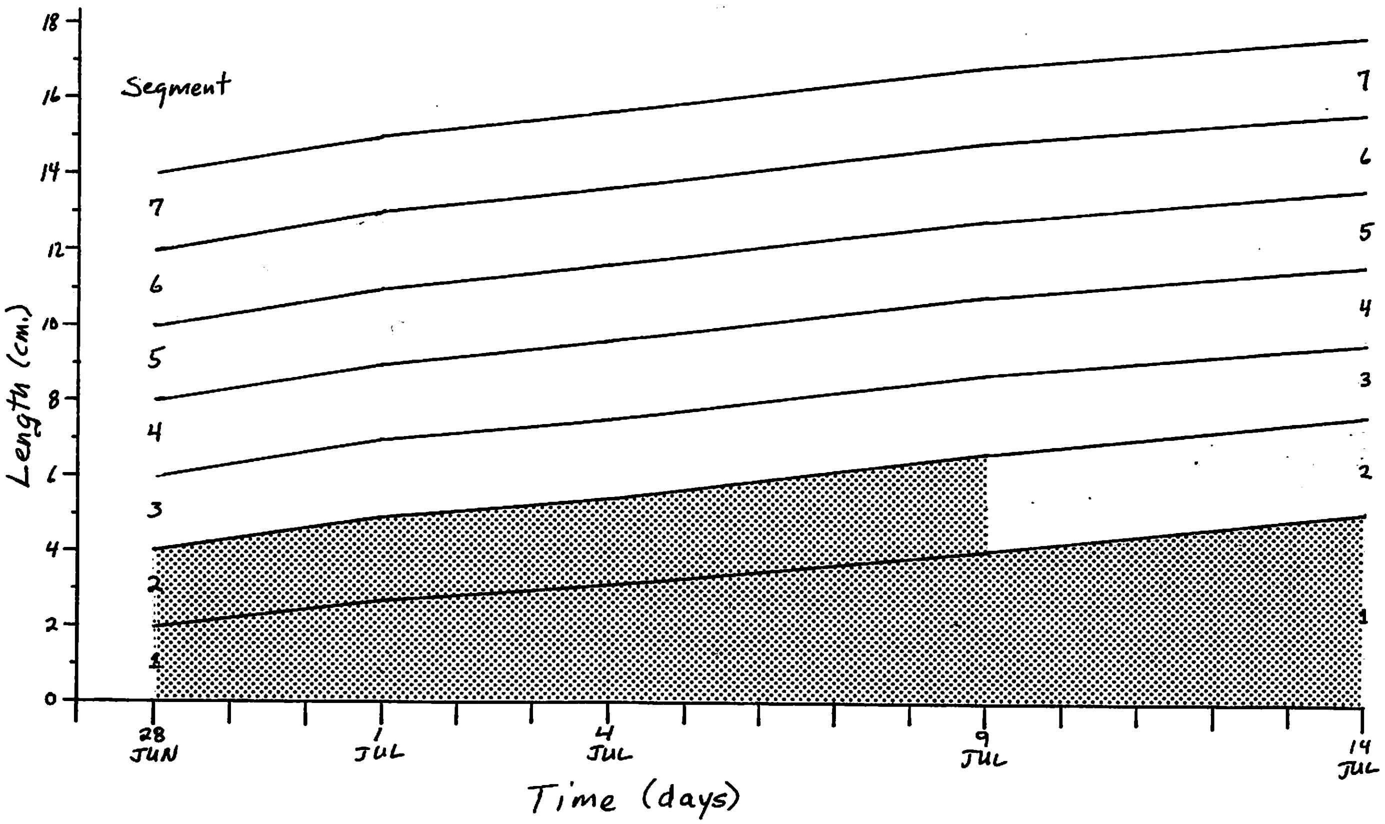


Figure I-13. Length of Blade Segments: *Pterygophora californica* (#4)



APPENDIX II

Investigator	Species	Method	Overall Elongation		Region of Max. Elongation	
			$\mathrm{cm} \cdot d^{-1}$ *	$\% \cdot d^{-1}$ *	$\mathrm{cm} \cdot d^{-1}$ *	$\% \cdot d^{-1}$ *
Burrows 1964	*Laminaria saccharina*	Measured changes in overall length of blade.	0.14–0.29	—	Not reported. Found that shape of base of frond is function of growth rate.	
Calvin & Ellis 1981	*L. groenlandica*	Hole-punching: added holes as blade grew.	JUL (n≅50)		Variable. Some plants grew throughout the blade; in others, growth confined to basal 10cm.	
			shallow (3–7m): ~0.05-0.07	—		
			deep (7–12m): ~0.03	—		
			Maximum: APR (n≅50)			
			shallow: ~0.45-0.75	—		
			deep: ~0.2-0.55	—		
Chapman & Craigie 1977	*L. longicruris*	Measured changes in overall length of blade. (1 hole punched to monitor erosion.)	JUN-JUL (n=10-20)		Not reported.	
			6m depth: ~0.45-0.6	—		
			9m depth: ~0.5-0.7	—		
			18 m depth: ~0.7-0.8	—		
			Maximum: (n=10-20)			
			6m depth (APR-MAY): ~0.6	—		
			9m depth (MAY-JUN): ~0.9	—		
			18m depth (APR-JUL): ~0.8	—		
Clendenning 1964 (reporting work of W.J. North)	*Macrocystis pyrifera*	Measured length of internodes	≤45	—	Greater growth in more deeply submerged internodes. (Note: Not necessarily greatest growth rate.)	

* Unless otherwise specified.

APPENDIX II (Cont'd.)

Investigator	Species	Method	Overall Elongation $cm \cdot d^{-1}$*	Overall Elongation %$\cdot d^{-1}$*	Region of Max. Elongation $cm \cdot d^{-1}$*	Region of Max. Elongation %$\cdot d^{-1}$*
Fallis 1915	*Nereocystis luetkeana* (fronds)	a) measured growth of fronds cut at various initial lengths	JUN 28 - JUL 21 (n=10) 3' lengths: 5.1	5.5	Greatest percentage increase in fronds trimmed to 6" 2.9	18.8
		b) measured growth of 3" segments of single frond	JUL 8 - JUL 20 (n=?) 3.6	5.9	Greatest increase in 4th 3"-segment 0.84	11.1
			control frond: 5.8	9.5		
		c) Hole-punching 1 set of holes @ 3" spacing @ t_0	JUL 19 - AUG 1 (n=10) 8.2	4.0	Greatest increase in 2nd 3"-segment 3.0	39.7
Fallis 1916	*Laminaria* spp.	a) measured growth of fronds cut at various initial lengths	JUN 15 - AUG 6 +(40) 1.11	2.8	+(5) 1.3	26.7
	Costaria costata		(50) 1.75	3.5	(10) 1.0	10.0
	Agarum fimbriatum		(35) 0.59	1.7	(5) 0.3	5.2
	Cymathere triplicata		(190) 0.82	0.4	(10) 0.2	2.5
	Egregia menziesii		— —	—	— —	—
	Alaria costata		(10) 5.45	4.9	(5) 1.3	25.6
	Laminaria spp.	b) measured growth between scratch marks made @ 1-10 cm spacing down center of blade or midrib.	JUN 15 - AUG 6 2.85	7.1	++(2nd, 1cm) 0.62	62
	Costaria costata		2.04	5.8	(4th, 1cm) 0.36	36
	Agarum fimbriatum		0.58	1.3	(2nd, 1cm) 0.12	12
	Cymathere triplicata		0.81	0.4	(1st, 10cm) 0.25	2.5
	Egregia menziesii		0.98	2.4	(1st, 10cm) 0.61	6.1
	Alaria costata		2.23	6.4	(1st, 1cm) 1.4	140
John 1970	*L. hyperborea* (Continued)	Measured distance from base of blade to collar. (Punched hole to account for (Continued)	7.6 m depth —	4	Not reported.	
			13.7m depth —	1 (Continued)		

*Unless otherwise specified. + cm. initial length of frond. ++ Blade segment position and length.

APPENDIX II (Cont'd.)

Investigator	Species	Method	Overall Elongation $cm \cdot d^{-1}$* $\% \cdot d^{-1}$*	Region of Max. Elongation $cm \cdot d^{-1}$* $\% \cdot d^{-1}$*
John 1970 (continued)	*L. digitata*	erosion of blade, but did not use correction in growth rates.)	1.5 & 7.6 m depth — ~3	Not reported.
	L. saccharina		1.5 m depth — ~8 7.6 m depth — ~13 13.7 m depth — ~6	Not reported.
Kain 1976a	*L. hyperborea*	Measured distance from base of blade to collar, Punched one hole to monitor erosion.	Central fingers of frond: JUL ~0.04-0.09 — Maximum: APR ~0.6-0.94 Mean frond length: fast growth ~0.13-0.38 slow growth ~0.01-0.10	By observation of relative position of hole punched ~10 cm from base of blade, growth appeared to not be limited to basal 10 cm.
Kain 1976b	*L. hyperborea*	Hole-punching. a) median line of holes b) median line of holes & perpendicular line of holes at widest part of blade c) grid of holes	$^+K_e \cong$ 0.1-0.3 FEB-MAR <0.1-0.27 MAR-APR 0.03-0.20 APR-MAY 0.0-0.03 MAY-JUN — —	~5 cm from base of blade ~10 cm " " " " ~15 cm " " " " diffuse throughout blade growth faster near edges than in center, particularly in distal portion of blade. Folds or fingers "break away" at edges. Near base lateral growth > longitudinal, ∴ width increases basally.

* Unless otherwise specified. + $K_e = \frac{\log_e L_t - \log_e L_0}{t}$, L_t = length at time t, L_0 = length at t_0, t = period of time
Note: $K_e = 0.5 \propto$ doubling in 10d.

APPENDIX II (Cont'd.)

Investigator	Species	Method	Overall Elongation $cm \cdot d^{-1}$ * $\% \cdot d^{-1}$ *	Region of Max. Elongation $cm \cdot d^{-1}$ * $\% \cdot d^{-1}$ *
Mann 1972	<u>Laminaria longicruris</u> <u>L. digitata</u> <u>Agarum cribrosum</u>	Hole-punching. Added holes 5-10cm from base as blade grew.	<u>JUL-AUG</u> + 0.11-0.24 n = 8-47 0.09-0.17 n = 12-50 0.13-0.14 n = 31-59	Not reported.
North 1971	<u>Macrocystis pyrifera</u>	Measured length of internodes.	<u>Older (~15m) plants</u> 2.5 ~1 <u>Younger (~1m) plants</u> 27.5 ~6 (n = 4)	Not reported. (Decreased growth rate as frond lengthens.)
Parke 1948	<u>L. saccharina</u>	Hole-punching. 1 set of holes @ 2.5 cm spacing @ t_0	<u>JUN16-AUG16 (n = 1)</u> 1.0 34.1 <u>Maximum: (FEB-APR)</u> 1.4-2.1 58.1-80.7	Primary growth (0-2.5 cm above base of blade) 16 JUN - 16 AUG 0.65 26 Maximum: (FEB-APR) 1.34-1.74 54.0-69.5
Sargent & Lantrip 1952 (reporting work of C. Martin)	<u>Macrocystis pyrifera</u>	Cut off growing tips of plants (~1m), anchored and monitored growth.	<u>JUN-AUG (n = 43)</u> 7.1 —	Not reported.
Sheldon 1915 (reporting work of D. Clark)	<u>Nereocystis luetkeana</u> (stipe)	Notched stipe at 2' intervals, measured elongation between notches.	<u>AUG 23 - SEP 13 (n = 2)</u> 1.0 0.3 (Note: In another study, Sheldon observed 2.5 $cm \cdot d^{-1}$ elongation.)	#(3rd, 2') 0.33 1.4 (i.e., hollow portion of stipe below bulb.)
Sundene 1964	<u>L. digitata</u>	Hole-punching. 1 set of holes @ 5 cm spacing @ t_0	<u>JUN 24 - JUL 21 (n = 7)</u> ~0.11 — <u>Maximum (24 MAR - 22 APR)</u> (n = 29) ~0.76 —	JUN 24 - JUL 21 #(1st, 5cm) 0.05 1.1 no measurable elongation after 3rd 5cm-segment. (Continued)

* Unless otherwise specified. + Instantaneous growth rate (monthly basis). # Stipe segment position from base of fronds & length.