The Development of an Edible Kelp Culture Technology for British Columbia

II. Second Annual Report

Louis D. Druehl

marine resources branch
Ministry of Environment
Province of British Columbia

THE DEVELOPMENT OF AN EDIBLE KELP CULTURE TECHNOLOGY FOR BRITISH COLUMBIA

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by

Louis D. Druehl

Simon Fraser University
and
Bamfield Marine Station

Fisheries Development Report No. 26

Marine Resources Branch
Ministry of Environment
Province of British Columbia

First Printing September,

Second Printing December,

Canadian Cataloguing in Publication Data

Druehl, Louis D., 1936-
The development of an edible kelp culture technology for British Columbia : II, Second annual report.

(Fisheries development report ; no. 26)

Bibliography: p.
ISBN 0-7719-8414-6

1. Kelp. 2. Marine algae culture - British Columbia. 3. Marine algae as food. I. British Columbia. Marine Resources Branch. II. Title. III. Series

SH390.5.C3D782 639 C80-092162-3

ABSTRACT

This second annual report records progress made in designing and evaluating the technology necessary for rearing the edible kelp (kombu) species *Laminaria groenlandica, Cymathere triplicata* and *Pleurophycus gardneri* in British Columbia. The life histories, seasonal changes in morphologies, and air-drying characteristics of the studied species are reported herein. A harvesting strategy is proposed for each species on the basis of this information. Seed for planting kombu farms can now be produced through part of the year. A method for extending the season for seed production has been successfuly tested. Further, cloning methods which should result in a genetically uniform product are being developed. A structure suitable for kombu farms has been developed. Several of these farms are now in production and the first harvests will be made in the summer of 1980.

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ACKNOWLEDGEMENTS

The following individuals and institutions contributed to this study. This assistance is greatly appreciated.

Drs. Y. Hasegawa and Y. Sanbonsuga of the Hoddaido Reg. Fish. Res. Lab., Kushiro, Japan, and Dr. L. Hanic of the Univ. Prince Edward Island contributed suggestions valuable to the development of culture techniques. Ms. R. Boal is senior technician on the project and Ms. K. Lloyd and S. Smith-Pakula contributed to all stages of this study. The W. Wenstob family provided security for the kombu farms near Tzartus I. and life-saving services on a number of occasions. Ms. V. Moan kept track of the Sointula kombu farm and acted as liaison with the village of Sointula. Mr. J. Boom provided emergency services throughout the year.

Throughout this study we enjoyed the services of the Bamfield Marine Station. Canadian Benthic Ltd. co-operated in field studies and assisted in establishing the new kelp farms. Simon Fraser University provided services and allowed me a leave of absence for part of the year to oversee this programme.

The Marine Resources Branch, Ministry of Environment, provided financial support and Mr. M. Coon of that Branch provided helpful suggestions. Research on nutrient and light requirements was funded by the National Research Council of Canada.

INTRODUCTION

This is the second annual report, recording progress in developing the biotechnology necessary for an edible kelp industry. The edible kelp is known as kombu and consists of some of the smaller species in the Order Laminariales. Presently, we are investigating three species which have characteristics of good kombu (*Laminaria groenlandica* Rosenvinge, *Cymathere triplicata* [Postels and Ruprecht] J. Agardh, *Pleurophycus gardneri* Setchell and Saunders). The products of these species are intended for domestic and foreign markets.

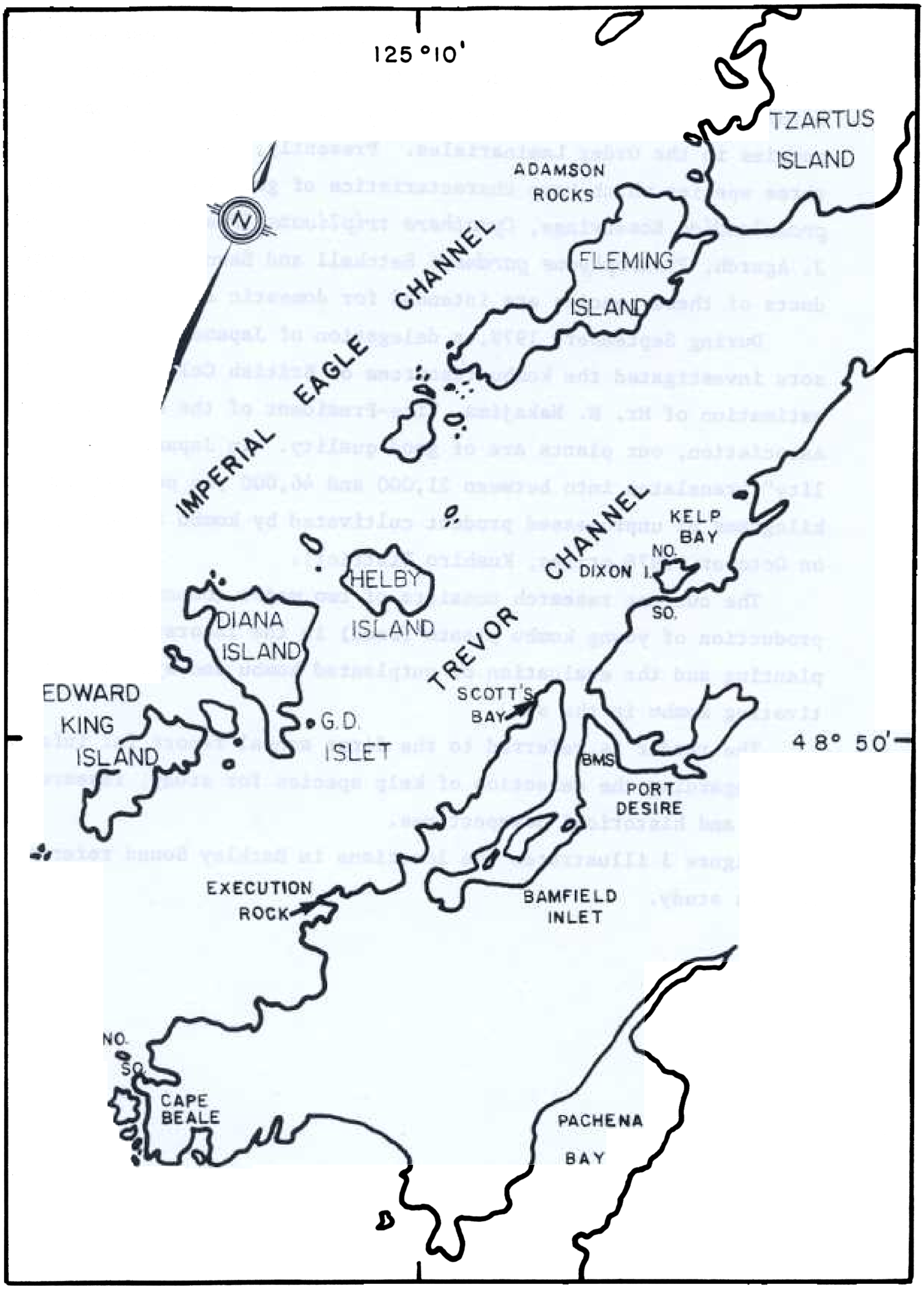
During September, 1979, a delegation of Japanese kombu processors investigated the kombu resources of British Columbia. In the estimation of Mr. N. Nakajima, Vice-President of the Kombu Makers' Association, our plants are of good quality. In Japan, "good quality" translates into between 21,000 and 46,000 yen per fifteen dry kilograms of unprocessed product cultivated by kombu farmers (based on October, 1978 prices, Kushiro District).

The current research consists of two major components: the production of young kombu plants (seed) in the laboratory for outplanting and the evaluation of outplanted kombu and systems for cultivating kombu in the sea.

The reader is referred to the first annual report for information regarding the selection of kelp species for study, research approach and historical perspectives.

Figure 1 illustrates the locations in Barkley Sound referred to in this study.

Figure 1. Locations in Barkley Sound referred to in this study



LIFE HISTORIES

At the initiation of this project the life histories of *Cymathere triplicata* and *Pleurophycus gardneri* were not known, and while the major events in the life history of *Laminaria groenlandica* were understood, the morphological features associated with different year classes were unknown. It is essential to understand these aspects of kombu biology before any attempt is made to commercially exploit these species.

1. *Cymathere triplicata*

Wild sporophytes of *C. triplicata* first became evident in April, and demonstrated net growth until August. Sori were initiated in August and all of the plants were fertile by September (Table 1). This generation persisted until December. On the basis of these observations, *C. triplicata* can be described as an annual species.

2. *Pleurophycus gardneri*

The suggestion made in the last annual report that *P. gardneri* is a perennial and not an annual species, as suggested by Setchell and Gardner (1925), is now confirmed (Druehl, 1980). From October until January, *P. gardneri* sporophytes persisted as stipes terminated by small basal blade portions (Figure 2). These blade rem-

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Figure 2. Developmental stages of *Pleurophycus gardneri*. a. Stipe of older plant at beginning of growth season (March). b. Young plant prior to development of mid-rib (March). c. Fully grown plant prior to blade erosion (May). d. (August) and e. (September) older plants demonstrating blade erosion. a and b are to the same scale and c, d, and e are to the same scale (following page).

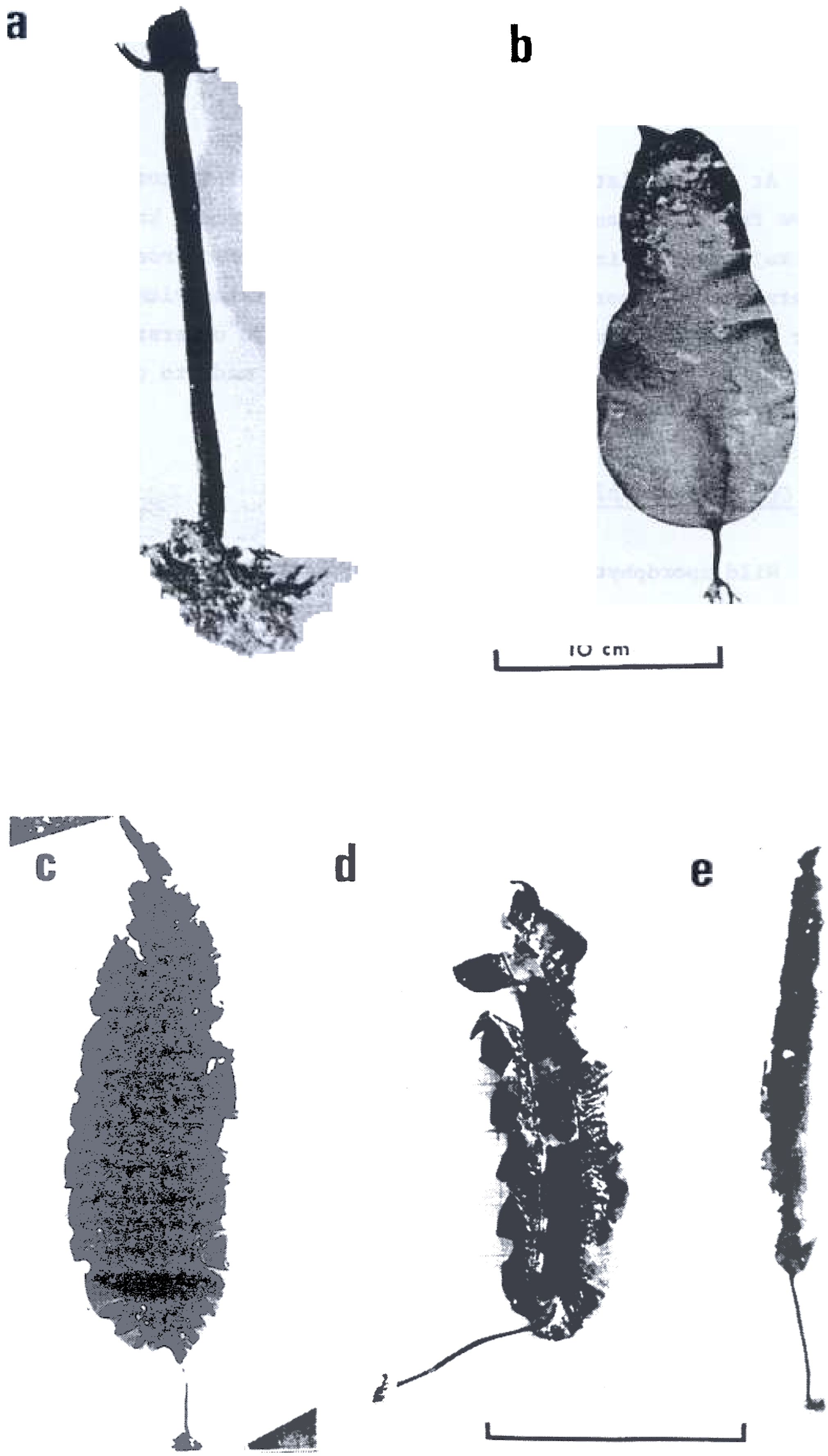

a
b
10 cm
c
d
e

Table 1. Presence (+) and absence (-) of sori from April, 1979 until March, 1980 for the three studied species in Barkley Sound.

	Laminaria groenlandica	*Pleurophycus gardneri*	*Cymathere triplicata*
April		+	
May	+	+	
June	+	+	
July		+	
August		+	+
September	+	+	+
October	+		+
November	+		+
December	+		+
January	+		
February	+	+	
March	+	+	

nants initiated rapid growth in January, and net growth continued until May. Sori were initiated in February and all of the plants were fertile by July (Table 1).

New sporophytes became evident in January and passed through at least part of the growing season without the distinctive midrib (Figure 2). A number of obvious first year sporophytes were tagged in the field in January, 1980 in order to determine the longevity of individual plants and the duration of the non-midrib state.

3. *Laminaria groenlandica*

Young sporophytes of *L. groenlandica* first became apparent in January, produced net growth into June, and became fertile in the fall. These plants entered their second year in January, when they initiated new net growth. Some of these plants produced sori in May/June (Table 1). There was no net growth from June until the following January when these plants entered their third year. All plants became fertile in the fall.

The three year classes can be distinguished morphologically (Figure 3). No one feature, however, clearly distinguishes between the age classes. Generally, first year plants have the lowest stipe width to thickness ratios (as measured midway between the holdfast and blade base), the thinnest and narrowest blades (as measured 10 cm from the blade base) and most acute blade base angles (Table 2). These dimensions increase with age class. Further, the age classes can be separated on the basis of colour and degree of splitting of the blades. Third year plants are heavily split and the stipes are darker than the blades. The younger plants have less severely split blades and the stipes and blades are the same colour.

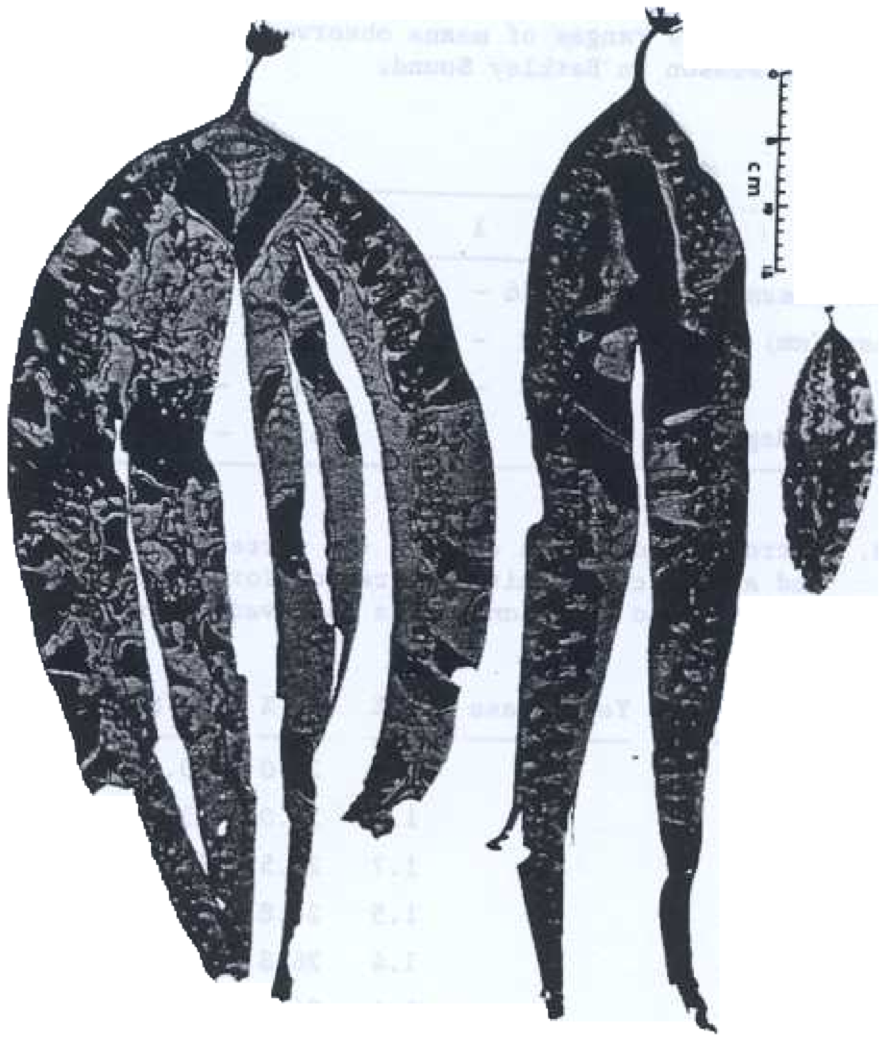
The three year classes behave differently chemically. Generally, the amount of nitrogen decreases and carbon increases with year class (Table 3).

4. Conclusions

The life histories of the three studied species are now reasonably well known. *Laminaria groenlandica* and *P. gardneri* are perennial species and *C. triplicata* is an annual. The times of sorus production are known for the three species. Since the presence of sori decreases the commercial value of plants, knowledge of the time of occurrence is important in determining harvesting strategy.

Laminaria groenlandica exists as at least three year classes. These year classes can be distinguished, but with some difficulty. Chemical and morphological features influencing the economic value

Figure 3. Three year classes of *Laminaria groenlandica* from Diana Island; March, 1980.



of the plants vary between age classes. These features appear to be most optimal in second year plants and for this reason harvesting should concentrate on this year class.

Table 2. Comparative measurements of morphological features used to distinguish year classes of *Laminaria groenlandica*. Values are ranges of means observed throughout the growing season in Barkley Sound.

	Year Class		
	1	2	3
Stipe width/thickness ratio	1.16 - 1.57	1.15 - 1.61	1.44 - 2.20
Blade thickness (mm)	0.2 - 1.0	0.4 - 1.2	0.8 - 1.6
Blade width (cm)	1.8 - 10.2	10.5 - 18.6	16.8 - 36.0
Blade base angle (degrees)	31 - 63	51 - 77	79 - 86

Table 3. Nitrogen and carbon content (as percentage of dry weight) and atomic carbon/nitrogen ratios for three year classes of *Laminaria groenlandica* as observed during 1979 (n = 1)

Month	Year Class	N %	C %	C/N
July	1	2.1	26.0	10.6
July	2	1.8	32.0	15.3
July	3	1.7	27.5	13.9
August	1	1.5	24.8	14.1
August	2	1.4	26.8	16.4
August	3	1.1	34.3	26.7
September	1	2.2	26.5	10.3
September	2	2.2	35.4	13.8
September	3	1.9	35.6	16.0

SEASONAL CHANGE OF MORPHOLOGICAL FEATURES

Several features assist in determining the economic value of kombu: weight, size, blade thickness, and weight per unit area. We have monitored these in the field on a monthly basis in an attempt to distinguish the optimal time for harvesting. The plants selected for this study were randomly selected from the larger plants available. This size range most closely approximates plants that would be harvested commercially.

Blade width, thickness of the blade margin and centre, and wet weight per unit area at the margin and centre were determined 20 - 30 cm from the blade base. All samples were composed of 10 - 13 plants.

Laminaria groenlandica and *P. gardneri* were measured monthly at Diana Island. Occasional measurements were made on these two species at other localities. Monthly measurements of *C. triplicata* were made on plants collected near Execution Rock.

1. *Laminaria groenlandica*

At Diana Island the year's largest plants were collected in June (Table 4). However, this population continued to increase in blade thickness until October and in wet weight per unit area until November. Figure 4 illustrates plants from four different months.

For seven months of the year, plants from Diana Island were compared with plants from Execution Rock. The latter population belongs to the *L. groenlandica* long stipe form and the former to the *L. groenlandica* short stipe form (Druehl, 1968). Throughout the observation period the Execution Rock plants were larger and thicker than the Diana Island plants (Table 5). Further, the Execution Rock plants did not lose as much weight during the winter as did the Diana Island plants, but they did not initiate new

Figure 4. *Laminaria groenlandica* as observed at Diana I. in: March (a), April (b), August (c), and January (d). All photographs are to the same scale.

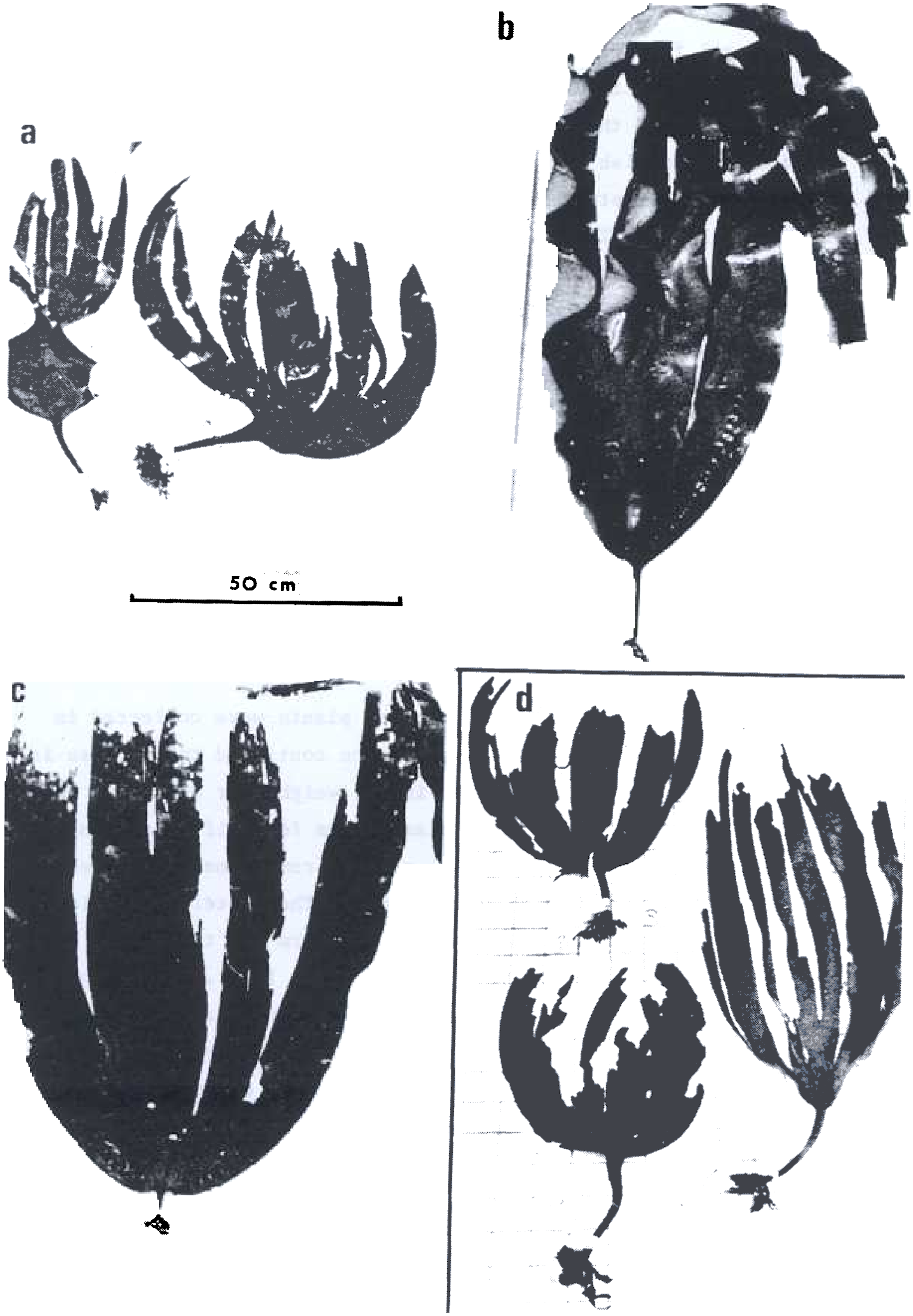


Table 4. Monthly means and standard deviations for selected morphological features of *Laminaria groenlandica* from Diana I., April, 1979 to March, 1980 (n = 10-13).

		Blade (cm)		Wet Weight/Unit Area (mg/cm^2)		Blade Thickness (mm)	
Month	Wet Weight (g)	Length	Width	Centre	Margin	Centre	Margin
	385 ± 129	128 ± 25	39 ± 11			0.9 ± 0.1	1.0 ± 0.1
	507 ± 138	139 ± 30	44 ± 8			0.9 ± 0.1	1.0 ± 0.1
	531 ± 154	149 ± 27	40 ± 16			1.0 ± 0.1	1.1 ± 0.1
	281 ± 138	87 ± 24	32 ± 12			1.0 ± 0.1	1.0 ± 0.1
	387 ± 115	92 ± 34	40 ± 10			1.0 ± 0.1	1.1 ± 0.1
	258 ± 95	77 ± 28	38 ± 8			1.1 ± 0.1	1.1 ± 0.1
	177 ± 55	55 ± 13	30 ± 11			1.3 ± 0.2	1.3 ± 0.1
	113 ± 48	41 ± 12	22 ± 8			1.3 ± 0.2	1.3 ± 0.2
	137 ± 41	47 ± 8	19 ± 8			1.2 ± 0.2	1.2 ± 0.1
	56 ± 6	23 ± 3	16 ± 1			1.2 ± 0.0	1.2 ± 0.1
	71 ± 34	53 ± 11	11 ± 5			1.2 ± 0.3	1.1 ± 0.3
	129 ± 30	83 ± 15	24 ± 4			1.0 ± 0.1	1.0 ± 0.1

Table 5. Comparative means with standard deviations for selected morphological features of *Laminaria groenlandica* collected from Execution Rock and Diana I. between August, 1979 and March, 1980 (n = 10-13).

Month	Wet Weight (g)		Blade Length (cm)		Blade Thickness (centre) (mm)	
	Execution Rock	Diana I.	Execution Rock	Diana I.	Execution Rock	Diana I.
August	323 ± 170	387 ± 115	106 ± 23	92 ± 34	1.2 ± 0.1	1.0 ± 0.1
September	297 ± 90	258 ± 95	84 ± 17	77 ± 28	1.2 ± 0.1	1.1 ± 0.1
October	226 ± 166	177 ± 55	49 ± 17	55 ± 13	1.5 ± 0.2	1.1 ± 0.1
November	203 ± 85	113 ± 48	41 ± 5	41 ± 12	1.5 ± 0.2	1.3 ± 0.2
December	223 ± 76	137 ± 41	51 ± 14	47 ± 8	1.2 ± 0.1	1.2 ± 0.2
January	216 ± 73	56 ± 6	48 ± 14	23 ± 3	1.4 ± 0.2	1.2 ± 0.1
March	211 ± 77	129 ± 30	61 ± 13	83 ± 15	1.5 ± 0.2	0.9 ± 0.1

growth as early in 1980 as the Diana Island plants.

During July, morphological variation in *L. groenlandica* from six locations was compared. The largest plants having the thickest blades and greatest wet weight per unit area were collected from Edward King Island (Table 6). These plants were of the *L. groenlandica* long stipe form.

2. *Pleurophycus gardneri*

The heaviest plants of the year from Diana Island were collected in May and the longest plants in July (Table 7). From May until July the blade margins eroded while the midrib continued to grow in length (Figure 2). Blade thickness and wet weight per unit area continued to increase until July. From October until December there were no blades.

During July, morphological variation in *P. gardneri* was compared between six locations (Table 8). The largest plants were collected from the wave-exposed south side of GD Islet, and the plants having the thickest blades with the greatest wet weight per unit area came from Cape Beale south.

3. *Cymathere triplicata*

The heaviest plants of the year were collected in August, and the longest plants in July from Execution Rock (Table 9). These plants continued to increase in blade thickness and wet weight per unit area until December. All plants were gone by January, 1980. Figure 5 illustrates changes in plants observed through 1979.

Figure 5. *Cymathere triplicata* as observed at Execution Rock in: April (a), June (b), August (c), and December (d). All photographs are to the same scale (following page).

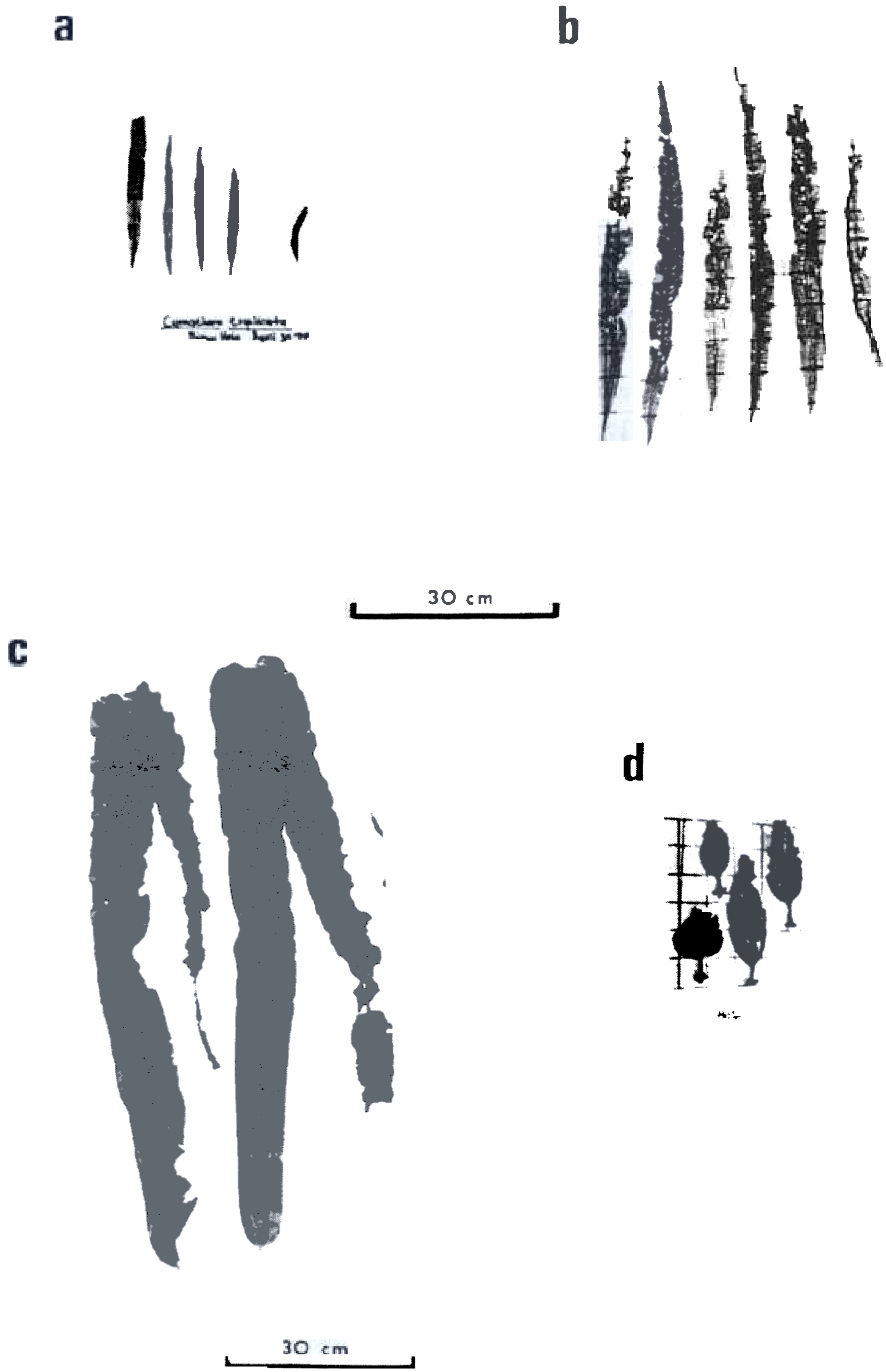

a
b
30 cm
c
d
30 cm

Table 6. Means and standard deviations for selected morphological features of *Laminaria groenlandica* as observed during July, 1979 at several sites in Barkley Sound (n = 10-13).

Location	Wet Weight (g)	Blade (cm)		Wet Weight/Unit Area (mg/cm^2)		Blade Thickness (mm)	
		Length	Width	Centre	Margin	Centre	Margin
Grappler Inlet	198 ± 51		6	126 ± 12	134 ± 16	1.0 ± 0.1	
Edward King Island	347 ± 154	113 ± 14	31 ± 11	148 ± 9	167 ± 10	1.2 ± 0.1	1.3 ± 0.1
Helby Island	298 ± 97	95 ± 11	34 ± 11	138 ± 11	143 ± 9	1.0 ± 0.1	1.0 ± 0.1
Dixon Island South	246 ± 53	73 ± 12	41 ± 8	143 ± 18	150 ± 14	1.0 ± 0.1	1.0 ± 0.1
Dixon Island North	196 ± 34	75 ± 11	31 ± 5	124 ± 8	129 ± 8	1.0 ± 0.1	1.0 ± 0.1
Diana Island	281 ± 138	87 ± 24	32 ± 12	137 ± 9	138 ± 9	1.0 ± 0.1	1.0 ± 0.1

Table 7. Monthly means and standard deviations for selected morphological features of *Pleurophycus gardneri* from Diana I., April, 1979 to March, 1980 (n = 10-13).

Month	Wet Weight (g)	Blade (cm)		Wet Weight/Unit Area (mg/cm^2)		Blade Thickness (mm)	
		Length	Width	Centre	Margin	Centre	Margin
April	278 ± 68		30 ± 3	160 18	76 ± 8	1.4 .3	0.6 ± 0.1
May	284 ± 107		32 ± 10	143 20	73 ± 9	1.2 .2	0.5 ± 0.1
June	254 ± 52		20 ± 3	158 12	73 ± 9	1.3 .2	0.7 ± 0.1
July	223 ± 49		21 ± 4	174 12	92 ± 13	1.6 .1	0.7 ± 0.1
August	130 ± 52		13 ± 6	154 18	80 ± 20	1.5 .2	0.7 ± 0.2
September	120 ± 40		13 ± 6	171 15	74 ± 14	1.5 .2	0.5 ± 0.1
January	41 ± 30		11 ± 1	131 19	74 ± 15	1.0 .1	0.4 ± 0.1
February	65 ± 19		16 ± 5	112 20	47 ± 9	1.1 .1	0.6 ± 0.1
March	93 ± 25		19 ± 5	140 17	69 ± 11	1.3 .2	0.6 ± 0.1

Table 8. Means and standard deviations for selected morphological features of *Pleurophycus gardneri* during July, 1979 at several sites in Barkley Sound (n = 10-13).

Location	Wet Weight (g)	Blade (cm)		Wet Weight/Unit Area (mg/cm^2)		Blade Thickness (mm)	
		Length	Width	Centre	Margin	Centre	Margin
Cape Beale North	203 ± 68	77 ± 33	19 ± 3	177 ± 15	101 ± 12	1.7 ± 0.2	0.9 ± 0.1
Cape Beale South	240 ± 74	96 ± 25	19 ± 2	198 ± 26	126 ± 24	1.7 ± 0.2	0.9 ± 0.2
Edward King Island	289 ± 65	108 ± 29	21 ± 2	175 ± 22	111 ± 11	1.3 ± 0.4	1.0 ± 0.4
GD Islet, South	309 ± 107	109 ± 20	23 ± 5	170 ± 12	84 ± 18	1.6 ± 0.1	0.8 ± 0.1
GD Islet, North	240 ± 104	93 ± 26	21 ± 3	165 ± 17	87 ± 9	1.5 ± 0.2	0.7 ± 0.1
Diana Island	223 ± 49	111 ± 17	21 ± 4	174 ± 12	92 ± 13	1.6 ± 0.1	0.7 ± 0.1

Table 9. Monthly means and standard deviations for selected morphological features of *Cymathere triplicata* from Execution Rock, May to December, 1979 (n = 10-13).

Month	Wet Weight (g)	Blade (cm)		Wet Weight/Unit Area (mg/cm^2)		Blade Thickness (mm)	
		Length	Width	Centre	Margin	Centre	Margin
May 28	5.7 ± 6	45 :: 27	4 ± 2				
June 20	25 ± 12	80 :: 19	10 ± 4	101 ± 16	32 ± 7	1.0 ± 0.2	0.2 ± 0.1
July 6	81 ± 51	146 :: 42	9 ± 3	155 ± 27	46 ± 10	1.2 ± 0.2	0.4 ± 0.2
July 31	118 ± 37	187 :: 27	9 ± 3	189 ± 23	63 ± 20	2.2 ± 0.3	0.6 ± 0.1
August 15	132 ± 49	157 :: 38	10 ± 2	195 ± 25	60 ± 7	2.0 ± 0.2	0.5 ± 1.0
September 12	91 ± 39	121 :: 38	8 ± 2	184 ± 33	77 ± 21	2.0 ± 0.3	0.5 ± 0.2
November 2	44 ± 9	32 :: 5	4 ± 1	225 ± 34	114 ± 34	2.0 ± 0.2	1.0 ± 0.3
November 13	36 ± 17	24 :: 8	8 ± 2	245 ± 47	223 ± 72	2.2 ± 0.4	2.0 ± 1.0
December 10	20 ± 6	15 :: 4	5 ± 2	297 ± 67	307 ± 26	2.3 ± 0.2	2.0 ± 0.1

4. Conclusions

Generally, the plants became less desirable commercially from the month of their greatest fresh weight. From this time on the plants became tattered and epiphytized. However, wet weight per unit area and blade thickness became most desirable 1-3 months after plants obtained their greatest fresh weight. This was most pronounced at Diana Island. In more wave-exposed areas the degree of epiphytism and blade damage was not as great, suggesting that a later harvest might be feasible at such sites. Under these conditions a more desirable wet weight per unit area and blade thickness may be obtained.

DRYING CHARACTERISTICS

Representative specimens of commercial size plants of the three studied species were dried out-of-doors to determine the feasibility of this inexpensive form of drying. All dryings were conducted on the roof of the Bamfield Marine Station during rain-free days. The plants were placed on a rack consisting of chicken wire stretched over a wooden frame (Figure 6). Weights were measured at time zero (usually 9:00 a.m.) and then hourly until the plants had obtained a constant dry weight. At each weighing the air temperature near the plants was recorded.

1. Results

From March to October, the time required to achieve constant dry weight never exceeded six hours for the three species (Table 10). The percentage of moisture lost decreased regularly from March to October for *L. groenlandica*, and remained relatively constant for *P. gardneri* from April to July. Acceptable plants of *C. triplicata* were available for drying during July and August only.

2. Conclusions

It is possible to air dry the three species out-of-doors in six hours or less throughout their growing season.

The regular change in weight loss upon drying for *L. groenlandica* from March to October indicates a lower moisture content in the older plants. Thus, the later the harvest the greater the dry weight return per unit wet weight harvested. This observation, combined with the conclusions derived in the previous section, implies that the later the harvest the better the product and the greater the return.

Figure 6. The frame employed in air-drying the three studied species

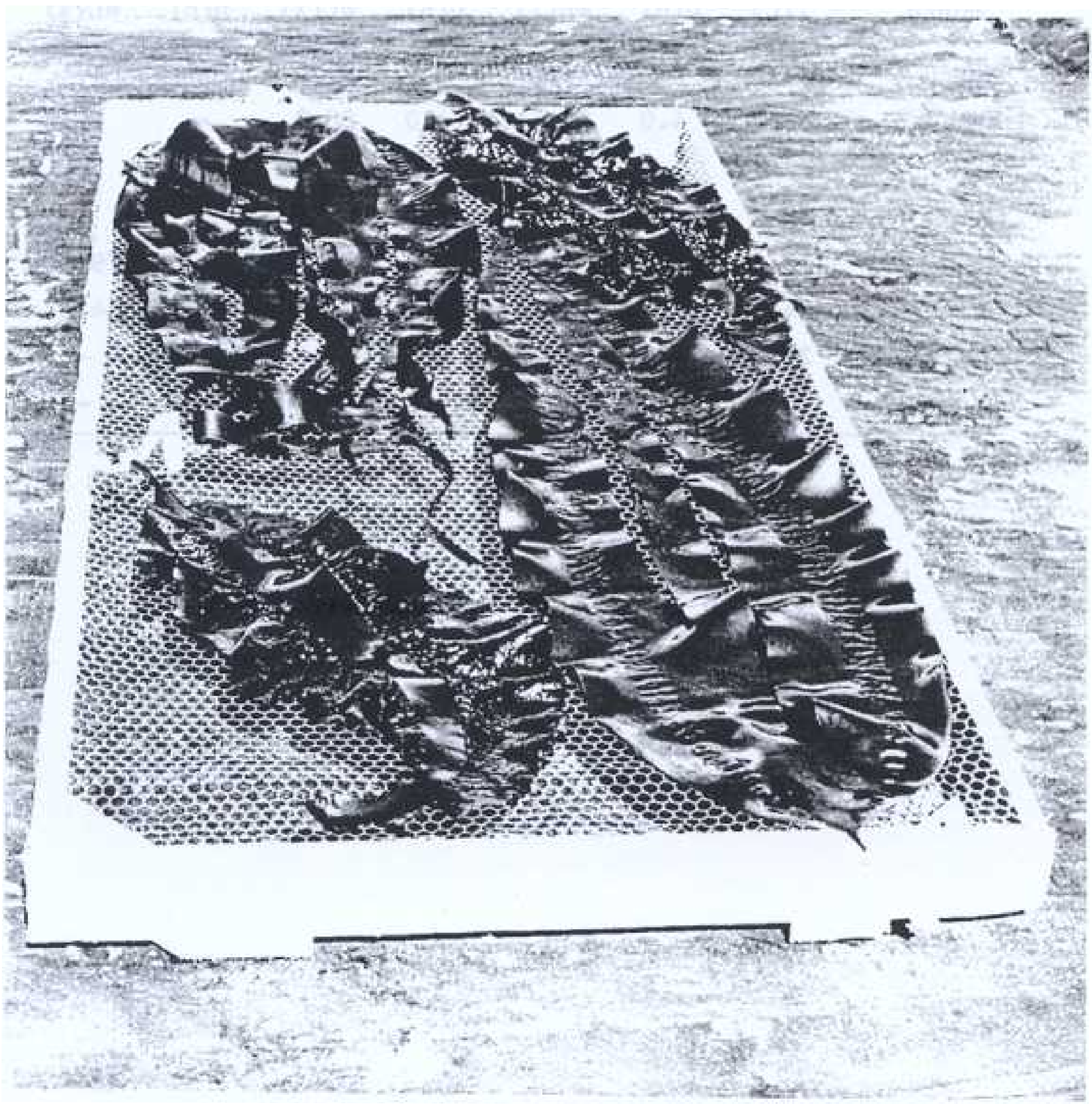


Table 10. Air drying of the three studied species: (T) mean temperature during drying period, (D) hours required to achieve a constant dry weight, (WL) percentage weight lost (n = 2).

		Laminaria groenlandica		*Pleurophycus gardneri*		*Cymathere triplicata*	
Month	T(C)	D(h)	WL(%)	D(h)	WL(%)	D(h)	WL(%)
	11.0		88.5		69.0		
	20.2		87.5		88.0		
	21.8		85.5		87.0		
	20.7		82.0		85.5		
	30.8		79.5		82.5	4.0	79.5
	24.1		75.0			4.0	84.0
	24.0		72.0				
	13.5		66.5				

HARVESTING STRATEGY

The following suggestions are based upon observations made on wild plants of *L. groenlandica* and *P. gardneri* from Diana Island and *C. triplicata* from Execution Rock. To determine the optimal time for harvesting, several features which affect the economic quality of the plants were used (Table 11). The wet weight determines the amount of plant material left after drying for processing and marketing. The greater the blade length and thickness, the better the quality of the plant. The presence of sori and epiphytes, and the laceration and erosion of the blades detract from the blades' economic value.

Table 11 lists the months which correspond to the extreme upper values for blade dimensions and wet weight, and the extreme lower values for moisture content. The times of occurrence of developed sori and marked deterioration of the blade (epiphytes and lacerations) are also listed. From this it can be seen that there is no month when all of the features used to evaluate edible kelp are optimal. Thus, the choice of the proper time to harvest must be a compromise. The suggested months for harvesting have been selected considering the weight of the harvested plants and the condition of the blades (sori, epiphytes and erosion). The blade thickness and moisture content indicated that a late harvest was preferable, provided the condition and weight of the blades were satisfactory.

Table 12 provides selected blade conditions for the suggested harvest months. *Laminaria groenlandica* was relatively thin during the month of greatest wet weight (June); however, to delay harvest for one month would result in plants weighing half as much due to loss of distal blade tissue. *Pleurophycus gardneri* obtained its greatest wet weight in May; however, all of the plants had well-developed sori, an undesirable feature. In April, the suggested month of harvest for this species, there is a slight wet weight loss and essentially sorus-free blades. August is the month recommended for harvesting *C. triplicata*. During this month the plants achieve their greatest wet weight and have a reasonable thickness. Later, soral production would depreciate the plants' value.

The suggestions above are based on observations made at a few localities in Barkley Sound over one year. There is an indication that later harvests could be achieved in more wave-exposed localities and in more northern waters.

Table 11. Features selected to evaluate plant quality. The months given in the first four columns indicate the period of optimal conditions, and those in the last two columns indicate the onset of adverse conditions. Poor blade condition results from epiphytism and blade erosion.

Species	Greatest Wet Weight	Greatest Blade Length	Greatest Blade Thickness	Lowest Moisture Content	Fully Developed Sori	Poor Blade Conditions
Laminaria groenlandica	June (531 g)	June (149 cm)	October (1.3 mm)	October (66%)	August	October
Pleurophycus gardneri	May (284 g)	July (111 cm)	July (1.6 mm)	July (83%)	May	August
Cymathere triplicata	August (132 g)	July (187 cm)	December (2.3 mm)	July (79%)	August	October

Table 12. Mean values for features used to evaluate plant quality during the month suggested for harvesting (n = 10-13, except for moisture content where n = 2).

Species	Harvest Month	Wet Weight (g)	Blade Length (cm)	Blade Thickness (mm)	Moisture Content (%)
Laminaria groenlandica	June	531	149	1.0	82
Pleurophycus gardneri	April	278	102	1.4	88
Cymathere triplicata	August	132	157	1.7	84

SEED PRODUCTION

"Seed" is the term applied to the young plants which are produced in the laboratory and eventually planted in the sea.

The production of seed involves: (1) the inducement of spore release from a sorus on a reproductively mature sporophyte, (2) the rearing of gametophytes to sexual maturity, and (3) the production and rearing of young sporophytes to approximately 5 mm length. The methodology necessary to achieve these steps is recorded in the first annual report (Druehl, 1980).

In addition to this sequence of seed production, we are exploring ways to extend the period of seed production and to reduce genetic variability of the product.

1. Normal Seed Production

Normal seed production is achieved by allowing spores collected from wild plants to develop directly into fertile gametophytes, which then produce the desired sporophytes for seed. Usually, this process requires 45 days under greenhouse natural light conditions. However, when initiated in December, approximately 65 days were required. This time was shortened by extending the photo-period by three hours using artificial light.

Acceptable spore release was achieved for *L. groenlandica* from September to February, for *P. gardneri* from June to September, and for *C. triplicata* from September to December. Normally, only poor spore releases were achieved during the early part of the year when sori were present (Table 1). Spore releases during the latter months of each species' period of maturity were usually contaminated by diatoms. This contamination reflected the heavy degree of epiphytism on the sori during these latter months.

Seed has been repeatedly produced for all three species; however, frequently the gametophytes of *C. triplicata* remained vegetative, producing macroscopic filamentous plants. We are presently attempting to determine the conditions necessary for inducing sexual reproduction.

2. Extension of the Seed Production Season

The following approaches are presently being investigated to extend the season over which seed can be produced.

Lüning and Dring (1975) have demonstrated that blue light is essential for sexuality of some kelp gametophytes. We have successfully grown gametophytes of the three test species under red light. Gametophytes under these conditions have obtained macroscopic size and remained asexual. One set of gametophytes has been fragmented. The resultant fragments adhered to the string substrate and produced a new generation of sporophytes. On the basis of this trial run it appears that we will be able to produce seed throughout the year, beginning in 1980.

Another approach to the delay of seed production is to slow down the maturation of the spore-bearing sporophyte and the development of gametophytes and young (microscopic) sporophytes. We are attempting to slow down these developmental processes by holding the various stages in the dark. Preliminary evidence indicates that spores and young gametophytes can persist in darkness for at least one month, whereas older gametophytes cannot. Results on the sporophyte stages are not yet available.

3. Control of Genetic Variability

We are presently exploring three ways to control genetic variation. The first involves cloning of the gametophyte generation so that the resulting male-female crosses give rise to genetically identical sporophytes. This is achieved by rearing isolated gametophytes under red light conditions. When these plants reach a macroscopic size, they are gently fragmented and males and females mixed. Gametogenesis is induced in these fragments by providing blue light. The resultant sporophytes are genetically identical. We have successfully completed these procedures using gametophytes of *Laminaria saccharina* from Germany. Figure 7 illustrates the various stages in the production of genetically identical sporophytes. Gametophytes reared from isolates of our studied species have not yet obtained large enough size to fragment.

The remaining approaches to controlling genetic variation involve cloning the sporophyte generation. This has the advantage of allowing the mariculturist to select the final product directly. While the gametophyte cloning provides genetically identical sporophytes, the features of these plants are not known until the plants reach maturity.

In September, a plant of *L. groenlandica* with adventitious haptera along the blade margin was collected from Execution Rock (Figure 7). This plant opened the possibility of cloning the diploid generation. The blade margin was cut into small segments, each bearing some haptera. Some segments were cultured in the greenhouse and some were outplanted on ropes. Eventually, all plant segments died after showing some growth. However, we now

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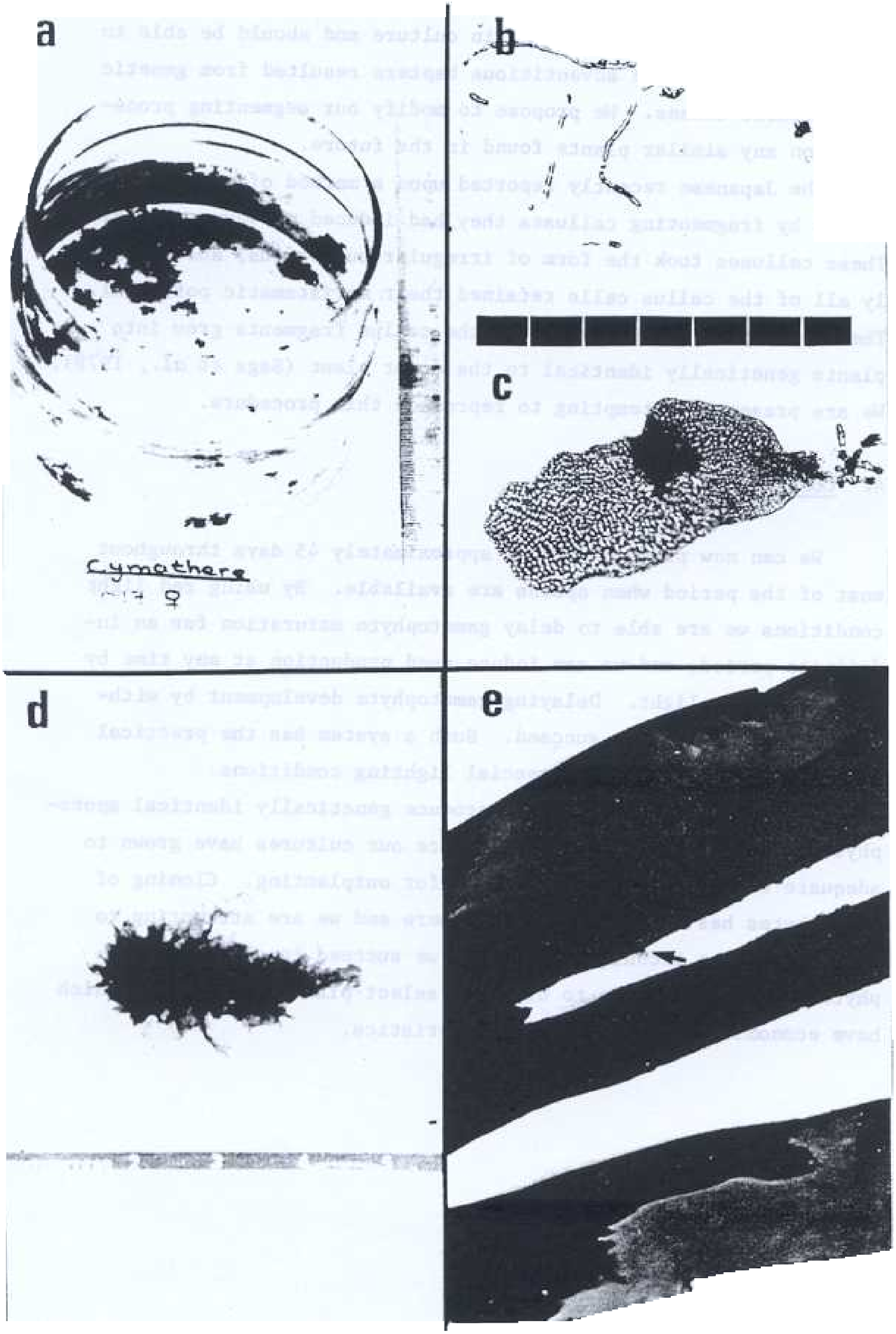
Figure 7. Stages in the production of genetically identical sporophytes: (a) a single macroscopic female gametophyte; (b) fragments of a female gametophyte (scale 0-1 mm); (c) microscopic sporophyte grown from a fertilized female fragment; (d) genetically uniform seed (macroscopic sporophyte) from fertilized female fragments; and (e) *Laminaria groenlandica* showing adventitious haptera (following page).


a
b
c
d
e
Cymothere

have gametophytes of this plant in culture and should be able to determine whether the adventitious haptera resulted from genetic or extrinsic causes. We propose to modify our segmenting procedures on any similar plants found in the future.

The Japanese recently reported upon a method of cloning sporophytes by fragmenting calluses they had induced on young plants. These calluses took the form of irregular outgrowths, and apparently all of the callus cells retained their meristematic potential. The sporophytes which arose from the callus fragments grew into plants genetically identical to the donor plant (Saga *et al.*, 1978) We are presently attempting to reproduce this procedure.

4. Conclusions

We can now produce seed in approximately 45 days throughout most of the period when spores are available. By using red light conditions we are able to delay gametophyte maturation for an indefinite period, and we can induce seed production at any time by providing blue light. Delaying gametophyte development by withholding light may also succeed. Such a system has the practical advantage of not requiring special lighting conditions.

We now have the ability to produce genetically identical sporophytes from cloned gametophytes. Once our cultures have grown to adequate size, we will produce seed for outplanting. Cloning of sporophytes has been achieved elsewhere and we are attempting to reproduce these techniques. Should we succeed in cloning sporophytes, we will be able to directly select plants for cloning which have economically desirable characteristics.

KOMBU FARMS

Kombu farms have been established in several locations to evaluate the response of the three studied species to different environments. From this study we will determine the optimal life history stage (spores, gametophytes or sporophytes), and the optimal times and environments for outplanting. The locations and dates of establishment for kombu farms in Barkley Sound are given in Table 13. One farm, 120 m in length, was established near Sointula in Broughton Strait during January, 1980.

1. Structure

Initially, long beach-combed logs were used to support a series of vertical lines bearing plants. This system was inexpensive and could be constructed from readily available materials. Further, the chance of the logs being stolen by souvenir-hunters was very slight.

This system was unsatisfactory, however, for two reasons. First, the logs became fouled with floating kelp and other debris, and second, the logs tended to roll, coiling the vertical lines around them and severely abrading the attached plants.

Presently, we are using rope systems buoyed by commercial floats (Figure 8). So far, this system has proven successful. To discourage souvenir-hunters, the floats are attached to half-inch steel cable which joins the rope system at approximately 2 m depth. The cost for materials for this system is given in Table 14.

2. Wild Plant Transplants

Wild plants of the three studied species in their first year (1979) of growth were transplanted to farms where their growth could

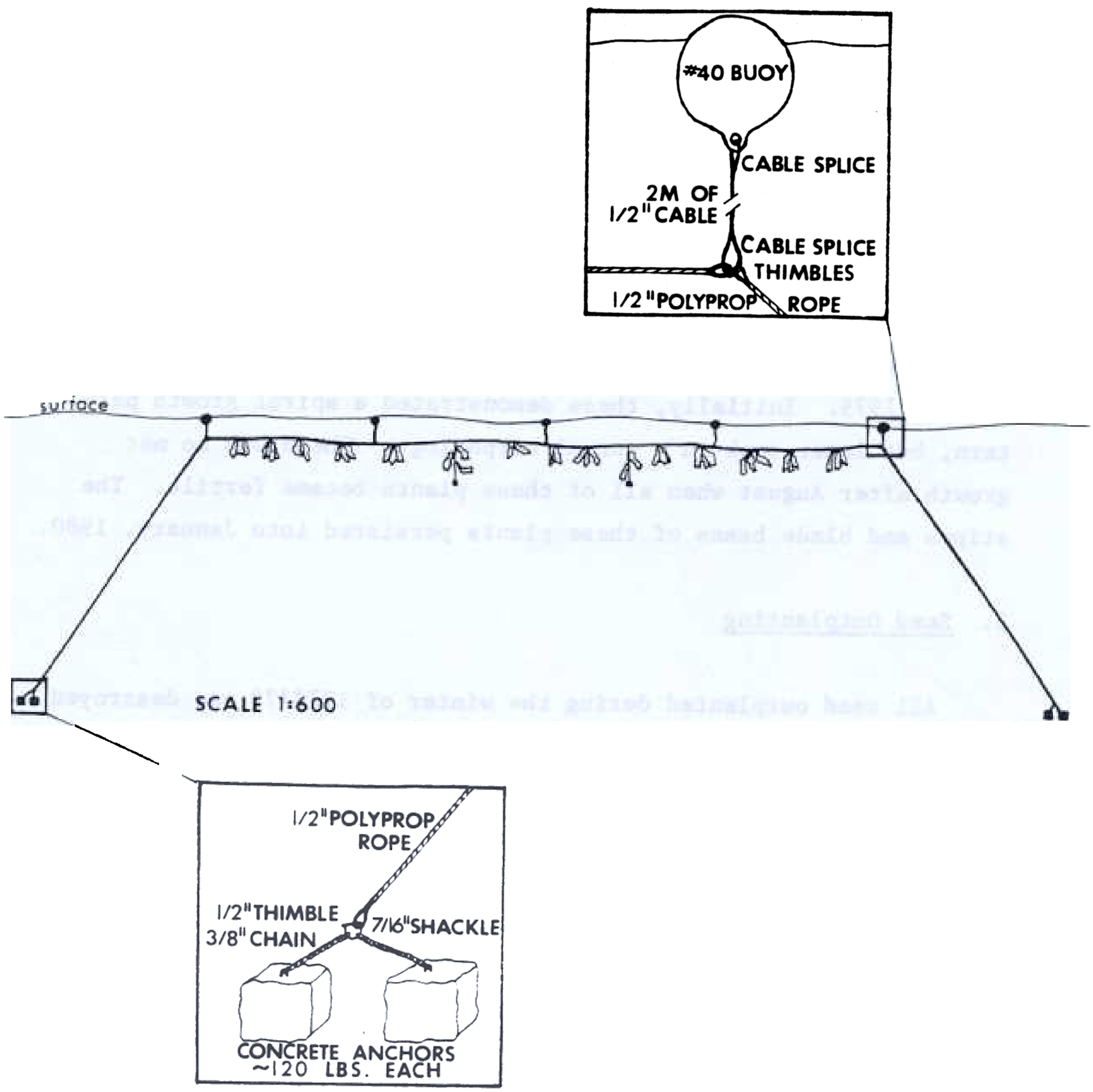
Table 13. Location of kombu farms in Barkley Sound, their date of establishment and their length of seeded culture line, as of March, 1980.

Location	Established	Length
Kelp Bay	March, 1979	240 m
Tzartus Island	February, 1979	60 m
Helby Island	September, 1979	90 m
Adamson's Rocks	January, 1980	300 m
Scott's Bay	February, 1980	60 m
Execution Rock	February, 1980	60 m

Table 14. Cost of materials for one 60 m long kombu farm. Costs are local Bamfield charges in January, 1980, with no provincial tax added. This list does not include vertical lines which are sometimes used to test depth response of the plants.

Materials	Cost
125 m, ½" Polyprop rope	$ 47.00
6 m, ½" cable (steel)	13.60
4 m, 3/8" chain (galvanized)	20.68
6 thimbles (galvanized)	9.60
2, 7/16" shackles (galvanized)	4.20
2, #40 buoys	41.30
3, 6600-20 floats	8.85
1.4 bags cement for anchors	10.00
Total materials/kombu farm	$155.23

Figure 8. Schematic illustrating the construction of a kombu farm



be monitored. Surviving plants will be harvested in May/June, 1980 and the products from the various locations compared.

Wild plants of *L. groenlandica* were transplanted to Kelp Bay rope farms in May, 1979. Initially these plants were heavily epiphytized by bryozoans, tunicates and mussels. The mussels abraded many of the stipes and some of these plants were lost. Those surviving grew slowly until October and by December were nearly free of epiphytes. In January, 1980, the plants were entering a rapid growth phase, and by March had grown considerably.

In September, 1979, wild plants were established at Kelp Bay, Helby Island and Tzartus Island. These plants followed the same growth pattern as described for the May plantings; however, Kelp Bay plants were less heavily epiphytized than were plants at the other two sites (Figure 9).

In May, 1979, wild plants of *P. gardneri* were planted at Tzartus Island on ropes attached to logs. These were all lost.

Wild *C. triplicata* plants were established at Tzartus Island in May, 1979. Initially, these demonstrated a spiral growth pattern, but later took on a normal morphology. There was no net growth after August when all of these plants became fertile. The stipes and blade bases of these plants persisted into January, 1980.

3. Seed Outplanting

All seed outplanted during the winter of 1978/79 was destroyed by the summer of 1979. This loss was due primarily to the initial use of logs as a support system. Subsequently, several sets of seed have been established at different sites (Table 15). This

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Figure 9. Seed of the three studied species as observed in March, 1980: *Cymathere triplicata* (a) and *Laminaria groenlandica* (b), outplanted in January, 1980; and *Pleurophycus gardneri* (c) outplanted September, 1979. Wild plants of *Laminaria groenlandica* at time of transplant to Kelp Bay September, 1979 (d) and one of these plants as observed in March, 1980 (e), (a), (b) and (c) are to the same scale and (d) and (e) are to the same scale (following page).


a
b
c
d
e

Table 15. The location, date and stage of outplant (1N = gametophyte, 2N = sporophyte) and positionion (V = vertical ropes, H = horizontal ropes) for seed of the three studied species.

Location	Date	Stage of Outplant	Positioning
		Laminaria groenlandica	
Tzartus Island	19 Jan 80	asexual 1N	V
	19 Jan 80	microscopic 2N	V
	19 Jan 80	macroscopic 2N	V
	27 Feb 80	macroscopic 2N	V/H
Kelp Bay	10 Jan 80	asexual 1N	V
	10 Jan 80	sexual 1N	V
	10 Jan 80	microscopic 2N	V
	10 Jan 80	macroscopic 2N	V
	6 Feb 80	macroscopic 2N	H
	13 Feb 80	macroscopic 2N	V
Adamson's Rocks	27 Jan 80	macroscopic 2N	H
	20 Feb 80	macroscopic 2N	H
Helby Island	19 Jan 80	macroscopic 2N	H
	25 Jan 80	macroscopic 2N	V
Scott's Bay	21 Feb 80	macroscopic 2N	V
Execution Rock	21 Feb 80	macroscopic 2N	V
Sointula	30 Jan 80	macroscopic 2N	V
		Pleurophycus gardneri	
Tzartus Island	9 & 29 Sep 79	macroscopic 2N	H
Kelp Bay	9 & 29 Sep 79	macroscopic 2N	H
Helby Island	9 & 29 Sep 79	macroscopic 2N	H
		Cymathere triplicata	
Tzartus Island	19 Jan 80	asexual 1N	H
Kelp Bay	10 Jan 80	macroscopic 2N	H
Helby Island	19 Jan 80	asexual 1N	H

seed has been placed along horizontal ropes positioned 2 m below the surface. Further, the seed has been spaced at different intervals along the ropes (30 or 50 cm). The density of plants allowed to persist at each interval will be altered (1, 2, or 3 plants) when the plants have grown to *ca* 10 cm length.

The *C. triplicata* and *L. groenlandica* seed has grown well until March, 1980. However, it would be premature to draw conclusions regarding suitability of habitat or life history stage at outplanting at the time of writing.

Seed of *P. gardneri* was outplanted in early and late September, 1979 at Tzartus Island, Kelp Bay and Helby Island. The early September seed became heavily epiphytized (*Obelia* and diatoms) by November at Tzartus Island and Helby Island but not at Kelp Bay. The late September seed was only lightly epiphytized at all three sites. However, this seed was heavily grazed (isopods?) at Tzartus Island and Helby Island. The Kelp Bay seed has grown well until March, 1980.

Figure 9 illustrates the conditions of seed for the three species as observed in March, 1980.

4. Environmental Monitoring

Weekly observations are made at each kombu farm located in Barkley Sound covering the following parameters: irradiance (above surface, just below surface and at 1, 2, 3, 4, 5 m below the surface), temperature and salinity (at 0, 1, 2, and 4 m depth), nutrients (NO_3, NO_2, NH_4, PO_4 at 0, 1, and 4 m depth), and water transparency (secchi depth) (Table 16).

Nutrient data have been collected jointly with Canadian Benthic Limited, in Bamfield Inlet. These data indicate a pattern of change in nutrient concentrations with season and depth (Table 17). In the top 10 m total nitrogen and phosphate are low from April to September and high for the rest of the year. Below 10 m these values remain relatively high throughout the year.

Table 18 gives selected environmental data for the three oldest kombu farms. Environmental data will be subjected to detailed analy-

Table 16. Environmental monitoring stations, starting dates of the weekly observations, and subjective evaluation of water motion.

Monitoring Station Location	Starting Date	Water Motion
Kelp Bay	8 Mar 1979	Moderately wave sheltered
Tzartus Island	8 Mar 1979	Tidal currents
Scott's Bay	8 Mar 1979	Moderately wave exposed
Helby Island	13 Sep 1979	Moderately wave exposed
Adamson's Rocks	11 Jan 1980	Fully wave exposed
Execution Rock	15 Feb 1980	Fully wave exposed

Table 17. Total inorganic nitrogen (N) and phosphate (P) concentrations for three depths in Bamfield Inlet during 1979. Values are μg-at$\cdot l^{-1}$.

	Depth					
	1m		5m		15m	
Date	N	P	N	P	N	P
January	19.2	1.8	19.8	1.9	19.9	1.9
February	15.4	1.5	15.9	1.6	18.1	1.7
March	11.3	1.0	15.0	1.3	15.3	1.3
April	0.2	0.3	8.8	1.1	13.7	1.6
May	0.3	0.2	0.5	0.4	0.9	0.5
June	2.9	0.4	4.1	0.7	10.7	0.9
July	14.6	1.5	16.5	1.6	16.5	1.6
August	0.6	0.2	15.6	1.7	16.8	1.9
September	0.7	0.2	10.2	1.2	15.5	1.6
October	15.7	1.5	20.5	2.3	23.0	2.2
November	12.6	1.2	12.2	1.4	12.7	1.4

Table 18. Selected environmental data for the oldest kombu farms. Values are means and standard deviations based on weekly observations through the periods indicated.

	Kelp Bay	Tzartus I.	Helby I.
Temp. at 1 m (C)			
Apr 79 - Sep 79	13.7 ± 3.5	13.6 ± 3.4	
Oct 79 - Mar 80	9.9 ± 1.9	9.8 ± 2.1	9.8 ± 2.0
Salinity at 1 m (°/oo)			
Apr 79 - Sep 79	27.2 ± 3.0	26.2 ± 3.2	
Oct 79 - Mar 80	28.7 ± 2.7	26.6 ± 4.0	27.6 ± 3.5
Secchi (depth in m)			
Apr 79 - Sep 79	5.3 ± 2.3	5.6 ± 2.0	
Oct 79 - Mar 80	8.9 ± 2.7	7.7 ± 2.5	7.7 ± 3.0
Irradiance at 1 m ($\mu E \cdot m^{-2} \cdot sec^{-1}$)			
Apr 79 - Sep 79	337.4 ± 244.0	327.5 ± 227.6	
Oct 79 - Mar 80	102.4 ± 104.6	111.5 ± 106.2	116.9 ± 116.8

sis following our first kombu farm harvest. Nutrient samples have been frozen and will be analyzed by Canadian Benthic Ltd. in the near future.

The various locations supporting kombu farms were selected, in part, to reflect different water movement conditions. The Japanese feel that water motion is a very important factor influencing the quality of cultivated kombu (Hasegawa, pers. comm.). We have subjectively classified the type of water motion at each farm (Table 16). However, an objective classification is required. At present, we are attempting to employ a system of plaster-of-Paris balls, whose erosion rates reflect the degree of water movement (Muus, 1968).

5. Conclusions

Kombu farms, constructed of anchored ropes which are buoyed at the surface by commercial floats, appear to be best suited to rearing edible kelp in the sea. Drifting kelp (*Macrocystis* and *Nereocystis*) will foul this system, but not as severely as the log system earlier employed.

There are two growth seasons for the plants: the season of rapid growth (January-June) and the season of slow growth (July-December). During the season of slow growth, the plants are most susceptible to epiphytism. We hypothesize that the presence of epiphytes does not initiate the slow growth season, but rather that epiphytes take advantage of the plants' decreased rate of growth by settling in greater numbers. The season of slow growth probably reflects the low nutrient levels observed during the summer months, and possibly intrinsic factors (Table 18; Lüning, 1979).

The developing epiphyte community, however, does compete with the plants for nutrients, occludes light, and adds stress, in the form of greater resistance to water movement. Thus, the greater the degree of epiphytism, the poorer the state of the plant as it enters the January-June rapid growth season. In Germany, Lüning (1969) has shown that the *Laminaria* blade that overwinters provides energy for the growth of the new blade the following season, and that this new growth is in part proportional to the size of the old blade. From this it can be hypothesized that epiphytes indirectly affect new growth as a plant enters its second year. This is the last period of rapid growth before the plants are harvested. On the basis of the above, the major factors which are likely to identify an ideal kombu farm are high nutrient levels and little epiphytism.

General observations indicate that epiphytism decreases with increasing wave exposure. This could mean that the physical action of the waves is cleaning the plants. Also, the wave action could be moving water up from depths where nutrient concentrations are greater, thus allowing for a prolonged rapid growth (and epiphyte-free) season.

Kombu farms in fully wave-exposed locations were established in January, 1980. Growth data from these and more sheltered locations, combined with nutrient data, should narrow kombu farm site options considerably.

PHYSIOLOGICAL STUDIES

Two studies, closely related to the edible kelp research, have been initiated: (1) nutritional requirements of *L. groenlandica* with Dr. P.J. Harrison (Oceanography, University of British Columbia); and (2) photosynthetic requirements of *L. groenlandica* with Mr. S. Villeneuve (graduate student, Simon Fraser University). These studies are financed by a National Research Council grant. Following is a condensation of results from our nutritional studies. The photosynthetic studies have just begun.

Ammonium and nitrate uptake kinetics were studied for three year classes of *L. groenlandica* collected from nitrogen depleted waters in Barkley Sound. First, second and third year plants had active uptake components which saturated near 20μM NO_3 or NH_4. Beyond 20μM the uptake rate was linear in the second and third year plants, suggesting a passive or diffusive uptake. At 20μM NO_3 or NH_4, the uptake rate of the first year plants was the highest (1.1 to 1.6μM gm dry wt. $^{-1}h^{-1}$). The uptake rates of the second and third year plants were considerably reduced. At night the uptake rate of NO_3 was sharply reduced, while the uptake of NH_4 was similar or higher than rates during the day (Table 19).

Table 19. Day and night uptake rates (μM·gm dry wt·$^{-1}$·h^{-1}) of three year classes of *Laminaria groenlandica* cultured in 30μM NH_4 and NO_3 (n = 4).

	NO_3		NH_4	
Year Class	Day	Night	Day	Night
1	1.10		1.60	2.90
2	0.25		0.40	0.73
3	0.10		0.15	0.22

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