# THE DEVELOPMENT OF AN EDIBLE KELP CULTURE TECHNOLOGY FOR BRITISH COLUMBIA

## I. Preliminary Studies

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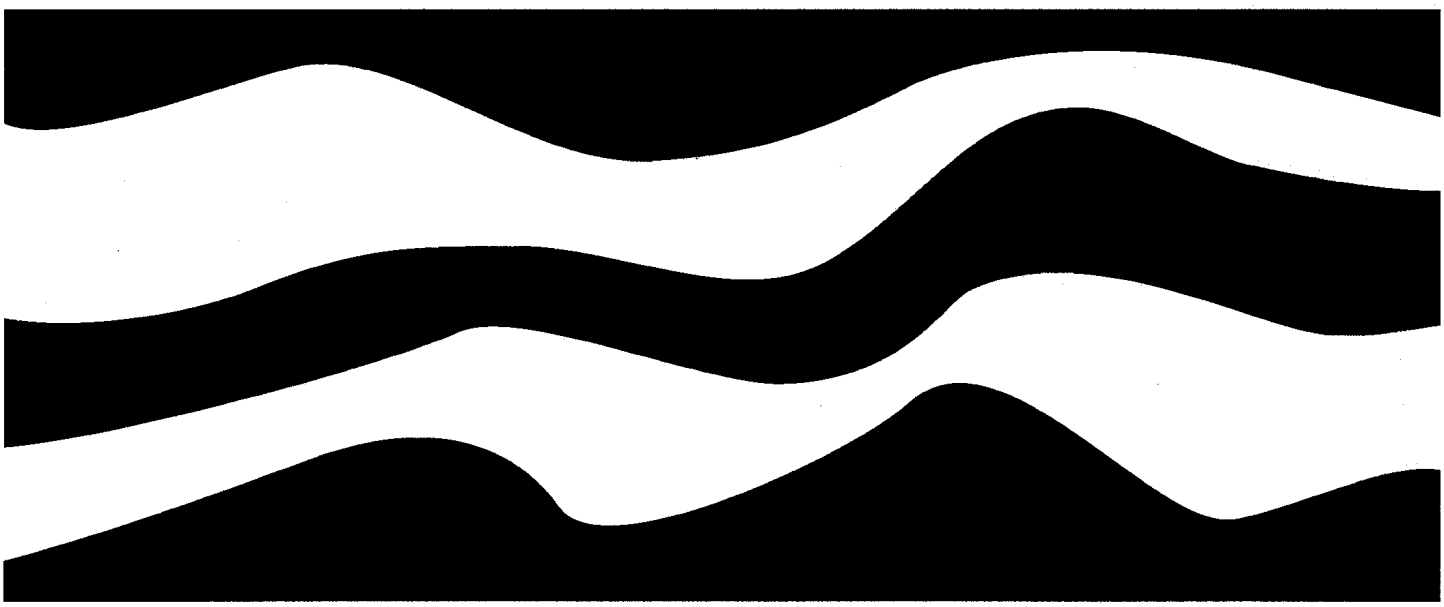
**marine resources branch**
Ministry of Environment
Province of British Columbia



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# THE DEVELOPMENT OF AN EDIBLE KELP CULTURE TECHNOLOGY FOR BRITISH COLUMBIA

## I. Preliminary Studies

by

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Simon Fraser University
and
Bamfield Marine Station

*Fisheries Development Report No. 24*

*Marine Resources Branch*
*Ministry of* Environment
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ABSTRACT

A research program, designed to develop the necessary technology for rearing edible kelp has been initiated. This program is based upon the time-tested methods employed by the Japanese in kombu culture and recent findings regarding reproductive biology of kelp.

The taxonomies, distributions and life histories of the three studied species (*Laminaria groenlandica* Rosenvinge, *Cymathere triplicata* [P&R] J. Agardh, and *Pleurophycus gardneri* Setchell & Gardner) are reviewed.

The three studied species appear to have physical features desirable for Japanese kombu. Further, we have been able to culture gametophytes through to the young sporophyte stage, which can be used as seed for kombu farms.

Cloning experiments have been initiated. These will allow us to eventually produce a genetically uniform crop.

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## ACKNOWLEDGEMENTS

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# INTRODUCTION

Various species of British Columbia Laminariales (kelp) may be suitable for human consumption. In the Orient, where similar species are commonly eaten, these plants are referred to as kombu (Japanese) and haidai (Chinese),. The most important kombu species include *Laminaria japonica* Areshung,, *L. diabolica* Miyabe and *L. augustata* Kjellman. Haidai usually refers to *L. japonica* and man-manipulated genetic variations of this species (Anon., 1976).

None of the above mentioned species is known, for certain, to exist in British Columbia coastal waters. A comparison of the northeast and northwest Pacific kelp floras indicates that we share only two species which are commercially exploited in Japan: *Cymathere triplicata* (Postels and Ruprecht) J. Agardh and *Laminaria yezoensis* Miyabe (Miyabe 1902, Druehl 1970). The latter species is poorly rated in Japan.

Initially, I am investigating three kelp species: *Cymathere triplicata*, *Laminaria groenlandica* Rosenvinge and *Pleurophycus gardneri* Setchell and Saunders. The first species was selected as it is a proven kombu species in Japan. The remaining two species, which do not occur in Japan, appear to have some of the physical attributes of good kombu (Kawashima 1972).

The purpose of this study is to develop the biological skills essential to the establishment of a kombu industry in British Columbia. The present manuscript reports on researches conducted from June 1, 1978 through March 4, 1979.

## HISTORICAL PERSPECTIVES AND RESEARCH APPROACH

Enhancement of Oriental kelp productivity dates back into the 17th Century. However, practices resulting in some maricultural control have evolved only within the past few decades.

The Japanese presently collect fertile kelp in the fall or earlier, and establish gametophyte cultures from these plants on string. Gametophyte and subsequent young sporophyte development on string takes place indoors under artificial light and in vigorously aerated, nutrient-enriched seawater (Hasegawa, 1972). Young sporophytes, attached to string, are laced into rope and suspended in the sea. Once placed in the sea, the rope cultures are cleared periodically of epiphytes and the number of sporophytes reduced to about three for every 30 cm segment of rope. Some attempts at fertilizing these plants with nutrients are being made (T. Kaneko, pers. comm.).

The Chinese employ a system similar to that developed by the Japanese; however, they have to deal with a shorter growing season, and limited coastline having suitable temperatures for year-round kelp growth. To overcome these problems, an extensive program of inbreeding, X-ray treatment and selection was undertaken (Fang et al., 1963). New varieties of *Laminaria japonica* resulted from this research which were resistant to higher temperatures (Anon., 1976). Further, the Chinese have extended the range of kelp production through the application of fertilizers (Tseng et al., 1955).

Generally, the Chinese and Japanese have mastered many of the problems associated with the laboratory production of sporophytes, and with the subsequent cultivation of these plants in the sea.

However, when their systems are compared with modern agricultural practices, some obvious shortcomings are apparent. First, they are essentially locked in to the natural cycle of kelp growth and maturation. This results from the fact that it is not yet possible to predictably induce meiosis. Thus, they must initiate their string cultures when meiospores are naturally available. A second drawback is that they have no applied method for reducing genetic variability in the cultured plants.

Recent advances in our understanding of kelp gametophyte biology make possible the delay of gametogenesis and the cloning of gametophytes. Most kelp gametophytes require blue light for gametogenesis (Lüning & Dring, 1975; Lüning & Neushul, 1978). Thus, growth of the gametophyte can be allowed to continue and gametogenesis delayed by providing only red light. This allows one to break out of the kelp's natural cycle and produce new sporophytes for

planting in the sea at will. Further, if isolated gametophytes are grown asexually in this manner, they reach large size and can be cloned by fragmentation (Markham et al., 1979). Crossing of these results in a large number of genetically uniform sporophytes. Further each crossing can be repeated indefinitely from cloned gametophytes maintained in red light.

My research approach is to adapt the time-tested methodology of Japanese kelp production to local species and conditions. At the same time I am attempting to apply the recently discovered techniques of gametogenesis control and cloning to the production of sporophytes for cultivation in the sea.

## THE STUDIED SPECIES

### 1. *LAMINARIA GROENLANDICA* Rosenvinge

#### a. Taxonomy

Prior to 1968, this species was referred to as *Laminaria cuneifolia* J. Ag. and *L. platymeris* De la Pyl. (e.g. Setchell & Garnder, 1925; Scagel, 1957). However, Druehl (1968) placed these two species in the *L. groenlandica* complex. Further, he recognized four ecological forms of *L. groenlandica* for the northeast Pacific: flat stipe, short stipe, long stipe and shade form. These forms do not appear to be genetically distinct. The present study deals primarily with the latter two forms. The shade form when grown outside of a heavy kelp canopy behaves the same as the long stipe form.

#### b. Description (after Druehl, 1968)

Blades up to 2 m long and 0.5 m wide, with or without bullae, often torn (Figure 1A, B). Stipe usually 10 to 30 cm long, slightly flattened or terete, holdfast of many branched haptera.

#### c. Distribution

*L. groenlandica* is known from Attu Island (52°55'N, 173°14'E), Alaska to Cape Blanco (42°50'N, 124°33'W), Oregon, with isolated populations in central California (Druehl, 1970). The presently known distribution of *L. groenlandica* in British Columbia is shown in Figure 2 (Scagel *in herb.).*

Generally, the distribution of *L. groenlandica is* restricted to wave-exposed and moderately wave-exposed shores, where it occurs from the low intertidal to 2-4 m below low water. In some wave-sheltered areas, this species is known from the subtidal region only (Druehl, 1967). In Barkley Sound, plants tend to be widely spaced throughout suitable habitats.

#### d. Life History

The life histories of the shade form and long stipe form are shown in Figure 3 (Druehl, 1965). This species is perennial and each subsequent year class has a broader blade. Plants younger than one

year do not produce sori in the early summer but all plants produce sori during the winter.

2. *CYMATHERE TRIPLICATA* (Postels & Ruprecht) J. Agardh

a. Taxonomy

*Cymathere triplicata* is a monotypic genus on the west coast of North America (Setchell & Gardner, 1925). A second species, *C. gibrosa,* may occur in northern Japan; however, contemporary opinion is that this is a species of *Laminaria* (Hasegawa pers. comm.).

b. Description (after Setchell & Gardner, 1925)

Blades up to 4 m long and 20 cm wide, entire, three folds running the full blade length (Figure 4A). Stipes 5-25 cm long, cylindrical to slightly flattened near blade base. Holdfast a disc.

c. Distribution

*C. triplicata* is known from Attu Island, Alaska, to Whidbey Island (48°N, 122°30'W), Washington (Druehl, 1970). Its presently known distribution in British Columbia is shown in Figure 5 (Scagel *in herb.*). Very little is known regarding local conditions necessary to support growth of this species. In Barkley Sound it is known from only one location (Blow Hole, Figure 7) where it is restricted to the lower intertidal and subtidal regions. Plants form dense patches which are sporadically spaced.

d. Life History

There is no published account of the life history of northeast Pacific *C. triplicata.* A survey of plants in the University of British Columbia Herbarium (Scagel in herb.) indicates that plants may produce sori as early as August and that these plants will remain fertile as long as they persist. Once sori have been initiated, vegetative growth may cease (Figure 3).

3. *PLEUROPHYCUS GARDNERI* Setchell & Saunders

a. Taxonomy

*Pleurophycus gardneri* is a monotypic genus known only from the northeast Pacific.

b. Description (after Setchell & Gardner, 1925)

Blade up to 1 m long and 40 cm wide, having a broad (up to 15 cm) midrib (Figure 1C, D). Stipe up to 50 cm long, terete at the base and slightly flattened at the blade base. Holdfast consists of branched haptera.

c. Distribution

*P. gardneri* is known from Montague Island (59°58'N, 147°22'W), Alaska to Coos Bay (43°25'N, 124°20'W), Oregon (Druehl, 1970),. The presently known British Columbia distribution is shown in Figure 6 (Scagel *in herb.*) it appears to grow a little higher in intertidal region and in a wider spectrum of wave conditions than does *C. triplicata.*

d. Life History

There is no published account of the life history of *P. gardneri.* On the basis of herbarium specimens (Scagel *in herb.*) it appears that sori may be produced as early as March (Figure 3). My observations suggest that vegetative growth ceases with the initiation of sorus production. Plants observed in March 1979 indicate that *P. gardneri* is a perennial and not an annual as stated by Setchell and Gardner (1925).

4. Conclusions

The taxonomy of the three studied species is well understood. Further, with a little practice, it-is easy to distinguish the three species from each other and from other British Columbia kelp.

On the basis of our present knowledge, all three species are relatively common throughout British Columbia coastal waters, with the exception of the Strait of Georgia. The gaps in distribution shown in Figures 2, 5, and 6

represent areas poorly known phycologically and probably do not reflect the complete distributional pattern.

The life history of *L. groenlandica* is relatively well known. *C. triplicata* and *P. gardneri* have not been previously studied. *L. groenlandica,* a perennial, produces sori in the summer and in the *fall.* *C. triplicata* is apparently an annual, producing sori from maturity until the death of the plant. The life span of *P. gardneri* is not known, but may involve several years.

Figure 1. Different growth forms of two of the test species. A. *Laminaria groenlandica* from Diana Island, July 1978. Total plant length 1.6 m. B. *L. groenlandica*, shade form from Grappler Inlet, July 1978. Total Plant length 0.3 m. C. *Pleurophycus gardneri*, wave-sheltered form from Diana Island, July 1978. Total plant length 1.5 m. D. *P. gardneri*, wave exposed form from Tzartus Island, July 1978. Total plant length 0.7m.

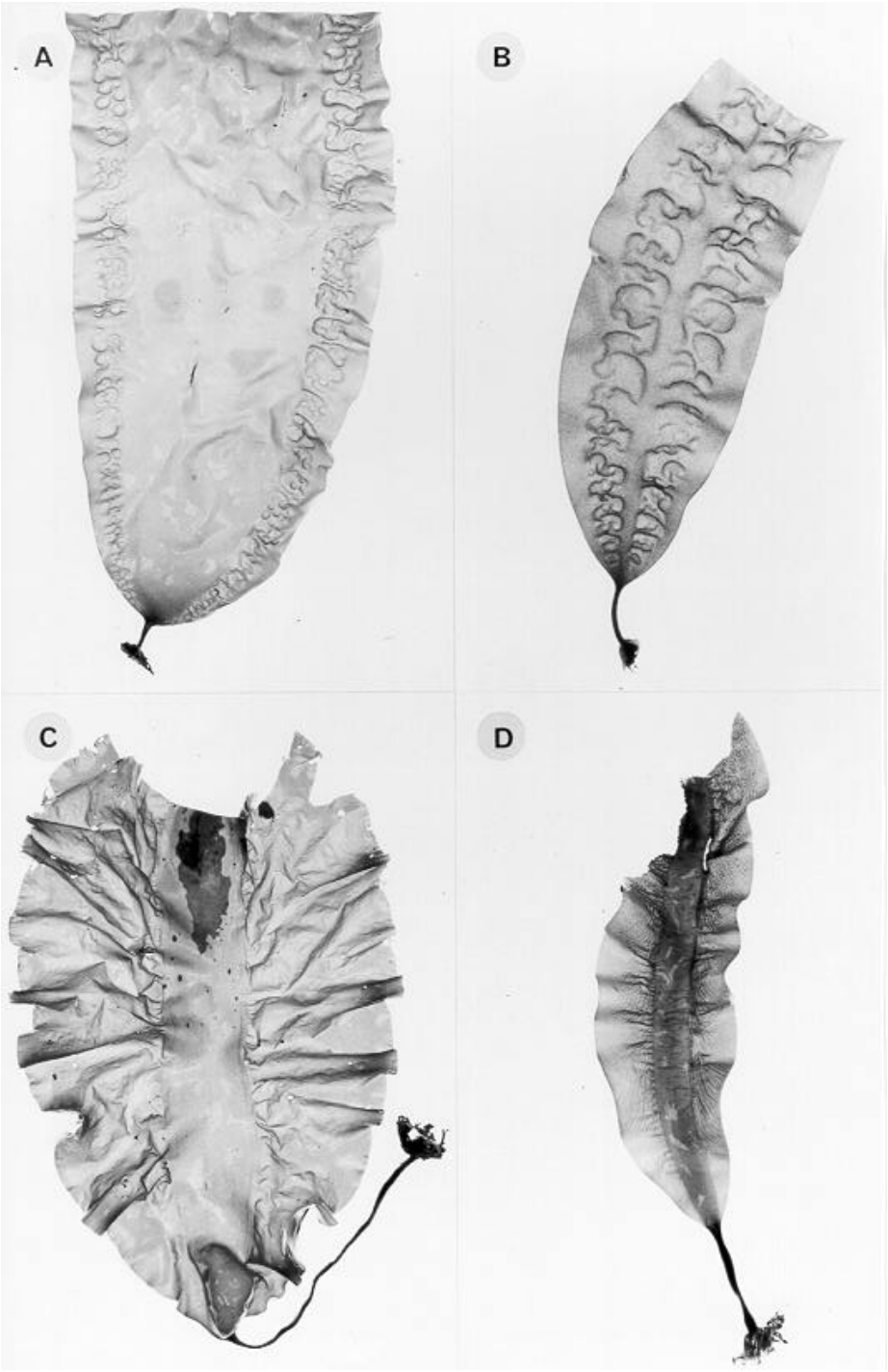
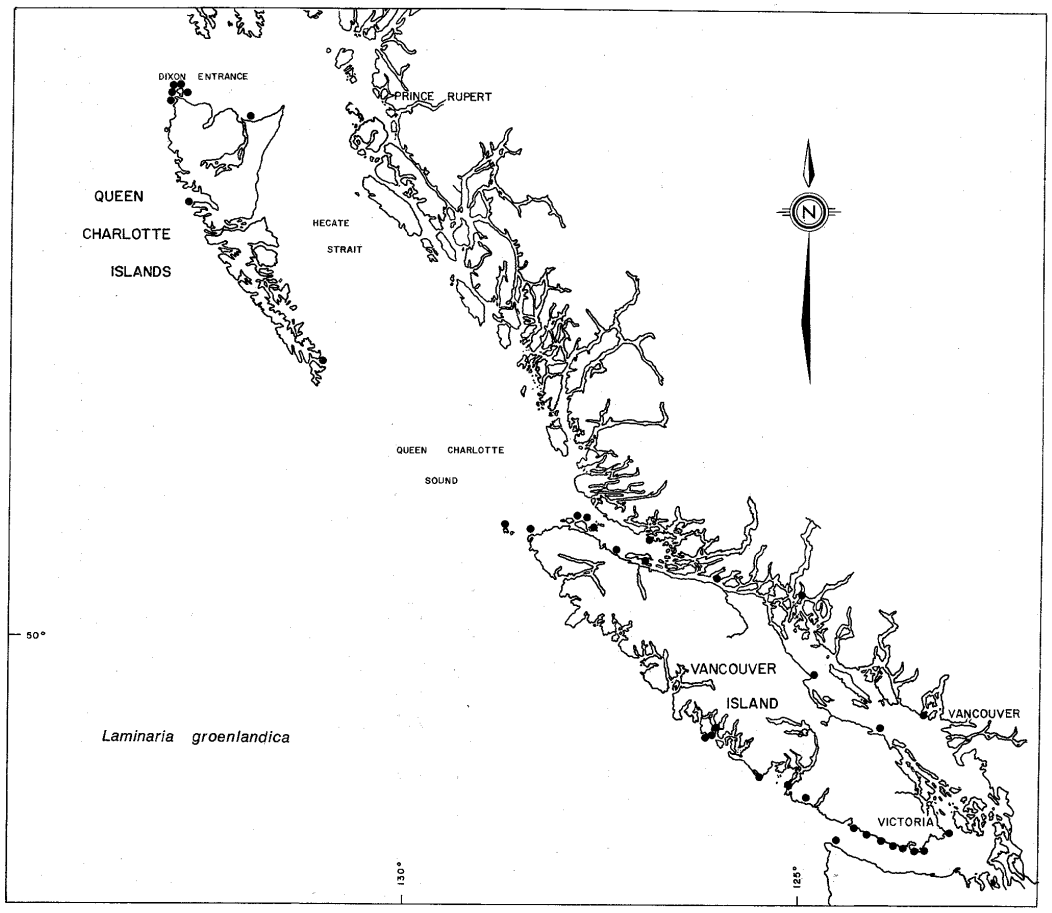


Figure 2. The presently known distribution of *Laminaria groenlandica* in British Columbia (Scagel *in herb*.).



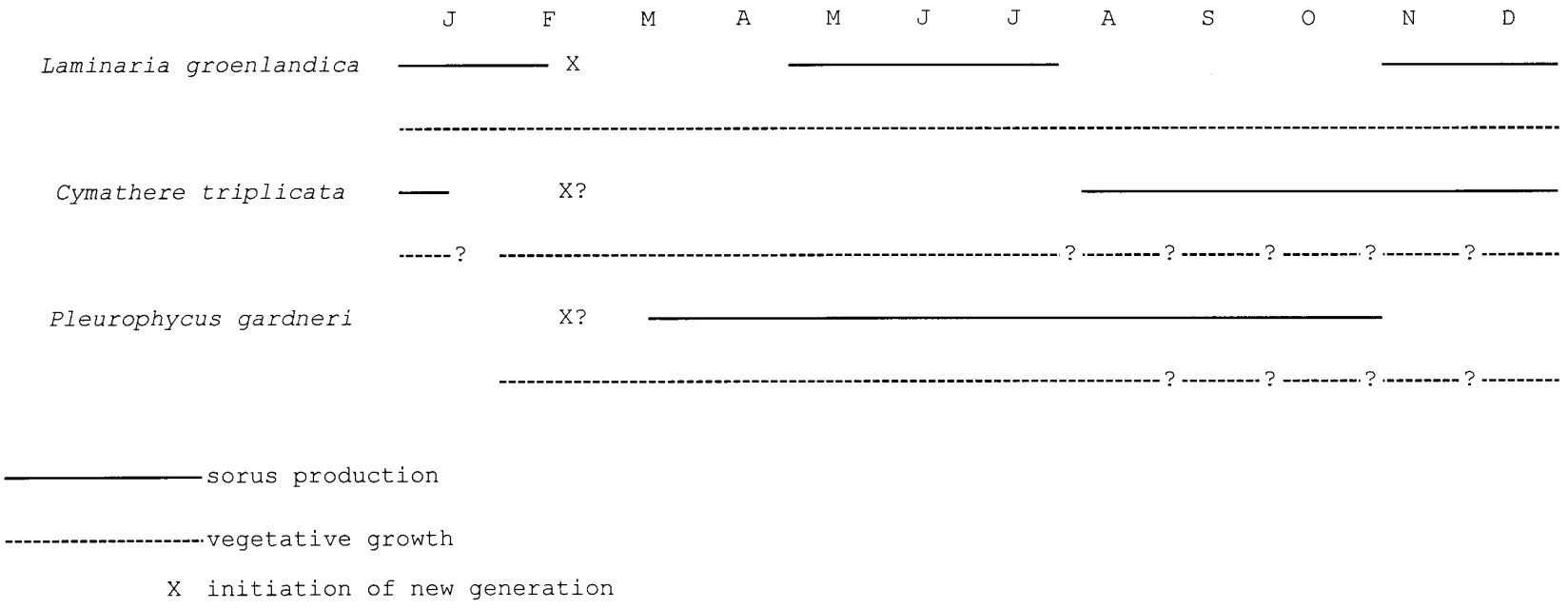



Figure 3. Life histories of *Laminaria groenlandica*, *Pleurophycus gardneri* and *Cymathere triplicata*.

Figure 4. A. *Cymathere triplicata* collected from the Blow Hole, July 1978. Total plant length, 1.2m. B. *Laminaria diabolica* (?) collected from Keagh Shoale, Queen Charlotte Strait, July 1978. Total plant length, 0.8m. Note the presence of haptera above disc.

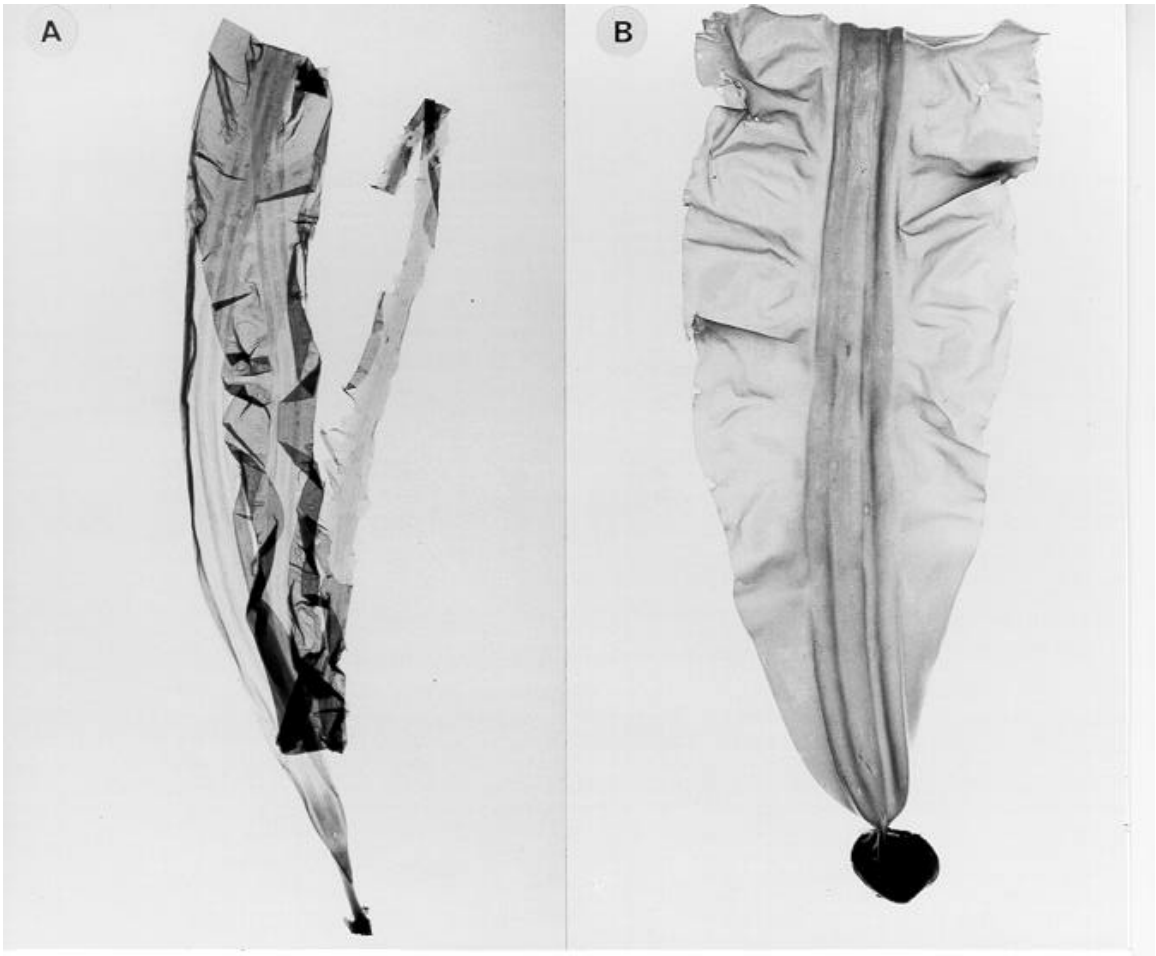


Figure 5. The presently known distribution of Cymathere *triplicata* in British Columbia (Scagel *in herb*.).

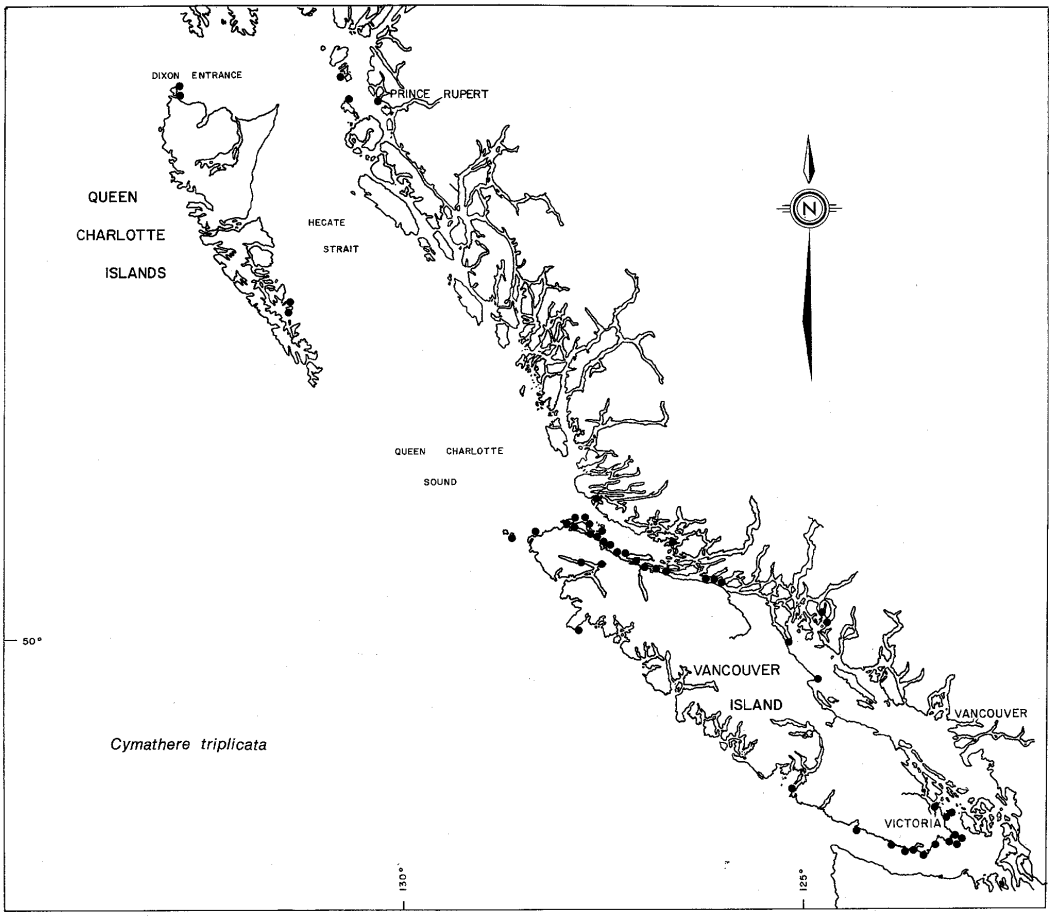
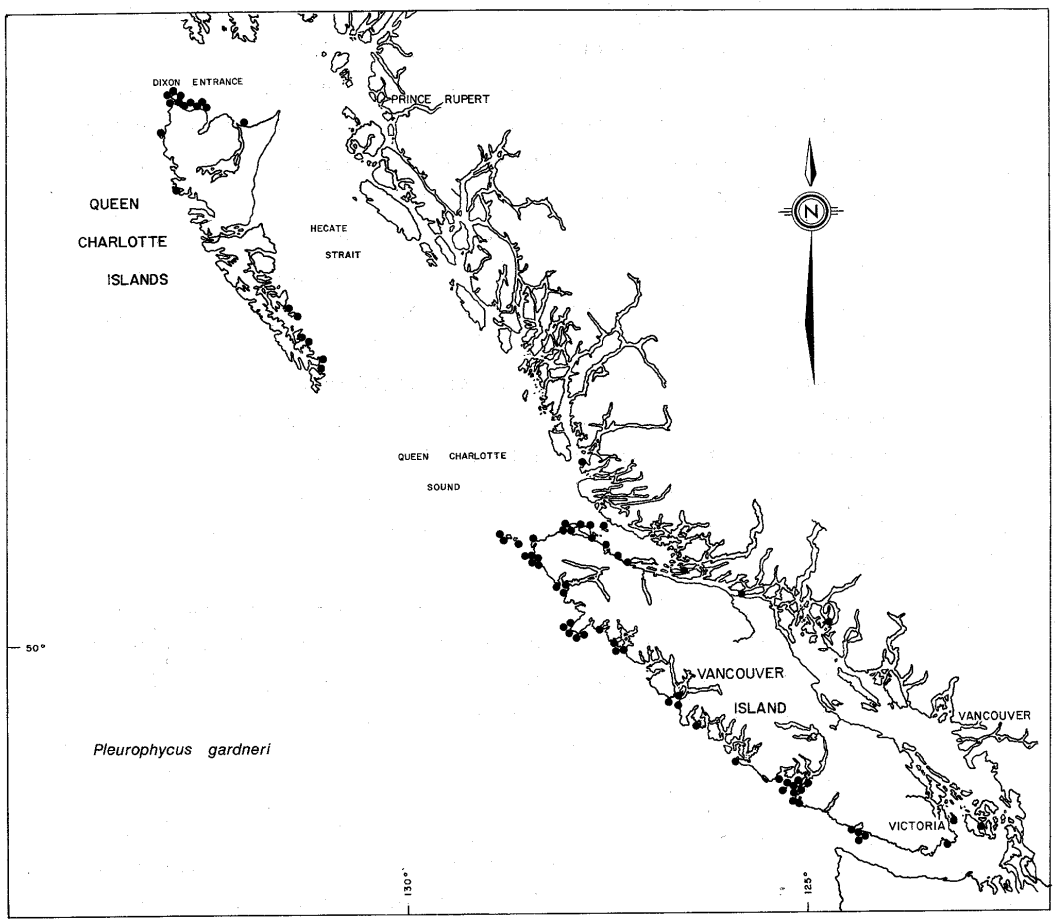


Figure 6. The presently known distribution of *Pleurophycus gardneri* in British Columbia (Scagel *in herb*.).



## SELECTED PHYSICAL AND CHEMICAL FEATURES OF THE THREE STUDIED SPECIES

Several features help in assessing potential kombu species. *Laminaria angustata var. longissma,* an excellent kombu species, is characterized as having a long (up to 8 m), narrow (usually less than 12 cm), fairly thick blade and weighing up to 1.5 kg (substantiality 140 mg wet weight/$cm^2$) (Kawashima, 1972).

In the present study I have employed plant size (Wet weight, length, width and thickness), moisture, carbon and nitrogen composition, and substantiality (mg wet weight/$cm^2$) to characterize potential British Columbia kombu species.

For comparison I have included some measurements of *Macrocystis integrifolia* Bory and *Laminaria saccharine* (L.) Lamouroux. These two species are not considered, by me, to be good kombu species.

Where possible, I have attempted to evaluate the effects of location and time on some of the studied features. However, limited distribution (particularly of *Cymathere triplicata)* and relative phasing of occurrence of sporophytes have hindered this task. The locations of places referred to in this section are shown in Figure 7.

The collections were made in a manner consistent with commercial harvesting. Small plants were not collected and the largest plants not selectively sought. Thus the collecting was random within the larger plants of the standing crop.

### 1. Blade Dimension

Relative blade sizes, as observed at one time, are presented in Table 1. Generally, the three studied species were larger and thicker than either *M. integrifolia* or *L. saccharina. C. triplicata* and *Pleurophycus gardneri* tended to be long and narrow. Thickness varied between the margin and centre in *C. triplicata* and P. *gardneri.* This feature is shared with most of the Japanese kombu species (Tokida, Nakamura & Druehl, in prep.).

2. Plant Weight and Substantiality

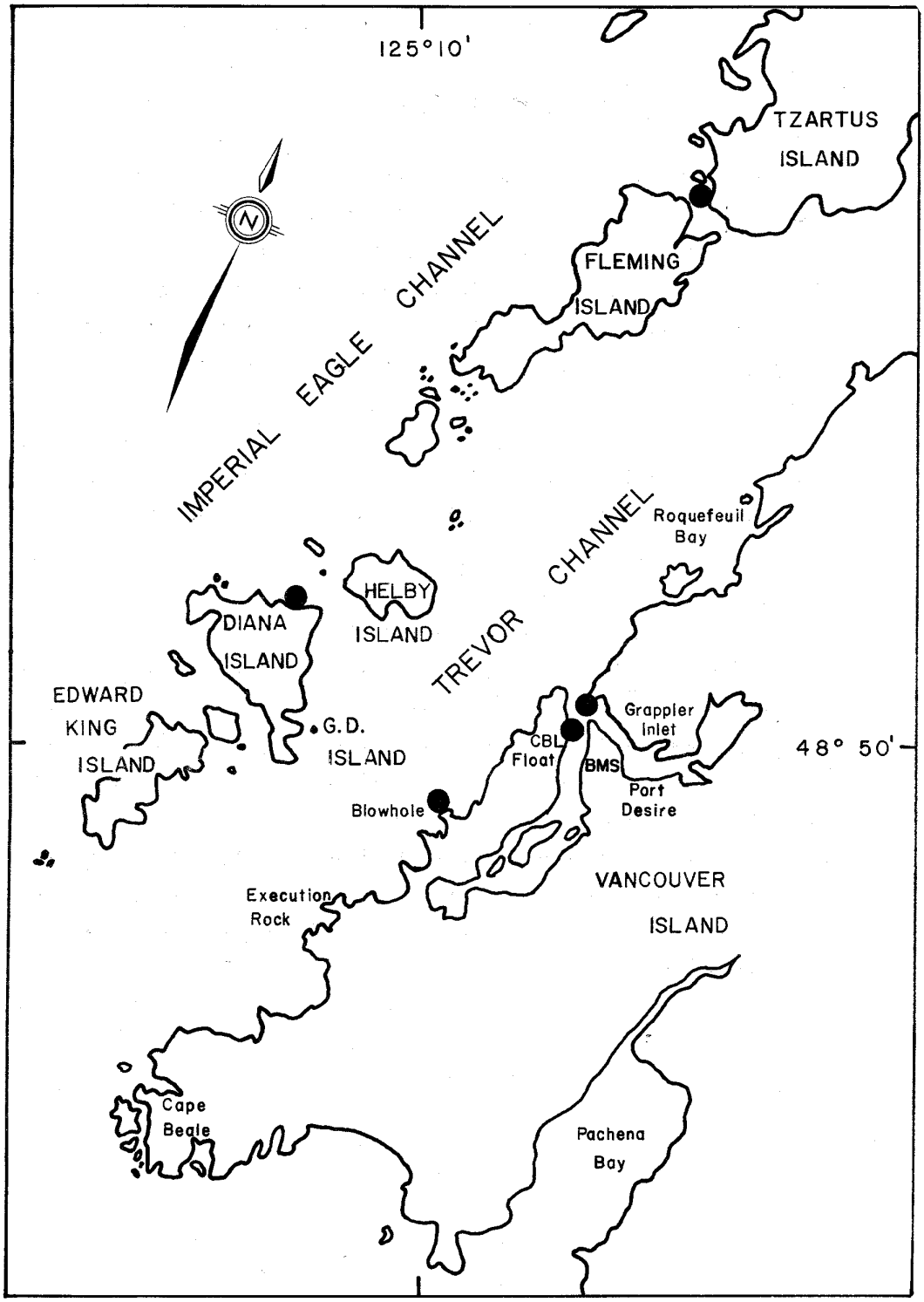
Whole plant wet weight and substantiality for plants from a variety of locations were compared (Table 2). Generally, whole plant weight was greatest for *L. groenlandica* and least for *C. triplicata*. Weights of *P. gardneri* with wave exposure corresponded to a morphological change (Figure IC, D). The more sheltered plants were broader, and had a more truncate base than did the wave-exposed plants.

Substantiality decreased distally from the blade base for *L. groenlandica* and *M. integrifolia,* and remained near constant for *P. gardneri*, *C. triplicata* and *L. saccharina* (Table 3). *C. triplicata*, *P. gardneri* and *L. saccharina* had a greater substantiality and all three of the studied species had substantialities greater than *M. integrifolia* and *L. saccharina.*

Substantiality was relatively uniform between plants from different locations (Table 2). Comparison of samples taken at different times indicated that substantiality for *L. groenlandica* remained fairly constant and *C. triplicata* and *P. gardneri* increased through the growing season (Tables 2 and 3).

Populations of the three studied species achieved a substantiality of greater than 150 mg wet weight/cm. This value is considered excellent for *L. angustata,* lower values being less desirable (Kawashima, 1972).

Figure 7. Map of eastern Barkley Sound illustrating locations referred to in the text.



## 3. Moisture, Carbon and Nitrogen Content

Moisture content was determined by air drying (24 hours at 55C) previously weighed wet tissue, and reweighing. The lowest moisture content occurred in *L. groenlandica* (81-84%); the other species were uniformly higher (85-92%, Table 3).

Carbon and nitrogen compositions were determined from air dried (24 hours at 55C) tissue using a Perken-Elmer CHN analyser. Carbon content was highest in *L. groenlandica* and lowest in *C. triplicata* (Table 4). Nitrogen content was also highest in *L. groenlandica* but lowest in *M. integrifolia.* There was little difference between the nitrogen and carbon content of the blade margin and centre except for *C. triplicata* where larger percentages were observed in the margin.

## 4. Conclusions

Direct comparison of the three studied species was difficult due to the fact that their development was not synchronized. Further, the ephemeral nature of *C. triplicata* and the restricted distribution of this species in our study area hindered comparisons.

On the basis of the presented data, the studied species shared several features in common with Japanese kombu. The blades of *C. triplicata* and *P. gardneri* were relatively long and narrow, being appreciably thicker in the central portion of the blade. Although *L. groenlandica* did not show an appreciable difference in thickness between blade margin and centre, it had a reasonably desirable thickness throughout. Substantiality was excellent for all three species.

Table 1. Mean blade size + 1 standard deviation as observed on June 6, 1978. Blade thickness were measured 20 cm from blade, n = 7.

| Species | Blade size (m) | | Blade thickness (cm) | |
|---|---|---|---|---|
| | length | width | center | margin |
| *Laminaria groenlandica* | 1.26 | 0.31 | 0.094 | 0.100 |
| Diana Island | +0.30 | +0.11 | +0.010 | +0.009 |
| *Pleurophycus gardneri* | 0.91 | 0.18 | 0.131 | 0.088 |
| Diana Island | +0.26 | +0.02 | +0.013 | +0.017 |
| *Cymathere triplicata* | 1.08 | 0.10 | 0.070 | 0.009 |
| Blow Hole | +0.28 | +0.02 | +0.012 | +0.004 |
| *Laminaria saccharina* | 0.66 | 0.28 | 0.036 | 0.020 |
| Bamfield Inlet | +0.30 | +0.12 | +0.007 | +0.006 |
| *Macrocystis integrifolia* | 1.04 | 0.18 | 0.025 | 0.023 |
| Grappler Inlet | +0.20 | +0.01 | +0.004 | +0.005 |

Table 2. Mean wet weight and substantiality values ($mg/cm^2$) ± 1 standard deviation for the test species. Substantiality values were taken 20 cm from blade base, n = 8.

| | Date | Collection site | Whole plant (g) | Substantiality Centre ($mg/cm^2$) | Substantiality Margin ($mg/cm^2$) |
|---|---|---|---|---|---|
| *Laminaria groenlandica* | June 22, 1978 | Dixon Island | 146 ± 103 | 156 ± 16 | 169 ± 11 |
| | | Scott's Bay | 223 ± 108 | 150 ± 10 | 174 ± 7 |
| | | G.D. Island | 338 ± 207 | 157 ± 21 | 175 ± 16 |
| | | Edward King Island | 229 ± 60 | 164 ± 16 | 164 ± 16 |
| | July 20, 1978 | Dixon Island | 147 ± 76 | 157 ± 33 | 164 ± 21 |
| | | Roquefeuil Bay | 171 ± 84 | 157 ± 22 | 171 ± 19 |
| *Pleurophycus gardneri* | June 22, 1978 | G.D. Island | 230 ± 81 | 192 ± 21 | 116 ± 18 |
| | | Edward King Island | 107 ± 46 | 181 ± 17 | 135 ± 16 |
| | July 20, 1978 | Blow Hole | 95 ± 40 | 196 ± 35 | 143 ± 24 |
| | | Execution Rock | 270 ± 111 | 201 ± 24 | 113 ± 21 |
| | | Cape Beale | 149 ± 59 | 185 ± 11 | 111 ± 10 |
| *Cymathere triplicata* | August 5, 1978 | Blow Hole | 143 ± 29 | 228 ± 18 | 65 ± 9 |

Table 3. Substantiality values ($mg/cm^2$) and percentage moisture ± 1 standard deviation for different regions of blades collected June 6, 1978, n = 7.

| Species & Location | Units | Blade Region | | | | | |
|---|---|---|---|---|---|---|---|
| | | Base - 10 cm | | 10 - 30 cm | | 30 - 50 cm | |
| | | centre | margin | center | margin | centre | margin |
| *Laminaria groenlandica* | Wet weight $mg/cm^2$ | 150 ± 11 | 152 ± 9.0 | 120 ± 4.0 | 124 ± 40 | 111 ± 6.0 | 115 ± 6.0 |
| Diana Island | % moisture | 81.5 ± 3.5 | 81.3 ± 4.5 | 81.6 ± 4.3 | 82.3 ± 3.7 | 81.7 ± 5.0 | 83.9 ± 3.3 |
| *Pleurophycus gardneri* | Wet weight $mg/cm^2$ | 151 ± 18 | 107 ± 11 | 155 ± 10 | 100 ± 18 | 153 ± 16 | 75.9 ± 8.0 |
| Diana Island | % moisture | 86.0 ± 1.2 | 88.4 ± 0.8 | 85.5 ± 1.6 | 88.0 ± 1.4 | 84.8 ± 1.9 | 86.8 ± 1.0 |
| *Cymathere triplicata* | Wet weight $mg/cm^2$ | 99.7 ± 24 | 29.6 ± 7.4 | 106 ± 29 | 32.9 ± 17 | 101 ± 42 | 24.8 ± 5.3 |
| Blow Hole | % moisture | 90.0 ± 0.6 | 88.2 ± 1.4 | 91.7 ± 0.6 | 88.8 ± 1.7 | 91.5 ± 0.6 | 87.8 ± 1.4 |
| *Laminaria saccharina* | Wet weight $mg/cm^2$ | 78.4 ± 12 | 45.4 ± 6.6 | 65.8 ± 9.0 | 45.9 ± 5.9 | 69.7 ± 11.7 | 53.1 ± 18.6 |
| Bamfield Inlet | % moisture | 89.5 ± 0.7 | 90.1 ± 0.7 | 90.0 ± 0.8 | 89.7 ± 1.1 | 90.3 ± 1.2 | 90.1 ± 1.3 |
| *Macrocystis integrifolia* | Wet weight $mg/cm^2$ | 72.6 ± 17 | 65.0 ± 12 | 48.7 ± 5.9 | 45.2 ± 3.9 | 44.4 ± 5.9 | 39.7 ± 5.5 |
| Grappler Inlet | % moisture | 87.4 ± 1.1 | 86.9 ± 1.5 | 87.7 ± 1.2 | 87.2 ± 13 | 89.0 ± 1.0 | 88.6 ± 1.2 |

Table 4. Carbon and nitrogen content, as a percentage of dry weight, for plants collected June 6, 1978. Analyses were conducted on composite samples (n = 7) taken 20 cm from blade base.

| | %C | | %N | |
|---|---|---|---|---|
| Species/Location | Center | Margin | Center | Margin |
| *Laminaria groenlandica* Diana Island | 36.1 | 35.3 | 1.67 | 1.58 |
| *Pleurophycus gardneri* Diana Island | 31.6 | 29.1 | 1.13 | 1.08 |
| *Cymathere triplicata* Blow Hole | 22.3 | 28.1 | 1.03 | 1.86 |
| *Laminaria* saccharin Bamfield Inlet | 26.9 | 24.8 | 0.83 | 1.00 |
| *Macrocystis integrifolia* Grappler Inlet | 30.0 | 29.1 | 0.85 | 0.83 |

## CULTURE STUDIES ON GAMETOPHYTES AND SPOROPHYTES

The production of young sporophytes for seeding kelp lines in kombu farms requires laboratory control of the microscopic sexual generation (the gametophytes). Further, laboratory treatment of young sporophytes may considerably shorten the residency time required to rear outplanted kombu (Hasegawa, 1976).

### 1. Culture Facilities

The culture facilities, which are located in Kombu House at the Bamfield Marine Station, were developed specifically for kombu culture work (Figure 8A). The greenhouse portion of Kombu House contains four seawater trays which are used for cooling Plexiglas chambers containing kombu cultures (Figure 8B, D). Untreated seawater from the Bamfield Marine Station seawater system is used as the coolant. Each tray is supplied with seawater filtered to 1µ (Figure 8C). This is achieved by first sand filtering to approximately 14 µ (Jacuzzi Sand Filter, Model 20 FM/A, Jacuzzi Canada Ltd., Rexdale, Ont.), and then filtration through a series of Cuno MicroWynd 11 filter cartridges (AMF Cuno Division, Meriden, Conn., U.S.A.). This filter system can deliver 250l/minute. Compressed air and fluorescent lighting (G.E. Model F40-D) are provided for each tray.

Attached to the greenhouse is an incubator room containing three Percival incubators (Model 1.35LL, Boone, Iowa, U.S.A.) and a walk-in cooler with temperature and light controls (Figure 9A, C). The incubators are operated with red (G.E. bodel F2OT12-R) and white fluorescent light (G.E. Model F40-D). The walk-in cooler serves as a laboratory, allowing us to work on the cultures under conditions ambient to the incubators.

### 2. Culture Methods

To achieve spore release, sori are first vigorously wiped with paper towelling and then hung in a cool, dark place for up to 24 hours. If the sori are relatively free of epiphytes, they are placed directly into PES (Provasoli's Enriched Seawater; Provasoli 1968) enriched with 80pg-at I/l as KI (Hasaio and Druehl, 1973). Heavily epiphytized sori may be treated with 1% bleach in seawater for 20 minutes, rinsed five times in seawater and then

transferred to PES (Druehl and Hasaio, 1969). Spore release usually occurs within 1 hour.

The spore suspension is then poured through several layers of cheesecloth to remove foreign matter. The spore suspension is diluted with a larger volume of PES and placed with the desired substrate for settling in darkness for at least 10 hours at 10-12 C. After 10 hours the spores have settled. The PES is changed and the cultures are transferred to either an incubator or the greenhouse and provided with aeration.

To culture gametophytes for cloning or to delay gametogenesis, the developing spores are placed under continuous red light (1 nE/$cm^2$/sec) at 12C (Lüning & Dring, 1975). To obtain sexual plants, the spores are placed in either white light (1.5 nE/$cm^2$/sec) at 12C, or in the greenhouse.

The developing cultures are monitored weekly and their media changed every 10 days.

The methods for isolating and cloning kelp gametophytes were obtained first-hand from Dr. James Markham (in Helgoland, Germany) and from the literature (Markham *et al.*, 1978). These techniques are presently being modified to suit our conditions.

To isolate gametophytes, 1 ml of spore suspension is added to 50 ml of PES, to which has been added 1 ml saturated aqueous solution of $GeO_2$/l, in petri dishes (100 x 15 mm). The media is changed after 10 hours and then every 10 days. Germanium dioxide is employed for the first 10 days only. When the gametophytes have reached the 10 cell stage, individual male and female plants are isolated in petri dishes and cultured until visible clumps are produced. The individual plants are then transferred to larger petri dishes (100 x 80 mm) and cultured until time to clone.

Cloning is achieved by gently grinding the individual plants with a mortar and pestle. The resulting male and female fragments are then mixed and transferred to white light or greenhouse conditions where they become fertile.

## 3. Results and Discussion

Gametophytes of the three studied species have been cultured through to maturity and sporophytes have been produced (Figure 10A-F). The shortest time required for sporophyte production from spores was 9 days. The usual time, how-ever, was 2-3 weeks. The time required for sporophyte production appeared to be a function of light intensity; at lower intensifies (0.5 nE/$cm^2$/sec) *Pleurophycus gardneri* required in excess of 30 days for sporophyte production.

The Japanese culture their plants for 45 days before outplanting (Hasegawa, pers. comm.). Thus, if we require 21 days for sporophyte production we are left with 24 days of sporophyte growth before outplanting. This period is long enough to produce macroscopic sporophytes of *Laminaria groenlandica* (204 mm long). We have not grown sporophytes of *Cymathere triplicata* or *P. gardneri* since September, 1978 and *L. groenlandica* since November, 1978. Some cultures have become fertile, apparently resulting from white light contamination introduced when opening the incubators (Lüning, pers. comm.).

Isolates of the three studied species, were obtained in Helgoland by Ms. Robin Boal. Some of these have been cloned and are presently being cultured under white light conditions.

## 4. Conclusions

All three species have been cultured through the various stages required to establish kombu farms. However, *L. groenlandica* was the only species we were able to grow to macroscopic size in Kombu House. All three species require considerable study before we can obtain the level of control necessary for reliable production.

The isolation, cloning and control of gametogenesis appear to be techniques amenable to commercial application. Thus we may soon be in a position to produce a genetically uniform crop.

Figure 8. Kombu House at Bamfield Marine Station. A. Exterior. B. Interior showing seawater trays and services. C. Seawater filter system; sand filter in lower right and cartridge filter in upper left. D. Plexiglas tanks for rearing young sporophytes on string. Sporophytes of *Laminaria groenlandica* can be seen projecting from the string. E. String with young sporophyte being inserted into half inch blue twisted polypropylene rope.

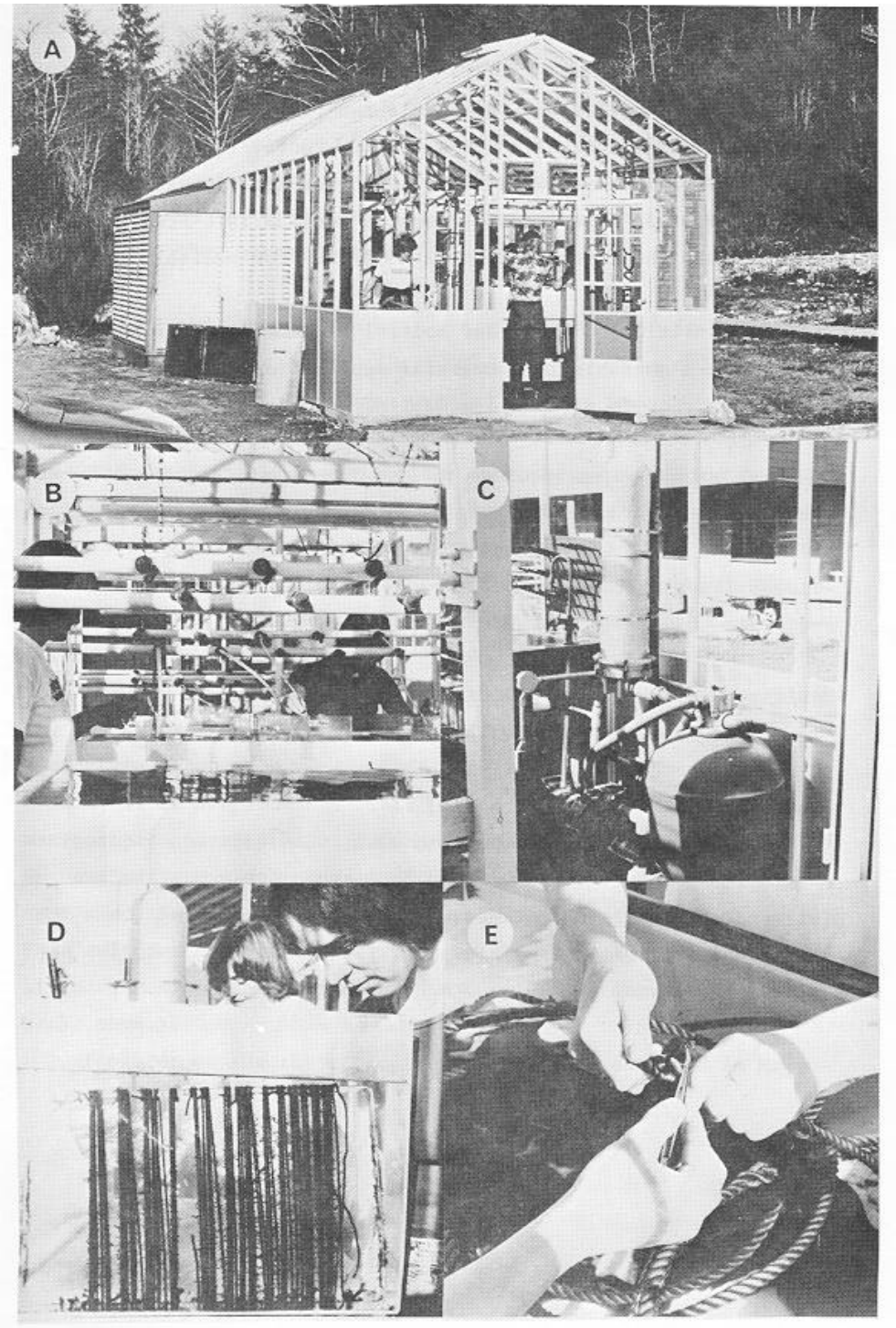


Figure 9. Incubator room for manipulating gametophytes and sporophytes. A. Walk-in cooler. B. Red light incubator for delaying gametogenesis. C. White light incubator for promoting gametogenesis.

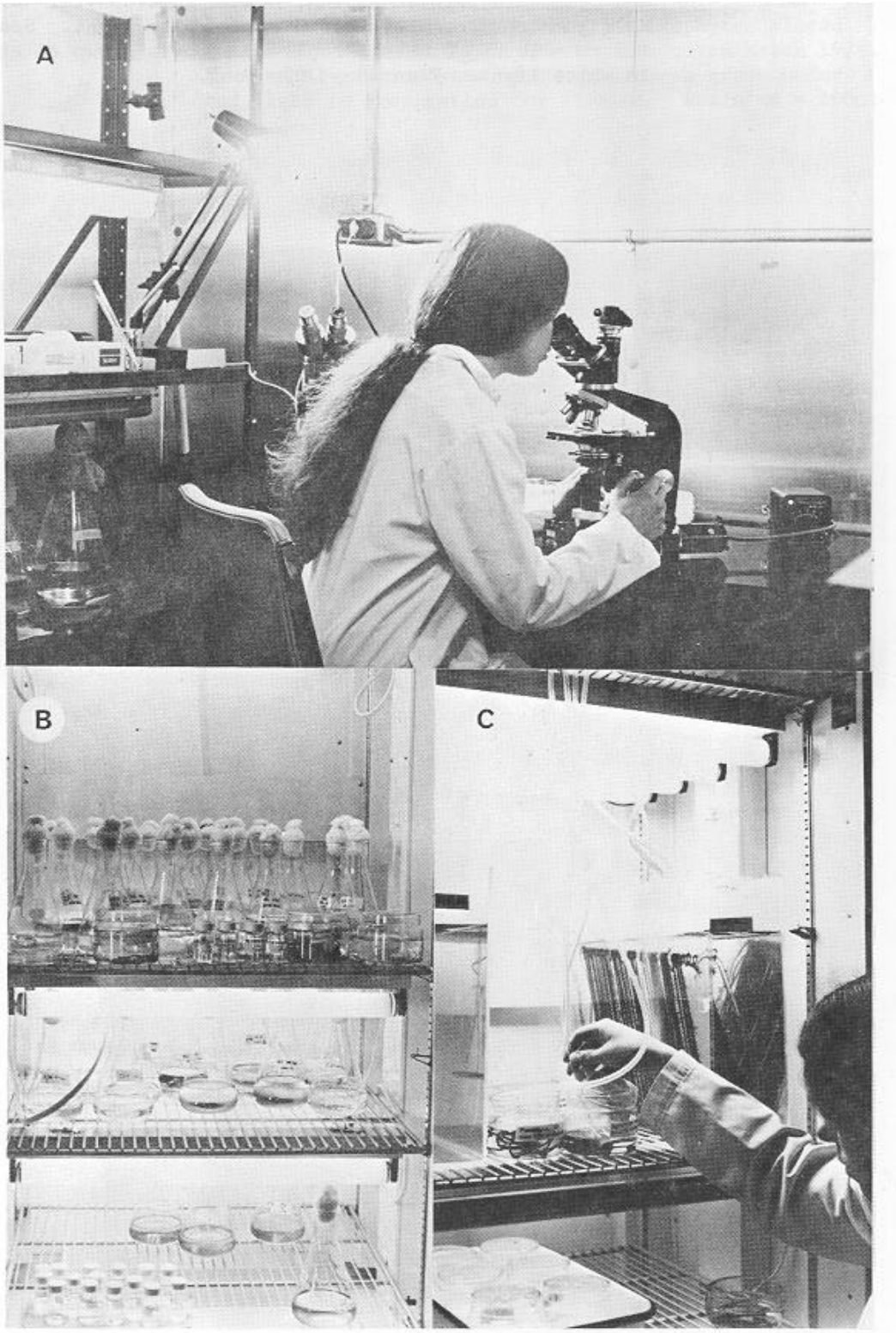


Figure 10. A. Female gametophyte of *Laminaria groenlandica* grown under white light. B. Male gametophyte of *L. groenlandica* grown under white light. Scale 1.5 cm = 100µ. C. Microscopic *L. groenlandica* sporophytes grown on string in white light. Plants 5-700µ long.

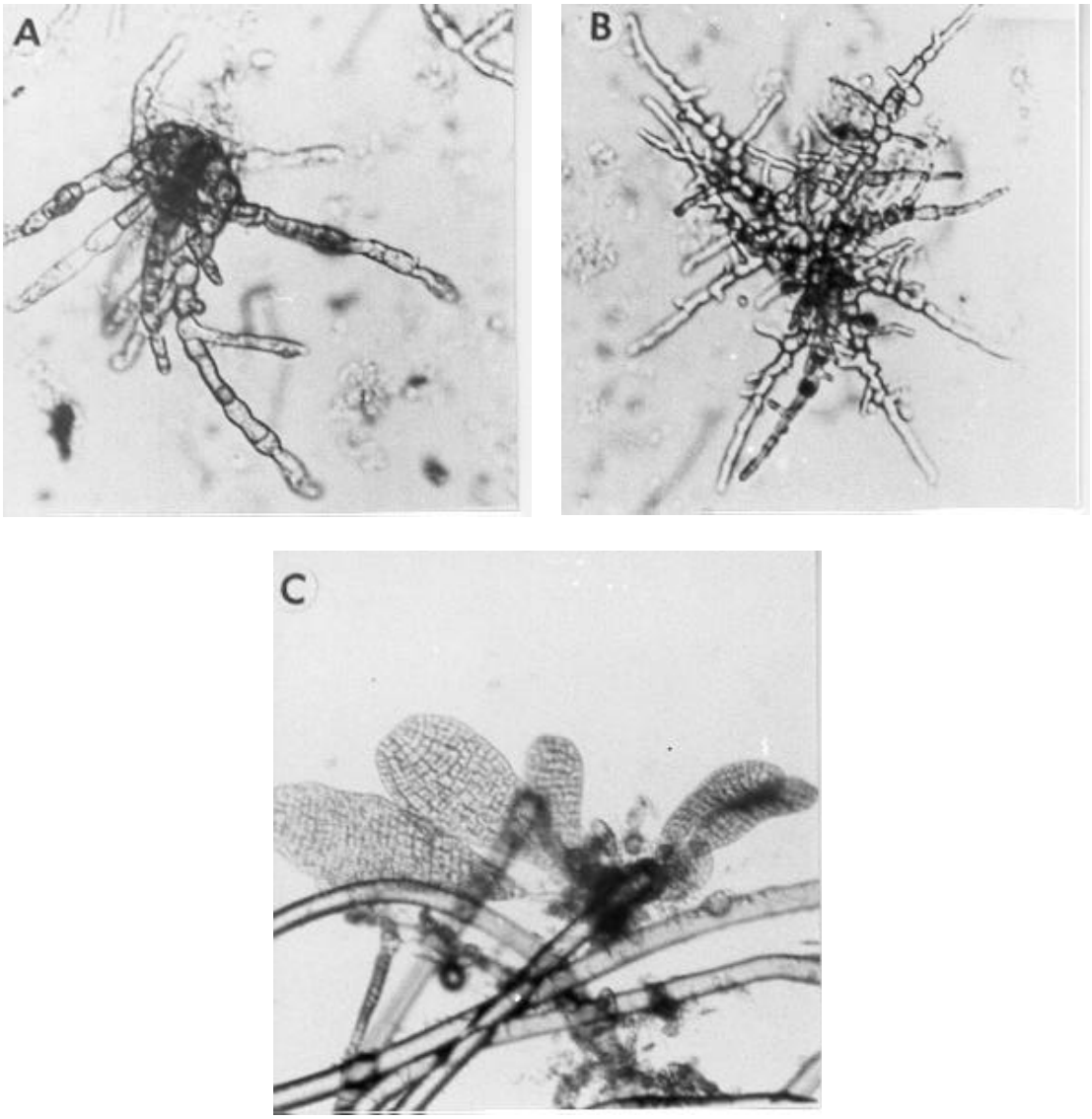
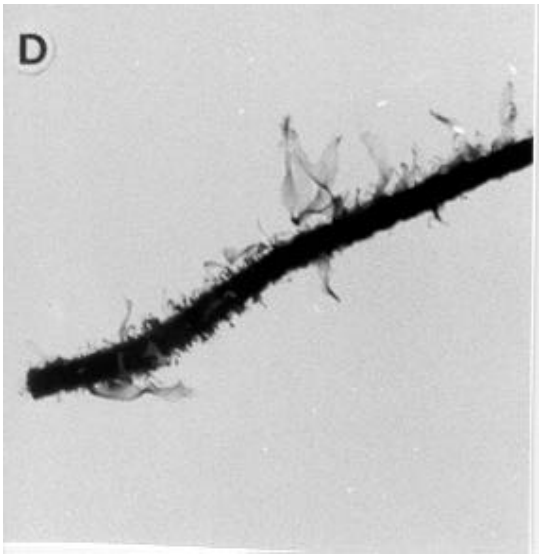
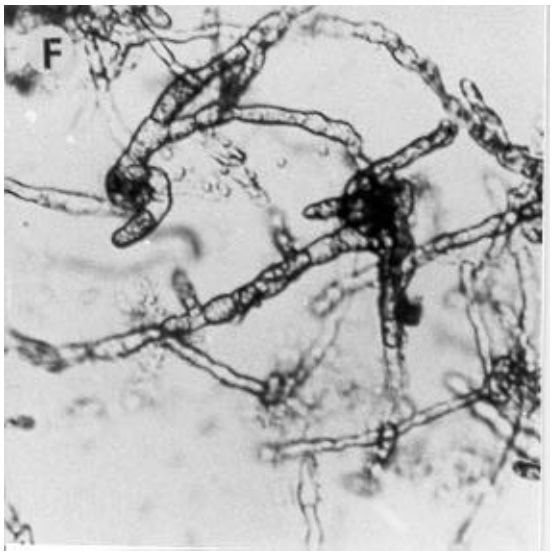


Figure 10 (cont'd). D. Young *L. groenlandica* grown on string in white light. The longest plants are approximately 5 mm long. E. *L. groenlandica* sporophytes placed in the sea in December, 1978. Photograph taken March, 1979. F. Asexual gametophytes of *C. triplicata* grown under red light in preparation for cloning. Scale cm = 100µ.







## OUTPLANTING OF KELP SEED

Once gametophytes become established on a substrate they can be outplanted. The Japanese usually outplant young sporophytes but gametophytes may also be outplanted (,Hasegawa, pers. comm.). Any stage outplanted is referred to as seed.

We have outplanted gametophytes and sporophytes of the three studied species in order to determine their survival at different stages of development.

### 1. Methods

Spores are allowed to settle and develop on the desired substrate; usually, hydrophilic string. The string is soaked for 24 hours in fresh water then autoclaved for 30 minutes at 15 psi before introducing the spores (Kaneko, pers. comm.).

Once the plants have reached the desired stage of development, the string is cut into 3-4 cm lengths and inserted through the warp of half inch blue twisted polypropylene rope (Figure 8E). Pieces of string are inserted every 30 cm along a 4-5 m rope. The rope is shackeled to an anchored log or float and maintained vertically in the water column by a 3-8 kg concrete weight.

### 2. Results and Discussion

Three types of nylon string were tested to determine their influence on settlement and development of *Laminaria groenlandica* gametophytes (Cremona string [Japanese], Dupon "Permagrip" type 66, and green nylon cod line). Under culture conditions all three types of string supported essentially equivalent gametophyte populations. however, the latter two types unravelled when outplanted. The effect this has on subsequent in situ development is presently being studied.

All three species were outplanted in the young gametophyte stage, December, 1978. *L. groenlandica and Pleurophycus gardneri* were attached to green nylon string and outplanted to Canadian Benthic's float (Figure 7, as CBL Float). *Cymathere triplicata* was seeded onto pottery chips because of its discoid holdfast, and outplanted at Scott's Bay.

As of March, 1979, macroscopic sporophytes of *L. groenlandica* had developed to about 3 cm long (Figure 10E). *P. gardneri* had sporophytes up to

0.5 cm long. Sporophytes of *C. triplicata* are not yet apparent. However, wild plants of this species have not yet appeared.

Macroscopic sporophytes (up to 3 mm) of *L. groenlandica* were outplanted at Tzartus Island early in February and again early in March. Two days after the first outplant, the kelp lines were subjected to a severe storm and all macroscopic plants lost. The second outplants are still growing.

## 3. Conclusions

Outplanted *L. groenlandica* appeared to grow well during the winter but the young plants could be severely damaged during winter storms. Winter outplanting might best be done in wave-sheltered areas followed by relocation to more wave-exposed areas in the spring. In some areas of Japan, the outplanted seed is lowered to considerable depth during the winter to avoid surface conditions (Hasegawa, pers. comm.).

*P. gardneri* developed to macroscopic size after outplanting. However, its growth was considerably slower than that noted for *L. groenlandica*. *C. triplicata* has not yet produced macroscopic sporophytes on the outplanted substrate or in nature.

Considerable work remains to define the best time and place to initiate seed outplants. The absence of earlier studies on the life histories of *P. gardneri* and *C. triplicata* has hindered our progress on these two species.

## TRANSPLANTATION OF WILD PLANTS

Sporophytes collected from nature were transplanted to different locations, where their growth and development were monitored. The objectives of these experiments were to define types of areas best suited for kelp agronomy. Further, various types of rope were tested to determine their suitability as substrate for kelp growth. I assumed that the wild plants would behave in a manner similar to those produced in Kombu House, once the cultured plants had obtained the size of the wild plants.

### 1. Methods

Three plants were placed at each of five depths (+2, 0, -2, -4 and -6 m relative to 0 tide level) in a wave-sheltered area (Port Desire), a moderately wave-sheltered area (mouth of Grappler Inlet), a moderately wave-exposed area (Scott's Bay), and a moderately wave-exposed area subjected to tidal currents (Tzartus Island) (Figure 7).

Three hydrophobic ropes (3/8" hollow yellow polypropylene, 1/4" twisted yellow polypropylene and 1/2" white nylon) were employed. Ropes, 8.5 m long, were attached to anchors weighing approximately 25 kg. These were placed 6 m below 0 tide and the ropes were buoyed to the surface by styrofoam floats.

Shade form plants of *Laminaria groenlandica,* measuring approximately 10 cm long and 4 cm wide, were transplanted from their natural habitat near the Grappler study site to the various study sites on July 10, 1978. *Cymathere triplicata*, collected from the Blow Hole, were cut to 80 cm blade lengths and transplanted to the study sites on July 13, 1978. *Pleurophycus gardneri*, collected from Diana Island were marked with a hole 10 cm distal to the blade base and transplanted to the study sites on July 15, 1978. These plants ranged in blade length from 80 to 110 cm.

### 2. Results and Discussion

a. Survival of kelp lines and plant responses to kelp lines

The initial and final disposition of kelp lines is shown in Table 5. All of the plants attached to the yellow hollow polypropylene rope had abraded off at the stipe by the time of the first observations following transplantation (August, 1978). These lines were removed. The loss of other lines was attributed to

vandalism, except for one line at Port Desire, which was caught in the propeller of a tourist boat. The persistence of lines at Tzartus Island probably reflects lower tourist traffic and the vigilance of the Wenstob family that lives near the study site.

b. Growth and development of *L. groenlandica*

One month after transplantation, all of the plants positioned 2 m above 0 tide level were lost or severely damaged (Table 6). This loss reflected surface conditions encountered (high insulation, surface seawater temperatures and salinities). In the more wave-sheltered areas, plants at 0 tide level were also lost; probably reflecting temperature and salinity stratification of these waters.

By the time of the final observation reported here, the vertical distribution of *L. groenlandica* on ropes was limited to a 2-4 m spread (Table 6). This approximates the vertical range in water depth exploited by the Japanese in their rope cultures of kombu (Hasegawa, pers. comm.).

From July until September, 1978, surviving plants increased 3-6 times in length and 2.5 - 4.7 times in width (Tables 6, 7). Coincident with the fall storms there was a slight erosion of length while width remained essentially the same, suggesting that there was little growth from end of summer through fall.

Sori were first evident in November, 1978. Sori on the shallow plants developed before those on the deeper plants. From November until the last measurement (February 27, 1979) the plants increased approximately 64% in length and 60% in width. The plants observed in February, 1979 retained remnants of the previous year's blade (Figure 11B). The new blade was appreciably wider than the older one.

The pattern of growth observed here closely follows that of plants in their natural habitat (Druehl, 1965). It appears that the period of rapid growth is initiated during the winter and persists until June or July. Thereafter, growth decreases until sorus production the following winter.

Growth of *L. groenlandica* at Port Desire and Grappler was poor relative to the two other study sites (Tables 6, 7). On the basis of acquired length it appears that Scott's Bay plants grew best during the late summer - early fall growth period and the winter

growth period (Table 6). However, there was a differential in width increase between the Tzartus Island and Scott's Bay plants. The Tzartus Island plants increased in width more than the Scott's Bay plants during the winter growth period (Table 7). By considering the blade of *L. groenlandica* to approximate a rectangle, we can multiply width by length to estimate surface area. Using this value to judge growth between the study sites leads to the conclusion that Scott's Bay conditions are optimal for growth during the late summer and Tzartus Island conditions are optimal in winter (Table 8).

Overall, it appears that Tzartus Island was the best environment tested for *L. groenlandica* agronomy. This conclusion is based on acquired plant material and the depth range through which plants survived.

c. Growth and development of *C. triplicata* and *P. gardneri*

Plants of both species were lost from the 2 m above 0 tide depth at all study sites. *P. gardneri* were all lost by September, 1978. Neither species grew during the observation period. All of the plants available for transplantation at that time were large and many had already produced sori. Growth of many kelp ceases upon sorus initiation (annual species), or is considerably decreased (perennial species).

Plants without sori at the time of collection (-in July, 1978) had produced sori during the first few months following transplantation. Residual plants of *C. triplicata* retained viable sori until February, 1979.

d. Epiphytes and grazers

The major epiphytes observed on *L. groenlandica* during November, 1978 are listed in Table 9. The new growthacquired between November and February, 1979 was free of epiphytes, whereas the older growth was epiphytized with diatoms, *Spirobis* (a tube dwelling polychaete worm), *Tricellaria* (a non-encrusting bryozoan), and *Ulva* (a green algae). Epiphytes, when present, were usually most abundant on one side of the plant.

The only grazers observed on the rope cultures were *Pugettia* (a kelp crab) and *Idothea* (an isopod). *Strongylocentrotus franciscanus*

(the red urchin) and *Haliotis kamtschatkana* (the pinto abalone) were observed on the anchors.

3. Conclusions

Three of the four rope types tested proved to be adequate substrates for kelp growth. No difference was, observed between hydrophilic (nylon) and hydrophobic (polypropylene) ropes. Hollow rope severely eroded the attached plants and was discarded as a substrate type. We have elected to use 1/2" twisted polypropylene rope because it is less expensive than nylon, and although it is more expensive than the 3/8" rope, it is easier to handle.

Transplanted *L. groenlandica* grew at all study sites, but grew best at Tzartus Island and Scott's Bay. At these two sites good growth was sustained through a 2-4 m vertical water column. These findings indicate that *L. groenlandica* grows best in areas of vigorous water motion and that it will be possible to rear many plants per unit area. These are the conditions of kombu farming in Japan (Hasegawa, pers. comm.). However, the response of very small kelp (i.e., less than 4 mm long) to these conditions must be ascertained before a final decision on an optimal farming environment can be made.

Due to the early growth and maturation of *C. triplicata* and *P. gardneri,* I was unable to learn much about their response to the studied environments. *C. triplicata* presents unique problems due to its discoid holdfast; how will this organ respond to rope substrate? The uncertainties of the life history of *P. gardneri* must be clarified if agronomy of this species is to proceed.

Epiphytism does not appear to be a major problem providing that harvesting is conducted during or near the end of an active growth period.

Table 5. Numbers of kelp lines set out July 1978 and those remaining in February 1979. *Laminaria groenlandica* kelp lines at Tzartus Island and Grappler Inlet consisted of four types of rope (see text). The remaining lines were half inch blue twisted polypropylene rope.

| | | Tzartus Island | Port Desire | Grappler Inlet | Scott's Bay |
|---|---|---|---|---|---|
| *Laminaria groenlandica* | initial 1978 | 4 | 2 | 4 | 2 |
| | final 1979 | 3 | 1 | 0 | 1 |
| *Cymathere triplicata* | initial 1978 | 2 | 2 | 2 | 2 |
| | final 1979 | 2 | 0 | 1 | 0 |
| *Pleurophycus gardneri* | initial 1978 | 2 | 2 | 2 | 2 |
| | final 1979 | 2 | 0 | 0 | 0 |

Table 6. Mean blade lengths ± 1 standard deviation of *Laminaria groenlandica* grown on kelp lines. These plants were 10 cm long in July 1978 when the experiment was started.

| | | Tzartus Island | | Scott's Bay | | Port Desire | | Grappler Inlet | |
|---|---|---|---|---|---|---|---|---|---|
| | Depth (m) | (n) | Length (cm) | (n) | Length (cm) | (n) | Length (cm) | (n) | Length (cm) |
| Aug. 22 1978 | +2 | (1) | 4 | (0) | | (0) | | (0) | |
| | 0 | (4) | 17 ± 5 | (3) | 29 ± 5 | (0) | | (0) | |
| | -2 | (11) | 26 ± 7 | (3) | 41 ± 5 | (0) | | (3) | 19 ± 3 |
| | -4 | (7) | 32 ± 8 | (3) | 30 ± 5 | (3) | 22 ± 7 | (3) | 13 ± 8 |
| | -6 | (9) | 24 ± 5 | (0) | | (4) | 18 ± 3 | (3) | 20 ± 1 |
| Sept. 22 | +2 | (0) | | (0) | | (0) | | | |
| | 0 | (0) | | (3) | 40 ± 10 | (0) | | | |
| | -2 | (6) | 34 ± 12 | (3) | 59 ± 8 | (0) | | | |
| | -4 | (6) | 42 ± 14 | (3) | 41 ± 2 | (3) | 30 ± 14 | | |
| | -6 | (7) | 30 ± 10 | (0) | | (4) | 34 ± 6 | | |
| Nov. 13 | +2 | (0) | | (0) | | (0) | | | |
| | 0 | (0) | | (3) | 21 ± 1 | (0) | | | |
| | -2 | (6) | 37 ± 10 | (3) | 49 ± 19 | (0) | | | |
| | -4 | (6) | 42 ± 11 | (1) | 40 | (3) | 29 ± 9 | | |
| | -6 | (7) | 33 ± 9 | (0) | | (1) | 13 | | |
| Feb. 4 1979 | +2 | (0) | | (0) | | (0) | | | |
| | 0 | (0) | | (3) | 39 ± 7 | (0) | | | |
| | -2 | (6) | 53 ± 23 | (3) | 44 ± 14 | (0) | | | |
| | -4 | (6) | 68 ± 12 | (1) | 4 | (2) | 39 ± 2 | | |
| | -6 | (7) | 59 ± 15 | (0) | | (1) | 22 | | |
| Feb. 27 | +2 | (0) | | (0) | | (0) | | | |
| | 0 | (0) | | (3) | 58 ± 12 | (0) | | | |
| | -2 | (5) | 52 ± 35 | (3) | 68 ± 16 | (0) | | | |
| | -4 | (6) | 67 ± 18 | (0) | | (2) | 48 ± 9 | | |
| | -6 | (5) | 57 ± 21 | (0) | | (1) | 26 | | |

Table 7. Mean blade widths ± 1 standard deviation of *Laminaria groenlandica* grown on kelp lines. These plants were 4 cm wide in July 1978 when the experiment was started.

| | | Tzartus Island | | Scott's Bay | | Port Desire | |
|---|---|---|---|---|---|---|---|
| | Depth (m) | (n) | Width (cm) | (n) | Width (cm) | (n) | Width (cm) |
| Sept. 22 | 0 | (0) | | (3) | 15 ± 3 | (0) | |
| 1978 | -2 | (6) | 11 ± 3 | (3) | 19 ± 1 | (0) | |
| | -4 | (6) | 13 ± 3 | (3) | 13 ± 3 | (3) | 10 ± 4 |
| | -6 | (7) | 10 ± 1 | (0) | | (4) | 10 ± 1 |
| Nov. 13 | 0 | (0) | | (3) | 15 ± 1 | (0) | |
| | -2 | (6) | 11 ± 2 | (3) | 16 ± 3 | (0) | |
| | -4 | (6) | 13 ± 2 | (3) | 14 ± 5 | (3) | 10 ± 1 |
| | -6 | (7) | 10 ± 2 | (0) | | (4) | 9 |
| Feb. 4 | 0 | (0) | | (3) | 15 ± 5 | (0) | |
| 1979 | -2 | (6) | 14 ± 8 | (3) | 11 ± 3 | (0) | |
| | -4 | (6) | 19 ± 4 | (1) | 3 | (3) | 15 ± 5 |
| | -6 | (7) | 19 ± 4 | (0) | | (1) | 9 |
| Feb. 27 | 0 | (0) | | (3) | 20 ± 4 | (0) | |
| | -2 | (5) | 20 ± 10 | (3) | 17 ± 3 | (0) | |
| | -4 | (6) | 26 ± 3 | (0) | | (2) | 18 ± 9 |
| | -6 | (5) | 23 ± 4 | (0) | | (1) | 12 |

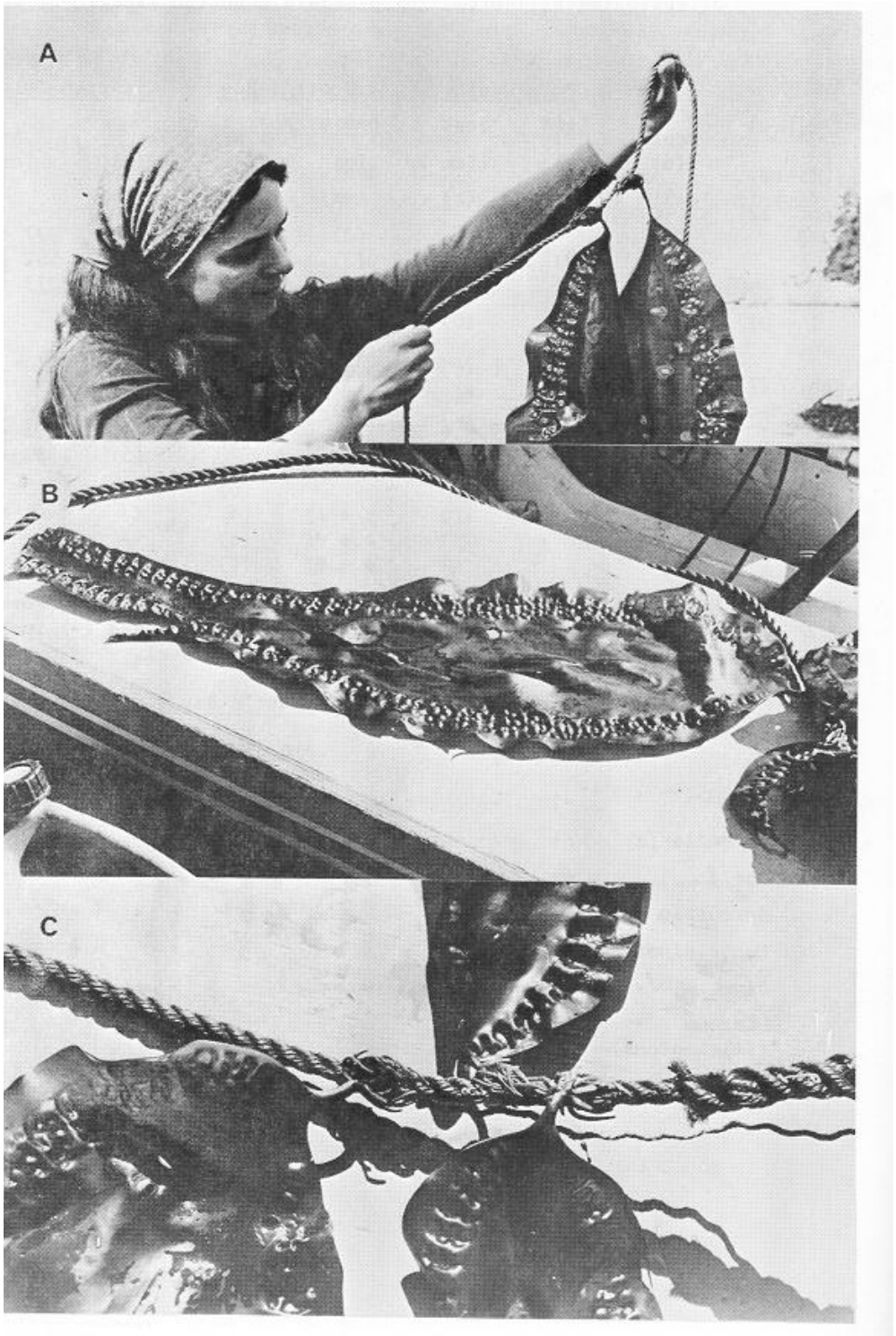
Table 8. Approximate surface areas ± 1 standard deviation of *Laminaria groenlandica* grown on kelp lines. These plants had a surface area of approximately 40 cm 2 in July 1978 when the experiment was started.

| | | Port Desire | | Scott's Bay | | Tzartus Island | |
|---|---|---|---|---|---|---|---|
| | Depth (m) | (n) | Area ($cm^2$) | (n) | Area ($cm^2$) | (n) | Area ($cm^2$) |
| Sept. 22 1978 | 0 | | | | | | |
| | -2 | | | (3) | 604 ± 216 | (6) | 377 ± 179 |
| | -4 | (3) | 335 ± 269 | (3) | 1104 ± 110 | (6) | 562 ± 287 |
| | -6 | (5) | 367 ± 104 | (3) | 520 ± 106 | (7) | 313 ± 126 |
| Feb. 27 | 0 | | | (3) | 1159 ± 454 | | |
| | -2 | | | (3) | 1128 ± 319 | (5) | 1308 ± 125 |
| | -4 | (2) | 900 ± 560 | | | (6) | 1701 ± 484 |
| | -6 | (1) | 312 | | | (5) | 1352 ± 631 |

Table 9. Epiphytes on *Laminaria groenlandica* as observed during November 1978. (+) indicates present, (++) indicates abundant and (-) indicates not present. Depth is in meters below 0 tide level.

| | Tzartus Island | | | Scott's Bay | | | Port Desire | |
|---|---|---|---|---|---|---|---|---|
| | Depth | | | Depth | | | Depth | |
| | -2 | -4 | -6 | 0 | -2 | -4 | -4 | -6 |
| *Tricellaria* | + | + | - | - | + | - | + | + |
| *Obelia* | ++ | - | - | + | + | - | - | - |
| *Spirorbis* | - | - | - | - | - | - | - | + |
| *Membranipora* | - | - | - | - | + | + | + | - |
| *Ulva* | - | - | - | + | - | - | - | - |
| Diatoms (filamentous) | + | - | - | - | - | - | ++ | - |

Figure 11. *Laminaria groenlandica* A. Wild plants after 8 months' growth on rope at Tzartus Island. B. Plant approximately 1 m long. Note the increase in blade width subsequent to the early winter slow growth period. C. Holdfasts attached to blue twisted half-inch polypropylene rope.



## OTHER POSSIBLE KOMBU SPECIES

In July, 1978, one plant resembling *Laminaria diabolica* Miyabe was collected from Malcolm Island (Figure 4B). This species is highly regarded in Japan as kombu. A subsequent visit to Malcolm Island failed to rediscover this plant (Coon, pers. com.).

We intend to take every opportunity to seek additional species, native to British Columbia, which have kombu potential.

## SUMMARY AND PROJECTIONS

Three species have been identified as potential marine crop plants for human consumption *(Laminaria groenlandica, Cymathere triplicata and Pleurophycus gardneri).* All three species share characteristics which distinguish good Japanese kombu and *C. triplicata* is presently harvested as a kombu species in Japan.

Although our knowledge of their distributions within British Columbia coastal waters is limited, it appears that populations of the three species are fairly common. Further, these species are relatively easy to distinguish in the field. These facts facilitate exploitation by potential entrepreneurs.

Establishment of kombu agronomy requires an understanding of the following topics related to the biology of potential species.

1. The Natural Histories of the Species to be Cultivated

The natural history of *L. groenlandica* is reasonably well understood; however, little is known regarding the other two species. This coming year, the natural histories of *C. triplicata* and *P. gardneri* will be revealed.

2. Laboratory Conditions for the Rearing of Gametophytes and Young Sporophytes for Seeding Kombu Farms

Conditions necessary for the production of gametophytes and young sporophytes of the three species for seeding kombu farms have been defined. However, the techniques must be refined, particularly for *C. triplicata* and *P. gardneri.* This work will continue through the coming year.

3. Optimal Field Conditions and Developmental Stages for Initiating Cultures in the Sea

Macroscopic sporophytes of *L. groenlandica* and *P. gardneri* have been produced in the sea from laboratory reared gametophytes. However, this has only been accomplished during the winter. These tests will continue into other seasons so as to define optimal times and developmental stages for outplanting and subsequent growth.

4. Kombu Quality Evaluation and Control

Ultimately, quality evaluation must be conducted by the consumer. Considering an Oriental market, it is naive to assume that a westerner's palate can discern good kombu. A procedure for preparing kombu for evaluation and a contact who can perform the evaluation are required.

Once we have a good kombu genotype, we have the proven techniques to reproduce that genotype year after year. Thus, we are assured of the genetic control of quality and we can produce a uniform product.

5. Suitable Substrates and Structures for Maintaining Cultivated Plants in Kombu Farms

We are not yet prepared to extensively explore the engineering of kombu farms. However, information on desirable substrates for farming is being accumulated. Further, problems unique to our shores are being defined (floating logs, kelp and boating tourists as fouling agents).

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