PART I

POLYMORPHISM IN <u>MACROCYSTIS</u> <u>INTEGRIFOLIA</u> BORY IN RELATION TO WATER MOTION

PART II

CONTROL OF DIATOM CONTAMINATION IN FIELD CULTURE OF GAMETOPHYTIC AND EARLY SPOROPHYTIC PHASES IN THE LAMINARIALES

by

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ABSTRACT

A field study in three parts was undertaken to investigate the relationships between the habit of Macrocystis integrifolia Bory and water movement.

Morphological variation in time within populations was related to seasonal changes in wind direction and speed. Four sites covering a wide range of exposures, yet characterized by essentially the same water properties were sampled throughout a period of one year.

In this way valid comparisons could also be made between populations. The sites were all situated in the vacinity of Bamfield on the west coast of Vancouver Island, B.C.

A spot sampling study was undertaken to determine whether the trends established in the above study apply consistently throughout the range of exposures covered by Macrocystis in local waters. Samples were obtained from Ucluelet on Vancouver Island to Warren Island in southern Alaska.

Through these studies several aspects of the habit were found to reflect the prevailing dynamic conditions to which the plant had been subjected throughout its development.

A transplant study was carried out among the continuous sampling sites to establish the mechanism of response to dynamic conditions. Growth data obtained through the transplant study indicated that stipe elongation and blade initiation vary directly with water movement. Growth of individual blades appears to be independent of this factor.

The results of the transplant study supported by variations observed within plants and within populations with time strongly suggest that the mechanism of response is phenotypic plasticity.

However, plants observed on the outer coast were, in some respects, markedly distinct. Thus the possibility of a second mechanism operating under conditions of genetic isolation has not been discounted.

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INTRODUCTION

The purpose of this study was to investigate the relationships between polymorphism in the sporophyte of *Macrocystis integrifolia* Bory and varying degrees of water movement.

Much attention has been paid to this question with respect to those members of the Laminariales other than the Lessoniaceae by several authors including Burrows (1958), Druehl (1967), Kain (1962, 1971), MacFarlane (1961), Norton (1969), Parke (1948) and Sundene (1958, 1961a, 1961b, 1964). From these works a general pattern has emerged which can best be explained by considering a typical laminarialean plant consisting of a lamina, stipe and holdfast.

Proceeding from a sheltered locality to one exposed to the direct force of the open sea, the lamina narrows and the tissues thicken, becoming tough yet flexible. The base of the lamina becomes more cuneate and if the species is characterized by the formation of longitudinal splits these become more prominent, often being absent entirely from plants of the same species in quieter waters. The length of the lamina is a highly variable feature and is determined by all of the factors which contribute to terminal erosion as well as growth rate and age.

Stipes from exposed sites are usually longer and smaller in diameter but are also stronger and more flexible.

Parke (1948) treated in detail the subject of morphological variation with respect to the holdfast of *Laminaria saccharina* (Linnaeus) Lamour. However, she attributed the occurrence of different forms to the nature of the substratum rather than exposure. She noted that plants growing on solid substrates bore compact and thick holdfasts with slightly branched haptera whereas plants growing on soft silt had holdfasts with thin, long, copiously branched haptera. However, other features of the plants used as examples along with the fact that turbulent waters seldom lend themselves to the accumulation of silt, strongly suggest that this effect was more closely related to exposure.

Through transplant studies it has been demonstrated that, given the proper stimulus, individual plants are capable of assuming a form appropriate to a particular set of environmental conditions. This type of response is termed "phenotypic plasticity".

Although certain aspects of the thallus of Macrocystis can be compared directly with the generalized laminarialean plant described above, there are features peculiar to this growth which set it apart and create new opportunities for morphological variation. The continuous production of fronds from the same holdfast throughout the year permits the plant to adjust to seasonal environmental changes. The potential for a more immediate response is created by the continuous production of new blades at the apex of the frond. The extent of water movement can vary significantly over short periods of time and with depth. This creates the posibility for a wide variation in the form of mature blades occurring on the same stipe. The isolation of reproductive and somatic regions, which also occurs to a lesser extent in the Alariaceae, enables the plant to make the necessary morphological adjustments to the environment without interferring with its ability to reproduce.

There are other features characteristic of Macrocystis which are not commonly found throughout the order. These include the spines along the periphery of the blades and the pneumatocysts at the junction between blade and stipe.

Morphological variation in Macrocystis has long been the subject of speculation. So variable are the form and dimensions of the juvenile and mature blades, within as well as between plants, that Hooker (1847) dismissed them as taxonomically unimportant beyond the generic level. Thus he considered the previously described species as synonyms of Macrocystis pyrifera Agardh. He recognized six varieties but pointed out that their characteristics were influenced considerably by the rigours of preservation and natural causes such as exposure. In this latter respect he related variation in the abundance of spines, corrugation, texture and breadth of the laminae, dimensions of the pneumatocysts and diameter of the stipe to the degree of water movement.

Skottsberg (1907) observed a relationship among certain features such as spines, pneumatocysts and exposure. However, his conclusions were limited by the high degree of variation within localities.

A relationship between morphology and exposure was first defined for the overall plant by Brandt (1923). He described two extreme forms of Macrocystis pyrifera (Linnaeus) C. Agardh as follows: an exposed form characterized by very stout stipes, long, tapering cysts with thick walls and long, narrow, thick, tough blades; and a sheltered form characterized by slender stipes, nearly globular, thin-walled cysts and broad, thin, brittle blades which were more or less heart-shaped at the base. The colour also varied, being much darker in the exposed form. Macrocystis integrifolia Bory on the other hand did not show this range of forms although from his descriptions it appears to have occurred in the same general locality.

Womersley (1954) noted the occurrence throughout the genus of a similar relationship with respect to a number of features. These included the surface texture (rugosity) of the blades, the presence or absence of spines along the margins, the demensions of the pneumatocysts and the form of the distal lamina.

Macrocystis has received a great deal of attention over much of its geographic range, due largely to its economic importance. Studies dealing specifically with the effects of exposure on morphology have not been carried out and the observations cited above have not been tested quantitatively.

The present work was directed toward the following basic questions:

(1) How readily does the morphology reflect spatial and temporal changes in exposure? Four sites for regular sampling were selected in close proximity to one another so that all would be characterized by essentially the same water properties except exposure. This technique has already been successfully applied by Norton (1969).

(2) How consistently do the finding in (1) apply throughout the range of exposures covered by *Macrocystis* in local waters? Samples were obtained from a variety of sites covering a wide geographic range. As these localities were expected to vary with respect to a variety of factors the relative importance of exposure would be reflected in the results.

(3) What is the genetic basis of morphological variation in the plant? Are only certain varieties or ecotypes able to thrive in certain habitats or are the features under consideration truly plastic? A transplant study within the four regular sampling sites was conducted and corresponding changes in growth rate and form were studied. An attempt was also made to culture the plant through the microscopic phase of its life cycle under natural conditions. It was hoped that a crop of young sporophytes of known parentage obtained in this manner would be availabe for transplantation. Unfortunately the technique was not developed in time to be of use to the present study. However, a complete description of the technique is included at the end of the thesis

as a separate section.

Based on results of the above three studies and those of previous workers, the functional significance of morphological variation in this species and the mechanism of environmental control are discussed.

MATERIALS AND METHODS

CONTINUOUS SAMPLING STUDY

Description of Sites

The choice of sites for continuous sampling was made mainly on the basis of exposure to waves generated by prevailing winds. During the Spring and Summer months winds in the Trevor Channel region of Barkeley Sound tend to blow most consistently from the south-east and west (Fig. 1). This creates wave forces travelling up Trevor Channel, whereas during Fall and Winter months surface waves are driven predominantly in the opposite direction.

A more consistent, but gentler type of water movement, is created by tidal currents. The flow is hardly noticeable at sites bordering Trevor Channel but is amplified by narrow inlets especially if these empty into large basins. Such is the case in Bamfield Inlet. Imbalances in the tidal flow caused by the projection of the Deer group of islands into Barkeley Sound are suspected as being responsible for the strong currents associated with many of the channels in that region.

With the highly irregular shore-line characteristic of the Bamfield area it was possible to choose four sites differing widely in exposure to water motion, yet lying within a radius of only 0.9 nautical miles. The four sites shown in Figure 2(b) are described as follows:-

Wizard Islet

-moderately exposed throughout all months of the year. Subject to deflected waves off Helby Island during Spring and Summer and slightly protected from the full force of waves travelling down Trevor Channel by a series of partially submerged peaks.

-subject to moderate tidal currents.

-substrate: solid bed-rock giving way slightly beyond the kelp zone to sand and broken shell with occasional bedrock outcroppings.

-slope: about 20-30 degrees at the collecting site.

Scott's Bay*

-moderately sheltered throughout all months of the year. Subject only to deflected waves except for those travelling from a northerly direction.

-not subject to noticeable tidal currents.

-substrate: solid bed-rock giving way to sand and broken rock at about -10 feet.

-slope: about 10 degrees.

Bamfield Inlet

-sheltered throughout all months of the year. Subject only to deflected waves except for those travelling from a northerly direction.

-subject to strong tidal currents flowing along Bamfield Inlet.

-substrate: solid and broken bed-rock giving way at about 15 feet to silt. Occasional plants extending into this zone on scattered refuse.

-slope: about 20-40 degrees.

Dixon Island

-sheltered throughout Spring and Summer. Moderately exposed during Fall and Winter months, being subject to only slightly deflected waves travelling down Trevor Channel. This results in a cropping back of the outer limits of the kelp bed during these seasons.

-not subject to noticeable tidal currents.

-substrate: cobble and loose rock giving way to sand with scattered stone and shell at about 15 feet.

-slope: about 10 degrees.

* After Mr. R. Bruce Scott, a former resident of Bamfield and local historian.

Data on position, open angle (to onshore waves) and maximum and minimum fetch are presented for the above localities in Table 1.

Oceanographic Data

This study was based on the assumption that all four sites were subjected to the same water mass such that differences between sites would not be significant. In order to test this contention near-surface temperature and salinity measurements were taken at one meter intervals throughout the month of August 1970. A Beckman in situ Salinometer (Model # RS 503 Beckman Instruments Inc. Cedar Grove, N.J.) which measured temperature and derived salinity from conductivity to two decimal places was used. Weekly measurements were taken at all four sites within a period of time not exceeding 100 minutes. The data are presented in the form of T/S diagrams in Figure 3. Although some variation occurred from week to week among the four sites, particularly in the upper two to four meters this did not follow any consistent trend. Hence, the sites were not considered to be significantly different from one another with respect to these two factors.

Data covering the entire year 1970 as well as part of 1969 are available for the entrance to Bamfield Inlet. Weekly temperature measurements from 1 and 5 meters are plotted in Figure 4 along with surface salinities.

Frond Sampling and Measurement

Regular monthly collections were made over the period February through August 1970. Additional collections were made in October and December.

Five mature fronds were usually collected from each site although this varied at times from three to six. Special care was taken to ensure that not more than one frond was obtained from each plant during a collection. Samples were always taken from subtidal plants and usually from the same general position in the bed. Therefore, it was very possible that more than one frond was collected from some plants throughout the study. All collections were made with the aid of SCUBA.

Fronds bearing intact distal meristematic regions were selected on the basis of their having reached the surface, thereby being subjected to the full force of surface waves. However, on several occasions fronds trailing along the surface at Dixon Island and Bamfield Inlet were scarce and it was necessary to select from those near the bottom. In such instances fronds of sufficient length to reach the surface were usually selected.

Each stipe was cut at the point of contact with the holdfast. The material was placed in a plastic bag and either transported on ice to Vancouver or measured in Bamfield. A meter stick was used to obtain the following measurements from outstretched fronds:

-length of the stipe from the holdfast to the most distal split in the apical meristematic region (discrepancies caused by twisting of older stipes were not considered significant).

-maximum diameter of the stipe, in millimeters,

-number of terminal splits (incomplete young blades at the distal meristematic region),

-number of blades, including those indicated by terminal splits,

-maximum width of every second blade from the apex of the frond.

Samples were taken of blades from positions 1/2 and 3/4 of the distance from the base of each frond to the apex and were preserved in 5% formalin/seawater. The dimensions of the pneumatocysts were later obtained from these samples. While some shrinkage or swelling of the tissue may have resulted from this treatment the effect would have been generally uniform. Hence, the basis for comparison would probably not have been altered.

Blade and cortex thickness measurements were taken at a standard distance of 15 cm from the pneumatocyst. It was felt that at this distance the tissue would be mature yet still intact in a large number of blades. The tissue was sectioned by hand and examined under a Nikon compound microscope with phase attachments. An excess of seawater was used under the coverslip to prevent distortion. Measurements to 1.0 microns were obtained using a calibrated ocular micrometer. The cortex was taken to include the meristoderm as well as the larger, non-pigmented cortical cells.

Due to erosion and decay of blades, which was most prevalent during Fall and Winter months, it was not always possible to obtain a complete set of measurements. Older laminae were usually reduced considerably in length and were often absent entirely, leaving only a small cluster of blades at the distal end of the stipe.

Care was always taken not to damage the plants during sampling but fronds from Bamfield Inlet and Dixon Island were particularly delicate and easily torn, even by their own weight when lifted out of water. This factor also tended to minimize the available data.

SPOT SAMPLING STUDY

A total of 33 samples were taken from 10 kelp beds covering a wide range in both time and space (Table 1, Fig. 2a).

Exposure was determined by several factors including the angle through which the site was subjected to unimpeded winds and the maximum and minimum fetch. From these data along with qualitative observations at the sites a judgement of the exposure was made as a basis for comparison with the regular sampling study.

Material collected at Amphitrite Point was measured in Vancouver, whereas all other material was measured on board ship. The techniques of sampling and measurement were the same as those used during the regular sampling study. However, sample blades were only obtained from the 1/2 position from each frond.

TRANSPLANT STUDY

Methods

A short time before the transplant study was begun a number of large, smooth stones varing in diameter from about 5 to 8 inches were chosen as substrates for the young plants. The surfaces were brushed and washed clean of all scale and loose material. Two adjacent parallel strips of liquid silicone rubber (General Electric Co.) were applied over the upper surface of each stone. The middle section of the strips was prevented from actually bonding to the surface by a narrow sheet of waxed paper. The strips were able to bond to each other along the entire length and to the stone at either end. After the rubber had set the waxed paper was removed and the strips were separated from one another along the middle section with a knife.

The prepared stones were numbered then placed in position at the selected sites. At Wizard Islet and Scotts's Bay it was necessary to cement them to the bottom. A waterproof cement supplied by the All-Crete Mfg. Co. of Woodland, California was used for this purpose.

The transplant sites at Wizard Islet, Bamfield Inlet and Dixon Island were all located at a depth of about -1.3 feet. The depth at the Scott's Bay site was about -8 feet.

In order to prepare the site to receive the new plants a small area of the bottom was cleared of all large plant and animal material. It was hoped that this would reduce competition among the plants. The site was marked with surveyor's tape so that it could be easily located in times of poor visibility.

After the stones were in place the young plants were attached by inserting the base of the stipe between the rubber strips so that the haptera were held firmly against the surface. An advantage of using this technique is that it enables one to use the same substrate a number of times in a continuous transplant study after the mature plants have been removed (Fig 5).

Description of Transplants

The plants used in the study were, in most cases, at about the same stage of development when the transplants were made. These bore a single lamina and were, in appearance, similar to various other young laminarialeans. However, they were easily distinguished by the presence of a short primary longitudinal split. The first set of plants transferred to Scott's Bay were at a slightly more advanced stage of development and in all but one instance had completed the first dichotomy. This was taken into consideration in the computation of growth data.

The major phase of the transplant study consisted of transferring plants from Wizard Islet to the remaining three sites. Five plants were transferred to Scott's Bay on May 17 in order to test the technique. On May 30 two more plants were added to this site and five were transferred to each of the remaining localities. An additional five, serving as controls, were positioned at Wizard Islet. Hence, the total number of plants initially involved in the study was twenty-two. Losses due to physical and biological factors caused this number to drop gradually throughout the study although replenishments were made during the early stages. The only successful replacement was plant number 5 at Wizard Islet which was positioned on June 15.

A reciprocal transplant was attempted between Dixon Island and Wizard Islet but was not successful. Within a few weeks all plants transferred to Wizard Islet were either severely damaged or entirely lost. Although these plants were at about the same stage of development as those collected from Wizard Islet their laminae were considerably wider and the texture appeared to be somewhat more delicate. These factors, combined with the greatly increased exposure at Wizard Islet, appear to have led to their deterioration. This problem was also encountered by Norton (1969) when he attempted to transfer plants from a sheltered to an exposed locality. It was concluded from the present attempt that such a transfer might have been successful if the plants had been obtained at a comsiderably earlier stage of development. As such plants were no longer available, the reciprocal transplant study was discontinued.

Evaluation of Plant Response

Measurements were taken *in situ* at two week intervals over a period which varied with the condition of each plant. All data were recorded in pencil on a sheet of sanded perspex. The following information was obtained from a single primary frond from each plant:

-stipe length,

-number of blades,

-number of terminal splits,

-dimensions of every second blade from the base of the stipe.

The growth rate in length of individual blades was assessed by punching a small hole with a cork borer in the center of the blade at a distance of 10 cm from the pneumatocyst. It was necessary to place the holes at 5 cm in very young blades in order to prevent their loss due to terminal erosion between measurements. Expansion of the perforations indicated that secondary growth was probably still occuring between 5 and 10 cm as has been demonstrated for *Macrocystis pyrifera* by Cribb (1953). However, this was not considered sufficient to alter the results appreciably.

RESULTS

CONTINUOUS SAMPLING STUDY

Changes Recorded in Time and Place

Individual frond samples did not yield sufficient data to enable direct comparisons to be made between localities. Nevertheless, by presenting each aspect of the habit on a month to month basis certain trends are often revealed and the consistency with which they are maintained throughout the year bears pointing out.

Stipe Diameter

Mean stipe diameter and length are plotted against time in Figure 6. However, before these graphs can be interpreted it is necessary to understand how these two features relate to one another.

A linear regression analysis was conducted for each locality to determine whether a linear relationship between maximum diameter and length could be defined on the basis of the combined data for each site. If such a relationship was determined it was assigned an R value (linear correlation coefficient) which is an expression of confidence and has a maximum value of 1.

Linear plots are superimposed on scatter diagrams for all localities except Dixon Island in Figure 7, and R values are included for each site. In all cases the regression was significant at the 0.01 level.

Covariance analyses were used to determine whether the linear relationships described in Figure 6 were significantly different from one another. A linear relationship between two variables is defined by the expression:

$y = a + b(x - \bar{x})$,

a is the mean value for y as determined from the data;

b represents the slope.

Considering only Bamfield Inlet and Scott's Bay, the probability of their a values and slopes being different from one another were 0.0540 and 0.1624 respectively. In neither case were these values considered significant. Hence, the data for the two sites were combined and compared with Wizard Islet by the same procedure. In this case the probability values for a and b were 0.2693 and 0.6583 respectively. Thus the three sites did not differ significantly from one another. The failure to define a linear relationship for Dixon Island will be considered in the DISCUSSION.

Returning to Figure 6 it is now apparent that although the R values given in Figure 7 were generally low the observed fluctuations in stipe length cannot be ruled out as contributing to the corresponding fluctuations in diameter within the sites with the exception of Dixon Island. The closeness of fit between these two factors in Figure 6 strengthens this conclusion.

Variation in stipe diameter throughout the study area was tested by applying Student's paired t-test to the monthly means. Due to the relatively narrow range of values obtained only those sites representing the outer limits of exposure (which also corresponded to the outer limits of mean stipe diameters) were considered. Probability values for common means are as follows:

Wizard Islet vs. Bamfield Inlet 0.1

Wizard Islet vs. Dixon Island 0.01

Bamfield Inlet did not differ significantly in mean stipe diameter from Wizard Islet. This may be related to the similarity of their mean stipe lengths, particularly as a linear relationship has been defined for both sites (Figure 7). As a linear relationship was not defined for Dixon Island the significant difference in mean diameter from Wizard Islet strongly suggests that the diameter of the stipe is related to the amount of water movement. This relationship is demonstrated consistently throughout the year.

The seasonality in stipe diameter shown by Dixon Island in Figure 6 can be explained in light of the above conclusions. As the maximum growth period for an individual frond is about six months (see pg. 21 and also North, 1971) the occurrence of the largest values for mean stipe diameter through late spring and early summer represents a response to dynamic conditions as they existed during the previous winter and early spring (Table 1). Hence, these fronds differed least from those sampled at Wizard Islet during this period of the year. The late summer and fall decrease in diameter can be attributed to the fact that the plants collected at this time of year would have grown under very sheltered conditions.

Stipe Length

Variations within and between localities with respect to stipe length was too large to reveal any pattern, with the possible exception of Dixon Island where values remained generally low throughout the year. Samples were not alway obtained from exactly the same depth, particularly at Bamfield Inlet.

Other variables which were not controlled include age and possibly growth rate. Hence, further consideration of the relationships between water movement and stipe length must await the results of the remaining two studies.

Distal Meristematic Region

The mean number of terminal splits for fronds from each locality is plotted against time in Figure 8 and maximum and minimum values are included.

Student's paried t-test was run and probability values for common means are as follows:

Wizard Islet vs. Scott's Bay 0.2
Wizard Islet vs. Bamfield Inlet 0.05
Wizard Islet vs. Dixon Island 0.05

These data suggest that rapid water movement enhances the accumulation of terminal splits and this trend is consistent throughout the year. However, the common seasonality indicated by all sites, with the exception of Dixon Island, does not appear to be related to this effect as dynamic conditions were least severe during spring and summer months. The importance of other variables such as age and tissue deterioration will be considered in the DISCUSSION.

Blades:

Number in relation to stipe length

The analysis applied to stipe diameter was used to test for a linear relationship between the number of laminae and the length of the stipe for each locality. The results are presented diagramatically in Figure 9 and R values are included. In all instances the probability of the slope being equal to 0 was less than 0.01.

Covariance analyses were used to compare localities and probabilites for significant differences between sites with respect to values a and b were as follows:

	a	b
Bamfield Inlet vs. Wizard Islet	0.0386	0.5491
B.I. + W.I. vs. Scott's Bay	0.0661	0.3385
B.I. + W.I. vs. Dixon Island	0.2286	0.4576

Thus there was found to be no significant variation among the four localities.

Width of Mature Laminae

There were several problems involved in comparing data on width of mature blades. To reduce the effect of variation within each plant it was necessary that as many blades as possible be considered. If the comparison was to be based on individual samples, then as many plants as possible would have to be included.

It was also essential that the same amount of data be drawn from each locality. The matter was further complicated by the high degree of variability in the number of intact blades present on each frond. However, it was possible to obtain sufficient data to test for variation among populations while taking all of these factors into consideration.

During April, for example, the most badly deteriorated fronds were collected from Bamfield Inlet. From that sample two fronds bore three intact mature blades, one bore two mature blades and the fourth frond did not bear any. In order to use a maximum number of frond only the first two mature blades on each of the first three fronds were considered. This standard number of fronds and blades was then applied to the remaining three samples from the April collection.

From the June collection, on the other hand, a standard number of four fronds and four blades was used. Due to the nature of this analysis there were always some samples for which there were more suitable fronds than could be used. In such cases the last fronds on the data sheets were eliminated first as they were measured in random order.

The data obtained in this way for each collection were subjected to a three factor analysis of variance. Variation among plants due to genotype or to environment-plant interactions were later ignored as the object of the study was to test for significant differences among the overall populations.

The actual variance among the four localities was compared with the calculated variance based on the null hypothesis (that the population means are equal). The ratio of the recorded variance to the calculated variance is termed the variance ratio and is designated F. The F values and probability values for common means for the final seven collections are presented along with sample sizes in Table 2. Due to insufficient data the first two collections were not included in the analysis. Mean blade width is plotted against time for each locality in Figure 10.

A significant level of variation is shown to occur among the four sites over much of the year. The nature of this variation is such as to suggest that increasing exposure is directly related to the formation of narrower blades.

Assuming this to be the case, the set of values obtained from Dixon Island are somewhat lower than expected. This may be related to the generally poor condition of the fronds collected from that site. Figure 11 shows a typical situation wherein all of the middle to lower blades have deteriorated by the time the frond has reached the surface. Sargent and Lantrip (1952) and Parker (1965) demonstrated that young blades near the apex of the frond depend to a large extent on the importation of metabolites from lower regions of the plant. In the absence of this source of metabolites the development of young blades near the surface may have been arrested.

As in the case of terminal splits a seasonality is indicated which is not directly correlated with exposure conditions and alternative explanations must await the results of the remaining studies.

Changes Recorded Only in Space

Due to blade atrophy it was not possible to obtain a complete set of samples from every collection. Blades were obtained most consistently from all localities during the months of April, May and June. Hence, comparisons were made on the basis of one overall collection covering this period.

For each set of data obtained from the sample blades a one factor analysis of variance was conducted. Variance ratios were determined and Scheffe's test for multiple comparisons with samples of unequal size was conducted. This permitted comparisons to be made between specific localities. The results of this test are expressed in the form of probability values for common means.

These analyses were used to test for significant variation in the following features: blade thickness, cortex:medulla ratio, mean spine length and pneumatocyst length and diameter. Only the lower blades were used and the results of the analyses are presented in Table 3. Mean values for the 1/2 and 3/4 positions are included along with standard errors of the means and maxima and minima.

These data were expected to reflect environmental conditions as they existed in the Bamfield region throughout early and late spring. Due to the high degree of variation within individual plants it is difficult to draw other than tentative conclusions. There was usually an absence of any pattern to this variation relative to exposure. However, it is very likely that some discrepancy may have been caused by the inclusion of immature blades from the 3/4 position.

Those features which appear to be most responsive to water movement are lamina thickness, spine length and pneumatocyst diameter. The occurrance of the shortest bladders at Dixon Island is consistent with the findings regarding pneumatocyst diameter. Hence, a trend toward the formation of pneumatocysts of smaller capacity in less exposed environments is suggested. This does not necessarily conflict with earlier statements regarding seasonal effects of exposure on stipe diameter at Dixon Island as the characteristics of individual blades would be expected to reflect a more recent dynamic environment.

Spot Sampling Study

Data obtained from the spot sampling study are presented along with mean values in Tables 4 and 5. Only the first three to four blades from each frond were considered in the determination of blade widths in order to facilitate comparisons with the results of the continuous sampling study.

Those features which seem to be most directly influenced by exposure conditions are stipe diameter, blade width and thickness and pneumatocyst dimensions. The data do not indicate a direct correlation of the following with exposure: the relative number of blades, terminal splits, cortex:medulla ratios and spine length. The same also applies to stipe length but in this instance the depth of water and age must also be considered.

Transplant Study

The transplant study was terminated by frond atrophy at all localities except Wizard Islet. However, all final measurements of stipe length and blade number are valid as the plants still contained complete primary fronds although the individual blades and apical meristematic regions were often badly deteriorated. The last remaining plants were collected from all localities on December 5 after 199 days.

Growth of Primary Fronds

The lengths of the primary fronds are plotted against time for each locality in Figure 12. Student's paired t-test was applied to the mean slopes of the exponential phase for all sites. Values for Wizard Islet differed significantly from those of all other sites $_{(p = 0.05)}$. Mean daily increments in cm per day and initiation rates for new blades were determined from consecutive readings and are presented in Table 6.

The growth rates of individual fronds show a distinct correlation with exposure. Neither the greater depth nor the density of the surface canopy appear to have affected the growth rate of stipes at Scott's Bay. This is consistent with the findings of Neushul and Haxo (1963). Growth was characterized at all sites by a slow growth phase followed by a period of rapid elongation and blade initiation. The maximum elongation rate of 8 cm/day throughout the exponential phase compares favorably with the previous maximum recording of about 7.9 cm/day for the same species in local waters (Scagel, 1948).

Growth of Laminae

Maximum daily increments of individual blades were determined from the distance covered by reference marks between consecutive readings and are presented in Table 7. A one factor analysis of variance was used to test for significant differences between localities. Only the fastest growing blade from each plant was considered in each case as this was assumed to represent the optimal rate of growth. The results are also presented in the above table. These data do not indicate a relationship between growth of laminae and water movement. The consistent occurence of low values for Scott's Bay suggests that light may be the controlling factor in blade elongation as these plants were growing in deeper water than the others and were also influenced to a greater extent by the surface canopy.

Blades Per Unit Lenght of Stipe

It is apparent from Table 4 that the number of blades per unit length of stipe is not a function of exposure. Although plants growing in more exposed localities are characterized by higher blade initiation rates (Table 6), this is counteracted by corresponding differences in the growth of the stipes. In Table 8 the age of the frond is considered and the ratio of the number of blades to the length of the stipe is shown to decrease steadily with time. This supports the contention that differences between localities with respect to this feature cannot be directly attributed to exposure conditions.

Lamina Width

Direct comparisons between localities with respect to blade dimensions could not be made at the time of each recording as the development of the plants was out of phase. Plants with larger numbers of blades were naturally closer to the surface so that blades forming near the apex were subject to different environmental conditions. Comparisons could, however, be made by considering only mature blades at the same position on each plant. This was only made possible by the short period of time over which the experiment was conducted. Maximum widths are given for mature blades numbered from the base of the frond for all localities in Table 9. These data support the previous findings regarding blade width and suggest that the wide range of values recorded throughout the Bamfield region are within the capacity of the individual plant.

DISCUSSION

Considering the relatively narrow range of exposure covered by the continuous sampling study it is not surprising that the morphological differences between consecutive localities were not more distinct. In most cases the trends established through the continuous sampling study have proven to be consistent over a wide geographic range. When only those localities representing the extremes of exposure are considered the trends are often more clearly defined. In such instances the occurrence of intermediate values at the remaining sites suggests the presence of a highly sensitive controlling mechanism.

Although each of the three studies deals with a particular aspect of the relationship between morphology and exposure, the full significance of the results can only be appreciated by incorporating them into an overall treatment of the problem. The following discussion deals with individual aspects of the morphology in this manner.

Stipe Diameter

The importance of exposure in the determination of stipe diameter can only be seen in its proper perspective if the effects of other factors such as the age of the plant and the length of the stipe are understood.

Clendenning (1971) has described a relationship between the age of the plant and diameter of the stipes it produces. First generation fronds of *Macrocystis pyrifera* were generally thinner, darker coloured and less likely to withstand the forces generated by strong wave action than later fronds on the same plant. Fronds were collected throughout the present study without regard to hierarchy and it is likely that if such a relationship does apply to this species, it would have affected the recorded values for stipe diameter uniformly. First generation fronds of control plants at Wizard Islet, however, did manage to reach the surface and appeared no less vigorous than secondary and tertiary fronds on the same plants.

Although it is possible to define a linear relationship between stipe diameter and length (Fig. 7) it has been demonstrated that this cannot always be used to explain seasonal fluctuations within specific localities. At Dixon Island these aberrant fluctuations are attributed to seasonal changes in exposure.

While the linear relationship could also be used to explain differences which occur between localities there are situations where this does not apply. The mean stipe length was similar for Amphitrite Point, Raglan Point and Port Hardy. However, the diameters varied in such a way as to suggest that decreasing exposure results in narrower stipes The same conclusion can be drawn from a comparison between Scott's Bay and Wizard Islet and the more exposed site at Hope Island.

The narrow range of stipe diameters recorded from many sites and the fact that the R values in Figure 7 are not significantly large suggest that it may be possible to compare many localities without regard to stipe length. Under these conditions a relationship between stipe diameter and exposure becomes more apparent. Table 4 demonstrates that this relationship applies over a wide geographic area while in the Bamfield region (Fig. 6), it has been shown to apply relatively consistently throughout the year. Due to frond atrophy data supporting these conclusions were not obtained from the transplant study.

The functional significance of this relationship is obvious but is complicated by the fact that in most cases the thickest region of the stipe occurred some distance from the holdfast. The same phenomenon has also been reported by Scagel (1948) and Cribb

(1954). However, this does not necessarily conflict with the assertion that stipe diameter is related to exposure.

By the time a frond has become subjected to the full force of surface waves its lower portions have already ceased to elongate. The plant may allow for this by producing a stipe of much greater strength during the earlier phases of growth than is actually required at that time. A less teleological interpretation, on the other hand, is that a stipe of sufficient diameter to withstand the stresses occurring during its formation is later strengthened through the incorporation of metabolites from other regions of the plant. Sargent and Lantrip (1952) and Parker (1965) found that various substances were transported downward from more distal regions of the plant. Stipe tissue forming further from the holdfast and during the rapid phase of elongation would become subjected to stronger dynamic forces before it had matured, resulting in a corresponding increase in thickness.

In accordance with the above interpretation it is now apparent that plants subjected to maximum exposure during early stages of growth would not show a linear relationship between stipe diameter and length later in their development. At Amphitrite Point, for example, the plants were collected slightly subtidally in a surge channel and the diameters of their stipes were large in spite of their short lengths. The plants at Dixon Island, on the other hand, were not subject to turbulence during summer months. This resulted in the formation of uniformly narrow stipes and a linear relationship was not defined. That this applies to a lesser extent throughout the Bamfield region is demonstrated by Figure 7. A large range of values for stipe length is always associated with a relatively narrow range of diameters and the linear relationships were weak.

The maximum diameter recorded throughout the overall study is expected to reflect dynamic condition rather than stipe length. Hence, a value of 12 mm was recorded for two fronds which differed in length by a factor of two. This appears to be the optimal stipe diameter for the species in Canadain waters (see also Scagel, 1948).

Stipe Length

The length of the stipe is a function of both depth and exposure. As the plant grows through the water column it comes increasingly under the influence of wave action and tidal currents. The resistance created by the blades causes the stipe to assume a diagonal position relative to the surface. Hence, by the time the apex has reached the surface the length of the stipe is much greater than the depth of water over the holdfast. Throughout the entire study there was found to be very little variation in the length of that portion of the frond trailing along the surface other than that which could be attributed to the varied position of the tide, along with possible seasonal effects. Growth, therefore, appears to cease shortly after the frond has reached the surface, regardless of dynamic conditions.

In sheltered localities the fronds were able to reach the surface over a shorter distance, thus accounting for the relatively low set of values for mean stipe length recorded for Dixon Island throughout the year.

Due to the fact that depth readings were not taken throughout the spot sampling study it is not possible to apply the above hypothesis to every site visited. However, there are some obvious exceptions. Fronds collected from Amphitrite Point, Raglan Point and Masterman Island were all growing at or near the low tide level and yielded low values for mean stipe length.

Distal Meristematic Region

Cribb (1953) has suggested that the number of terminal splits may be related to the growth rate of the frond. According to this theory a plant with a high level of meristematic activity would tend to initiate new blades more quickly than they could separate into individual units.

This interpretation is supported by both the continuous sampling study and transplant study. More exposed localities were characterized by plants having larger number of terminal splits, faster growth rates of fronds and higher blade initiation rates. As the growth rate of individual blades does not seem to be related to water movement they would tend to accumulate distally on fronds in such localities.

The importance of water movement in promoting the formation of terminal splits, hence the growth of the frond, is suggested indirectly by work on other members of the order. For example, direct correlation between turbulance and the degree of digitation of the lamina in Saccorhiza, Laminaria and Hedophyllum has been demonstrated by Norton (1969), Sundene (1961a) and Widdowson (1964) respectively. Norton (l.c.) points out that the series of meristematic events preceding digit formation in Saccorhiza polyschides are the same as those described for Macrocystis pyrifera. Hence it is not unreasonable to expect these events to be initiated by a common set of stimuli.

If water movement was the only controlling factor one would not expect the seasonality indicated in Figure 8. The Wizard Inlet site was subjected to fairly strong wave action throughout the year, yet during the Winter months was characterized by about the same mean number of terminal splits as the other localities in the Bamfield region. Seasonal changes in light and temperature followed the same general pattern as that shown by the terminal splits. It is beyond the scope of the present study, however, to contemplate the significance of these factors relative to one another. The low values encountered at Dixon Island during late summer suggest that high surface temperatures in combination with a low level of water movement may have adversely affected the plants in that area. Clendenning (1971) attributed a late summer decline in photosythetic capacity of distal blades of *Macrocystis pyrifera* to high surface temperatures.

Age is also important as older fronds terminate their growth through a reduction in the blade initiation rate with the eventual formation of single terminal lamina (Scagel, 1948), (see also Figure 12). However, the only direct correlation between stipe length and number of terminal splits which could be attributed to this factor occurred during the Winter of 1970.

Whereas exposure may promote the formation of terminal splits it may at times be so severe as to reduce this number by prematurely separating young blades. This is in agreement with Womersley (1954) who observed that "plants growing in denser beds have a broader (terminal) blade with more segments than where the fronds are more exposed to broken water." He also noted a depth effect but this appears to be explained by the fact that submerged terminal blades would most likely be undergoing rapid growth but would not be subjected to the full force of surface waves.

Considering all of the factors which can influence the number of terminal splits along with the fact that the fronds were collected for the spot sampling study at different periods of the year, the lack of correlation throughout this study with exposure is not entirely unexpected.

The shape of the terminal blade is also highly variable. Those collected from moderately exposed to sheltered areas were broadly falcate with a length 3 to 5 times the breadth (Figure 13). Terminal blades from exposed locations were narrowly falcate and 6 to 10 times as long as broad (Figure 14). These descriptions are the same as those used by Smith (1944) in delineating _Macrocystis integrifolia_ and _pyrifera_ respectively. It is obvious from Figure 14, however, that the terminal blade has been modified by the tearing action on surface waves. Smith's note on local distribution suggests that he based his conclusions only on plants subjected to this sort of treatment. The extent to which these differences represent genuine responses to environmental conditions is, therfore, open to question.

Blades

Number in Relation to Stipe Length

Although the linear relationship between blade number and stipe length was defined for all localities in the Bamfield region the slope and position did not vary significantly within the study area. The number of blades per unit length of stipe steadily decreased throughout the life of the frond. Since fronds collected throughout the spot sampling study had achieved a highly varied level of development the degree of variation occurring among populations is understandable and is not related to water movement.

Width of Mature Laminae

The results of the two sampling studies suggest that increasing exposure has a narrowing effect on blade width. In Figure 10 this relationship is shown to apply throughout the year while in Table 4 it is seen to apply over a wide geographic area.

A seasonal fluctuation in blade width followed a common pattern throughout the study area. The similarity between this pattern and that of the number of splits in the distal meristematic region suggests the influence of a common set of factors. The occurrence of narrower blades during winter months may be related to seasonal changes in wind direction and speed. Figure 1 demonstrates that weather conditions were most severe at all locations during this period of the year. On the other hand, dimishing light and possible temperature conditions may have caused the growth of young blades near the surface to cease before achieving maximum width. It is also possible that the formation of smaller blades is simply a manifestation of an overall slowing down of growth processes which occurs after the frond has reached the surface.

A final factor which should be taken into consideration is the length of the stipe. North (1971) found that the blades of greatest width usually occurred about half way between the base and apex of the frond. This phenomenon was observed many times throughout the present study particularly with respect to older fronds. This suggests that one could expect an inverse relationship between the width of youngest mature blades and the length of the stipe to apply to fronds which had passed the half-way point in their growth. A comparison of Figures 6 and 10 suggests that this relationship may explain some of the observed fluctuation in blade width.

On the basis of comparisons between blades occupying the same position on different fronds the results of the transplant study suggest the presence of a high level of environmental control over lamina width. The ability of the plant to respond in this manner may, under exposed conditions, conflict with its ability to produce a maximum surface area for photosynthetic activity. However, increased exposure was also characterized by the formation of more highly corrugated or rugose surfaces. This appears to meet the plant's photosynthetic needs while keeping resistance to water movement to a minimum. Calm forms were usually completely lacking in this feature. In this respect the type of water movement may also figure significantly. Although fronds collected from Browning Passage were similar in appearance to those collected from the most exposed sites many of the blades had completely smooth surfaces. This may be related to the fact that, as in Bamfield Inlet, tidal currents appeared to constitute the major form of water movement. A depth effect was also observed as blades further removed from the influence of surface wave action were generally less rugose.

In sheltered waters the formation of wider blades would not only provide a large surface area for photosynthetic activity but would also increase resistance to water movement. This may further increase the amount of light reaching the surface of the blades by causing the laminae to assume a parallel position relative to the surface of the water.

Norton (1969) has suggested two possible mechanisms by which turbulence may control blade width. Rapid water movement may act directly upon the meristematic zone through the creation of tension along the surface of the blade, or indirectly through its influence on the physiology of actively dividing cell. The latter possibility may explain the growth of narrow-bladed forms of <u>Alaria esculenta</u> in a laboratory culture system by South (1970). As he used a continuously flowing system it is possible that there was sufficient water circulation of elicit the observed response.

The shape of the base of the blade varied considerably and in a fashion similar to that described by Parke (1948). She attributed these variations to differences in the growth rates and described three main forms: a fast growing fusiform type, a slower growing cuneate type and very slow growing semicircular or subcordate type. These forms were found throughout the present study but their occurrence appeared to be more closely related to exposure as there was found to be no significant difference in the growth rates of blades at the same depth from one locality to an other. Examples of the three forms are shown in Figure 15, 16, and 17.

Exposure also plays an important role in the determination of blade length by promoting deterioration of the distal portions. Young laminae are able to counteract this through a high rate of production of new tissue. However, erosion continues after growth ceases and this usually results in the complete loss of older laminae. In Bamfield Inlet the nature of the water motion was such that decay was not promoted. This resulted in the formation of unusually long laminae which occupied much of the stipe.

Grazers and epiphytes are also considered to play in important role in the determination of blade length in more exposed localities. On the other hand, microbial decay may have been the major factor responsible for the short length of the laminae on plants collected from Dixon Island.

<u>Lamina Thickness</u>

A consideration of only the older laminae collected throughout the continuous sampling study suggests that blade thickness increases with exposure. This is further exemplified by the results of the spot sampling study and agrees with the findings of Norton (l.c.). There appear to be two distinct categories: a thick, tough outer coast form demonstrated by plants from Amphitrite Point, Browning Passage, Hope Island and Warren Island and a thinner, more delicate form characteristic of the remaining localities. While there was considerable variation within each form, there was a complete absence of intermediate values over a range of about 7 microns.

Although the cortex:medulla ratio did not vary significantly with exposure, the corresponding differences in the thickness of cortical tissue may have been partially responsible for the observed variation in texture.

Spines

The results of the continuous sampling study suggest an inverse relationship between spine length and exposure but this is not substantiated by the spot sampling study. Although the spines were, at times, much reduced in length or widely spaced, under no conditions were they found to be entirely absent from sterile laminae.

The position of the spines relative to the edge of the blade does appear to vary in a fixed pattern. Under exposed conditions the spines tend to lie close to the edge of the blade (Figure 18), possibly as a means of reducing friction. On the other hand, in sheltered localities they tend to project outward, often forming an angle of 90 degrees (Figure 19). In this case the effect would be to increase friction, thereby creating as much drag as possible along the edge of the blade. The possible benefit of increasing drag to the plant has already been discussed in the section on lamina width. Similar observations on the position of the spines of Macrocystis pyrifera were made by Brandt (1923).

Pneumatocysts

As in the case of blade thickness, the pneumatocysts seem to be distinguished by an outer and inner coast form. Skottsberg (1907) found the pneumatocysts from the most exposed localities to be of such large dimensions as to warrant the use of the special descriptive term "longibullata". This term would apply equally well to local plants from the outer coast. The occurrence of smaller pneumatocysts at Amphitrite Point is attributed to the immature status of the sample blades. Skottsberg also described a short, wide, pyriform type characteristic of less exposed sites. Similar forms were occasionally observed throughout the spot sampling study usually at localities described as moderately exposed. Generally the inner coast forms were very similar in appearance to outer coast forms and differed only in over all size.

The dimensions of the pneumatocysts varied throughout the entire range of exposure in such a way as to suggest that decreasing exposure has a reducing effect. However, with particular regard to length this variation was not as consistent as some of the other features considered.

The occurrence of stronger dynamic forces along with the greater thickness of the lamina in more exposed areas may be related to the formation of pneumatocysts of larger capacity as both these factors would tend to draw the fronds away from the surface.

The greater influence of wave action near the surface may have been responsible for the occurrence of larger pneumatocysts at the 3/4 position at all localities in the Bamfield region except Wizard Islet.

The very small pneumatocysts observed at Dixon Island seem to represent an anomalous situation characteristic of only the most sheltered waters. Similar pneumatocysts have been described for loose lying populations of Macrocystis pyrifera (Moore, 1962). Lindauer, Chapman and Aiken (1961) reported a complete absence of bladders on plants growing under similar conditions.

QUALITATIVE OBSERVATIONS

Holdfast

The morphology of the holdfast was generally uniform over a wide range of exposures with one notable exception. Instead of being closely adherent to the substrate the rhizomatous portions of the holdfasts of plants growing in Bamfield Inlet appeared to lose their polarity and gave rise to a compact network of long, narrow descending haptera. This resulted in the formation of a bee-hive shaped holdfast, the center of which was often completely decayed or eaten away.

Neushul (1971) reported a loss of geotropism in the holdfast of Macrocystis pyrifera but attributed this to the influence of warm water. This explanation does not appear to apply in the present situation as even the oldest holdfasts at other sites in the Bamfield region remained closely adherent to the substrate. Parke's (l.c.) suggestion of a substrate effect also does not seem to apply as most of the plants in Bamfield Inlet were growing on solid or broken bedrock.

Why the plants should respond in this way remains very much a mystery. Other laminarialeans growing in Bamfield Inlet responded in a similar manner, often forming large holdfasts which were very poorly attached. It is possible that the plant does not become firmly attached to the substrate because to do so would constitute an unnecessary expenditure of energy which could otherwise be diverted to storage processes in the haptera. A large, densely packed holdfast could also serve an anchoring function for a plant which is growing in very sheltered waters.

Sporophylls

Certain aspects of the morphology of the sporophylls tended to vary greatly within and among localities. While environmental factors may have had a modifying effect, the nature of this variation appeared to be more closely related to the nature of their precursors.

Those sporophylls which occupied the most proximal positions on the stipe were either derived from the first lamina to separate from the young frond or from a basal meristem which failed to give rise to a new lateral. As such, these sporophylls generally lacked pneumatocysts and were usually highly ramified. Neushul (1962) pointed out that the origin of the sporophylls was also reflected in the branching pattern, being symmetrical in the former case and asymmetrical in the latter. Sporophylls further removed from the base of the plant resembled more closely the sterile laminae.

Pneumatocysts and spines were usually present and branching became less prevalent with increased distance from the holdfast. These sporophylls tended to show the same general responses to exposure conditions as have already been described.

THE ENTIRE PLANT

This study has clearly demonstrated the wide range of morphologies which enable the plant to occupy many of the varied habitats characteristic of local waters. In many ways the nature of the relationship between form and environment has been shown to comply with the general pattern already established throughout the order.

The results of the transplant study suggest that the mechanism of morphological adaptation in the Bamfield region is phenotypic plasticity. The apparent ability of the plants to respond to seasonal fluctuations in dynamic conditions within a locality and also within individual fronds supports this conclusion.

Whether phenotypic plasticity is solely responsible for the entire range of morphologies which were observed is still very much open to question. The forms found growing on the outer coast were, in some respects, so strikingly different from inner coast forms as to suggest the presence of at least two categories of gene pools. North (1969) has demonstrated that the majority of the spores which are liberated from a mature sporophyte probably complete the life cycle within a short distance of the parent. Therefore, it is quite possible that the two forms have resulted through continuous inbreeding while the potential for a high degree of polymorphism has been maintained. This would continue to be essential in an environment where dynamic conditions can vary significantly over a very short distance. Whether genetic isolation has resulted in the establishment of separate species or varities has yet to be demonstrated.

SUMMARY

Several aspects of the habit of Macrocystis integrifolia Bory have proven to be highly variable. The overall morphology of the plant is related to the nature and extent of water movement to which it is subjected throughout its development.

Under exposed conditions the plant must assume a form which will enable it to withstand the stresses created by rapid water movement. Thus, plants growing in such areas are characterized by:

stipes of large diameter,
narrow, thick, tough laminae which are cuneate at the base,
spines set close to the margins of the laminae,
large, tapering pneumatocysts,
holdfasts which are closely adherent to the substrate and bear short, wide haptera.

As the horizontal component of water movement tends to draw the fronds away from the surface, plants subjected to broken water or strong tidal currents require pneumatocysts of larger capacity. The inclination of the fronds also necessitates the formation of longer stipes than would be required by plants growing at the same depth in sheltered waters. This is compensated by a higher rate of stipe elongation throughout the exponential phase of growth.

Rapid water movement also stimulates blade initiation. Hence the number of segments in the terminal lamina may provide an index of the developmental rate of the plant, particularly as the elongation of individual laminae does not appear to be a function of exposure. However, as the form of the termainal lamina can also be influenced considerably by the damaging effects of broken water and by the age of the frond, this feature is not considered to be a reliable indicator of dynamic conditions.

The laminae of plants from exposed sites also tend to be more highly rugose. This appears to serve the function of providing a

large surface area for light absorbtion without greatly increasing resistance to water movement.

In sheltered waters there is less emphasis on stress and friction. Thus plants growing under these conditions have:

narrow stipes,

wide, thin, delicate laminae which are cuculate at the base,

outward projecting spines,

small, often globular pneumatocysts,

holdfasts which are not closely adherrent to the substrate and bear long narrow haptera.

Besides providing a large surface area for light absorbtion the overall morphology of the laminae may also enchance photosyntheis by enabling them to take advantage of mild current flows in assuming a horizontal position in the water column.

The length of the mature laminae is a function of several factors in addition to water movement. These include tissue strength, grazing pressure, epiphytes, microbial activity and age. Thus, it is not possible do draw firm conclusions regarding the effects of water movement without greatly reducing the number of variables.

While the sporophlls tend generally to reflect dynamic conditions in much the same way as sterile laminae their morphology is also influended to a large extent by the nature of their precursors.

A wide range of intermediate forms have been described from sites subjected to less extreme conditions. The results of a transplant study, seasonal fluctuations within populations and variations within plants suggest that the mechanism of response is phenotypic plasticity. However, some aspects of the plants observed at the exposed or outer coast sites suggest that they may be genetically distinct from inner coast forms. The most consistent feature upon which a taxonomic separation might be based is lamina thickness. Further studies involving the two forms are recommended before such a step is taken.

Figure 1. Measurements of wind direction and speed (m.p.h.) taken throughout 1970 at Cape Beale at the south-east entrance to Barkeley Sound. Three recordings were taken daily at 0700, 1200 and 2000 hours. Wind speed is indicated as follows:- ...1 - 10; ...11 - 20; ...above 20. (These data were provided by the Government of Canada, Meteorlogical Service).

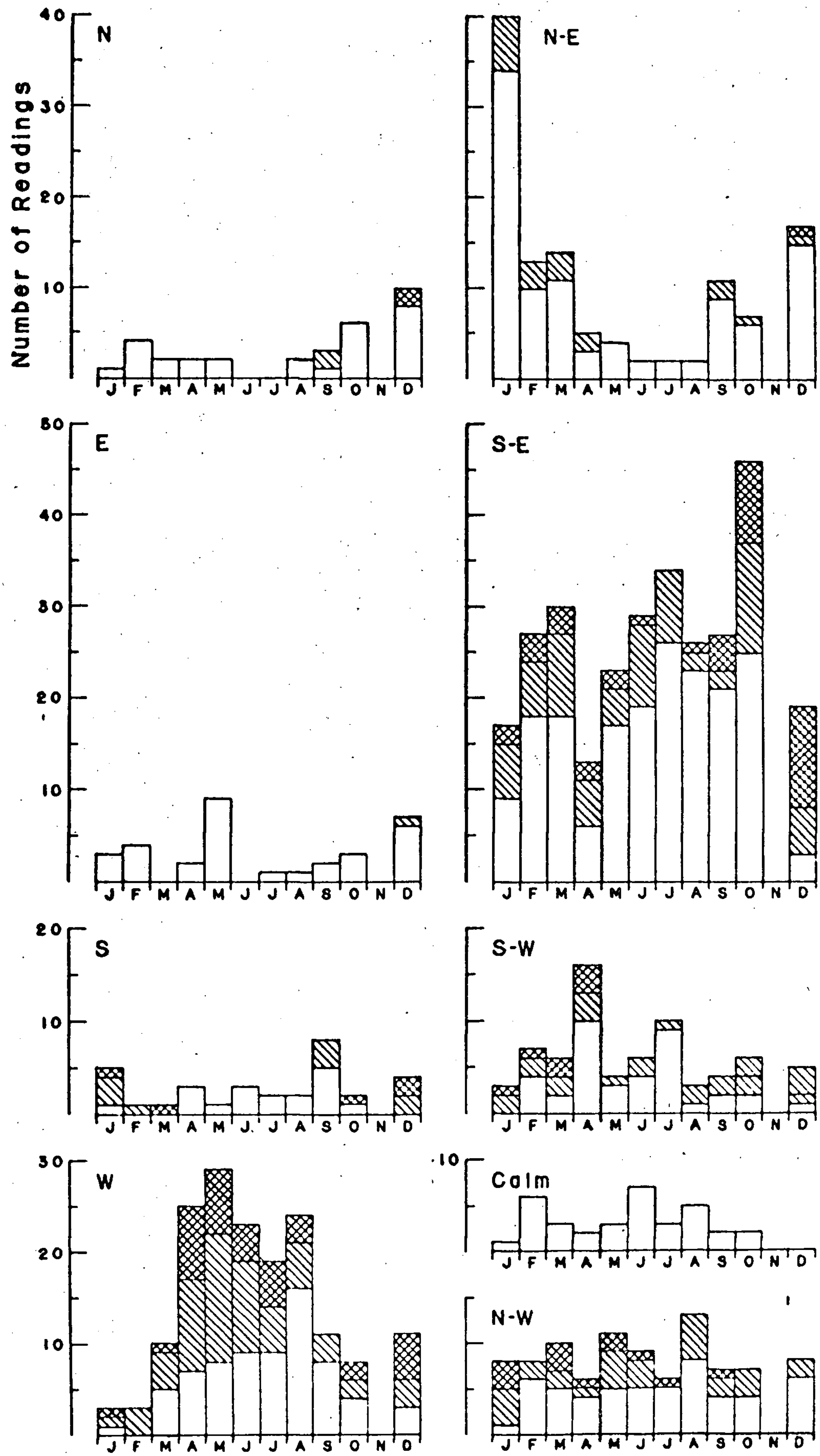

Number of Readings
N
N-E
E
S-E
S
S-W
W
Calm
N-W
J F M A M J J A S O N D
10
20
30
40
50

Figure 2a. Coast of British Columbia and Southern Alaska showing sites visited throughout the spot sampling study.

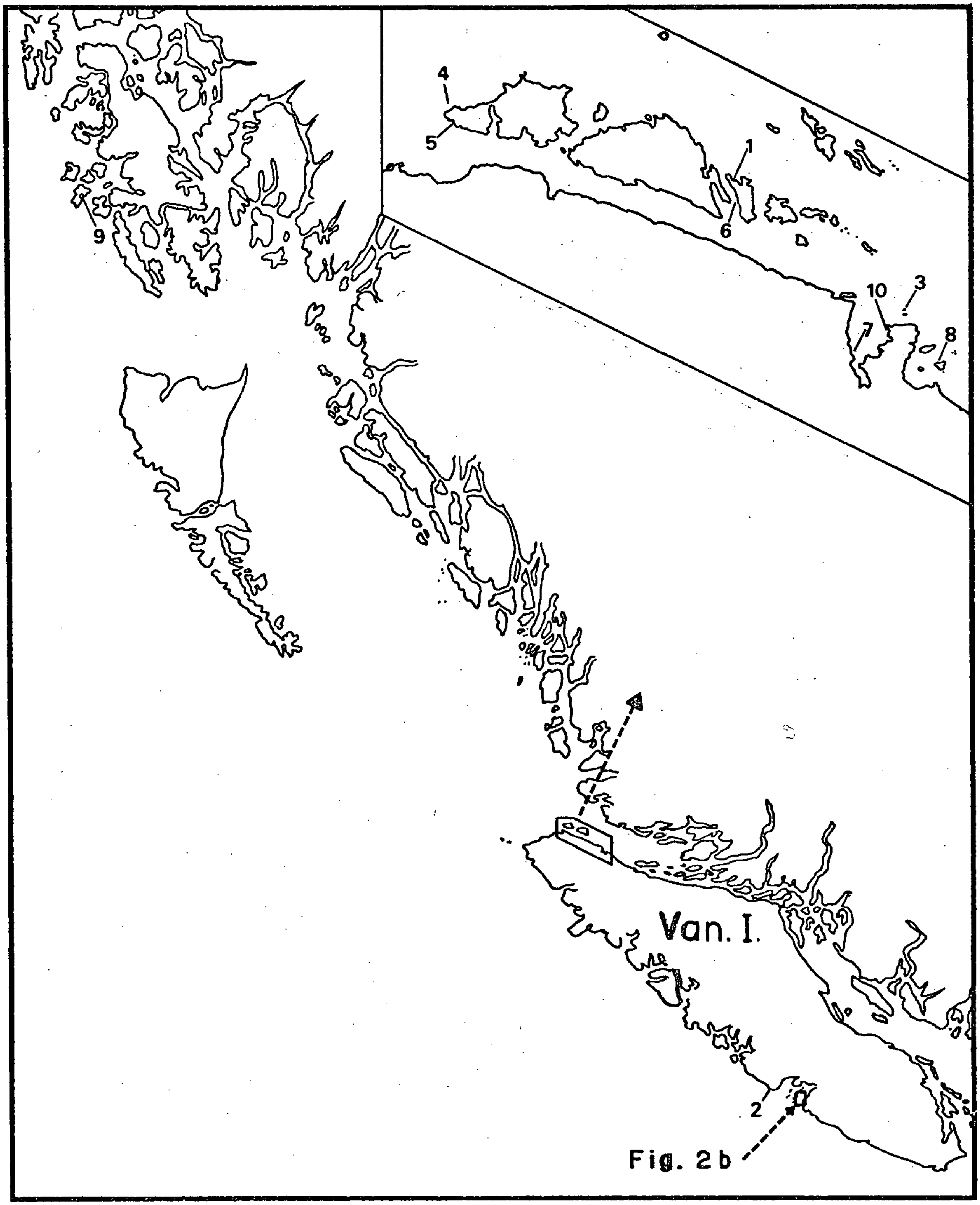

4
5
1
6
9
10
3
7
8
Van. I.
2
Fig. 2b

Figure 2b. Sites visited throughout the continuous sampling study.

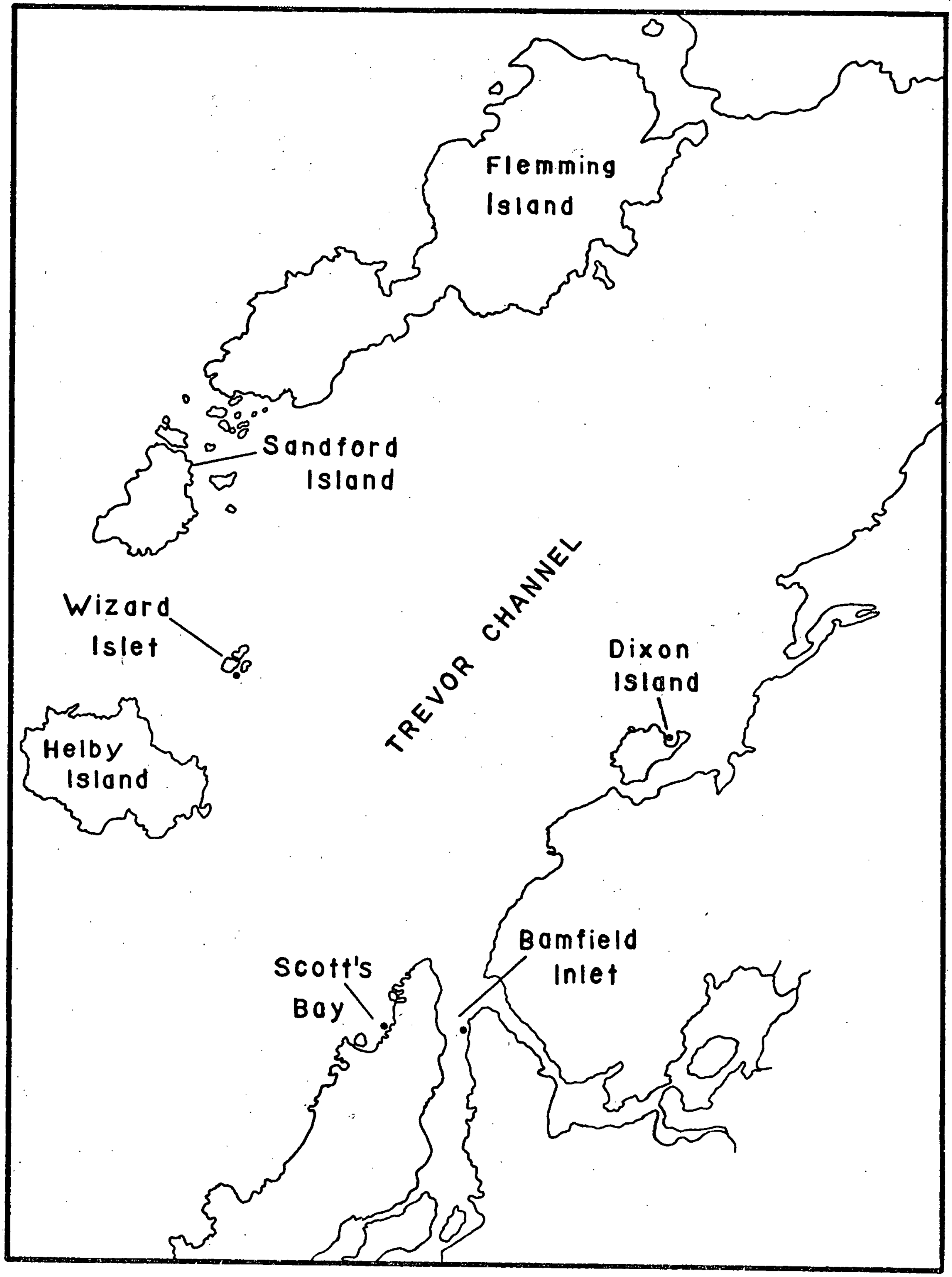
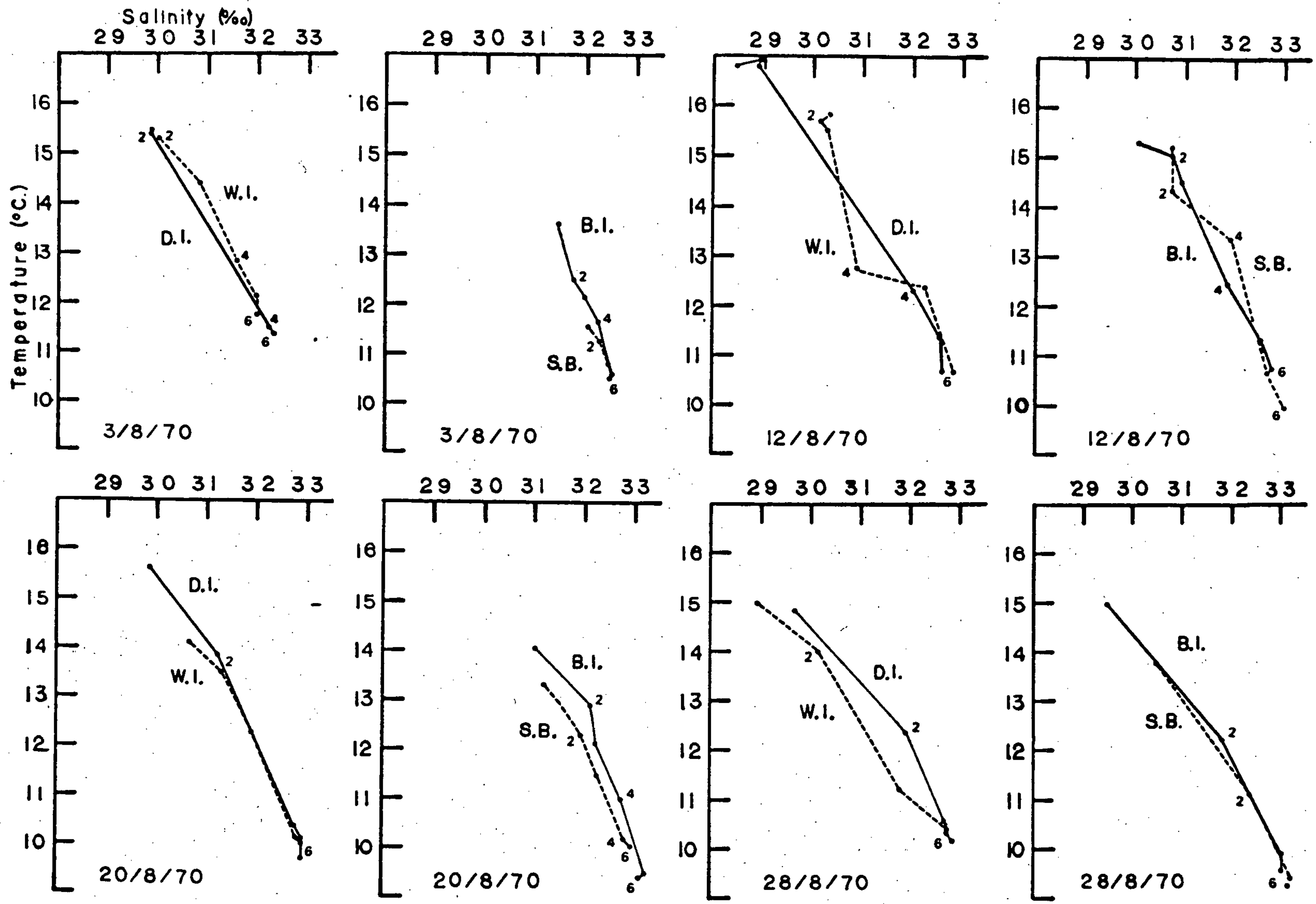

Flemming
Island
Sandford
Island
Wizard
Islet
TREVOR CHANNEL
Dixon
Island
Helby
Island
Bamfield
Inlet
Scott's
Bay

Figure 3. Near-surface temperature/salinity diagrams based on measurements taken at the four Bamfield sites throughout August, 1970. Depth readings are in meters.



Salinity (‰)
29 30 31 32 33
Temperature (°C.)
16 15 14 13 12 11 10
W.I.
D.I.
3/8/70
B.I.
S.B.
3/8/70
D.I.
W.I.
12/8/70
B.I.
S.B.
12/8/70
D.I.
W.I.
20/8/70
B.I.
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20/8/70
D.I.
W.I.
28/8/70
B.I.
S.B.
28/8/70

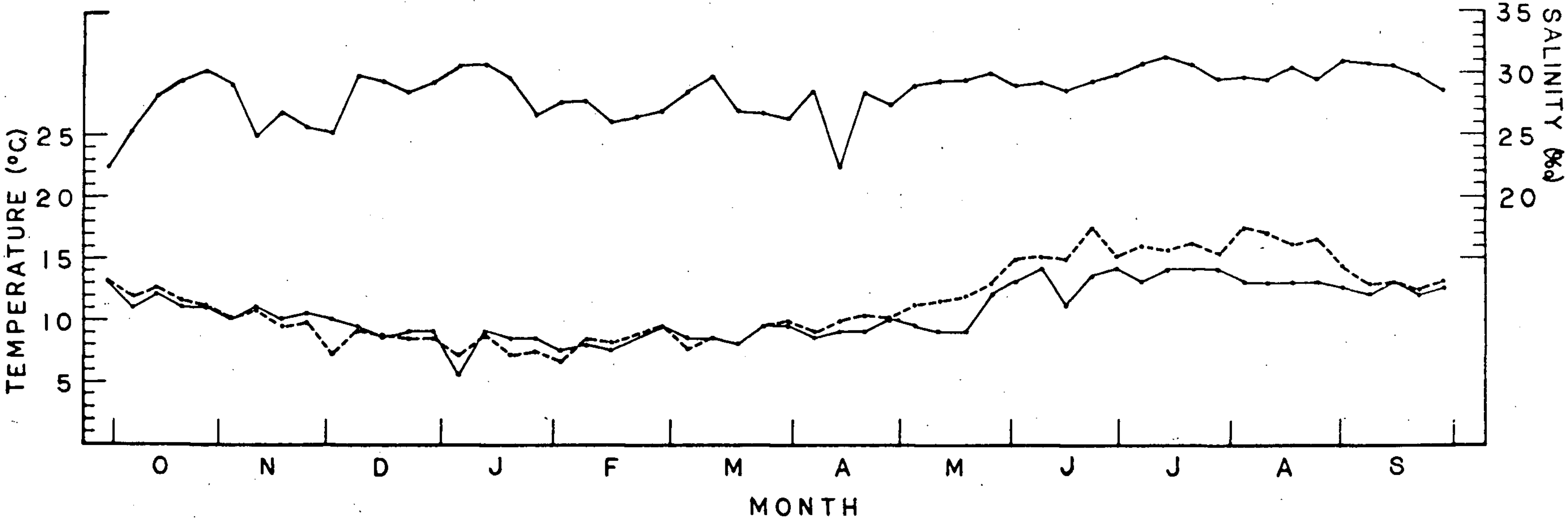
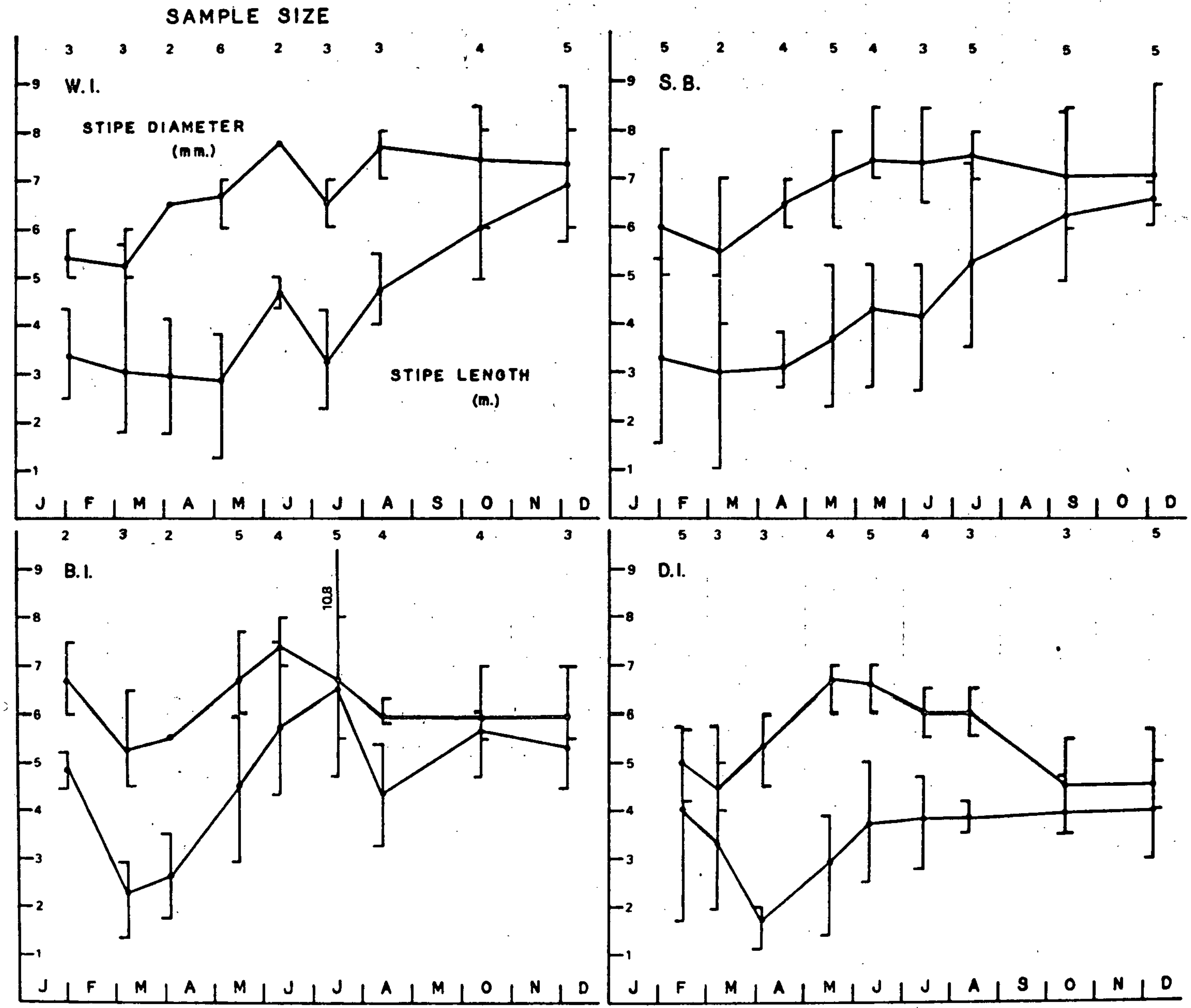


Figure 4. Variation with time of salinity at one meter and temperature at one meter (----) and five meters (——) in Bamfield Inlet through 1969-70. (Provided in part by the Bamfield Marine Station)

Figure 5. The Wizard Islet transplant site following the experiment. A transplant stone bearing the silicone rubber strips remains cemented in place in the foreground.

Figure 6. Variation with time of mean stipe diameter and length at the four continuous sampling sites throughout 1970. Maximum and minimum values are indicated by the horizontal bars.


SAMPLE SIZE
W. I.
STIPE DIAMETER
(mm.)
STIPE LENGTH
(m.)
S. B.
B. I.
D. I.
10.8
J F M A M J J A S O N D
J F M A M M J J A S O D
J F M A M J J A M O N D
J F M A M J J A S O N D

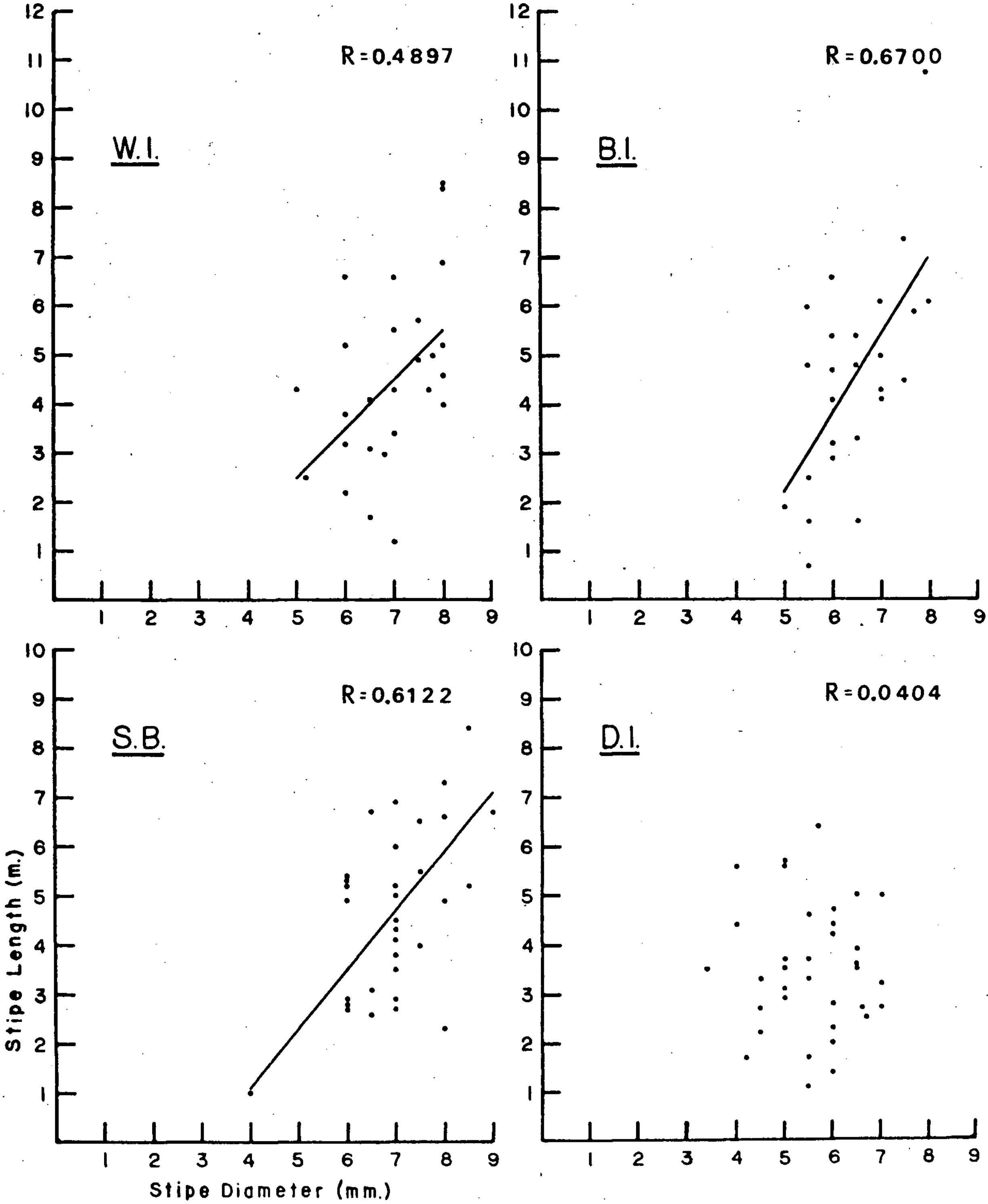
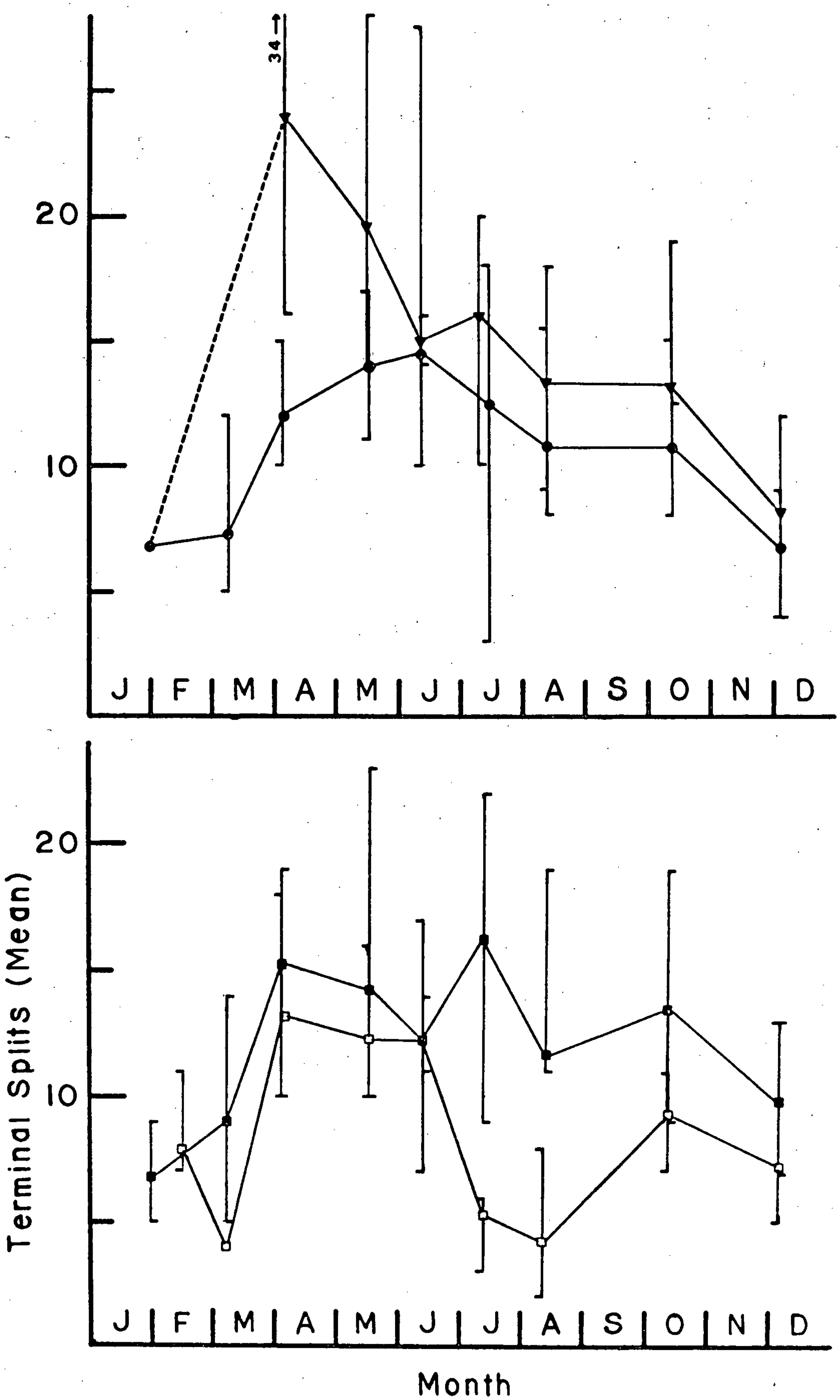


Figure 7. Recorded values of stipe diameter and length for fronds collected throughout the continuous sampling study. A linear relationship is expressed graphically for three of the sampling sites. The significance of the term "R" is explained in the RESULTS.

Figure 8. Variation with time of the mean number of terminal splits on fronds collected throughout the continuous sampling study. ▲ Wizard Islet, ■ Scott's Bay, ● Bamfield Inlet, □ Dixon Island. Maximum and minimum values are indicated by horizontal bars.


34
20
10
J
F
M
A
M
J
J
A
S
O
N
D
20
10
Terminal Splits (Mean)
J
F
M
A
M
J
J
A
S
O
N
D
Month

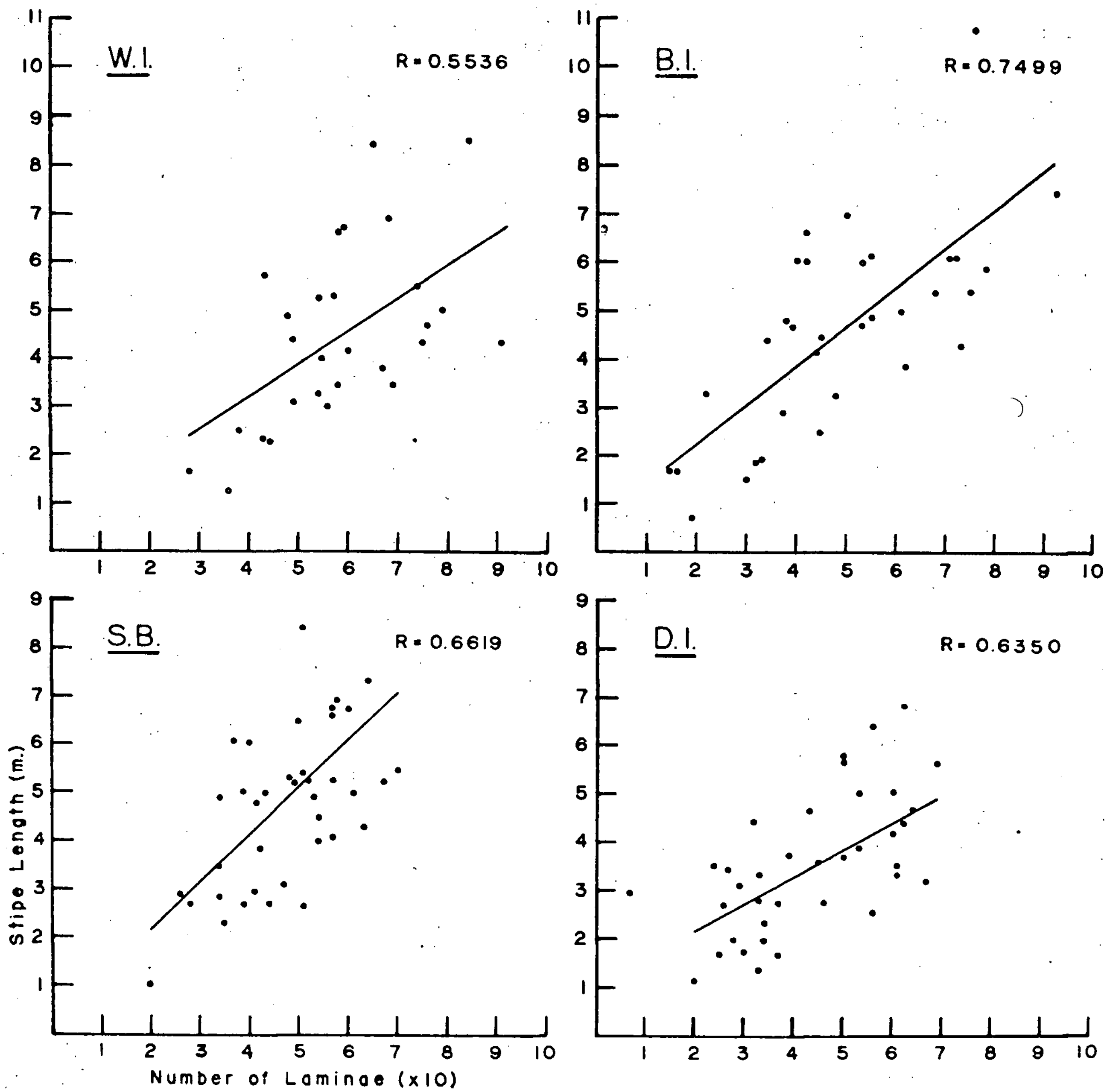


Figure 9. Recorded values of blade number and stipe length for fronds collected throughout the continuous sampling study. A linear relationship is expressed graphically for all sites. The significance of the term "R" is explained in the RESULTS.

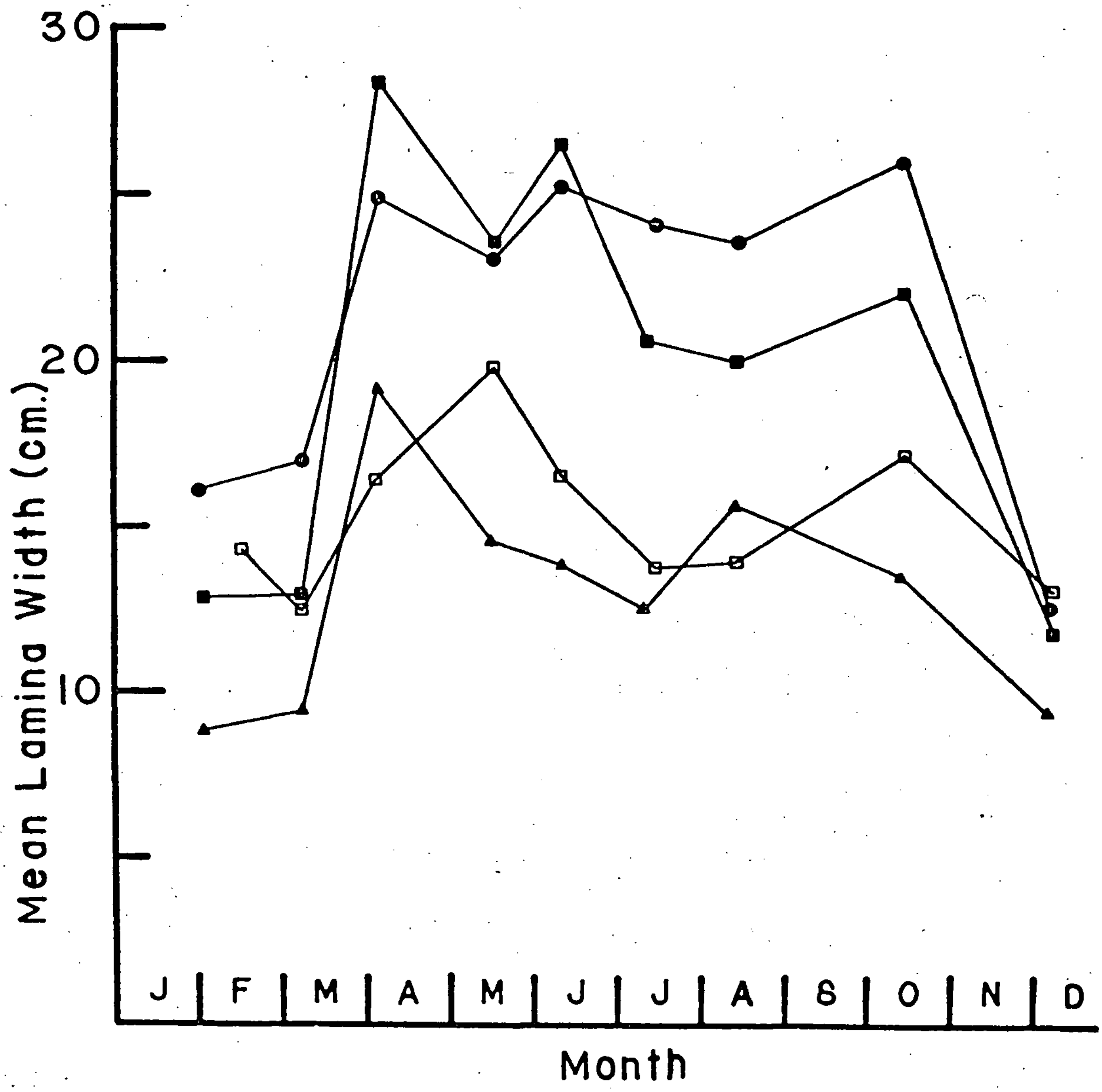


Figure 10. Variation with time of the mean width of young, mature laminae on fronds collected throughout the continuous sampling study. Legend is given in Figure 8.

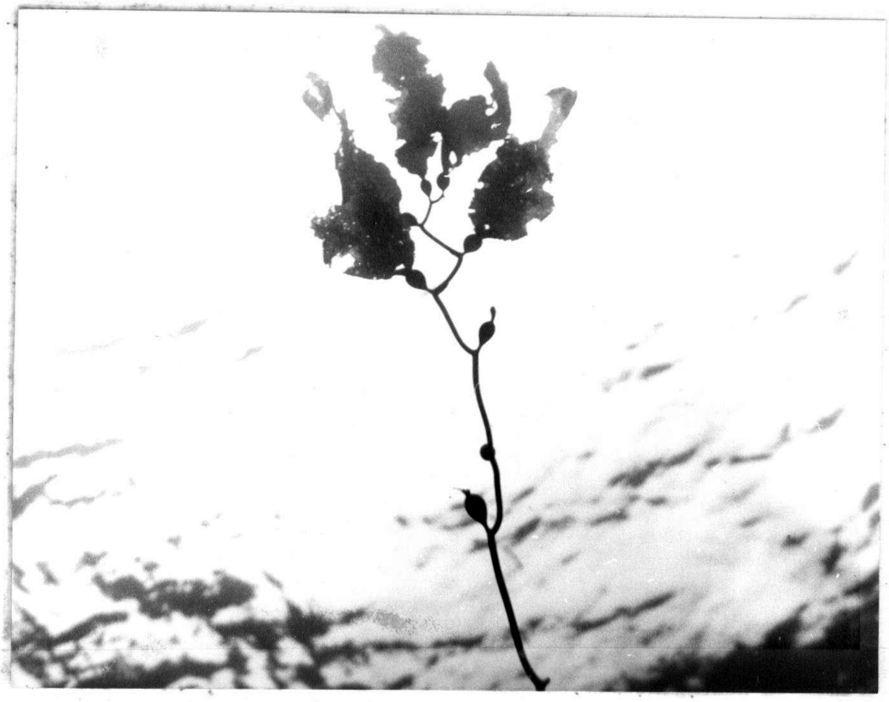
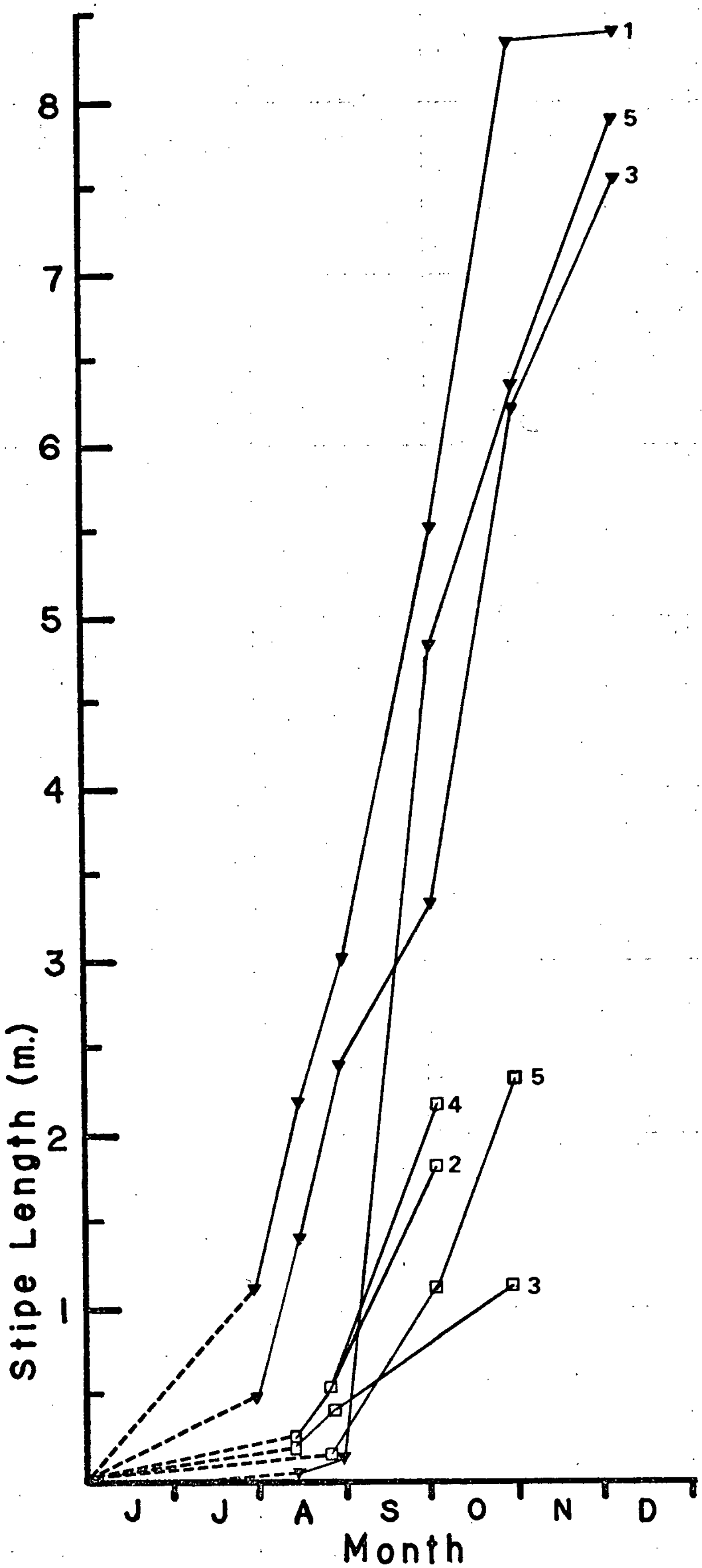


Figure 11. A badly deteriorated frond extended toward the surface at Dixon Island.

Figure 12. Transplant study. Growth in length of primary fronds.

▲ Wizard Islet, ■ Scott's Bay, ● Bamfield Inlet,

□ Dixon Island.


Stipe Length (m.)
8
7
6
5
4
3
2
1
J
J
A
S
O
N
D
Month
1
5
3
5
4
2
3

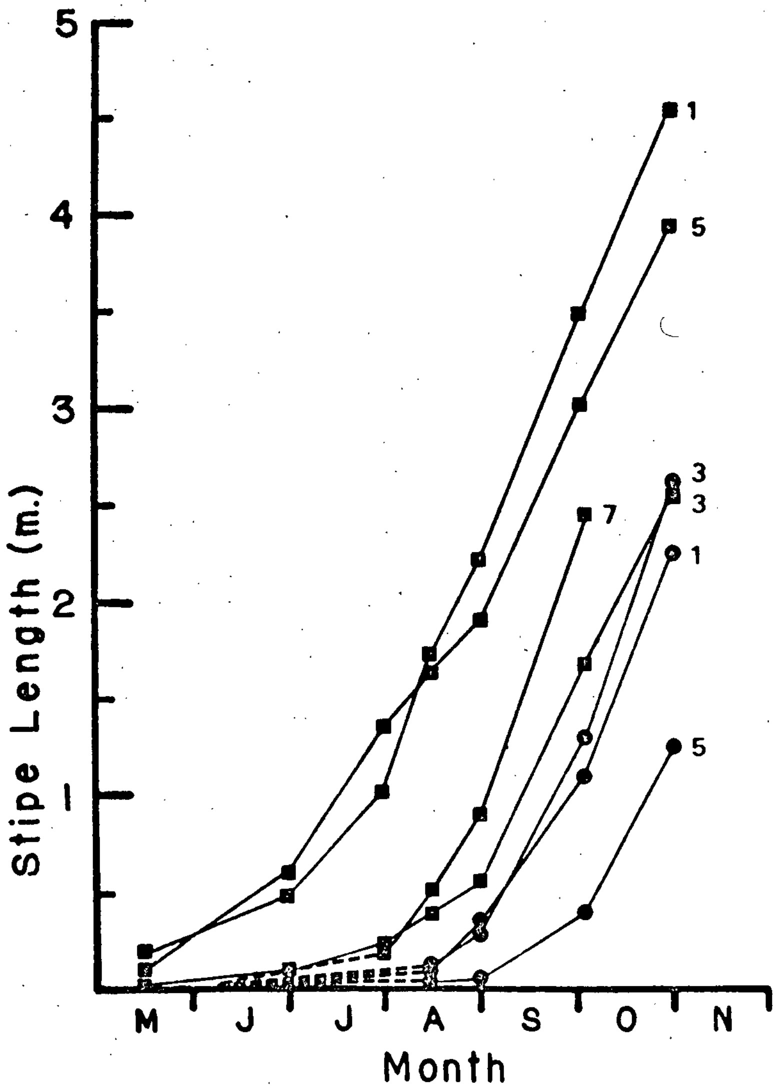


Figure 12. Continued

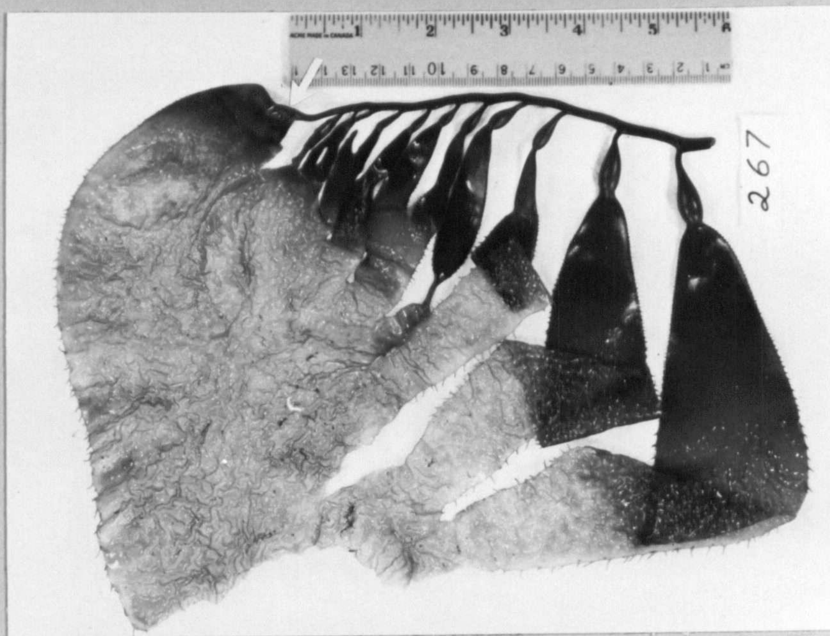


Figure 13. A distal meristematic region of a plant from Wizard Islet. Note the bladder which is beginning to form at the apex of the stipe.

Figure 14. A distal meristematic region of a plant from Hope Island.

Figures 15, 16, 17. Basal portions of blades collected during the sampling studies. The exposure categories are (from left to right): moderately sheltered, moderately exposed, exposed.

Figures 18, 19. Margins of laminae from an exposed (18) and moderately exposed (19) site. Note the outward projection of the spines on the lamina from the less exposed site.

Chart Number	Location	Date	Coordinates	Open Angle	Fetch (n.m.) Max.	Min.	Exposure Category	Comments
(Spot Sampling Study)								
1	Raglan Point, Balaklava Is.	10/04/70	N: 50°52' W:127°38.1'	Nil	-	-	M.S.	In a small cove, sheltered from the direct force of onshore winds.
2	Amphitrite Point	30/04/70	N: 55°23' W:125°32.52'	180°	Unlimited		E.	Plants growing at about zero tide level in a surge channel.
3	Masterman Island	12/09/70	N: 50°45.4' W:127°25.2'	80°	28.0	12.0	M.E.	
4	Hope Island	14/09/70	N: 50°55.2' W:127°59.5'	125°	Unl.	18	E.	Protected only by an offshore bed of *Nereocystis*
5	Hope Island	14/09/70	N: 50°54.3' W:127°59.1'	180°	2.8	2.8	E.	In a slight indentation of the shoreline. Subjected to large waves from the northwest. Slightly less exposed than (4).
6	Browning Passage	16/09/70	N: 50°51.35' W:127°38.25'	- -	- -	- -	E.	Subjected to unimpeded waves travelling along Browning Passage and to strong tidal flows.
7	Port Hardy	16/09/70	N: 49°43.3' W:127°29.15'	22°	12.0	12.0	S.	Probably sheltered during summer months, becoming moderately exposed throughout winter due to prevailing northerlies.

Table 1. Sites visited throughout the Spot Sampling and Continuous Sampling studies. Exposure terms are: E - exposed, S - sheltered, M - moderately. Fetch is given in nautical miles. The term "open angle" describes the angle through which the site is subjected to onshore waves.

Chart Number	Location	Date	Coordinates	Open Angle	Fetch (n.m.) Max.	Min.	Exposure Category	Comments
8	Deer Island	17/09/70	N: 50°43.3' W:127°23.0'	70°	12.0	12.0	M.E.	Force of waves somewhat dampened by an offshore series of emerged rocks.
9	Warren Island	14/01/71	N: 55°52'59" W:133°51'15"	25°	8.0	8.0	M.E.-E.	<u>Macrocystis</u> found only along the north side of Warren Cove. Rippling of the sand at about 40 feet suggests the occurrence of heavy surface wave action.
10	Cape Daphne	18/01/71	N: 50°44.65' W:127°26.9'	170°	7.0	0.7	M.E.	
(Continuous Sampling Study)								
	Wizard Island	- - -	N: 50°51.46' W:125° 9.4'	120°	2.7	1.3	M.E.	
	Scott's Bay	- - -	N: 48°50.15' W:125° 8.6'	120°	7.6	1.1	M.S.	
	Bamfield Inlet	- - -	N: 48°50.1' W:125° 8.1'	38°	2.8	2.2	S.	
	Dixon Island	- - -	N: 48°51.25' W:125° 6.95'	110°	3.7	0.6	S.-M.E.	

Table 1. Continued.

Month	Sample Size		Variance Ratio F	Probability
	Fronds per Site	Laminae per Frond		
April	3	2	15.32	0.0032
May	4	4	6.46	0.0126
June	4	4	7.29	0.0087
July	4	4	6.21	0.0141
August	3	3	2.56	0.1502
October	3	3	2.62	0.1450
December	3	3	1.10	0.4162

Table 2. Analysis of variance among the four continuous sampling sites for width of young mature laminae. The term "Variance Ratio" is explained in the RESULTS. Probability values are for common means.

Table 3. Analysis of data obtained from sample blades which were collected in the Bamfield region during April, May and June of 1970. Variance ratios and probability tables apply only to blades obtained half-way along the stipe from the holdfast.

Feature	Location	Position on Frond	Mean	Max.	Min.	Standard Error	Sample Size	Variance Ratio	Sheffe's Test Table of Probabilities			
Lamina	Wizard	1/2	367	433	264	24.63	7	2.42		W.I.	S.B.	B.I.
Thickness	Islet	3/4	314	401	249	21.94	8		S.B.	0.3919		
(u)	Scott's	1/2	327	390	242	17.47	10		B.I.	0.0874	0.8309	
	Bay	3/4	328	409	240	14.52	12		D.I	0.2849	0.9972	0.9096
	Bamfield	1/2	309	351	244	9.65	12					
	Inlet	3/4	329	381	263	11.37	11					
	Dixon	1/2	323	367	281	9.95	11					
	Island	3/4	286	349	224	17.93	8					
Cortex:	W.I.	1/2	2.45	3.58	1.76	0.23	7	6.28		W.I.	S.B.	B.I.
Medulla		3/4	2.71	5.05	0.59	0.51	8		S.B.	0.0018		
	S.B.	1/2	5.24	10.34	3.08	0.64	10		B.I.	0.0393	0.5454	
		3/4	2.78	4.54	1.10	0.29	11		D.I.	0.1046	0.2807	0.9616
	B.I.	1/2	4.40	6.32	2.64	0.31	11					
		3/4	5.41	16.50	1.66	1.43	11					
	D.I.	1/2	4.09	6.42	3.12	0.31	11					
		3/4	4.34	8.17	1.22	0.79	8					
Spine	W.I.	1/2	2.9	4.2	3.1	0.42	5	3.19		W.I.	S.B.	B.I.
Length		3/4	4.1	5.6	2.1	0.51	7		S.B.	0.1583		
(mm.)	S.B.	1/2	4.6	6.2	1.9	0.40	10		B.I.	0.0387	0.8876	
		3/4	4.9	6.8	3.8	0.24	11		D.I.	0.1307	0.9998	0.9120
	B.I.	1/2	5.0	8.8	2.8	0.51	11					
		3/4	5.2	7.7	3.0	0.48	11					
	D.I.	1/2	4.6	6.0	3.0	0.26	11					
		3/4	5.3	6.8	3.2	0.45	7					

Feature	Location	Posn. on Frond	Mean	Max.	Min.	Standard Error	Sample Size	Variance Ratio	Sheff's Test Table of Probabilities
Pneumato-	W.I.	1/2	55.8	70.0	40.0	4.73	6	4.83	W.I. S.B. B.I.
cyst		3/4	51.5	70.0	22.0	5.03	8		S.B. 0.8755
Length	S.B.	1/2	50.9	65.0	35.0	3.27	10		B.I. 0.9648 0.9844
(mm.)		3/4	63.5	85.0	45.0	4.02	10		D.I. 0.0329 0.0897 0.0293
	B.I.	1/2	52.8	75.0	25.0	4.17	12		
		3/4	61.3	85.0	35.0	4.63	11		
	D.I.	1/2	37.5	50.0	20.0	2.55	11		
		3/4	39.3	50.0	30.0	2.77	7		
Diameter	W.I.	1/2	16.7	23.0	13.0	1.41	6	6.35	W.I. S.B. B.I.
(mm.)		3/4	15.0	21.0	7.0	1.58	8		S.B. 0.9762
	S.B.	1/2	16.0	20.0	11.0	0.81	10		B.I. 0.1967 0.2608
		3/4	16.7	20.0	11.5	0.76	10		D.I. 0.0116 0.0105 0.4690
	B.I.	1/2	13.5	18.0	9.0	0.70	12		
		3/4	13.37	16.0	11.0	0.48	11		
	D.I.	1/2	11.5	15.0	6.0	0.91	11		
		3/4	12.1	13.0	11.0	0.30	7		

Location	Stipe Length (m.) Frond	Stipe Length (m.) Mean	Stipe Diameter (mm.) Frond	Stipe Diameter (mm.) Mean	Lamina Number Frond	Lamina Number Mean	Lamina #/L Ratio	Lamina Width Frond	Lamina Width Mean	Terminal Splits Frond	Terminal Splits Mean
7) Port Hardy	3.75	3.75	6.0	6.17	48	45.66	13.51	11.05	12.87	1	6.7
	2.40		6.0		39			15.45		14	
	4.00		6.5		50			12.10		5	
1) Raglan Point	2.90	3.37	7.5	6.90	53	50.4	14.96	21.03	29.67	27	23.2
	3.16		7.5		51			24.10		20	
	3.40		6.5		49			16.53		25	
	3.60		6.0		46			18.66		16	
	3.80		7.0		53			23.05		28	
3) Masterman Island	2.46	2.39	7.0	7.75	31	26.5	11.08	20.85	15.18	13	11.5
	2.32		8.5		22			9.50		10	
8) Deer Island	7.00	7.22	8.5	7.83	49	51.99	7.06	15.70	16.26	5	5.7
	8.42		8.5		62			16.82		11	
	6.25		6.5		42			--		1	
10) Cape Daphne	3.95	4.24	7.0	7.0	35	30.33	7.15	13.86	13.39	8	6.7
	6.00		7.0		29			14.60		5	
	2.78		7.0		27			11.70		7	
9) Warren Island	6.00	4.67	9.5	9.10	43	39.12	8.39	13.58	9.88	9	5.2
	2.80		9.0		35			6.85		-	
	6.40		10.0		46			9.03		12	
	7.30		11.0		53			10.05		5	
	0.87		6.0		19			--		-	

Table 4. Measurements of fronds collected throughout the spot sampling study. Arrangement is in order of increasing exposures.

Location	Stipe Length (m) Frond	Stipe Length (m) Mean	Stipe Diameter (mm.) Frond	Stipe Diameter (mm.) Mean	Lamina Number Frond	Lamina Number Mean	Lamina Ratio	Lamina Width Frond	Lamina Width Mean	Terminal Splits Frond	Terminal Splits Mean
2) Amphitrite Point	1.72	3.46	7.0	7.57	43	39.0	11.27	6.60	6.15	11	1.7
	4.82		8.5		54			6.50		5	
	5.16		7.0		49			5.60		-	
	2.48		8.0		20			7.75		-	
	2.35		8.0		23			6.58		2	
	5.52		7.5		49			4.98		2	
	2.20		7.0		35			5.05		3	
4) Hope Island	2.35	9.30	8.0	8.75	24	40.5	4.36	6.40	7.08	6	8.5
	6.95		9.5		57			8.75		11	
5) Hope Island	5.90	6.95	11.0	11.5	50	53.0	7.63	10.73	10.33	10	5.0
	8.00		12.0		56			9.83		-	
6) Browning Passage	10.75	13.97	10.0	11.0	65	70.00	5.01	8.08	9.21	2	1.0
	17.18		12.0		75			10.33		-	

Table 4. Continued.

Location	Lamina				Spine		Pneumatocyst			
	Thickness (u)		Cortex:Medulla		Length (mm.)		Length (mm.)		Diameter mm.)	
	Frond	Mean	Frond	Mean	Frond	Mean	Frond	Mean	Frond	Mean
7) Port Hardy	254	292	9.00	8.57	4.2	4.3	50	62.5	9	12.0
	330		7.13		4.4		75		15	
1) Raglan Point	367	347	6.01	6.14	6.2	5.7	65	62.4	25	23.8
	366		4.29		6.6		60		23	
	295		6.43		4.0		55		23	
	359		7.72		6.0		70		24	
3) Masterman Island	240	336	17.25	12.38	7.1	5.7	65	67.5	22	21.0
	432		7.50		4.4		70		20	
8) Deer Island	352	352	10.93	10.93	6.2	6.2	110	110.0	18	18.0
10) Cape Daphne	*(339	331	4.42	5.94	- -	- -	- -	73.3	-	23.7
	(- -		- -		- -		85		27	
	(- -		- -		- -		80		25	
	*(323		7.46		- -		- -		-	
	- -		- -		- -		55		19	
9) Warren Island	*(505	598	4.98	4.78	- -	4.8	- -	97.5	-	25.5
	(- -		- -		- -		95		26	
	i691		4.57		4.8		- -		-	
	- -		- -		- -		100		25	

Table 5. Measurements of blades obtained throughout the spot sampling study. Blades were taken from the 1/2 position when available. Those marked with an asterisk were taken from the 7/8 position. Other symbols used are: (, from the same plant; i, immature lamina.

Location	Lamina				Spine		Pneumatocyst			
	Thickness (u)		Cortex:Medulla		Length (mm.)		Length (mm.)		Diameter (mm.)	
	Frond	Mean	Frond	Mean	Frond	Mean	Frond	Mean	Frond	Mean
2) Amphitrite	440	491	5.46	5.39	5.0	4.3	50	52.0	14	14.9
	528		6.87		4.5		55		17.5	
	518		5.38		2.7		55		13	
	479		3.85		4.9		50		15	
	- -		- -		- -		50		14	
4) Hope Island	656	589	10.74	10.50	4.9	4.9	80	80.0	-	21.0
	521		9.46		- -		80		21	
5) Hope Island	571	580	10.70	10.65	6.5	6.5	90	80.0	23	19.5
	589		10.60		6.5		70		16	
6) Browning Passage	579	711	6.12	8.51	5.4	6.5	80	85.0	23	20.5
	843		10.90		7.0		90		18	

Table 5. Continued.

Location	Plant Number	Mean Daily Increment Through Exponential Phase	Maximum Daily Increment (cm./day)	Maximum Rate of Lamina Initiation per Day
Wizard Islet	1	7.80	9.83	0.59
	3	5.52	13.34	0.52
	5	8.00	14.24	0.79
Scott's Bay	1	2.80	3.82	0.32
	3	3.23	3.48	- -
	5	2.70	3.42	0.32
	7	- -	3.50	0.21
Bamfield Inlet	1	3.23	4.21	0.41
	3	3.76	4.70	0.41
	5	- -	3.00	0.25
Dixon Island	2	- -	3.53	0.42
	3	1.16	1.43	0.36
	4	- -	4.53	0.42
	5	3.30	4.25	0.27

Table 6. Growth of primary fronds and initiation rates for laminae recorded throughout the transplant study.

Location	Plant Number	Lamina Number	Max. Rate of Elongation (cm./day)	Variance Ratio (F)
Wizard Islet	1	5	4.17	3.78
		16	3.50	
	2	3	2.84	
		7	2.96	
	3	2	2.83	
		4	3.94	
		10	3.14	
Scott's Bay	1	5	0.44	
		10	2.00	
		12	1.71	
	2	7	2.95	
		11	2.78	
	4	12	3.20	
	5	5	0.92	
		9	1.28	
		13	1.86	
	7	1	1.20	
		3	3.29	
Bamfield Inlet	3	1	1.66	
		2	2.54	
Dixon Island	2	2	3.69	
		3	5.07	

Scheffe's Test: Table of Probabilities

	W.I.	S.B.	B.I.
S.B.	0.1710		
B.I.	0.7153	0.9984	
D.I.	0.7333	0.0522	0.2568

Table 7. Growth of laminae on plants involved in the transplant study. The term "variance ratio" is explained in the RESULTS. Probability values are for common means.

Table 8. Transplant study. Variation with time of the mean number of blades per meter of stipe for primary fronds.

Location	Wizard Islet			Scott's Bay			Bamfield Inlet			Dixon Island		
Plant #	1	3	5	1	5	7	1	3	5	3	4	5
1/07/70				26.32	17.74	38.46						
30/07/70	17.27	43.47	27.65									
31/07/70				13.72	10.94	20.00						
13/08/70										35.00	38.46	- - -
15/08/70	12.27	18.46	12.85									
16/08/70				9.82	10.97	11.76						
17/08/70							20.00	33.33	33.33			
27/08/70										22.50	29.09	43.75
29/08/70	11.33	12.93	9.58									
30/08/70				9.45	7.69	9.78	15.39	23.33	- - -			
2/10/70	9.82	7.81	9.73									
3/10/70				8.60	9.90	6.55	16.67	16.15	25.00	15.66	6.42	15.18
31/10/70	8.38	6.61	8.50	8.57	9.87	- - -	9.73	10.65	13.71	12.50	- - -	10.39
5/12/70	8.81	6.36	8.24									

Location-	Wizard Islet			Scott's Bay					Bamfield Inlet			Dixon Island				
Plant # -	1	3	5	1	2	3	5	7	1	3	5	1	2	3	4	5
Lamina # 1	11.5	7.0	8.2	- -	- -	- -	- -	11.8	14.1	17.0	13.5	8.0	11.3	7.2	7.0	- -
2	9.3	- -	9.0	- -	- -	- -	- -	14.0	15.1	- -	17.2	11.0	16.0	10.7	12.5	11.8
3	11.0	9.0	9.6	- -	- -	- -	7.4	16.5	15.0	21.0	15.0	11.0	19.6	- -	17.0	- -
4	- -	10.8	10.5	7.1	12.0	8.8	7.5	7.2	- -	- -	12.8	13.8	20.2	- -	21.0	10.8
5	13.3	12.2	11.1	9.8	12.5	13.3	- -	21.4	13.9	12.5	- -	13.0	17.1	- -	- -	- -
6	10.7	10.5	13.0	13.1	11.0	11.5	- -	21.9	16.6	10.6	- -	- -	- -	10.7	- -	- -
7	15.4	13.0	15.2	13.5	11.9	- -	7.6	22.2	10.3	13.0	- -	20.0	- -	- -	- -	17.0
8	17.0	14.0	15.2	13.4	- -	19.1	- -	14.4	- -	- -	- -	- -	- -	- -	- -	- -
9	15.7	16.0	18.5	15.4	14.6	- -	8.0	- -	- -	- -	- -	- -	- -	- -	14.3	- -
10	17.7	- -	17.0	14.8	16.8	8.6	9.7	- -	- -	- -	- -	- -	- -	- -	- -	- -

Table 9. Transplant study... maximum width,in centimeters,of mature laminae numbered from the base of the primary stipe.

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PART II

ABSTRACT

A field culture apparatus employing germanium dioxide to inhibit diatom growth was designed and tested. Through two experiments, set up at Bamfield on the west coast of Vancouver Island, a design was achieved which provided satisfactory control for up to 33 days on less than 1 gm of the inhibitor. Adverse effects on the microscopic phases of *Macrocystis integrifolia* Bory attributable to the presence of GeO_2 were not detected.

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INTRODUCTION

A common problem in culture studies of microscopic phases of marine algae has been the accumulation and growth of contaminants, the most notable of these being diatoms. High concentrations of diatoms impede microscopic examination of culture slides and may also inhibit normal growth and development of gametophytes in the Laminariales (North, 1970). This prospect is of particular significance as the present study was born out of an attempt to culture the gametophyte and early sporophyte of *Macrosystic integrifolia* Bory under natural conditions.

A major advancement in the control of diatom concentrations in laboratory culture studies has been the use of germanium dioxide (GeO_2) in concentrations of 1 to 10 mg/l (Lewin, 1966). In view of the high level of control attained it appeared feasible to extend this technique to field culture studies. Lewin has demonstrated that concentrations of GeO_2 as high as 64 mg/l did not affect the growth rate of *Chlamydomonas moewusii*, a non-silicified green flagellate. Similar evidence has not been obtained for the gametophyte or young sporophyte in the Laminariales. The use of GeO_2 in undetermined strength in the field however, attached new significance to this question. An experiment was conducted to detect and assess the extent of this problem.

MATERIALS AND METHODS

Apparatus

An *in situ* culture apparatus (Fig. 1) was designed which utilized natural tidal current flows in its operation. The research site chosed was located at the mouth of Bamfield Inlet on the west coast of Vancouver Island. A semi-diurnal tidal cycle provided a regular ebb and flow current with a maximum of about one knot.

The culture chamber consists of a perspex cylinder which accommodates six glass slides (37 x 25 mm) in series. These are held in place by a border of plastic tubing on each side. To the forward (inflow) and of the chamber is attached a GeO_2 diffuser consisting of a funnel which amplifies and directs the current through a Nalgene aspirator type vacuum pump. To the suction nozzle of the vacuum pump is attached a 60 ml. plastic bottle containing 1 g of GeO_2. Removal of a perforated rubber stopper placed at the outflow end of the chamber permits access to the culture slides.

Two apparati are positioned side by side on a plastic stand (Fig. 2). The forward end of the stand is attached directly, by swivel connectors, to a main positioning line running vertically between an anchor and a float. Two monofilament support lines serve to level the stand while a fin at the aft end keeps the diffusers facing into the current. The apparatus is assembled and maintained through the use of SCUBA.

Preparation and Analysis of Culture Slides

The culture slides were placed in the bottom of a plastic aquarium (21 x 31 cm) containing 1200 ml of filtered seawater. Cooling water was pumped continuously from Bamfield Inlet. The slides were innoculated from a fresh spore suspension and maintained for 24 hours in the dark. The young gametophytes were labelled for future identification with Calcofluor White fluourescent brightener at 0.01% for two hours (from Cole, 1964). This substance was taken up by the cell walls and could still be detected under U.V. light after 33 days in the sea.

The prepared slides were placed in the plastic guides and inserted into the culture chambers. These were transported in seawater to the site and placed in position on the culture stands.

Slides periodically collected from the chambers were placed in 5% formalin/seawater solution for future study. Efficiency of control was assessed by counting the total number of diatoms crossed by a 0.039 mm horizontal line at 400X over a vertical distance of 15 mm. Values given in the text represent the mean of three random samples.

Control of GeO_2 Level

The solution rate of the GeO_2 was adjusted by altering the size and position of an inflow port in the side of the inhibitor bottle. The diameter of this port determines the amount of water entering the chamber by way of this secondary route. The height of the port above the base of the bottle determines the extent to which eddy diffusion influences the solution rate. Two experiments were conducted with the aim of achieving a design that would provide a desirable level of diatom control with a minimal uptake of the inhibitor.

Experiment 1 was set up at a depth of 5 meters below zero tide level on August 5, 1970. Three variations in the size and position of the secondary intake port were tested (Table 1, test designs A,B,C). The rate of uptake of the inhibitor increases through designs A to C. Two crystalline forms of the inhibitor differing widely in solubility were employed. A relatively slow-dissolving form supplied by the Fisher Scientific Co. was used in test designs A, B and C, while a more soluble form supplied by the British Drug House was used only in designs A and B (designated D and E). All tests were run against a control lacking GeO_2. Slides were collected at intervals of 4 days.

Through the duration of experiment 1 a well defined plankton bloom occurred in Bamfield Inlet and adjacent waters. From visual observations made during the previous year and supported by Secche disc readings*, it was expected that this bloom would cease during late August or early September to be followed by a bloom lasting only about two weeks in early October. A second experiment set up in early Fall would therefore serve to demonstrate the relationship between the relative efficiency of each design and seasonal fluctuations in the concentration of diatoms in the water column.

Experiment 2 was set up on October 12 at the same depth and location as experiment 1. Test designs A, B and C employing only the less soluble form of the inhibitor were run against a control. One collection of slides was made after 33 days.

<u>Effect on the Gametophyte and Early Sporophyte</u>

The development of the gametophyte and early sporophyte was compared using plants treated with GeO_2 and controls. Control slides from the previous experiments could not be used for this purpose due to the high concentrations of diatoms present. A single stand was set up on August 6, 1970 at a depth of -7.5 meters. At this level a distinct increase in visibility was felt to indicate a decrease in diatom concentrations. A test apparatus of design C employing the Fisher form of GeO_2 was run against a control. Slides were collected at intervals of 4 days.

A system described by Norton and Burrows (1968) was used to assess the rate of development of the plants. The presence or absence of the following stages were recorded:

*Data of the Bamfield Station.

1) the ungerminated spore;
2) a spore with a germ tube, characteristically dumbell shaped;
3) a gametophyte with either antheridia or an extruded egg;
4) sporophyte produced but composed of only 10 cells or less;
5) sporophyte composed of 11 to 20 cells;
6) sporophyte composed of 21 to 30 cells;
7) sporophyte composed of 31 to 50 cells;
8) sporophyte composed of 51 to 100 cells.

Recordings were made of 40 plants on each culture slide, and the results of this experiment are presented in Table 3.

RESULTS AND DISCUSSION

Diatom concentrations were satisfactorily reduced in test designs B, C, D, E and A, B, C of experiments 1 and 2 respectively (Fig. 3, Table 2). That this effect can be attributed to the use of the inhibitor is demonstrated by the controls in both experiments.

In design A the inflow port was restricted in diameter and placed well above the bottom of the inhibitor bottle. The reduced circulation resulted in a low solution rate of the inhibitor and a high level of diatom contamination. The use of a more soluble form of the inhibitor in a bottle of the same design (D) in experiment 1 resulted in a definite suppression of diatom growth.

The uniformity of results obtained from tests B, C, D and E suggests a limit in the efficiency of the apparatus beyond which further percent reductions in diatom concentrations could not be achieved. The plankton bloom found to occur throughout experiment 1 resulted in the accumulation of relatively large numbers of diatoms on all slides. Although a short bloom did occur during the early stages of experiment 2 the water had completly cleared by the 33rd day. The results indicate that under such conditions almost complete elimination of diatoms could be achieved (Fig. 4a,b).

A visual inspection of all GeO_2 bottles in experiment 1 after 26 days indicated that only in test design C was replenishment required. The inhibitor was completely depleted from the same bottle in experiment 2 after 33 days. In light of this fact the discrepencies observed between tests B cnd C of experiment 2 are not considered significant.

An overall evaluation suggests that a large inflow port placed near the top of the inhibitor bottle (design B) provides optimal control. Further increases in the concentration of GeO_2

were achieved by lowering the level of the inflow port as in design C or by using a more soluble form of the inhibitor are not required.

Whereas plants grown on control slides tended not to produce sporophytes, those subjected to GeO_2 followed a developmental sequence characteristic of the order. This discrepancy may be related to adverse effects caused by the greater accumulation of diatoms on the control slides (North 1970). There is insufficient evidence to support the alternative possibility that the inhibitor had a stimulating effect on the plants under study.

As the apparatus was tested under a limited set of conditions, it is considered likely that factors even more conducive to diatom contamination could be encountered. For example, any increase in the concentration of diatoms in the water column would tend to cause a proportionate build-up of diatoms on the culture slides. The flow of seawater through the culture chamber could be subsequently reduced through such minor modifications in design as the use of a small intake funnel. Uptake of the inhibitor could be adjusted accordingly.

Contamination by organisms other than diatoms was not a significant problem in the experiments although a gradual thinning out of gametophytes strongly suggests the presence of herbivores. A large variety of zooplankters were observed from time to time but these tended to remain sparsely scattered over the slides. Seasonal variations in the nature and extent of this problem have been discussed by Jones and Dent (1970).

The basic principle behind the design and operation of the apparatus permits some degree of versatility. In some localities it may be more convenient to adapt the apparatus to current flows other than those generated by tides. These include rivers and vertical and horizontal components of wave motion.

Fig. 1. Schematic representation of the in situ culture apparatus. Current flow is from right to left. b. barrier screen, c.c. culture chamber, c.s. culture slides, f. funnel, i. inhibitor bottle, i.p. secondary inflow port, o.p. outflow port, v. vacuum pump.

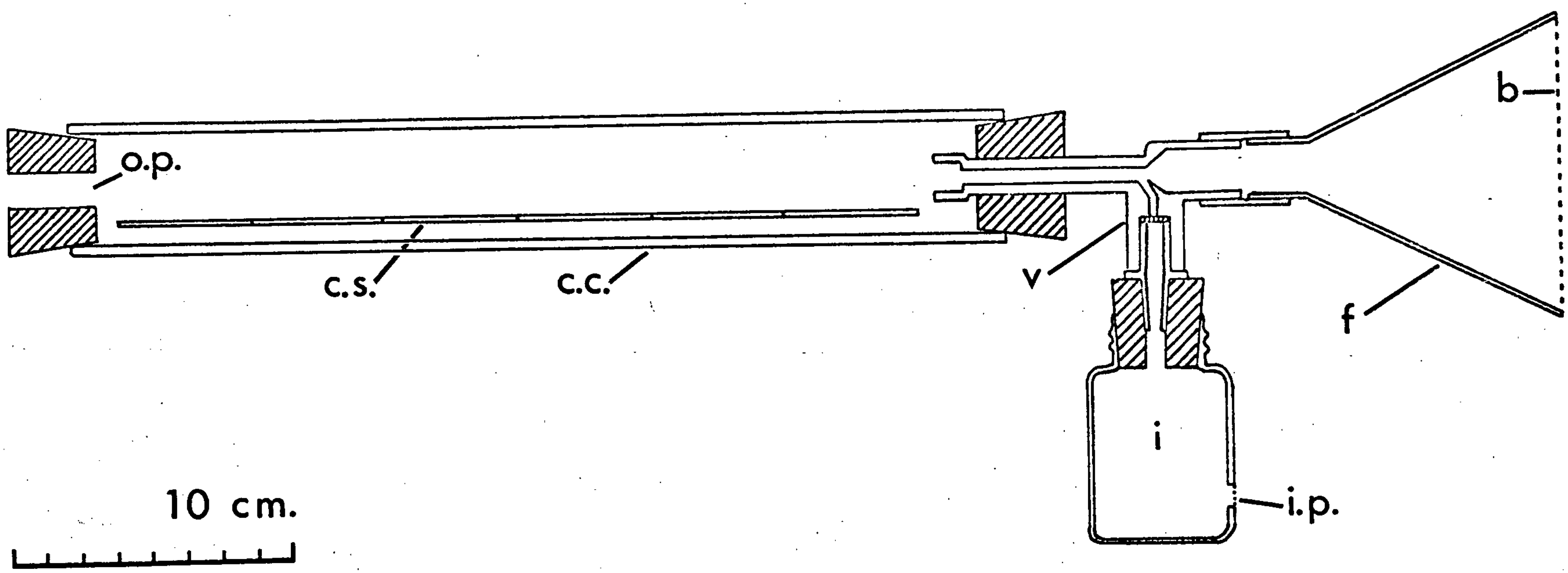

b
o.p.
c.s.
c.c.
v
f
i
i.p.
10 cm.

Fig. 2. The assembled culture apparatus.

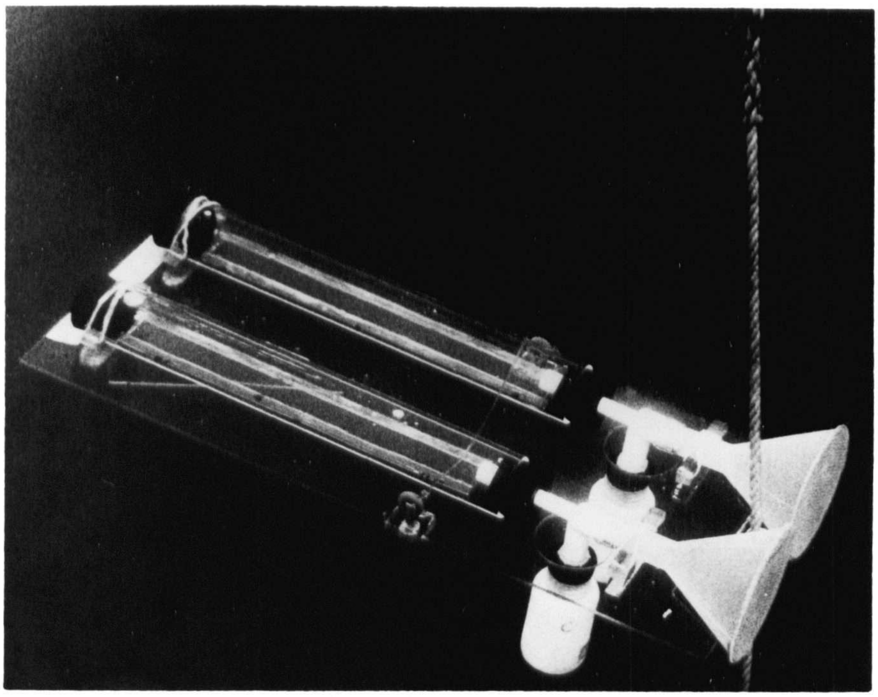
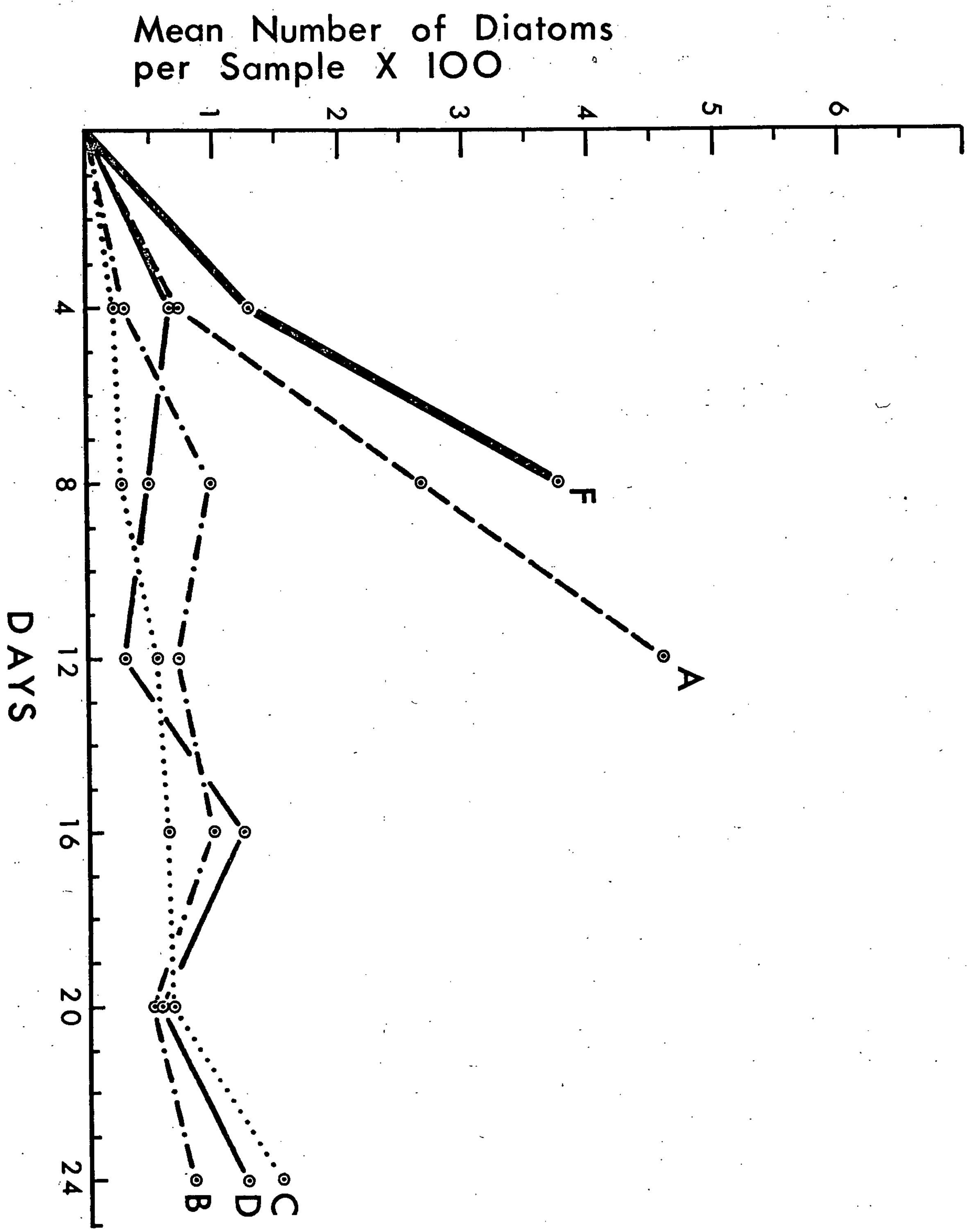


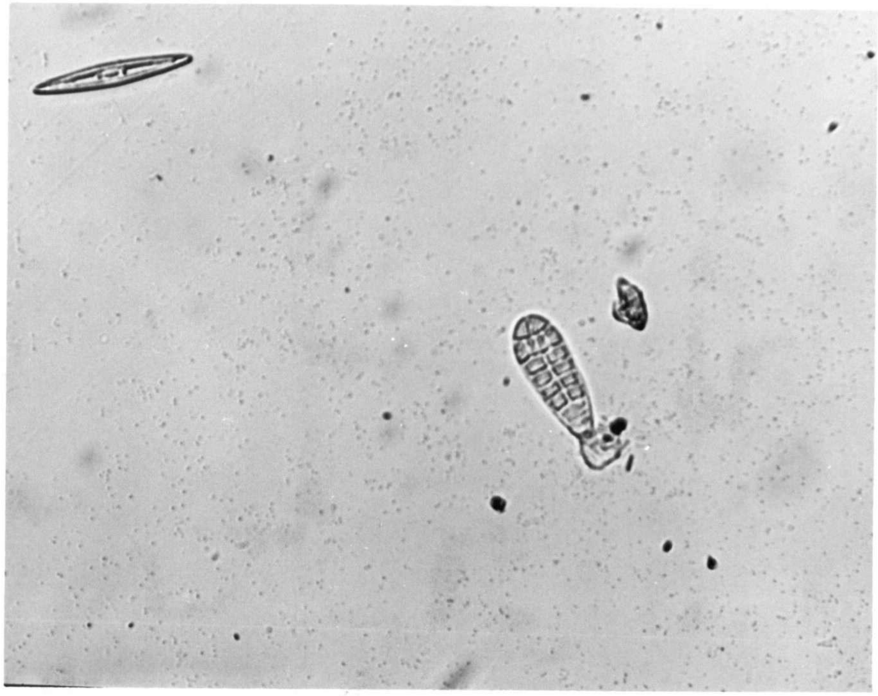
Fig. 3. Experiment 1. Mean number of diatoms per sample on slides collected from the field at 4 day intervals throughout the period August 6 to 30, 1970. Each line represents a different test design of the culture apparatus (refer to Table 1). GeO_2 was used in all designs except the control (F). Data for design E, for the purpose of clarity, was not included in the graph, having conformed closely with that of designs B, C, and D. Due to overlapping and clumping it was not possible to take samples from slides F and A after 8 and 12 days respectively.



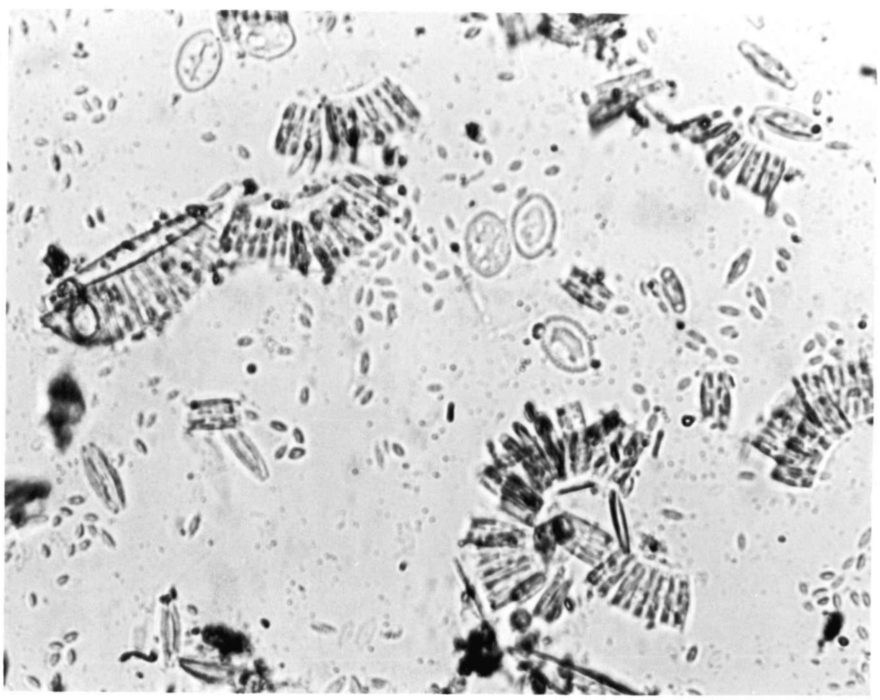
Mean Number of Diatoms
per Sample X 100
1
2
3
4
5
6
DAYS
4
8
12
16
20
24
F
A
C
D
B

Figure 4. Comparison of slides treated (a) and untreated (b) with GeO_2 over a period of 33 days in the sea. Note the young sporophyte which is attached to the empty oogonium in (a).

(a)



(b)



50μ

Table 1

Variations in the size and position of the secondary inflow port.

Test Design	Level of Inflow Port	Diameter of Inflow Port
A (D)	1 3/4"	1/32"
B (E)	1 3/4"	3/16"
C	1/2"	3/16"

Table 2

Mean number of diatoms per sample on slides collected after 33 days covering the period Oct. 12 - Nov. 14, 1970.

Test Design	Results
A	111.3
B	3.6
C	73.3
Control	748.5

Table 3

Development of gametophytes and young sporophytes at -7.5 meters.

Slide Category*	Days	Developmental Stage 1	2	3	4	5	6	7	8
C	5	1	39						
T	5	-	40						
C	9	-	40						
T	9	-	40						
C	13	-	23	17					
T	13	-	9	31					
C	17	-	1	39					
T	17	No Information							
C	21	No Information							
T	21	-	1	14	7	9	9		
C	25	-	1	38	1				
T	25	-	-	14	3	4	2	6	12

* C - control slides

T - test slides subjected to GeO_2

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