

## Alginate content and composition of *Macrocystis pyrifera* from New Zealand

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### Abstract

Tissue samples of *Macrocystis pyrifera* from 2 sites in southern New Zealand and harvested over a period of 12 months were analysed for alginate content and composition (M:G ratio). Plants were divided into three frond classes of different length and each frond was further separated into age categories of blades and stipes (viz young, mature and old blades; mature and old stipes). Within each size class, younger blades had higher alginate content than older blades. Stipes did not show such variation with age. Alginate from younger blades and stipes had higher proportions of mannuronic acid residues than those from old blades and stipes. The range of M:G ratios for age categories of either blades or stipes from longer fronds was greater than those for smaller fronds. Alginate content and M:G ratios of stipes were always higher than for blades. The difference between M:G ratios of blades and stipes was greater for smaller fronds than for longer ones. Differences between collection sites and seasonal trends are also discussed.

### Introduction

Alginic acid, the major structural polysaccharide of brown algae, is a linear copolymer of D-mannuronic and L-guluronic acid residues. It is extracted in commercial quantities from several brown seaweeds and has found numerous applications in the food and other industries (King, 1983; Brown *et al.*, 1989). An innovative use of calcium alginate is in the immobilization of enzymes and living cells (Margaritis & Merchant, 1984).

The seasonal variation in the alginate content

has been reported for a number of species belonging to the Laminariales (Black, 1950; Haug & Jensen, 1954; Wort, 1955; Whyte & Englar, 1978; Hay *et al.*, 1983) and Fucales (Daly & Prince, 1981; Chennubhotla *et al.*, 1982; Gorham & Lewey, 1984). In other studies variation in both alginate content and composition have been determined (Rosell & Srivistava, 1984; Ji Minghou *et al.*, 1984; Kim & Park, 1984). A limited number of investigators have measured the alginate content and M:G ratios of different parts of a seaweed and occasionally the age of the tissue has been considered (Haug *et al.*, 1974; Stockton

*et al.*, 1980; Craigie *et al.*, 1984; Cheshire & Hallam, 1985; Indergaard & Skjak-Braek, 1987). These studies have indicated that variations in alginic structure are related to morphological and environmental factors.

The genus *Macrocystis* has a bipolar distribution, occurring along the north-east Pacific as well as the temperate southern oceans (Chin *et al.*, 1991). In New Zealand, *M. pyrifera* is restricted to cooler southern shores (Rapson *et al.*, 1942). *M. pyrifera* is a major source of raw material for the production of alginic (McHugh & Lanier, 1983) yet despite this, there is limited published information on the alginic chemistry of this species. In this paper we report on a comprehensive study of alginic content and M:G ratios in *M. pyrifera* from New Zealand. Both within plant and seasonal variations were investigated. This forms part of a larger study on the alginic chemistry of New Zealand brown seaweeds.

## Materials and methods

*Macrocystis pyrifera* (L) C. Agardh. was collected approximately every month during the period June 1987 to May 1988, from Pudding Island (45° 50' 30'' S, 170° 38' 30'' E), a relatively sheltered site in Otago Harbour, and from Katiki Point (45° 23' 30'' S, 170° 52' 00'' E), an exposed coastal site. At each collection, five plants with actively growing apices were harvested, washed with seawater at the site of collection and any adhering extraneous matter removed by hand. Living fronds were classified into three size classes. Class 1 fronds were at least 2.0 m in length, reaching the surface at high tide and with the apical scimitar present and growing. Class 2 were > 1 m but < 2 m, sub-surface at high tide, with the apical scimitar present and growing. Class 3 were at least 0.25 m in length but less than 1 m with the apical scimitar present and growing. Each frond class was separated into blade (lamina and pneumatocyst) and stipe (except too little material was obtained for the apical scimitar stipes and they were not investigated). All samples from Pudding Island, (except December

1987) and the Katiki Point samples for June, July, August were further divided according to age: *young*; the apical scimitar with all attached blades, *mature*; the portion of fronds with blades showing no sign of distal erosion; *old*; the portion of fronds with blades showing no signs of damage except for distal erosion. This gave five parts for each size class of frond (young blade, mature blade, old blade, mature stipe and old stipe) and a total of 15 parts per plant. All severely damaged or decayed fronds and blades were discarded. The holdfast was not investigated.

Five plants were collected at each harvest. Corresponding parts from each of the 5 plants were combined, creating 15 separate pooled samples. The pooled samples were dried at 35 °C and ground in a Wiley mill (700 µm mesh) and stored until analysed. The exceptions were the December 1987 harvest from Pudding Is. in which the 5 plants were kept separate and subdivided only into blades and stipes, and the September–December 1987 and March 1988 samples from Katiki Pt., which were divided into size classes one, two and three and fronds were separated into blades and stipes but were not further subdivided according to age.

## Alginic extractions

Alginic was extracted by the method of Rosell and Srivastava (1984). Ground seaweed (1.02 to 1.05 g) was extracted twice by shaking with 0.1 M HCl (50 ml) for 30 min at room temperature. The residue was allowed to settle and the acid removed by aspiration. Alginic was extracted from the residue by 2% Na<sub>2</sub>CO<sub>3</sub> (50 ml) for 90 min at room temperature. The extraction was repeated twice. The extracts were centrifuged at 1200 g for 20 min at room temperature. The supernatants from the three extractions were pooled. If necessary, the pooled supernatants were neutralised with 0.5 M HCl for overnight storage. Alginic acid was precipitated by lowering the pH to 1.8–2.0 using 0.5 M HCl. After standing for 20–30 min the precipitate was separated by filtration through 2 layers of fine mesh, washed muslin. The precipitated alginic acid was washed with 0.5 M HCl

(400 ml) and then with cold distilled water (4 × 400 ml). The alginic acid was suspended in distilled water and the pH adjusted to pH 7 with 0.5 M NaOH. The resulting sodium alginate solution was freeze-dried, and weighed.

#### *M:G ratios*

The M:G ratios of the sodium alginate extracts were determined by a modification of the method of Grasdalen *et al.* (1979) The sodium alginate (10–15 mg) was treated with 2% trifluoroacetic acid (in D<sub>2</sub>O, 1 ml) at 75 °C for 1 h. The solution was neutralised with 4% NaOD in D<sub>2</sub>O. Proton NMR spectra were recorded at 75 °C with a Varian VXR-300 instrument in the pulsed Fourier-transform mode. Sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) was used as an internal reference. A 180-τ-90 water (HOD) suppression pulse sequence was used to suppress the HOD peak. The peak areas were determined by machine integration. M:G ratios were measured for Pudding Is. samples harvested every second month commencing in July 1987. M:G ratios were determined on Katiki Pt. samples collected in July, September, October, November, December 1987 and March 1988.

#### *Statistical analysis*

The detailed statistical analysis of alginate content and composition concerned the differences within plants and the effects of season and site on these differences. As plants from some harvests were divided differently from other periods, the statistical significance of differences between parts of plants were assessed using contrasts (Snedecor & Cochran, 1980, pp. 224–226). Where contrasts were suggested at the design stage of the study (such as the effect of site on the differences between blades and stipes) their statistical significance was assessed using F-Tests in the usual way. Contrasts that were suggested in the course of exploratory analysis of the data were assessed using Scheffe's test (Snedecor & Cochran, 1980, pp. 232–233). For example, the source of interaction of the difference between

blade and stipe alginate contents with season could be traced to a particular month using this approach.

As plant material was pooled each month (except December 1987, where alginate content of five separate plants was obtained) the data on whole plant alginate content and M:G ratios is confined to a single observation every month. In the absence of replication, site and seasonal effects on whole plants were confounded with variation between plants. Data from December 1987 gave an estimate of the variation between plants at Pudding Is. and this permitted a statistical assessment of seasonal changes using a least significant difference (LSD) calculated from the Studentized range distribution (Snedecor & Cochran, 1980, pp. 233–236). This comparison depends on the assumption that plant variation in December was typical of the whole year.

Usually a *P*-value <0.05 is taken as an indicator of a significant effect. Because the statistical analysis was exploratory in nature, the 0.05 level should not be rigidly applied: we have considered *P*-values as large as 0.10 as indicators of potentially significant effects that should be subjected to confirmatory experiments. Effects are described as non-significant (NS) only if *P* > 0.10.

#### **Results**

Sections (i) and (ii) address the differences within plants and the variation of those differences with site and season. In (iii) we report the seasonal variation of whole plants at Pudding Is. using December 1987 data to estimate the variation between plants. Alginate content was determined for every collection but M:G ratio was obtained every second month commencing July, 1987 at Pudding Is. and for July, September–December, 1987 and March, 1988 at Katiki Pt. In section (ii) age categories of blades and stipes were averaged to give data for three classes of blades and stipes. The averages were weighted according to the weights of the plant parts. This produced data comparable to that collected from September to December, 1987 and March, 1988 and allowed all

data relevant to differences between blades and stipes to be included in the analysis.

(i) *Variation within blades and stipes*

Average algininate contents and M:G ratios for the age categories of blades and stipes is given in Table 1. These are averages taken over all collections and all size classes. The standard deviations (SD) given in this table apply to differences: the differences in algininate content of young and old blades at Pudding Is. is  $28.0 \text{ mg g}^{-1}$  with a standard deviation of 7.8. Although it is valid to compare blades with stipes in Table 1, we defer such comparisons to section (ii) where additional data for Katiki Pt. is incorporated.

Algininate content of blades follows the general pattern of young blade > mature blade > old blade. The differences between age categories are statistically significant ( $P < 0.01$ ), as shown in Table 2. Examination of Fig. 1a suggests that the age effect is consistent over site and season. The observation is confirmed by the lack of significant interactions ( $P > 0.10$ ) of age with site or season.

Table 1. The average algininate contents ( $\text{mg g}^{-1}$ ) and M:G ratios of age categories of blades and stipes collected between June 1987 and May 1988 from Pudding Is. and June 1987 and August 1987 from Katiki Pt. (SD = standard deviation).

	Algininate content ( $\text{mg g}^{-1}$ )	M:G ratio
<i>Pudding Island</i>		
Young Blade	267.9	1.62
Mature Blade	255.4	1.42
Old Blade	239.9	1.10
Mature Stipe	343.6	1.92
Old Stipe	350.5	1.64
SD of any difference	7.8	0.05
<i>Katiki Point</i>		
Young Blade	291.8	1.74
Mature Blade	271.9	1.25
Old Blade	235.9	1.14
Mature Stipe	322.4	1.30
Old Stipe	323.6	1.25
SD of any difference	15.0	0.13

Similarly, size class does not have a significant influence on algininate content. We note the unusually high algininate levels for class 1 young and mature blades at Katiki Pt. in Table 3. These values arise as a result of high algininate content in July and August. However, they are not statistically significant ( $P > 0.10$ ). None of the factors considered (size, age, season or site) have a significant effect on algininate contents of stipes. This is evident in Fig. 1a by the lack of a systematic ordering of mature and old stipe contents.

In Fig. 1b M:G ratios follow the regular pattern young blade > mature blade > old blade and mature stipe > old stipe. These differences are significant for both blades and stipes as shown in Table 2 ( $P < 0.01$ ). Analysis of variance did not find significant effects for site or season. However, the size class does have a significant influence ( $P = 0.03$ ) on the differences between age categories of blades at Pudding Is. (Table 2). The nature of the interaction is seen from the means for each size class and age category in Table 3. The available data from Katiki Pt. is insufficient to judge the effect of size class at this site.

Table 2. P-values for effects of size class, season and site on algininate contents ( $\text{mg g}^{-1}$ ) and M:G ratios of age categories of blades and stipes collected between June 1987 and May 1988 from Pudding Is. and between June 1987 and August 1987 from Katiki Pt.

Source	Algininate content ( $\text{mg g}^{-1}$ )	M:G ratio
<i>Blades</i>		
Size class	NS	<0.01
Age × size class	NS	0.03
Age at Pudding Island	<0.01	<0.01
Age at Katiki Point	<0.01	<0.01
Age × month	NS	NS
Age × site	NS	NS
<i>Stipes</i>		
Size class	NS	NS
Age × size class	NS	NS
Age at Pudding Island	NS	<0.01
Age at Katiki Point	NS	NS
Age × month	NS	NS
Age × site	NS	NS

NS = not significant ( $P > 0.10$ ).

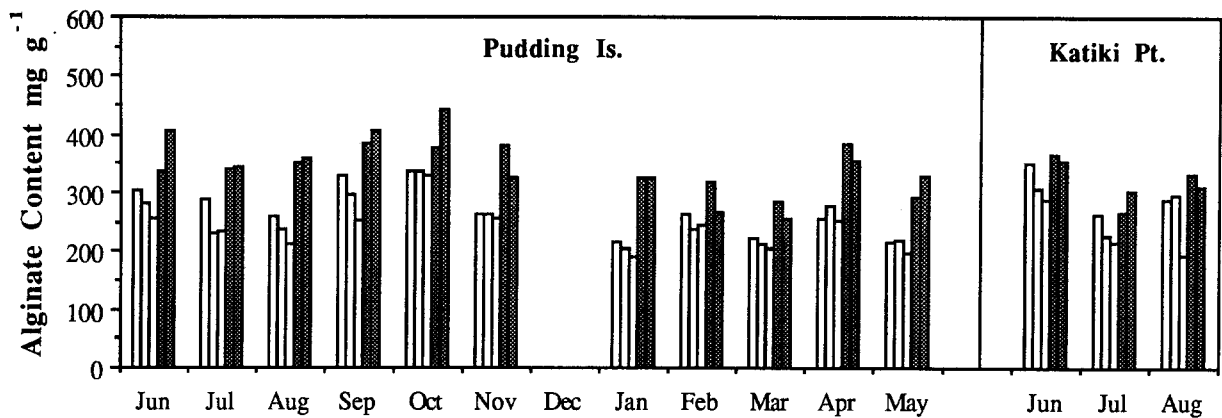


Fig. 1a. Alginate contents of different aged blades and stipes of *M. pyrifera* for monthly samples from the two collection sites. The alginate contents for each month are denoted by open bars for blades and stippled bars for stipes; the first bar is for young blades, the second bar is for mature blades, the third bar is for old blades, the fourth bar is for mature stipes and the fifth bar is for old stipes.

Table 3. The average alginate contents ( $\text{mg g}^{-1}$ ) and M:G ratios of blades and stipes categorized according to age and size class collected between June 1987 and May 1988 from Pudding Is. and between June and August 1987 from Katiki Pt.

		Alginate content ( $\text{mg g}^{-1}$ )			M:G ratio		
		Class 1	Class 2	Class 3	Class 1	Class 2	Class 3
<i>Pudding Island</i>							
Blade	Young	262.2	278.9	262.7	1.76	1.73	1.37
	Mature	257.3	258.1	250.8	1.60	1.43	1.25
	Old	240.4	239.1	240.2	1.18	1.07	1.06
Stipe	Mature	351.3	348.6	330.9	1.93	2.02	1.79
	Old	346.3	342.2	362.8	1.59	1.69	1.63
SD of any difference			13.6		0.09		
<i>Katiki Point</i>							
Blade	Young	332.4	258.6	287.5	1.71	1.56	1.96
	Mature	322.0	241.1	252.6	1.26	1.08	1.40
	Old	247.9	219.4	240.2	1.03	1.26	1.12
Stipe	Mature	346.7	301.6	318.8	1.37	1.24	1.29
	Old	307.3	342.0	321.5	1.07	1.36	1.32
SD of any difference			25.9		0.22		

(ii) Differences between blades and stipes

*P*-values for the statistical comparisons are given in Table 4. In this table, notation such as (blade – stipe)  $\times$  month refers to variation of the difference between blade and stipe with season. The mean alginate contents and M:G ratios of the

three size classes of blades and stipes at each site is given in Table 5. In Table 5, entries for Pudding Is. are obtained from Table 3 as weighted means (over age categories); entries for Katiki Pt. incorporate data not used in Table 3. Figure 2a shows that alginate content is greater for stipes than blades and that the difference between blades and

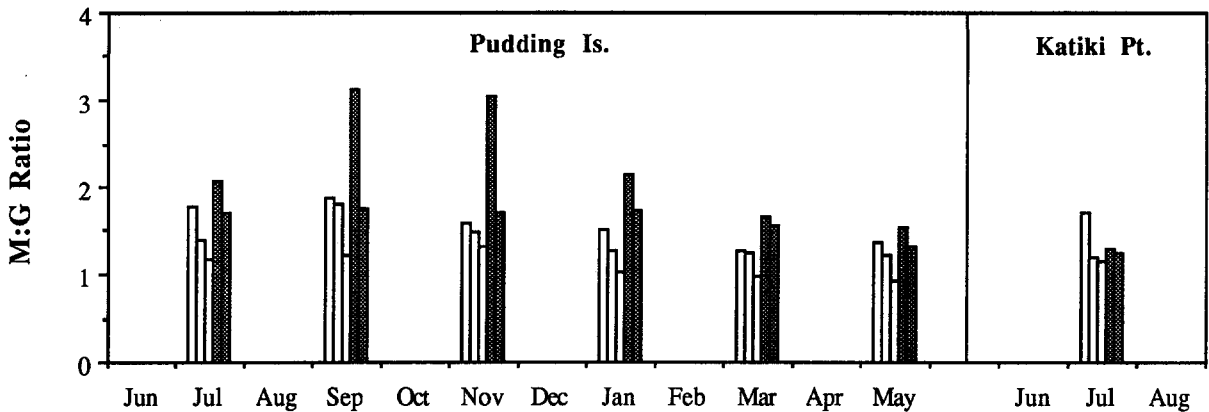


Fig. 1b. M:G ratios for different aged blades and stipes of *M. pyrifera* for monthly samples from the two collection sites. The M:G ratios for each month are denoted by open bars for blades and stippled bars for stipes; the first bar is for young blades, the second bar is for mature blades, the third bar is for old blades, the fourth bar is for mature stipes and the fifth bar is for old stipes. (Note the vertical axis is from 0 to 4.0 whereas it is 0 to 2.5 in Figs 2b, 3b).

Table 4. P-values for effects of size class, season and site on alginate contents ( $\text{mg g}^{-1}$ ) and M:G ratios of blades and stipes collected between June 1987 and May 1988 from Pudding Is. and Katiki Pt.

	Alginate content ( $\text{mg g}^{-1}$ )	M:G ratio
Size class effects		
Effect of class on blades	NS	NS
Effect of class on stipes	NS	NS
Class $\times$ Pudding Is. months	0.02	NS
Class $\times$ Katiki Pt. months	NS	NS
Differences between blades and stipes at Pudding Is.		
Blade - stipe	<0.01	<0.01
Size class $\times$ (blade - stipe)	NS	0.04
(Blade - stipe) $\times$ month	0.02	0.03
Differences between blades and stipes at Katiki Pt.		
Blade - stipe	<0.01	<0.01
Size class $\times$ (blade - stipe)	NS	NS
(Blade - stipe) $\times$ month	NS	NS
Contrasts for the differences between sites		
(Blade - stipe) $\times$ site	<0.01	<0.01

stipes is greater at Pudding Is. Figure 2b suggests similar effects for M:G ratios. In all of the above cases  $P < 0.01$ .

The blade-stipe difference also varies with season. At Pudding Is. there is a significant

(blade - stipe)  $\times$  month interaction for both alginate content ( $P = 0.02$ ) and M:G ratio ( $P = 0.03$ ). The interaction for alginate content arises largely because the difference between blades and stipes in February and March is considerably less than in the remaining months (Fig. 2a). This effect accounts for 66% of the interaction sum of squares. Similarly the interaction term for M:G ratios can be attributed to an unusually large difference between blades and stipes in January (Fig. 2b) which accounts for 74% of the interaction sum of squares. Because of the exploratory nature of the statistical analysis, Scheffe's test has low power and neither of these dominant components are statistically significant.

Size class also affects the difference between the M:G ratios of alginate from blades and stipes in the Pudding Is. data ( $P = 0.04$ ). From Table 5 the difference between blade and stipe M:G ratios increases from class 1 to class 3 fronds. A relative increase of a similar magnitude is observed for Katiki Pt. data, though the magnitude of the differences remain small and therefore not statistically significant. Another effect of class is an interaction with month at Pudding Is. ( $P = 0.02$ ). This may be traced to a single atypical month, October 1987, where the class 3 alginate content for both blades and stipes is significantly less than the corresponding class 1 and 2 contents. In Oc-

Table 5. The average alginate contents ( $\text{mg g}^{-1}$ ) and M:G ratios of blades and stipes from the three frond size classes collected between June 1987 and May 1988 from Pudding Is. and Katiki Pt. (values obtained from weight adjusted means of different aged blades and stipes).

	Alginate content			M:G ratio		
	Class 1	Class 2	Class 3	Class 1	Class 2	Class 3
<i>Pudding Island</i>						
Blade	249.0	252.1	247.4	1.38	1.28	1.19
Stipe	345.9	343.0	352.3	1.64	1.76	1.70
SD of any difference		9.8			0.05	
<i>Katiki Point</i>						
Blade	263.0	244.3	248.7	1.47	1.42	1.49
Stipe	302.1	312.4	320.0	1.54	1.55	1.63
SD of any difference		11.5			0.05	

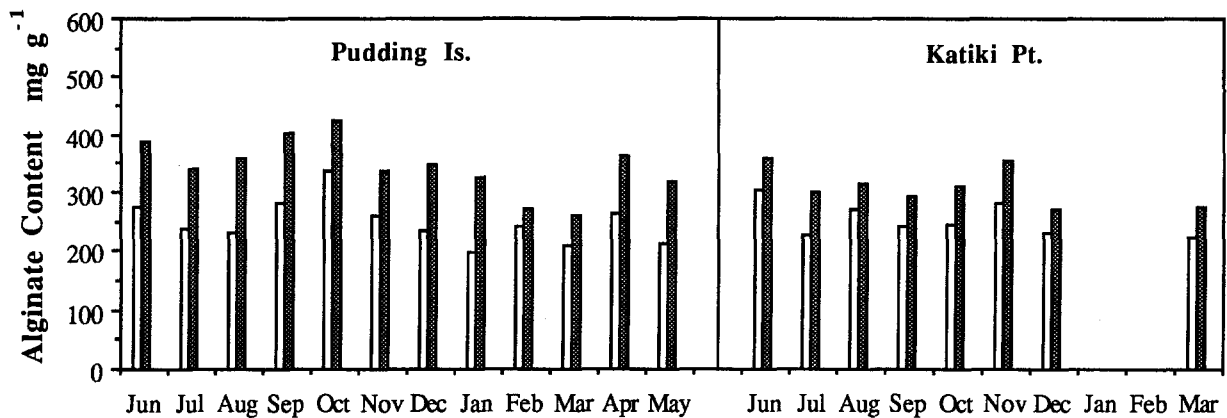


Fig. 2a. Average alginate contents for all blades and all stipes of *M. pyrifera*. For each month the first (open) bar denotes blades and the second (stippled) bar denotes stipes.

tober, the difference between class 3 and the average of classes 1 and 2 is  $85.5 \text{ mg g}^{-1}$  with a standard deviation of 16.5 whereas in all other months the difference is not significant. The effect of this single month accounts for 61% of the interaction sum of squares. The remaining part of the interaction sum of squares is not significant.

### (iii) Seasonal variation for whole plants

The alginate contents of the five plants sampled in December 1987 gives a least significant differ-

ence (LSD) at the 5% level of 68.0. Table 6 shows groupings of statistically similar months obtained for a Studentized range test. The seasonal trend is towards a peak in September and October (spring) (Fig. 3a). While data was not available for a similar analysis of alginate contents from Katiki Pt., no seasonal pattern is discernible (Fig. 3a). Similarly, no statistical analysis of M:G ratios for either site (Fig. 3b) is possible. However, in both cases the highest values were observed for the September, October and November samples.

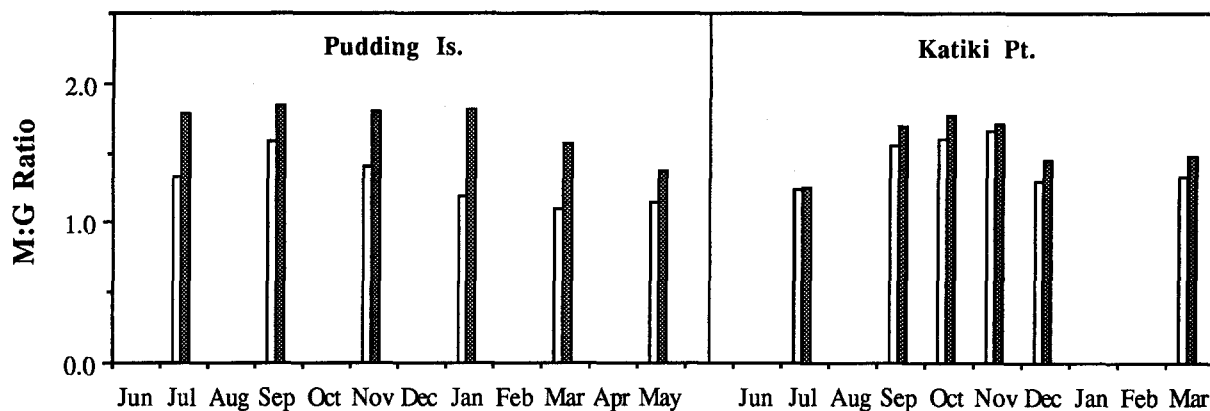


Fig. 2b. Average M:G ratios for all blades and all stipes of *M. pyrifera*. For each month the first (open) bar denotes blades and the second (stippled) bar denotes stipes.

Table 6. Seasonal variation of alginate contents ( $\text{mg g}^{-1}$ ) at Pudding Island (the months of June to December were in the year 1987 and the months January to May were in 1988). Months connected with an unbroken line are not significantly different.

Jan.	Mar.	May	Dec.	Aug.	Jul.	Feb.	Nov.	Apr.	Jun.	Sept.	Oct.
211.1	217.2	219.3	231.3	240.3	250.0	250.4	265.7	277.1	292.9	293.9	342.1

## Discussion

Despite the morphological and ontogenetic complexity of plants of the genus *Macrocystis* there are no reports in the literature on the alginate content and composition of fronds at different stages of development. In previous studies, stipe and blade material has been separated but age differences have not been considered e.g. Whyte and Englar (1978) and Rosell and Srivistava (1984) used tissue from the top 2 m of fronds, Hay *et al.*, (1983) used pooled material from whole fronds greater than 3 m long while Ji Minghou *et al.* (1984) worked with plants about 2 m in length. Moreover, different sized fronds are also exposed to different environmental conditions within the water column and this too might affect the alginate content and composition. In this study we have attempted to take into account the complexity of the plant by not only separating blades from

stipes but by analysing material of different developmental stage within fronds and comparing fronds of different size. Our results show how useful information may be lost when work is done only on whole plants.

A comparison of alginate content in blades of different age (Fig. 1a) shows that the content of young blades from the apical scimitar, although higher, is not significantly different ( $P > 0.10$ ) from that of mature blades (Table 1). Young and mature blades do, however, contain significantly more alginate than old blades (Table 1). The results of an early study on *M. pyrifera* from San Diego, California are in agreement with the trend reported here. Hoagland (1915) found that harvestable blades had a higher alginate content than non-harvestable (presumably old) blades, 18.4% and 16.1% respectively. A comparison with other brown seaweeds is limited because most authors do not provide statistical analyses of their data.



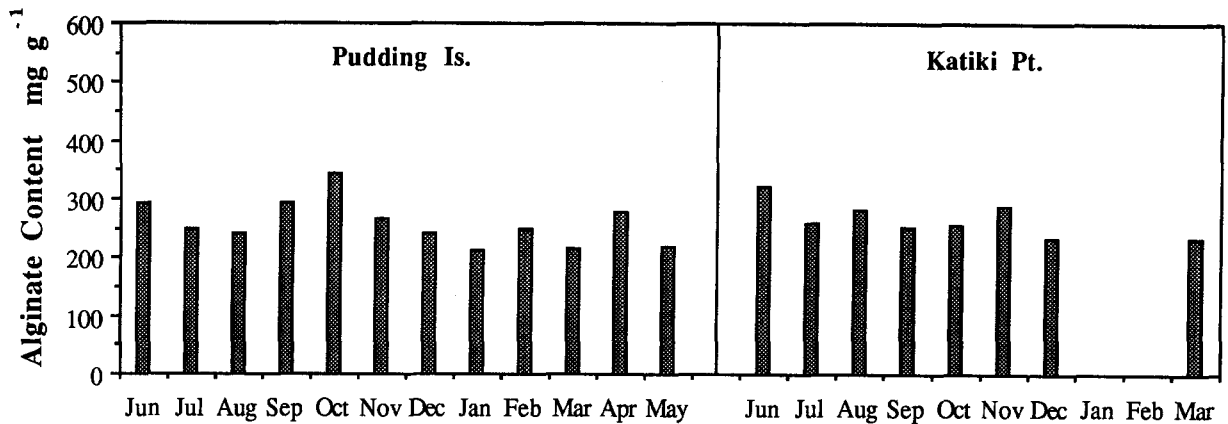


Fig. 3a. Average alginate contents of whole *M. pyrifera* plants for monthly samples taken at the two collection sites. The estimates were obtained from the parts (excluding the holdfast), the weights of the parts being taken into account.

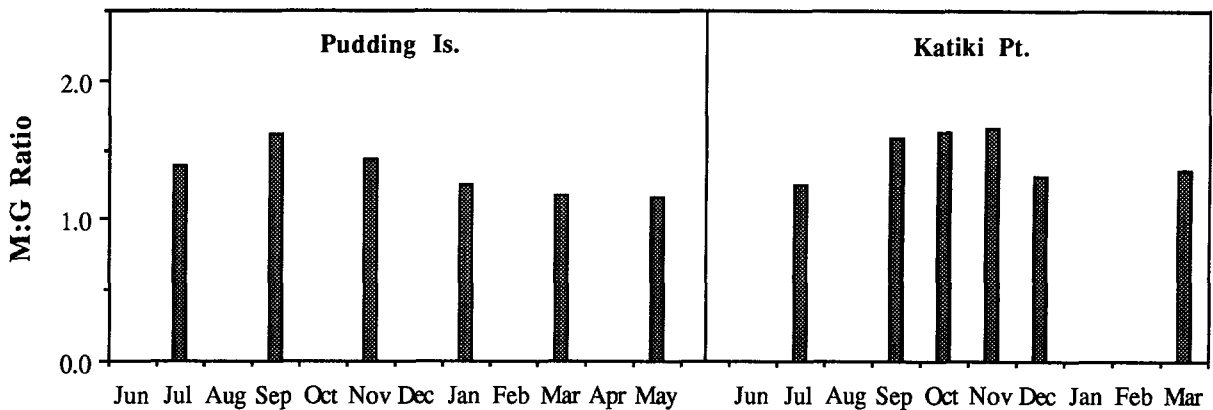


Fig. 3b. Average M:G ratios of whole *M. pyrifera* plants for monthly samples taken at the two collection sites. The estimates were obtained from the parts (excluding the holdfast), the weights of the parts being taken into account.

Thus, it is not known if apparent differences between different aged blade material are statistically significant (e.g. Haug *et al.* 1974; Ji Minghou *et al.*, 1984, Nishide *et al.*, 1988). The results from these and other studies on a range of brown seaweeds are inconsistent with the alginate content of younger tissue greater than older blade material (e.g. *Laminaria hyperborea*, Haug & Jensen, 1954; *Undaria pinnatifida*, Nishide *et al.*, 1988), less than older blade material (e.g. *Laminaria saccharina*, Black, 1954; Ji Minghou *et al.*, 1984), and similar to older blade material (e.g. *Durvillaea potatorum*, Cheshire & Hallam, 1985) being reported. The lower alginate content of

older blades may reflect the greater concentration of storage polysaccharides which are known to accumulate in these blades (Schmitz & Srivastava, 1979; Zimmerman & Kremer, 1986) or alternatively it may be related to differences in the ease of extraction of alginates of different composition (Cheshire & Hallam, 1985) reflecting anatomical changes associated with an increase in blade thickness with age (Kain, 1982).

In contrast to blades, stipes of different ages contain similar quantities of alginate (Fig. 1a; Table 1). These results are again in agreement with those reported by Hoagland (1915) for *M. pyrifera*, who found 19.3% and 18.8% for harvest-

able and non-harvestable stipes respectively. In studies on other plants, however, the amount of alginate in different parts of stipes has been shown to differ e.g. in *Laminaria hyperborea* a slight increase from basal portions to the stipe/blade junction has been observed (Haug 1964) whereas Hay *et al.*, (1983) found a decrease from the base to the top of the stipe in *Ecklonia maxima*.

The M:G ratios of blades of different ages differ significantly, with a higher proportion of mannuronic acid in young blades than in older ones. These differences between young, mature and old blades were maintained over the season and were similar at both sites (Fig. 1b). We have been unable to obtain any comparable published data for *Macrocystis* but one observation (M. Indergaard, pers. comm.) for *M. pyrifera* collected from California is in agreement with our results; M:G ratios of young and old laminae were 1.83 and 1.45 respectively and for young and old pneumatocysts, 1.83 and 1.74 respectively. Changes in the M:G ratio of blade material of different ages from various brown seaweeds follow a similar pattern to that reported here, with younger blades having higher M:G ratios than older blades (Haug *et al.*, 1974; Ji Minghou *et al.*, 1984; Cheshire & Hallam, 1985; Indergaard & Skjak-Braek, 1987).

The M:G ratios of different stipe fractions follow a similar trend to those of blades in that the mature stipes have a greater mannuronate content than the old stipes (Fig. 1b; Table 1) although the difference is not significant for Katiki Pt. plants (due probably to only a single month of data being available). Differences between stipes of different ages were statistically similar at each sampling time and there appears to be no difference between the two sites. Unpublished results (M. Indergaard, pers. comm.) for *M. pyrifera* from California show a similar trend to those presented here: the M:G ratio for young and old stipes were 1.98 and 1.61 respectively. Data published by Haug (1964) for *Laminaria hyperborea* stipes show an increase in the M:G ratio from the base of the stipe to the stipe/blade junction as do the results of the study by Indergaard *et al.* (1990) on *L. saccharina*.

If the range of values for M:G ratios within

each size class are compared, we find that at Pudding Is. the ranges for blades and stipes are greater in classes 1 and 2 than in class 3 (Table 3). There appear to be two possible explanations. If the small, class 3 fronds, are younger than the longer, class 1 and 2, fronds then the age gradient within class 3 is less and therefore one might expect the range of values within this frond class to be less. An alternative explanation is that all parts of class 3 fronds are exposed to similar environmental conditions, whereas class 1 and 2 fronds, which are growing through a greater portion of the water column, are exposed to a greater range of environmental conditions and this might affect their composition. Indergaard & Skjak-Braek (1987) and Indergaard *et al.* (1990) have found that nutrient concentrations and irradiance levels can influence alginate composition. Further work is required to distinguish between these two explanations.

In general the alginate content of blades from different sized fronds do not differ significantly. This is also true for stipes (Tables 4 and 5). There is no other comparable published data for *Macrocystis* but if we assume that the different frond classes can be equated to age then it allows us to compare our results with those of several other brown seaweeds of different ages. Several studies have indicated that while the alginate content of blades does not change with age, the content of stipes tends to decrease in older plants (Haug, 1964; South, 1979; Hay *et al.*, 1983). These results are, however, subject to the criticisms that size does not necessarily relate to age (South, 1979) and that the assay procedure may not work equally effectively on plants of different ages (Cheshire & Hallam, 1985).

Although for blades from Pudding Is. (Table 5) there appeared to be an increase in M:G ratios with increasing size (class 1 > class 2 > class 3), this was not statistically significant (Table 4). Indeed, the M:G ratios of blades from fronds of different sizes from both sites did not differ significantly. The same is true for stipes (Tables 4 and 5). In contrast, Haug (1964) found that small (assumed to be young) stipes of *Laminaria hyperborea* had a higher M:G ratio than those of larger

(assumed to be older) stipes. He concluded that these differences were primarily due to the fact that the outer cortex, which has a lower M:G ratio than the medulla and meristoderm, constitutes a greater proportion of large stipes compared with small ones. In *Macrocystis*, stipe diameter of different frond classes changes very little and this may account for the lack of difference between the frond size classes.

In this study we found that throughout the year and across different classes the algininate contents of stipes were significantly higher than those of blades (Fig. 2a & Table 3). The results in the literature are contradictory as to whether blades or stipes yield higher algininate contents. For *Macrocystis*, Rosell & Srivastava (1984) and Ji Minghou *et al.* (1984) found that the algininate content of the stipe was higher than that of the blade whereas Wort (1955) found that blades generally had higher levels than stipes throughout the year. Hay *et al.* (1983) found considerable fluctuation between blades and stipes through the year. These conflicting results have also been found in other species, e.g. Black (1950) reported that stipe yields were higher than blades through the season for *Laminaria digitata* and *L. saccharina*. Haug and Jensen (1954) and Haug (1964) also found that stipe contents were generally but not always higher than for blades of *L. digitata*, *L. saccharina* and *L. hyperborea* whereas Hay *et al.* (1983) found the opposite trend in their study on *L. pallida*. The differences found between the algininate contents of blades and stipes were greater at the more sheltered Pudding Is. site. This trend was also found by Black (1950) in his study on *Laminaria digitata* from sheltered sea lochs and open sea sites whereas no such differences were apparent for *L. digitata* in the study by Haug & Jensen (1954).

A comparison of the M:G ratios of stipes and blades shows that contrary to most published data the ratios for stipes were higher than those of blades (Fig. 2b). The single observation on *M. pyrifera* by Ji Minghou *et al.* (1984) contradicts our findings as does data presented by Rosell & Srivastava (1984), an average of 1.3 and 1.6 for stipes and blades respectively, but unpublished

data by Indergaard (pers. commun.) on three plants from California do agree with ours: young stipes and young blades have M:G ratios of 2.0 and 1.83 respectively and old stipes and old blades have M:G ratios of 1.61 and 1.45 respectively. In general, in most brown seaweeds it was found that M:G ratios of blades are greater than those of stipes, e.g. limited observations on *L. digitata* (Haug *et al.*, 1974; Stockton *et al.*, 1980; Craigie *et al.*, 1984), *L. hyperborea* (Haug *et al.*, 1974), *L. longicruris* (Craigie *et al.*, 1984), and *Durvillaea potatorum* (Cheshire & Hallam, 1985). Studies of the mechanical properties of algininate gels have shown that structural rigidity increases with increasing polyguluronate content. Thus one might expect the stipe, which tends to have greater mechanical rigidity than the more flexible blade to have a higher proportion of guluronic acid. In most of the species examined this is indeed true. However, stipe morphology of *M. pyrifera* is quite different being slender and flexible. This is reflected in their high M:G ratio. Clendenning (1964, 1971) suggested that their ability to withstand wave action is improved by entwining into bundles and because of their 'springiness'. The differences between blades and stipes were again greater at the sheltered Pudding Is. site. This appears to be due to the blade differences at the two sites. Craigie *et al.* (1984) have shown that algininate structure of *Laminaria longicruris* laminae alters depending on degree of exposure of the location and that these differences are reflected in morphological differences between the plants at the different sites (smaller and thicker at the more exposed site). They put forward three possible explanations to account for these differences: genetical variation, environmental adaptation or differences in growth rate due to the prevailing environmental conditions. Kain (1982) found morphological differences between the blades of *M. pyrifera* plants from an exposed and a sheltered site in New Zealand with the latter being narrower and longer but of similar thickness. She found that the morphology of plants from the exposed site were more similar to those from southern California than to plants from the more sheltered site in New Zealand. While growth

rates of plants from the exposed site were not measured, growth rates of the morphologically similar Californian plants were greater than those of the sheltered New Zealand plants. Thus the enzymatic conversion of mannuronate to guluronate may perhaps be more complete under the faster growing conditions (Craigie *et al.*, 1984). It is noted that for both sites the difference between M:G ratios of blades and stipes for the smaller size classes 2 and 3 are approximately double that of the longer size class 1 (Table 5). Further studies on the factors governing algininate composition are required.

The most common procedure for studying seasonal variation in algininate yields and M:G ratios is to analyse whole plants. To allow comparisons with other studies, bearing in mind the lack of replication in many of these, we have combined the data obtained from the various parts (excluding the holdfast) and taken into account the weights of these parts to arrive at analyses for the whole plant (Figs 3a and 3b). On this basis the maximum value for algininate content at Pudding Is. occurs in spring (September and October) which corresponds to a period of rapid growth as estimated by the rate of blade elongation (Chin, 1989). Apart from June, the values for the other months are not significantly different from each other. While the results for Katiki Pt. cannot be confirmed statistically, the algininate contents are similar to those of Pudding Is. although there appears to be no detectable seasonal trend. Spring peaks in algininate content have been reported for other *Macrocystis* species (Hay *et al.*, 1983; Rosell & Srivastava 1984) and for other brown seaweeds (Black, 1950; Haug & Jensen, 1954; Ji Minghou *et al.*, 1984). Other studies, however, have found either no obvious seasonal trend (Wort, 1955; Daly & Prince, 1981) or peaks at other times of the year (e.g. autumn Whyte & Englar, 1978; Jensen *et al.*, 1985). These peaks do not necessarily coincide with maximum growth rates.

At both sites the range of M:G ratios were similar (Fig. 3b). While the values for the M:G ratios cannot be compared statistically, the highest values were observed in spring with lower values in summer and autumn. Seasonal varia-

tions in M:G ratios have been studied less often than algininate content. Rossell and Srivastava (1984) found no obvious seasonal trend for *M. integrifolia* or *Nereocystis luetkeana*. In studies of other brown seaweeds Ji Minghou *et al.* (1984) reported the M:G ratios for *Laminaria japonica* and *Undaria pinnatifida* increased in the period March to July (northern hemisphere), but decreased for *Sargassum mijabei*, whereas Kim and Park (1984) found the M:G ratios for *Ecklonia cava* to be variable over a year.

With the exception of the work of Daly and Prince (1981) on *Sargassum pteropleuron*, seasonal studies have not generally been based on replicated observations. The variable results often observed in seasonal studies may be due, in part, to the sampling procedure. Future studies should include replicated observations to take into account the variation between plants.

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