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Biomass and agar assessment of three species of *Gracilaria* from Negros Island, central Philippines

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Abstract

Biomass, cover and agar quality of three species of *Gracilaria* were monitored monthly in three sites in Negros Oriental, central Philippines, during the period July 1992–June 1993. The biomass per unit area of *G. arcuata* peaked around March while *G. salicornia* was maximal around November. Seasonality in *G. blodgettii* is not obvious from our data as this is a highly harvested species and we may have sampled after gleaners have harvested the algae.

Agar yield values were for *G. arcuata* from Bais, 2.9 ± 7% to 21.7 ± 0.7% while the same species from Siaton yielded 2.6 ± 0.6% to 18.3 ± 0.5%; *G. salicornia* yielded 2.9 ± 0.1% to 15.7 ± 1.3% and *G. blodgettii*, 0.1% to 20.7 ± 5.9%. Gel strength and viscosity were variable. Gel strength values were: *G. arcuata* (Bais), 17-260 g cm^{-2}; *G. arcuata* (Siaton), 58-270 g cm^{-2}; *G. salicornia*, 29.5–147 g cm^{-2}; *G. blodgettii*, 29.6–235 g cm^{-2}, and related to the 3,6-anhydrogalactose and sulphate content of the algae.

Introduction

Because the major seaweed product in the Philippines is carrageenan from *Eucheuma* and *Kappaphycus* species, much of the phycocolloid research and product development have been geared towards this compound. However, carrageenan has limited applications and the need to develop mariculture technologies for agarophytes is high. Prerequisite to mariculture is basic research on the biology of the source algae and their agar content.

This study aimed at gathering information on *Gracilaria* beds in three sites on eastern Negros Island, central Philippines, specifically on the standing stock of their most abundant *Gracilaria* species, and their agar yield and quality. This is part of a larger study dealing with the assessment of *Gracilaria* beds in the Philippines, with a view towards mariculture and recommending options for the management of existing stocks. *Gracilaria* species are currently harvested from wild populations in the Philippines for food, animal feeds, fertilizers and phycocolloids.

Materials and methods

Study sites

Based on preliminary surveys of potential study sites, three areas were selected in Negros Oriental: (1) Lag-it, Capiñahan, South Bais Bay, (2) Canday-ong Dumaguete City, and (3) Malo, Siaton.

Lag-it, Bais City (9° 36′ N, 123° 10′ E) is a large intertidal flat of seagrasses covered most of the year by algae. Landward is a mangrove forest and seaward is a coral reef. The dominant seagrass is *Enhalus acoroides* (L.f.) Royle. The major substratum consists of a mixture of sand and silt. Species of *Gracilaria* found were *G. arcuata* Zanardini, *G. salicornia* (C. Ag.) Dawson and *G. eucheumoides* Harvey. The last occurred in negligible quantities and was not included in the monitoring. The area is highly fished and gleaned by people collecting fishes, invertebrates and algae.

Canday-ong, Dumaguete City (9° 25′ N, 123° 20′ E) is a seagrass-algal bed inside a back reef. It is located near the mouth of Banica River. Only one species, *G. blodgettii* Harvey, was found growing on pebbles. The area is highly fished and gleaning occurs, including the harvest of *G. blodgettii.*

Malo, Siaton (9° 6′ N, 122° 54′ E) is a narrow and rocky intertidal area exposed to the southwest monsoon. Surge channels are present as a result of the strong monsoon winds. Seaward is a coral reef. Species found were *G. arcuata* and *G. salicornia* but the latter occurred in negligible amounts and was not monitored. The area is highly fished but no gleaning occurs.

Biomass, cover and zonation

To determine abundance, cover and zonation, systematic sampling was employed. Two to four transect lines, 20 m apart, were laid from the shore seaward. Transect line length varied among sites, between 50 m and 150 m, depending on the width of the *Gracilaria* bed. In Lag-it, two 150 m transects were used; in Canday-ong, four 50 m transects and in Malo, two 100 m transects were used. Permanent metal quadrats measuring 0.5 m $\times$ 0.5 m, divided into 25 subquadrats, were set up at intervals of 10 m along each transect for monthly monitoring of cover and associated macroflora and fauna.

Biomass was quantified using 0.25 m^2 quadrats randomly thrown within the *Gracilaria* bed. Ten replicates were taken monthly from August 1992 to July 1993. All algae within the quadrat were collected and brought back to the laboratory for sorting to species, cleaning and weighing. Wet weights were obtained after blotting with cheesecloth to remove the excess water. The algae were then oven-dried at 60-70 °C to constant dry weight. Adhering dirt particles and other organisms are considered as contaminating material (admixture).

Environmental parameters

Salinity was measured using a hand-held temperature-compensated AO refractometer (American Optical), pH with an Orion portable pH meter (Model SA 250, Orion Research Incorporated, U.S.A.) and temperature using an ordinary mercury quick reading thermometer. Phosphate-phosphorus (PO_4^{-3}-P) and nitrate-nitrogen (NO_3^--N) were determined using ascorbic-molybdate method for the former (Koroleff, 1983) and cadmium reduction method for the latter (Grasshoff, 1983). Rainfall data were taken from the local Philippine Atmospheric Geophysical and Astronomical Services Administration (PAGASA). All measurements were made monthly.

Agar yield

For agar yield, about 1 kg was harvested monthly for extraction. Prior to extraction, the algae were sorted and cleaned in the laboratory. Extraction of agar followed the method modified from Hurtado-Ponce & Umezaki (1988) by treating 25 g dry algae with 5% NaOH solution for one hour at 90 °C. The sample was then washed and soaked for another hour in 0.5% acetic acid. After soaking, the sample was again washed. Extraction was done with boiling distilled water for one hour. The sample was then blended with an ordinary kitchen blender before squeezing through a 3-ply cheesecloth. The extract was then frozen overnight at 0 °C, thawed the following day, oven-dried to constant weight and stored in plastic bags prior to quality testing. The following agar qualities were determined: viscosity of 1.0% agar solution at 65 °C using a Brookfield dial reading viscometer (Spindle 2, 60 rpm, Brookfield Engineering Laboratories, Inc., MA., U.S.A.), gel strength of 1.5% agar solution set overnight using an MCD gel tester (Model G741, No. 181, Marine Colloids Division, FMC Corporation, Rockland, ME, U.S.A.), gelling and melting temperatures of 1.0% agar solution (modified from Whyte & Englar, 1980), sulphate content using the extraction method of Jackson & McCandles (1978) and turbidimetric method of determination (Golterman et al., 1978), and 3,6-anhydrogalactose content using the colorimetric method of Craigie & Leigh (1978).

Results

Biomass, abundance and cover

The mean monthly and cover biomass (Figure 1) expressed in per cent of three *Gracilaria* species in three sites exhibited a seasonal trend.

In Lag-it, the mean dry biomass of *G. arcuata* ranged from 1.7 $\pm$ 1.6 g m^{-2} in April 1993 to 25.7 $\pm$ 24.6 g m^{-2} in March 1993 (Figure 1). There was a gradual increase in biomass from November 1992, peaking in March 1993 and a sudden decline. Cover followed the same trend. On the other hand, the seasonal trend in the mean biomass of *G. salicornia* was not as distinct and did not coincide with cover data (Figure 1). Highest mean biomass was in November

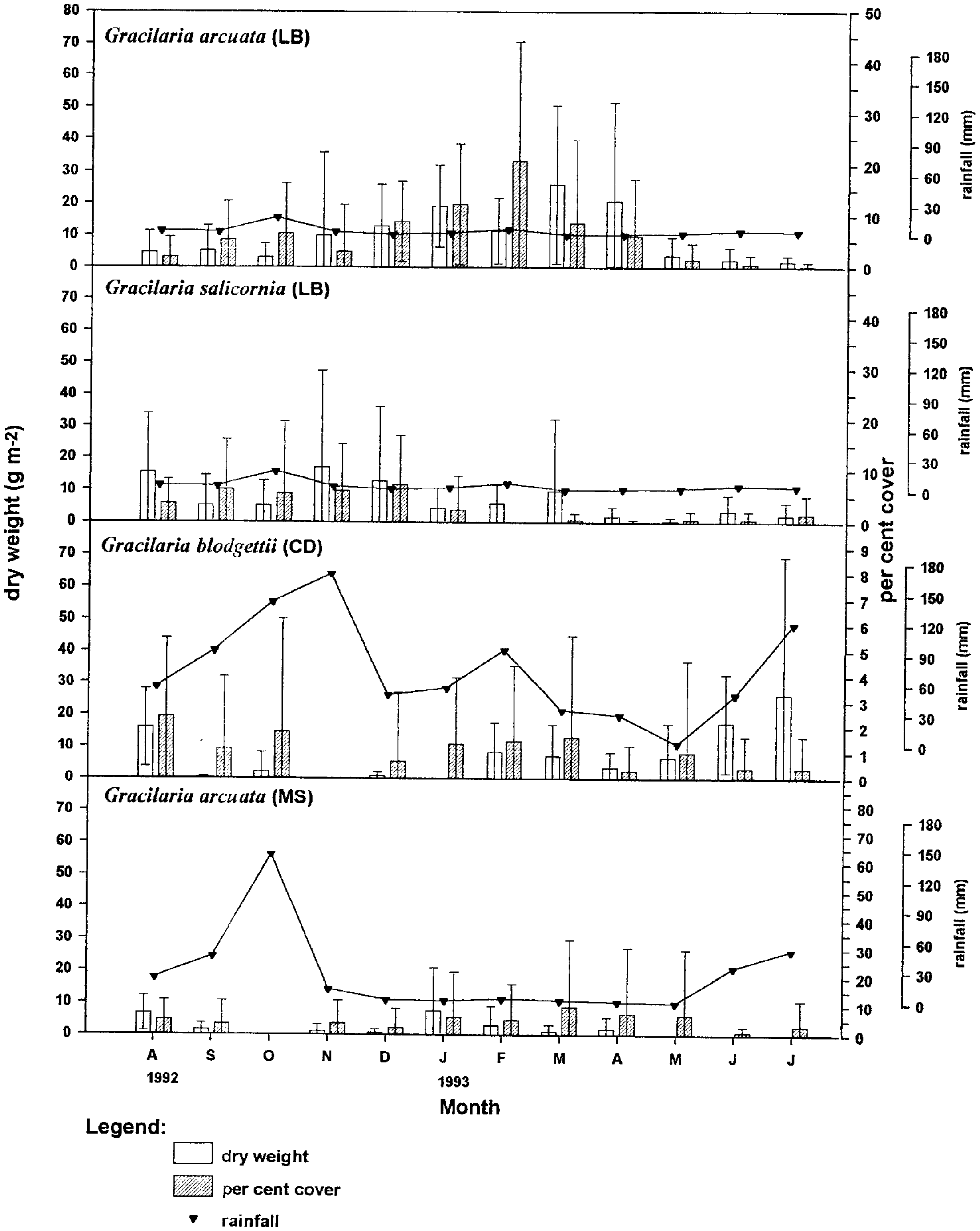

Figure 1. Monthly mean dry biomass in g m^{-2} (n = 10) and mean per cent cover (n = 30 for Lag-it; n = 20 for Canday-ong and Malo) of three species of *Gracilaria* from August 1992 to July 1993 in three sites plotted against rainfall (mm). (LB) Lag-it, Bais; (CD) Canday-ong, Dumaguete; (MS) Malo, Siaton. Bars represent standard deviation.

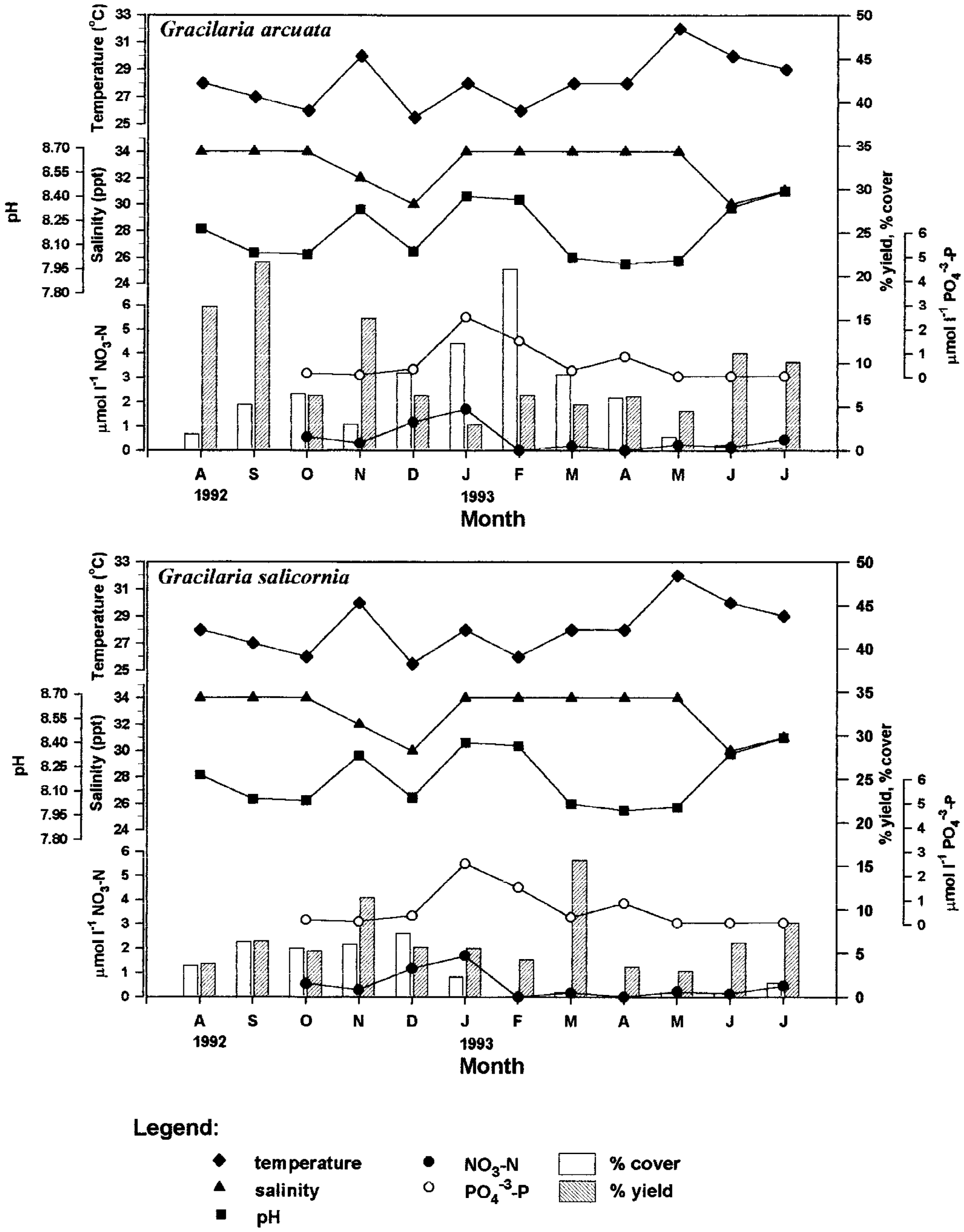

Figure 2. Monthly mean per cent cover and agar yield of *G. arcuata* and *G. salicornia* in Lag-it, Bais plotted with chemical parameters (pH, salinity, temperature, PO_4^{-3}-P and NO_3^--N).

1992 (22.3 ± 30.6 g m^{-2}) and lowest was in May 1993 (0.3 ± 1.1 g m^{-2}). Relatively high values were obtained in August and December with highest cover in December 1992. Occasional harvesting of *G. salicornia* was observed but not for *G. arcuata.* Rainfall in Bais during August 1992 to July 1993 was relatively uniform, below 10 mm per month, except for October 1992 when rainfall reached 18.3 mm. Water temperature ranged from 25 to 32 °C, salinity, 30 to 34 ‰, pH, 7.98 to 8.43, NO_3^--N from not detectable (ND) to 1.16 μmol l^{-1} and PO_4^{-3}-P, 0.05 to 2.5 μmol l^{-1} (Figure 2).

Gracilaria blodgettii biomass in Canday-ong was the lowest during the rainy months of September 1992 to January 1993 (0 to 0.2 ± 6.0 g dry wt m^{-2}) and the highest during the months of August 1992 and June–July 1993 (15.9 ± 12.2 to 26.1 ± 43.0 g dry wt m^{-2}). Rainfall was relatively high (above 25 mm) except for February–May 1993 (Figure 1). Water temperature ranged from 28 to 32 °C, salinity, 24 to 34 ‰, pH, 8.07 to 8.45, NO_3^--N, ND to 5.56 μmol l^{-1} and PO_4^{-3}-P, ND to 5.23 μmol l^{-1} (Figure 3). This species is heavily harvested for food by gleaners.

The biomass and cover data of *G. arcuata* in Malo suggest that the population starts to grow in January through May and starts to decline by June (Figure 1). No data were collected for October due to heavy rains and strong winds which resulted in zero underwater visibility. Water temperature ranged from 27 to 31 °C, salinity, 3 to 34 ‰, pH, 7.98 to 8.66, NO_3^--N, ND to 2.71 μmol l^{-1} and PO_4^{-3}-P, 0.55 to 2.25 μmol l^{-1} (Figure 3).

Reproduction

During the monthly monitoring of permanent quadrats over a year period, cystocarpic thalli of *G. blodgettii* in Canday-ong were observed only in August 1992, January 1993 and July 1993. In August 1992 and July 1993, cystocarps were present in the intertidal population only. In January 1993, both intertidal and subtidal populations bore cystocarps. No reproductive structures were observed on *G. arcuata* and *G. salicornia* in Bais.

Water content and contaminating material

Among the three species, *G. salicornia* had the highest water content, taking up 75% of its wet weight. The two species, *G. arcuata* and *G. salicornia* had about 50% moisture content each. There was no significant monthly variation among species and between sites.

Gracilaria blodgettii was the 'cleanest' among the three species. Much of its admixture was sand and adhering dirt. No significant monthly variation was discernible. No samples were obtained from Malo in October 1992.

Yield

Monthly variations in agar yield occurred in all species in all sites (Figure 4) but was not found significant (2-Way ANOVA, between months, p = 0.97043; between species, p = 0.54711, alpha = 0.05). Agar yield for *G. arcuata* from Lag-it ranged from 2.9 ± 7% to 21.7 ± 0.7% (Figure 2) while the same species from Malo yielded 2.6 ± 0.6% to 18.3 ± 0.5% (Figure 3). *G. salicornia* yielded 2.9 ± 0.1% to 15.7 ± 1.3% (Figure 2) while *G. blodgettii* yielded 0.1% to 20.7 ± 5.9% (Figure 3).

Gel strength and viscosity

The following gel strength values were obtained: *G. arcuata* (Lag-it), 17–260 g cm^{-2}; *G. arcuata* (Malo), 58–270 g cm^{-2}; *G. salicornia*, 29.5–147 g cm^{-2}; *G. blodgettii*, 29.6–235 g cm^{-2} (Figure 4). Monthly variations within species and between species were seen but were not significant (2-Way ANOVA, between months, p = 0.045687; between species, p = 0.02194, alpha = 0.05).

Agar extracted from *G. arcuata* from Lag-it ranged in viscosity from 0 to 42 cps while the same species from Malo had an agar viscosity range of 0 to 28.8 cps. *G. blodgettii* from Canday-ong had an agar viscosity range of 2.5 to 15 cps and *G. salicornia* 0 to 47 cps (Figure 4).

Gelling and melting temperatures

Gelling temperatures of the extracted agars were between 38 °C and 49.5 °C for the Bais species, *G. arcuata*, and between 44 °C and 53 °C for *G. salicornia*. For Canday-ong agar, it was between 42 °C and 49 °C for *G. blodgettii* and for Malo agar between 42 °C and 55.5 °C for *G. arcuata*.

Melting temperatures of the Bais agars were 59 °C – 81 °C for *G. arcuata* and 64 °C – 77 °C for *G. salicornia*; of the Malo agar it was 54 °C – 84.5 °C for *G. arcuata* and of the Canday-ong agar it was 52.5 °C – 78 °C.

Sulphate and 3,6-anhydrogalactose contents

Among the three species, *G. salicornia* recorded the lowest mean sulphate content (4.2% ± 1.5; n = 21) compared with *G. arcuata* (5.2 ± 1.1; n = 24) and *G. blodgettii* (5.3 ± 1.7; n = 21) (Figure 5).

As in sulphate content, the same pattern was found for 3,6-anhydrogalactose. *G. salicornia* recorded the

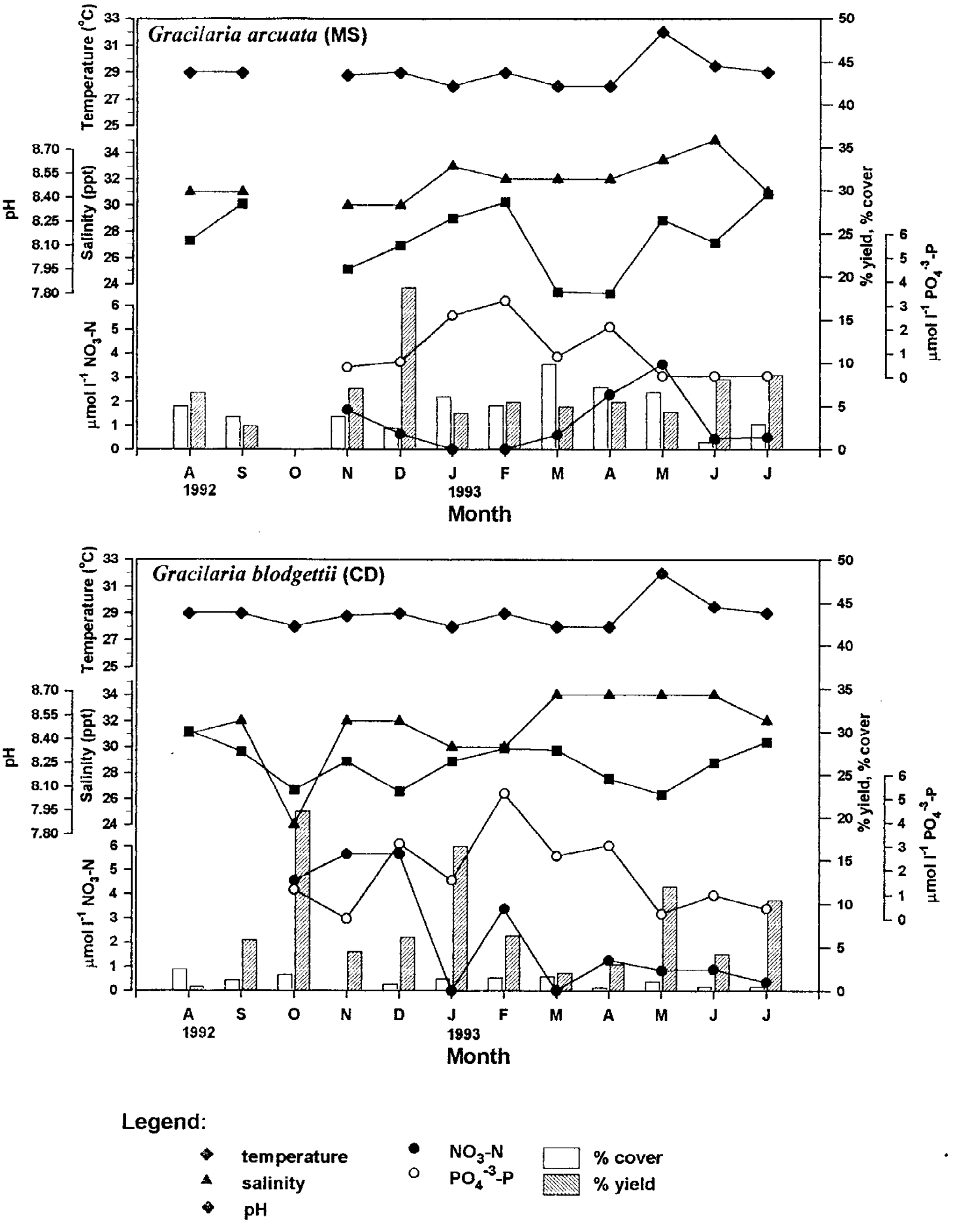

Figure 3. Monthly mean per cent cover and agar yield of *G. arcuata* in Malo, Siaton (MS) and *G. blodgettii* in Canday-ong, Dumaguete (CD) plotted against chemical parameters (pH, salinity, temperature, PO_4^{-3}-P and NO_3^--N).

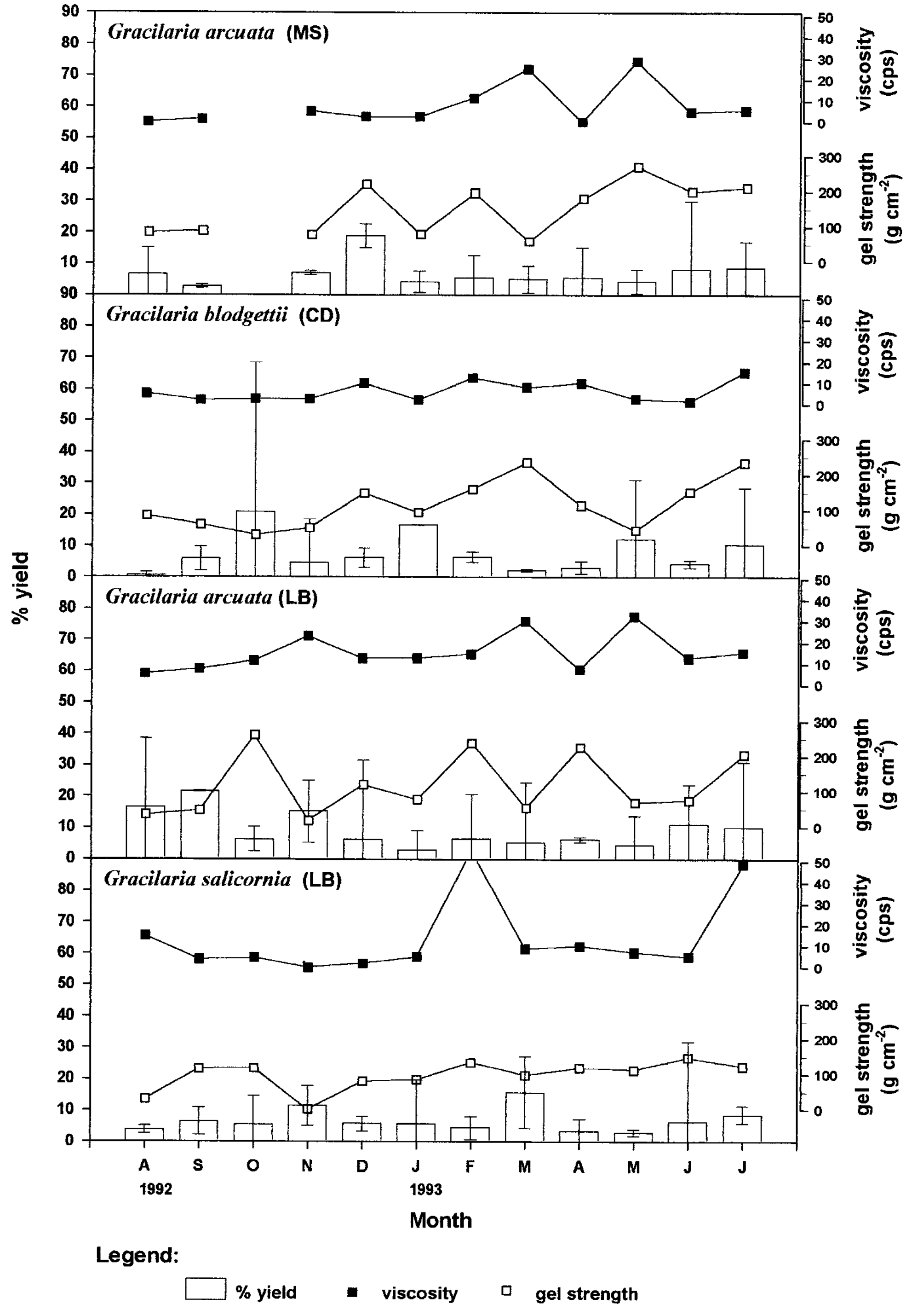

Figure 4. Monthly mean agar yield (n = 3), gel strength and viscosity of *G. arcuata* and *G. salicornia* in Lag-it, Bais, *G. arcuata* in Malo, Siaton (MS) and *G. blodgettii* in Canday-ong, Dumaguete (CD) from August 1992 to July 1993. Bars represent standard deviation.

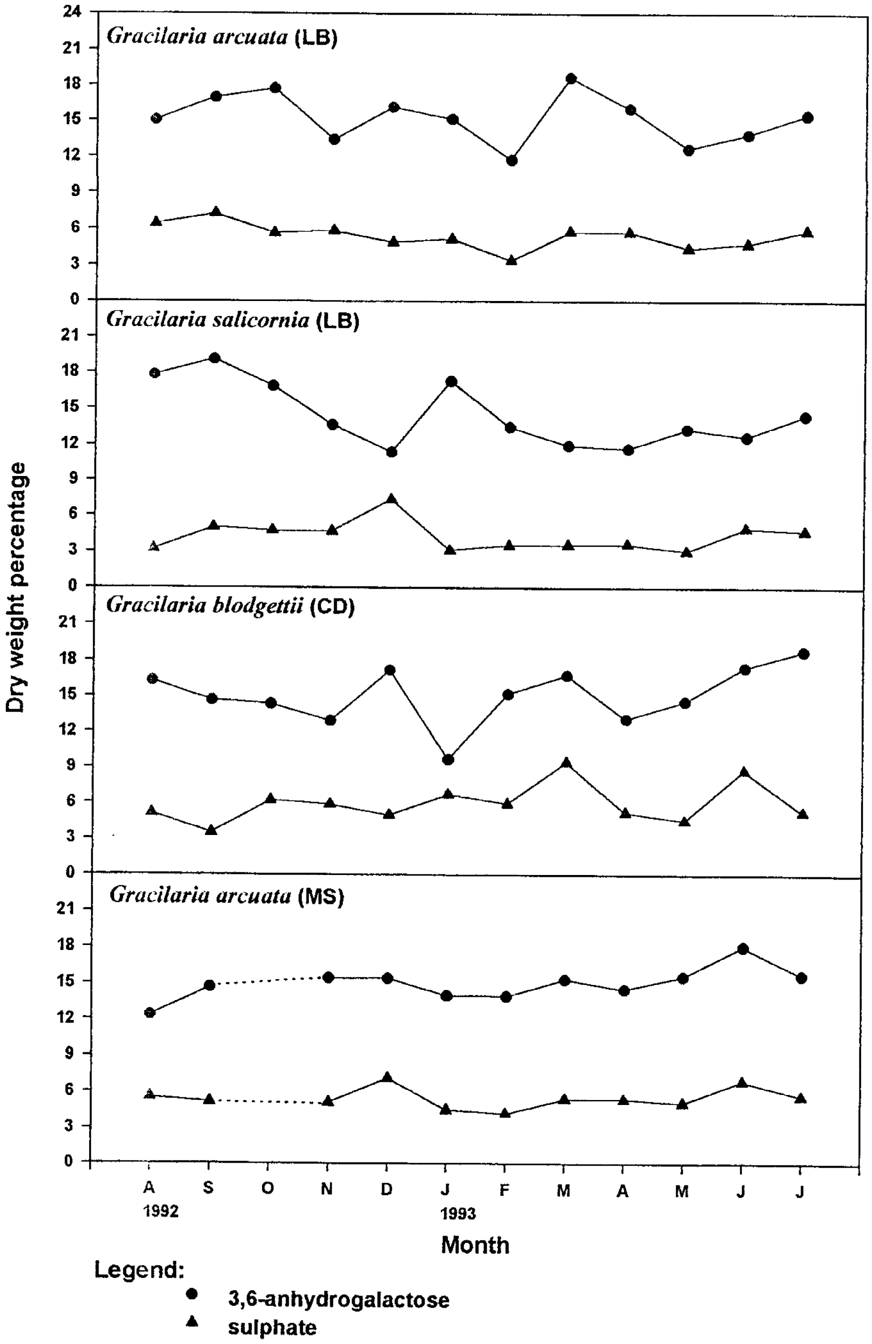

Figure 5. Dry weight percentages of agar 3,6-anhydrogalactose and sulphate contents of *G. arcuata* and *G. salicornia* in Lag-it, Bais (LB) and *G. arcuata* in Malo, Siaton (MS) and *G. blodgettii* in Canday-ong, Dumaguete (CD) from August 1992 to July 1993.

lowest mean anhydrogalactose content (13.9–2.4; n = 22) compared with *G. arcuata* (15.3 ± 1.8; n = 24) and *G. blodgettii* (15.1 ± 2.6; n = 20) (Figure 5).

Discussion

The two *Gracilaria* species in Lag-it, Bais seemed to reach growth peaks at different times with *G. arcuata* peaking at months when *G. salicornia* was declining (Figure 1) which may be a result of interspecific competition. Trono & Azanza-Corrales (1981) found that the biomass of *G. verrucosa* (Huds.) Pappenf. in Manila Bay declined when the biomass of *Acanthophora spicifera* (Vahl) Børg. was high.

For *Gracilaria arcuata* at Lag-it, Bais, a growth peak occurred after a period of higher PO_4^{-3}-P and NO_3^--N levels in the water (Figure 2). Sousa-Pinto et al. (1996) demonstrated that growth of *Gelidium robustum* (Gardn.) Hollen. & Abb. was directly proportional to increasing concentrations of phosphates and Macler & West (1987) found growth rate of *Gelidium coulteri* Harv. reduced to nearly zero after three weeks of nitrogen starvation. Seasonality in *G. blodgettii* is not obvious from our data as this is a highly harvested species and we may have sampled after gleaners have harvested the algae.

Other factors may have contributed to the seasonal growth. Lag-it Bais and Canday-ong, Dumaguete are both exposed to the northeast monsoon which occurs during the months of November through April while Malo, Siaton is exposed to the southwest monsoon. Trono & Azanza-Corrales (1981) found low biomass of *G. verrucosa* in Manila Bay during the southwest monsoon.

It was interesting to note that no reproductive thalli were collected during the whole sampling period, except for *G. blodgettii*. In Manila Bay, Trono & Azanza-Corrales (1981) found reproductive thalli of *G. verrucosa* the whole year round with a preponderance of reproductive over vegetative ones during months of high salinities.

Agar yield also showed variation but was not obviously correlated with other factors. This is in contrast to the findings of other workers (Macler & West, 1987; Mouradi-Givernaud et al., 1992; Chirapart & Ohno, 1993; Sousa-Pinto et al., 1996), where it has been correlated with the nutrient levels in the water.

Gels produced from the three species had monthly variations in gel strength and viscosity. Rees (1969) and Lahaye & Rochas (1991) have shown that the gel strength of agar is related to its 3,6 anhydrogalactose content and sulphate.

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