Seasonal variation on size and chemical constituents of *Sargassum sinicola* Setchell et Gardner from Bahía de La Paz, Baja California Sur, Mexico

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SUMMARY

Investigation on seasonal variation in size and chemical constituents of *Sargassum sinicola* Setchell et Gardner from Bahía de La Paz, Baja California Sur, Mexico, was carried out from a control bed and compared with an experimental bed with artificial nutrients added. No significant differences were found between the control and experimental thalli for size or chemical composition, except for iodine and raw fiber. For control thalli the results were: size 7.5–56.0 cm, alginate yield 7.2–13.7%, viscosity 58.7–191.7 millipascal seconds (mPa s), mannitol 2.9–8.1%, raw fiber 5.5–7.5% and iodine 0.020–0.141%; while in the experimental thalli the size ranged from 7.5 to 80.3 cm and the alginate yield was 7.8–10.4%, viscosity 41.4–163.4 mPa s, mannitol 2.9–8.3%, raw fiber 5.9–10.7% and iodine 0.021–0.098%. These variations were related to its natural growth cycle, and showed reductions during the senescence period. Results suggest that *S. sinicola* is not affected by relatively low nutrient concentrations, and could be considered as raw material for alginate production.

Key words: alginate, chemical constituents, iodine, mannitol, raw fiber, *Sargassum sinicola*, size.

INTRODUCTION

Sargassum sinicola Setchell et Gardner is the most common and abundant seaweed in the Gulf of California (Casas-Valdez *et al*. 1993; Nuñez-López & Casas-Valdez 1997, 1998; Pacheco-Ruíz *et al*. 1998). Potential uses of *Sargassum* species are as animal food complement, fertilizer, and as a source of antibacterial substances and alginates (Chapman & Chapman 1980; Ang 1987; Padmini-Sreenivasa-Rao *et al*. 1988; Marín *et al*. 2003). Total biomass estimated for all the species of the genus *Sargassum* from the western coast of the Gulf of California is 154 000 wet tons (Pacheco-Ruíz *et al*. 1998) and 18 900 wet tons in Bahía de La Paz (Hernández-Carmona *et al*. 1990; Fajardo-León 1994). However, in spite of its wide distribution and abundance, *Sargassum* species have not been harvested commercially in the Gulf of California.

Some macroalgae (e.g. *Macrocystis pyrifera* (Linnaeus) C. Agardh) are severely affected by high water temperature and low nutrient concentrations as occur during the El Niño Southern Oscillation (ENSO). Experimental studies have demonstrated that adding artificial nutrients helps to maintain *M. pyrifera* during ENSO conditions. In contrast, other species such as *Eisenia arborea* J.E. Areschoug is more resistant to low nutrient concentrations (Hernández-Carmona *et al*. 2001). However, the effects of nutrients on the chemical constituents of *S. sinicola* are unknown. In addition, studies on the natural variation of the main chemical constituents of the alga could determine the potential use of the algae. The goal of this study was to examine the seasonal variation in the content of four of the main chemical constituents (alginate, mannitol, raw fiber and iodine) of *S. sinicola* under two conditions; without artificial nutrients (control) and with artificial nutrients added (experimental).

Study area

Bahia de La Paz, Baja California Sur, Mexico is a coastal water body adjacent to the Gulf of California with a length of 90 km and a width of 60 km. The climate is very dry and warm. The average annual rainfall in the area is 188 mm and the wettest month (62 mm) is September (Jiménez-Illescas *et al*. 1997). The sea surface water temperature in winter (December–February) ranges from 19 to 21°C, in spring (March–May) from 21 to 24°C, in summer (June–August) from 24 to 28°C and in autumn (September–November) from 28 to 18°C (González-Navarro & Saldierna-Martínez 1997). El Sauzoso, located to the

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Table 1. Average total length (cm) of experimental *Sargassum sinicola* thalli with and without (control) nutrients added, from Bahía de La Paz, Baja California Sur, Mexico. Data are mean ± standard error ($n = 30$)

Treatment	Season			
	Autumn	Winter	Spring	Summer
Experimental	7.5 ± 2.8	40.3 ± 19.5	56.3 ± 29.8	11.0 ± 2.3
Control	7.5 ± 2.8	38.3 ± 22.1	80.3 ± 28.9	16.5 ± 5.1

west of the bay at 24°18′ 26′-N and 110°38′ 17′-W, is a semiprotected site having moderate surge, with a shallow slope (Cruz-Ayala *et al*. 1998).

MATERIALS AND METHODS

This experiment is part of a broader study of *S. sinicola* in El Sauzoso. We specifically examined the effect of nutrient availability on a population recruited on three quadrats of one square meter, where artificial nutrient (Osmocote: N 14%, P 14%, K 14%) was dispensed using PVC pipes attached to concrete blocks of 56 W × 36 L × 12 H cm (Hernández-Carmona *et al*. 2001). To avoid bias in sampling, quadrats were placed at random along the coast line from where *S. sinicola* thalli were sampled. We used another three quadrats for sampling thalli in areas without nutrient enrichment (control). Thirty thalli were tagged in each quadrat with numbered plastic tags and the total length was measured each month to determine changes in thallus size. *S. sinicola* was sampled from the control and experimental quadrats seasonally. Nitrate was determinate from bottom seawater, near the quadrats (1 m^2), using the cadmium reduction technique (Strickland & Parson 1972).

In alginate extraction, the method described by Arvizu-Higuera *et al*. (2002) was used. Twenty grams of the dried and milled algal materials (30 mesh with a sieve size of 0.5 mm) were hydrated overnight with 0.1% formaldehyde and then washed with HCl at pH 4. The alginate was extracted with Na_2CO_3 at pH 10 in a water bath at 80°C for 2 h. The paste was diluted and filtered under vacuum and the clarified solution was precipitated with ethanol at a proportion of 1:1. The alginate fibers were then dried at 50°C for 12 h. Alginate yield was expressed as percentage/dry weight. The apparent viscosity of the alginate was determined in 1% (w/v) solution at 22°C with a viscometer (Brookfield LVT, Middleboro, MA, USA), at 60 r.p.m. (6.28 rad·s^{-1}) using the appropriate spindle, adding 0.5% sodium hexametaphosphate as calcium sequester.

Mannitol, iodine and raw fibers were determined using the techniques described by Larsen (1978). Mannitol was determined by a fast oxidation of the polyol, titrating with $Na_2S_2O_3.5H_2O$, with starch solution as an indicator; iodine, by fusion in the presence of NaOH to release it, adding Br and titrating with $Na_2S_2O_3.5H_2O$; and raw fiber by extraction of the soluble material in acid and alkali at high temperature, and determining the organic part remaining by the weight lost.

Data were computed to obtain the average and standard error (±1 SE) with the significance level at 95%. ANOVA was used to detect significant differences among seasons and among treatments ($P < 0.05$), and Tukey test to detect significant differences between means (Zar 1999).

RESULTS

At the beginning of the experiment, the average seawater nitrate concentration was not significantly different between the treatments with (0.62 μM) and without nutrients (0.25 μM). During winter, the concentration was significantly higher in quadrats with nutrients (8.76 μM) than without (2.55 μM). During spring, there was a trend for higher nitrate concentrations in quadrats with nutrients (6.03 μM) than the controls (2.57 μM), but this trend was not significantly different. Near the end of the experiment in summer, nitrate concentrations were not significantly different, although concentrations were still higher with nutrients (6.83 μM) than without nutrients (4.34 μM).

The average initial size of *S. sinicola* thalli was 7.5 cm high (autumn) in both control and experimental thalli. Maximum average sizes reached in spring were 80.3 cm in control thalli and 56 cm in experimental thalli (Table 1). Size showed significant seasonal variation, but it was not significantly different between treatments (Table 2).

Sodium alginate yield (Fig. 1a) and viscosity (Fig. 1b) were significantly different between seasons but not significantly different between treatments (Table 2). The highest yield was obtained in control thalli during spring (13.7%), and the highest viscosity (191.7 mIlipascal seconds (mPa s)) in winter. Alginate viscosity showed the same trend for control and experimental thalli. The minimum viscosity occurred in spring with 58.7 mPa s in control thalli and 41.4 mPa s in experimental thalli.

Minimum mannitol value was 2.9% in autumn in both treatments and increased to a maximum value in winter with 8.1% in control thalli and 8.3% in the experimental thalli. Mannitol decreased in spring and

Table 2. ANOVA for significant differences between seasons and treatments in size, viscosity and chemical composition from *Sargassum sinicola*

	Effects	d.f. effect	MS effect	F	*P*
Size	Season	3	4 613.9800	17.92	0.000*
	Nutrients	1	283.5900	1.10	0.309
	S *vs* N	3	210.5900	0.82	0.502
Yield	Season	3	24.2287	2.96	0.042*
	Nutrients	1	32.9720	4.03	0.051
	S *vs* N	3	12.7741	1.56	0.212
Viscosity	Season	3	57 767.1731	70.47	0.000*
	Nutrients	1	133.9582	0.16	0.688
	S *vs* N	3	4 620.1986	5.64	0.002*
Mannitol	Season	3	58.3993	39.66	0.000*
	Nutrients	1	0.7151	0.49	0.489
	S *vs* N	3	7.0916	4.82	0.005*
Raw fiber	Season	3	12.8379	29.04	0.000*
	Nutrients	1	19.1330	43.28	0.000*
	S *vs* N	3	15.1331	34.23	0.000*
Iodine	Season	3	0.0257	36.03	0.000*
	Nutrients	1	0.0103	14.51	0.000*
	S *vs* N	3	0.0057	8.06	0.000*

*$P < 0.05$. d.f., degrees of freedom; MS, mean square.

summer in both treatments (Fig. 1c). Differences were significant between seasons, but not between treatments (Table 2).

In raw fiber, minimum values occurred in summer (5.5 and 5.9%) in both treatments. In contrast, the maximum values were in spring for the control thalli (7.5%) and autumn (10.7%) for experimental thalli (Fig. 1d). Significant differences were obtained between seasons and treatments (Table 2).

Iodine in control plants were lower from autumn to winter and increased significantly in spring and peaked in summer (0.141%). Experimental thalli had similar iodine values as control thalli from autumn to spring and decreasing significantly in summer (0.027%) (Fig. 1e). Significant differences between seasons and treatments were obtained (Table 2).

DISCUSSION

Although nutrient concentration was higher in the experimental quadrats during winter, thalli were not significantly different in size. The thalli length from autumn represents the average size of all populations at the beginning of the experiment. During spring, in which thalli reached their maximum size of 56 cm with nutrients and 80 cm without nutrients, it was possible that high nitrate levels during spring (15 μM) (Paúl-Chávez pers. comm. 2005) in experimental quadrats might have lowered its basic metabolic process (Lobban & Harrison 1994), resulting in lower growth rates and smaller thalli. In summer, all thalli deteriorated due to natural senescence. The large variation of thallus length during winter and spring was due to the mix of old thalli, that lost part of the fronds, with new thalli that regenerated or germinated from zygotes.

Seasonal variation in alginate yield from *S. sinicola* may be related with changes in light, wave exposure, currents, temperature (Espinoza-Avalos & Rodríguez-Garza 1986; Espinoza & Rodríguez 1987) or others. However our results suggest that nutrient concentration could not be the main factor affecting the size and chemical constituents and that the variation could be related to thallus growth. In control quadrats, the thalli collected from autumn to winter were juveniles with low content of alginate (11.7%). As they matured in the spring, the cell wall and intercellular matrix increased with alginate and reached a maximum of 13.7%. In summer, senescent thalli with residual fronds had lower levels of alginate (7.2%). Similar results were obtained by Aponte de Ataola *et al*. (1983) for *Sargassum* species from Puerto Rico with yields of 7–12% in January (winter) and 17.5–20% in May (spring) for *Sargassum vulgare* C. Agardh and *Sargassum polyceratium* Montagne, respectively. Also *Sargassum swartzii* C. Agardh and *Sargassum tenerrium* J. Agardh from India had alginate yields of 19.7 and 11%, respectively (Chauhan 1970). Hernández-Carmona (1985) found values of 16.5% in winter and 27% in spring for *S. sinicola* from La Paz, Baja California Sur, Mexico; Pérez-Reyes (1997) reported *Sargassum* spp. values of 6.6% in January (winter) and 11.8% in May (spring) from the same location as the present study. The experimental thalli exposed to nutrients followed a similar trend to control thalli.

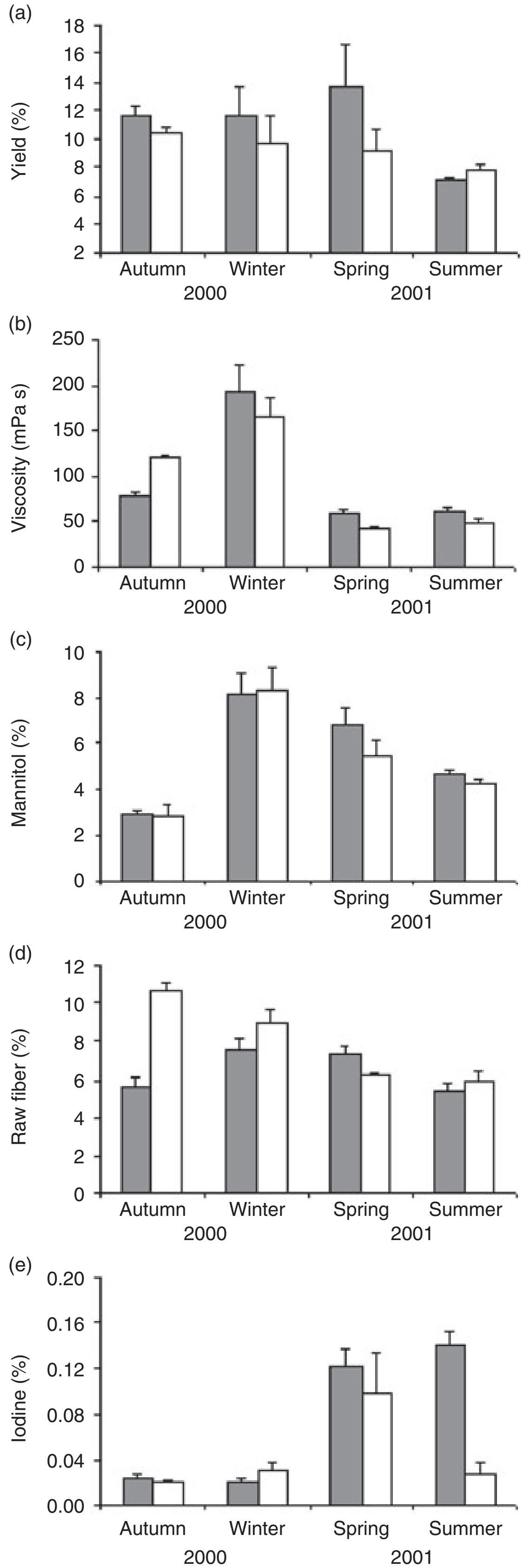

Fig. 1. Seasonal variation in average yield (a) and viscosity of 1% sodium alginate solution (b), mannitol (c), raw fiber (d) and iodine (e) of *Sargassum sinicola* collected from Bahía de La Paz, Baja California Sur, Mexico. □, experimental thalli; ■, control thalli. Vertical bars indicate the standard error.

Alginate viscosity was highly variable during the year. The lower values in the control thalli from spring to summer are correlated with senescence of thallus (Shah *et al*. 1970). The highest alginate viscosity of 191.7 mPa s in a 1% solution falls into the low viscosity type. This value was higher than that reported by Alankararao *et al*. (1988) for *S. vulgare* in India with 60 mPa s and lower than in *S. polycystum* C. Agardh with 70.8 mPa s in a 0.5% alginate solution (Saraswathi *et al*. 2003). The alginates extracted from *Sargassum* species contain a higher amount of guluronic acid blocks that produce stronger gels, as compared with alginates from *Macrocystis* (Shyamali *et al*. 1984; McHugh 1987; Saraswathi *et al*. 2003); therefore this type of alginate could be used in applications requiring the formation of strong gels.

Mannitol is a monomeric compound used by algae as a reserve and is remobilized from mature or old tissue to provide energy and carbon skeletons for growth in the meristems, and is influenced by osmotic potential (Lobban & Harrison 1994). Mannitol showed significant seasonal variation in thallus growth and reproductive condition. However, it was not significantly different between control and nourished thalli. Mannitol content in control thalli (2.9–8.1%) was, however, higher than those reported for *Sargassum ilicifolium* (Turner) C. Agardh 2–5% and *Sargassum myriocystum* J. Agardh (1.3–5.0%) in India (Chennubhotla *et al*. 1982).

The fiber method determines the amount of non-extractable organic material in the sample (Larsen 1978). In this study, the fiber content was significantly higher in experimental (5.9–10.7%) than control thalli (5.5–7.5%). These values, however, were lower than those reported by Pérez-Reyes (1997) for Mexican *Sargassum* spp. with 10.3–11.3% and by Huerta-Múzquiz *et al*. (1998) for *S. polyceratium* with 12% fiber content.

Iodine is located mainly in the stipe tissue (Kolb *et al*. 2004). Iodine content in *S. sinicola* ranged from 0.021 to 0.141%, and was higher in mature than in juvenile thalli. The iodine content for *S. sinicola* was higher than those obtained by Qing-xiang and Xiao (1998) for different species of *Sargassum* from China, including *Sargassum vachellianum* Greville (4.5×10^{-3}%), *Sargassum hemiphyllum* (Turner) C. Agardh (4.7×10^{-3}%), and *Sargassum assimile* Harvey (5.6×10^{-3}%). This suggests that *S. sinicola* has a higher capacity to store iodine than other *Sargassum* species.

In the present study it has been shown that there is an inverse relationship between the mannitol and iodine content (mannitol increasing – iodine decreasing) in all seasons and this relationship changed only in summer in experimental thalli.

Variations of the main chemical constituents may be related to the plant growth cycle and not strongly influenced by seawater nitrate concentrations. *S. sinicola* showed significant reductions during the senescence period. The results suggest that *S. sinicola* could be considered to be an important raw material for alginate production. Thus, *Sargassum* spp. should be harvested in spring, a period of maximum biomass and high yield. Although viscosity is lower in spring, gel strength could be high.

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