Aquaculture, 101 (1992) 7–16
Elsevier Science Publishers B.V., Amsterdam

Experimental tank cultivation of *Gracilaria chilensis* in central Chile

R. Ugarte[1] and B. Santelices[2]
Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile

(Accepted 16 April 1991)

ABSTRACT

Ugarte, R. and Santelices, B., 1992. Experimental tank cultivation of *Gracilaria chilensis* in central Chile. *Aquaculture*, 101: 7–16.

Gracilaria chilensis was grown continuously in tanks over a 13-month period, changing the water only every 15 days, and adding CO_2, air and nutrients. Biomass production was markedly seasonal, with a summer maximum of 100 g m^{-2} day^{-1} (wet) and a winter minimum of 10 g m^{-2} day^{-1}. Average production was 4.1 kg m^{-2} $year^{-1}$ (dry), fourfold greater than the production of wild beds. The material from tank cultivation contained 30–35% agar, as compared to 18% in wild *G. chilensis*. Epiphytism was the main problem in these cultures, and its control with chloride may reduce productivity by 40%. Pre-treatment of the water seems to be the best method to prevent epiphytes. The marked seasonality in biomass production suggests the need to change stock density, air bubbling frequency, levels of CO_2 and nutrient enrichment, and harvest frequency from summer to winter. It was estimated that such adjustments could increase the productivity of this system to 6 kg m^{-2} $year^{-1}$ of dry *Gracilaria chilensis* with an annual average agar yield of 30%.

INTRODUCTION

Growing seaweeds in tanks has several advantages over pond or open-water farming (Neish, 1979; Ryther et al., 1979; Bidwell et al., 1985). Productivity per unit area is higher than in the other types of farming, the whole production process can be effectively controlled and several steps can be mechanized, decreasing labour input. Specific types of seaweeds can be grown to be consumed as food, used as raw materials for valuable chemicals or as effectors of tertiary effluent treatment.

Due to the present market demands for *Gracilaria* (see Santelices and Doty, 1989, for a review), tank farming has been attempted at several latitudes. The

[1]Present address: Department of Fisheries and Oceans, 1707 Lower Water Street, B3L 2S7, Halifax, N.S., Canada.
[2]Address for questions and reprints

most extensive work, with *G. tikvahiae*, achieved production rates of 34.8 g dry weight m^{-2} day^{-1} (= 127 ton ha^{-2} $year^{-1}$; Hanisak and Ryther, 1986). The culture was developed in 55-l tanks, with water exchange rates of 30 times per day, vigorous air bubbling but no CO_2 additions. Subsequently, this system was scaled up to 24 000-l tanks, decreasing its production to 22–25 g dry weight m^{-2} day^{-1} (80–90 ton ha^{-1} $year^{-1}$). Although these production values are among the highest shown for any species of seaweeds, this tank farming exhibited two severe economic limitations. The method was very energy intensive because it required large amounts of flowing seawater and aeration. In addition, in the U.S.A. the requirements of large acreages of coastal land for land-based tank or raceway systems seemed to be economically prohibitive.

Tank *Gracilaria* farming in Chile has a few additional advantages that might reduce the above economic limitations. On a worldwide basis, the Chilean resource is reputed to be the *Gracilaria* crop with the highest quality gels (Guiseley, 1970; McLachlan and Bird, 1983). Successful tank farming in Chile would allow the use of the highly productive coastline of northern and central Chile, which receives high radiant energy and exhibits an abundance of upwelling areas, but whose steep slope and excessive wave exposure renders ocean farming unsuccessful except in a few, sheltered bays. Many of these are semidesertic coastal lands inadequate for agriculture or other productive activities and, therefore, of low cost.

The high investments required for tank farming of seaweeds (Hughenin, 1976) have lead to the suggestion that its true commercial success will depend on the development of polycultures in which seaweeds and other crops interact synergistically (Neish, 1979). Fish and shellfish cultivation have increased very significantly in Chile in recent years (SERNAP, 1989) and attempts are under way to cultivate salmon and turbot (Alvial et al., 1990) in tanks and raceways. The possibilities of polyfarming these species with species of *Gracilaria* is an attractive possibility that will further reduce the above economic limitations.

Tank farming of *Gracilaria* has been attempted in central Chile, but results are inconclusive. Using 200-l tanks, a water exchange rate of 25 times per day and constant air bubbling, Edding et al. (1987) obtained 15–17 g dry weight m^{-2} day^{-1} (daily mean over a year) with 2 kg wet weight m^{-2} of initial biomass. However, the inoculum of these experiments was renewed every month. Therefore, it is still unknown if this species (supposed to be *Gracilaria chilensis* Bird, McLachlan and Oliveira) could survive and grow in tank farming for extended periods. In addition, obtaining and transporting fresh *Gracilaria* from wild crops makes the frequent inoculum renewal of culture tanks economically unfeasible.

In this study we evaluate: first, if *Gracilaria chilensis* could be tank-cultivated without inoculum renewal for a 13-month period. Second, if the energy requirements of the system could be reduced, decreasing frequency of air bub-

bling and water exchange. Third, if other growth factors could be manipulated so as to optimize the biomass and agar production of this culture system.

MATERIALS AND METHODS

The experiments were performed from January 1987 to February 1988 at the coastal research station of the Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, in Las Cruces, Central Chile (33°31'S; 71°38'W). The inocula used were vegetative thalli of *Gracilaria chilensis* collected by scuba divers from wild crops at Coquimbo (30°S; 71°N) in December 1986 at 4 to 6 m depth.

Eight outdoor rectangular fiberglass tanks (1 m^3 in volume, in m^2 surface area) were used. Water was supplied by a 3-HP pump. Air was supplied by oil-free compressors during daylight hours. Air was distributed through 2.5-cm-diam PVC pipes, with 0.5-mm holes each 5.0 cm. The location of these pipes on the bottom of each tank, caused two circulation cells. A minimum flow of 40 l m^{-2} min^{-1} and a pressure of $435–725 \times 10^{-6}$ Pa was necessary to turn over 4 to 8 kg of *G. chilensis*, twice per min. CO_2 was supplied as a carbon source for the culture. The pH was maintained between 7.0 and 8.5, considered to be optimal for *Gracilaria* photosynthesis (DeBusk and Ryther, 1984). Whenever the pH rose over 8.5, CO_2 was injected by a diffuser into a water current which flowed through a PVC pipe system into the culture medium in the tanks. This design permitted the mixture of the gas with water, avoiding loss to the atmosphere (Bidwell et al., 1985).

Continuous culture

Four replicate tanks were used to examine the possibility of growing *Gracilaria chilensis* without inoculum renewal for a 13-month period. Initial stocking density was 6 kg m^{-2}. Every 15 days the biomass of each tank was put in a net for 10 min to drain excess water, then weighed to ± 10 g and the excess biomass was harvested. Simultaneously, the tanks were cleaned with fresh water and the culture medium was changed completely. The harvested biomass was used to extract agar and measure gel strength.

The culture medium was seawater enriched with $(NH_4)_2SO_4$ as a source of N and $NaOHP \times H_2O$ as a source of P in a ratio of 10:1. The quantity of nutrients added was based on the assumption that N was 5% of the dry weight of the seaweed, that the dry:wet weight relationship was 10:1, and that the species grew at 2% daily. Therefore, 0.80 g m^{-2} day^{-1} of P was added to the culture system.

As the culture aged, epiphytes developed in the tanks infesting the cultures. Three treatments were tried to control epiphytes. Hand-cleaning at the time of harvesting, covering the tanks with black plastic for 48 h and adding chlorine to the culture medium. The chlorine, in quantities of 240 ml per 1000 l

of culture medium, was maintained during 1 h in the absence of *G. chilensis*. Then 147.5 ml of sodium thiosulphate was added to neutralize the chlorine and the *G. chilensis* stocks were introduced into the tanks 15 min after neutralization.

Biomass production and agar yield of *Gracilaria chilensis* were correlated with the seasonally changing water temperature and total radiation. A daily register of tank-water temperature was obtained with a mercury thermometer, and data on total solar radiation (cal cm^{-2} day^{-1}) were obtained from the Departamento de Fisica, Universidad Santa Maria of Valparaiso, close to the site of our experiments.

Cultivation factors

The production effects of six factors were tested in short-term cultures of *Gracilaria chilensis*. In general all experiments started 21 days after acclimation of the seaweeds to outdoor conditions. The length of the experiments varied from 2 or 3 weeks to several months, depending on the factor tested. The factors studied were:

Initial inocula density: cultures were grown with 4, 6 or 8 kg m^{-2} of initial inoculum, at different seasons;

Aeration: during January 1988, cultures were grown with daily aeration periods of 2.5, 5.0 and 10.0 h;

Water exchange: 1.7 and 4.2 daily renewal volumes were tested during February 1988;

Salinity: 20‰ and 35‰ were compared in 21-day-long trials during February 1988;

Nitrogen: N additions of 0.5 and 1.0 g m^{-2} day^{-1} in the culture medium (maintaining the N:P ratio of 10:1).

Carbon dioxide: The normal thrice-daily addition of CO_2 (0.02 kg m^{-2} day^{-1}) during the summer, to maintain the pH between 7.0 and 8.5, was tested against once-daily addition (0.007 kg m^{-2} day^{-1}) during January 1988.

RESULTS

Continuous culture

Gracilaria chilensis could grow indefinitely under the outdoor growing conditions used in this study. The experiment was stopped after 13 months. During the study, the tanks and the experimental *Gracilaria* thalli were repeatedly colonized by epiphytes. Species of *Enteromorpha, Ectocarpus* and *Ulva* were the most frequent colonizers. *Ulva* spp., occurred first in the middle of February (summer) and appeared particularly at the base of the *Gracilaria chilensis* thalli. The density of *Ulva* was low and the original infestation could have come from the wild *G. chilensis* population. The rest of the year, juveniles of *Ulva* were not evident. *Enteromorpha* spp. appeared regularly

throughout the year, although they were more abundant in spring and summer. These algae grew very fast, completely covering the tank walls and much of the *Gracilaria* thalli in less than 20 days. *Ectocarpus* spp. appeared at the end of the winter, when the water temperature was over 14°C. These algae were attached to several parts of the thalli but their growth was more limited than that of *Enteromorpha*. Diatoms occurred frequently in the warm months of spring and summer, but they did not affect the growth of *Gracilaria*. They were easily cleaned from tank walls and thalli with water jets.

In general, epiphytism problems diminished considerably during winter, when the radiation and water temperature of tanks were low. *Gracilaria* thalli fragmented or damaged by manipulation or other factors provided more suitable substrata for epiphytes.

Several epiphyte removal techniques were successively applied to the replicate tanks, attempting to reduce infestation without damaging the *Gracilaria* crops. The first method used, removal by hand-cleaning, was labour intensive and would be impractical in large culture units. Moreover, excessive manipulation of thalli caused fragmentation and rupture, reducing growth.

Covering tanks with black plastic allowed the *Gracilaria* cultures to survive in darkness, eliminating *Ectocarpus* and reducing by 80% the quantity of *Enteromorpha*. However, *Gracilaria* did not grow. Treatment with 4–6% commercial chlorine for 6–10 h killed 80% of the *Enteromorpha* and *Ectocarpus* thalli. However, despite bleaching of *Ulva* fronds, the basal parts remained viable. These basal parts could subsequently regenerate the frond and the repeated use of chlorine produced injuries to the apical meristem of *Gracilaria*, reducing growth. After realizing this effect, the culture medium was treated with chlorine and then precipitated with thiosulphate prior to circulation in the culture in order to kill epiphyte propagules without damaging *Gracilaria*. However, the apical damage caused decreased growth during the spring (1987) and summer (1988) months.

Total yield in our tanks (Fig. 1) was 14.11 kg of dry matter m^{-2} (11.3 g m^{-2} of dry matter daily). The highest production values in our experimental tanks occurred during the first 4 months of culture, exceeding 100 g m^{-2} day^{-1} of wet matter (16 g dry weight). This occurred at a time of high solar radiation and high temperature. The lowest production was in winter, reaching 9–10 g m^{-2} day^{-1} of wet weight in July. Due to apical damage produced by epiphyte treatment, the growth values during spring and summer (1987–1988) did not reach those obtained during the first 4 months of culture, and the maximum production did not exceed 70 g m^{-2} day^{-1} of wet matter.

The agar gel yields of the material grown in tanks are substantially higher than those of wild crops (Fig. 1). Agar content of recently collected field material and of the tank-grown material, during the first month of culture was 18.0%. In the following months, the values for tank-grown *Gracilaria* rose to

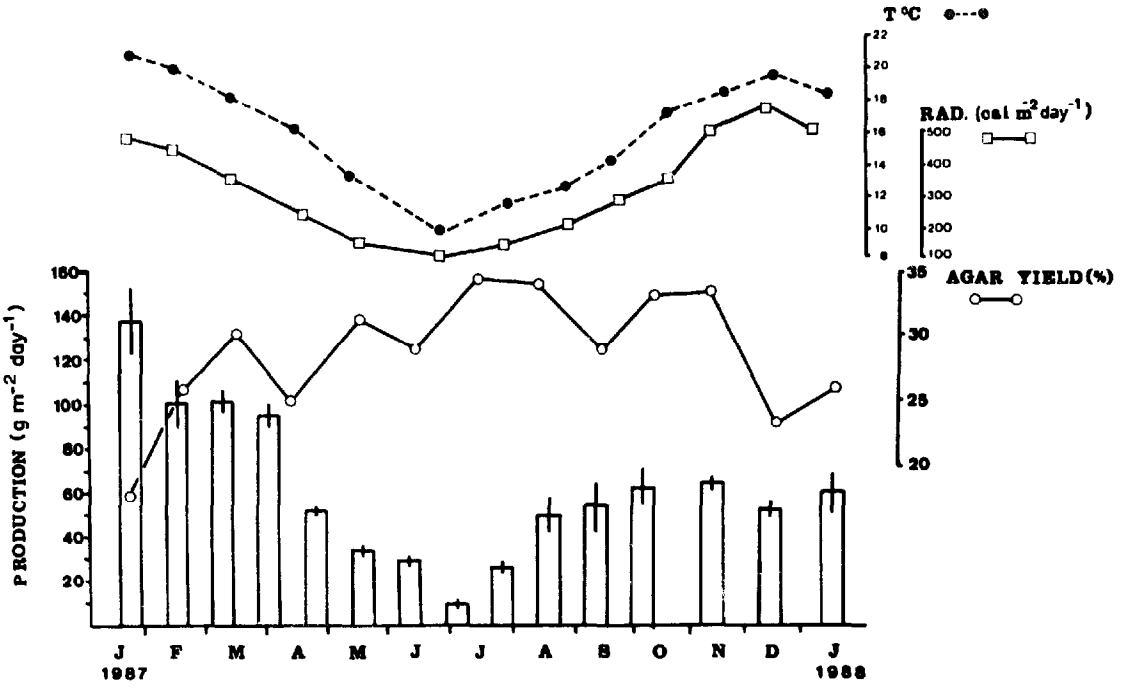

Fig. 1. Biomass production (wet matter) and agar yield of tank-cultivated *Gracilaria chilensis* as a function of monthly variations of light and temperatures.

34% and subsequently oscillated between 23.4% and 34.0%. Annual mean agar content was 29.1% and the total production of agar was 1.1 kg m^{-2} $year^{-1}$.

Effects of cultivation factors

Initial density of inocula. During the winter months, there were no differences between low (4 kg m^{-2}), medium (6 kg m^{-2}) and high (8 kg m^{-2}) inoculum density (Table 1). In spring, low inoculum density gave substantially greater growth (90 ± 4 g of wet matter m^{-2} day^{-1}) than other densities, while in summer high density stocking was more productive. Stocking densities of less than 3 kg m^{-2} enhanced epiphyte infestation and resulted in reduced *Gracilaria* growth at all seasons. Agar yields did not correlate with density variations. While in spring agar yields at high-inoculum densities were higher than agar yields produced by low-inoculum densities, the opposite trend occurred in summer.

In summary, the greatest growth of *Gracilaria* can be achieved in cultures with initial densities of 4 kg m^{-2} in fall, winter and spring and 8 kg m^{-2} during summer.

Aeration. Five h of air bubbling during daylight increased production only slightly above that obtained with 2.5 h (Table 1). Therefore, a reduction of almost 50% in air circulation costs would result in a reduction of only 15 to

TABLE I

Biomass production and agar yield in thalli of *Gracilaria chilensis* submitted to different levels of various factors

Experimental factor	Experimental level	Production ($g\ m^{-2}\ day^{-1}$)	Agar yield (%)
Inoculum density and season	Winter–low	34± 6	14.47
	Winter–high	35± 1	17.47
	Spring–low	90± 4	33.92
	Spring–high	70± 5	38.74
	Summer–low	8± 4	26.39
	Summer–high	120±10	21.13
Air bubbling (per day)	2.5 h	55± 5	26.10
	5.0 h	63± 4	26.07
	10.0 h	103± 8	27.90
Water exchange (vol.)	1/15 days	22± 4	23.01
	1.7/day	40± 5	28.95
	4/day	25± 3	28.29
Salinity (‰)	20	38± 3	22.63
	35	22± 2	30.71
Nutrients	N:0.5 $g\ m^{-2}\ day^{-1}$	60± 4	27.17
	N:1.0 $g\ m^{-2}\ day^{-1}$	62± 4	32.53
CO_2 ($kg\ m^{-2}\ month^{-1}$)	0.2	83± 4	22.57
	0.56	120± 6	21.13

20% in the production of *Gracilaria*. At 10 h daily bubbling, production was doubled from 63.0 ± 4 g m^{-2} day^{-1} to 102.5 ± 8 g of wet matter m^{-2} day^{-1}. Changes in bubbling frequency did not modify agar yields. Agar yield was 26% with 2.5 and 5.0 h of air bubbling, and 28% under the 10 h bubbling regime.

Water exchange. During the first 7 days of the experiment, greatest growth was observed in tanks with maximum water exchange. However, epiphyte growth was also greater in these tanks. After 3 weeks, the growth of *Gracilaria* with more frequent water exchange was similar to that obtained with no exchange (Table 1). The most productive system used was 1.7 volumes of daily water exchange. However, although the epiphyte density was low, it was greater than in tanks with no exchange of water. In winter, the epiphyte problem was not as great as in summer. Therefore, the frequency of water exchange could be increased in winter, while in summer water changes could be restricted to periods of harvest and tank cleaning. Nevertheless, it is necessary to evaluate whether the higher productivity obtained with daily exchange of water would compensate for the high pumping cost.

Salinity. 20‰ initial salinity of the culture medium produced a higher growth rate and lower agar yield of *Gracilaria chilensis* than 35‰ salinity (Table 1).

Nitrogen. In these experiments, increasing N concentration in the culture medium did not produce any noticeable increase in the growth rates of *Gracilaria* (Table 1). With both 0.5 and 1.0 g N m^{-2} day^{-1}, production values were close to 60 g m^{-2} day^{-1}.

Carbon dioxide. The reduction of CO_2 in the culture medium diminished production levels of *Gracilaria* by 30% (Table 1). The thalli of *Gracilaria* rapidly used the available CO_2, raising the pH values in the culture medium over 9.5 and producing necrosis in the apices of the seaweeds.

DISCUSSION

The results obtained in this research suggest that cultures of *Gracilaria chilensis* can be maintained for a long period with a water exchange every 15 days, CO_2, air, and nutrient additions and mechanisms of epiphyte control. The production pattern, although strongly seasonal, showed positive production at all times during the year. Repeated chlorine applications to control epiphytes, reduced the production by 40% at the end of spring and summer. Nevertheless, the production of *Gracilaria* in our experimental tanks reached up to 4.1 kg m^{-2} $year^{-1}$ of dry biomass, representing four times the production of natural and farmed beds of *Gracilaria* in Chile (Pizarro, 1986).

Agar yields of 29–30% of dry weight in cultured *Gracilaria* greatly exceed the 18–22% values for natural beds (Cancino et al., 1987). It is evident that the high gel yield of *Gracilaria* cultured in tanks can compensate for periods of low biomass production. There seem to be several reasons for the increase in gel content. The seaweeds produced in tanks are relatively free of sand, epiphytes, animals and other contaminants frequently found in wild crops. Moreover, the nutrient levels seem also to be optimal for gel formation. These findings are consistent with data on *Gracilaria* by Craigie et al. (1984) who report that the optimal N range to produce maximum biomass was coincident with the level required for maximum agar production.

Enteromorpha spp. epiphytism constituted the principal problem in our continuous culture system through the year. By the end of our experiments, it was evident that this pest can be managed in several ways; an integrated control strategy using several alternative methods should probably be implemented in the future. A first possible step is to obtain the water for culture from habitats where propagules of *Enteromorpha* are less abundant. A second measure, is to filter the seawater used. A third step, proven of value in our experiments, is to pretreat the water with chlorine and then to neutralize it

with thiosulphate. An effective and sustained application of these sterilizing mechanisms would permit control of epiphytes in cultures, as occurred in all of our experiments started after June 1987. The danger of infection by epiphytes is, however, a reality and should be considered not only in the management practices of these cultures, but also in tank design and other facilities.

The effect of environmental variation on the growth of *Gracilaria* suggests that alternative cultivation strategies should be applied in different seasons. To enhance summer production and reduce winter costs, culture conditions in high- and low-production seasons should be not be identical. Furthermore, a gradual change from one condition to the other must be made in spring and fall. During the low-production season, the optimal inoculum density should not exceed 4 kg m^{-2}, air bubbling should be 2.5 to 3.0 h daily, distributed in fractions of 15 to 30 min, consumption of CO_2 is close to 0.14 kg m^{-2} $week^{-1}$ while optimal nutrients are about 0.4 g m^{-2} day^{-1} of N and 0.04 g m^{-2} day^{-1} of P. As high salinities decrease production, special care should be taken to compensate for water evaporation in the tanks. During summer, optimal inoculum density should be 8 kg m^{-2}; air bubbling should be constant during the daylight and consumption of CO_2 will approach 0.21 kg m^{-2} $week^{-1}$. The N and P volumes added to the culture medium should be twice the winter values. However, if we consider that changes in initial density of inocula (8 kg) can increase by 20% the biomass produced in our continuous experiments, that air bubbling of 10 h daily will increase the production by 35% with respect to that obtained with 5 h, and that 20% salinity will further increase production by 30%, it is highly probable that total nutrient values to add to the system must be recalculated. In turn, these increments in biomass will force a more frequent harvest, perhaps weekly, during summer.

Some of the highest costs of maintaining seaweed in a tank culture system (i.e. for water renewal and air pumping) have been reduced considerably with *Gracilaria chilensis* in our experimental study. However, other factors such as CO_2 seem to be less flexible. The next necessary step is the construction of a commercial unit to evaluate the behavior of *G. chilensis* in large-scale tanks, and the technical and economic feasibility of such culture by itself or in polyculture systems.

ACKNOWLEDGEMENTS

This research was supported by research grants CORFO-CHILE and FONDECYT 803/90 to the second author. Our gratitude to A. Kalergis and A. Sanhueza for help during early stages of this study and to G. Sharp and K. Warkenting for reading and commenting on the manuscript. This paper was written while the senior author was in a training program at the Department of Fisheries and Oceans, Halifax, Canada. His acknowledgements to J.D. Pringle for support and facilities provided.

REFERENCES

Alvial, A., Vidal, L. and Wurman, C., 1990. Turbot: una nueva especie de cultivo en Chile. Aquanotic. Int., 6: 4–7.

Bidwell, R.G.S., McLachlan, J.M. and Lloyd, N.D., 1985. Tank cultivation of Irish moss, *Chondrus crispus* Stackh. Bot. Mar., 28: 87–97.

Cancino, J.M., Muñoz, M. and Orellana, M.C., 1987. Effects of epifauna on algal growth and quality of the agar produced by *Gracilaria verrucosa* (Hudson) Papenfuss. Hydrobiologia, 151/152: 233–237.

Craigie, J.S., Wen, Z.C. and Van der Meer, J., 1984. Interspecific, intraspecific and nutritionally determined variations in the composition of agars from *Gracilaria* spp. Bot. Mar., 27: 55–61.

DeBusk, T.A. and Ryther, J.H., 1984. Effects of seawater exchange, pH and carbon supply on the growth of *Gracilaria tikvahiae* (Rhodophyceae) in large-scale cultures. Bot. Mar., 27: 357–362.

Edding, M., Macchiavello, J. and Black, H., 1987. Culture of *Gracilaria* sp. in outdoor tanks: productivity. Hydrobiologia, 151/152: 369–374.

Guiseley, K.B., 1970. The relationship between methoxyl content and gelling temperature of agarose. Carbohydrate Res., 13: 247–256.

Hansiak, D. and Ryther, J.H., 1986. The experimental cultivation of the red seaweed *Gracilaria tikvahiae* as an "energy crop": an overview. In: W.R. Barclay and R.P. McIntosh (Editors), Algal Biomass Technologies. Beih. Nova Hedwigia 83. J. Cramer, Berlin, pp. 212–217.

Hughenin, J.E., 1976. An examination of problems and potentials for future large-scale intensive seaweed culture systems. Aquaculture, 9: 313–342.

McLachlan, J. and Bird, C.J., 1983. *Gracilaria* in the seaweed market: a prospectus. In: H.R. Fuentes, J.C. Castilla and L.H. Disalvo (Editors), Symposium Internacional Avances y Perspectivas de la Acuacultura en Chile. Universidad del Norte, Coquimbo, Chile, pp. 133–157.

Neish, I.C., 1979. Principles and perspectives of the cultivation of seaweeds in enclosed systems. In: B. Santelices (Editor), Actas del Primer Symposium sobre Algas Marinas Chilenas. Subsecretaría de Pesca, Ministerio de Economía, Fomento y Reconstrucción, Santiago, Chile, pp. 59–74.

Pizarro, A., 1986. Conocimiento actual y avances recientes sobre el manejo y cultivo de *Gracilaria* en Chile. Monografías Biológicas, 4: 63–96.

Ryther, J.H., DeBoer, J.A. and Lapointe, B.E., 1979. Cultivation of seaweed for hydrocolloids, waste treatment and biomass for energy conversion. Proc. Int. Seaweed Symp., 9: 1–16.

Santelices, B. and Doty, M.S., 1989. A review of *Gracilaria* farming. Aquaculture, 78: 95–133.

SERNAP, 1989. Anuario Estadístico de Pesca. Servicio Nacional de Pesca, Ministerio de Economía, Fomento y Reconstrucción, Santiago, Chile, 183 pp.