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Testing Open-Water Cultivation Techniques to *Gracilaria domingensis* (Rhodophyta, Gracilariales) in Santa Catarina, Brazil

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ABSTRACT

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The aim of this work was to develop field techniques to cultivate *Gracilaria domingensis* in association with mussel cultivation. This red algae that has been used as food, feed for marine animals and especially for agar production. The experiments were done at Armação do Itapocoroy Bay, Penha, Santa Catarina State, Brazil, where there is an extensive commercial mariculture of the mussel *Perna perna*. The rational behind this work is that the production of this seaweed, besides constituting an additional cash-crop to the fishermen that farm mussel, would also contribute to improve the water quality by absorbing excess nutrients, mainly nitrogen and phosphorous compounds, released by the mollusks. Different systems to attach seaweed thalli to suspended ropes were tested. Depth, growth period and location, inside and outside the bay, were also tested. Production was assessed through weight variation every week for four weeks. Results indicated that *G. domingensis* growth rates may be considered high in comparison with other red seaweed cultivated elsewhere, reaching up to 10 % day^{-1}. It was concluded that the cultivation of *G. domingensis* is technically feasible in the mussel cultivation grounds inside the bay, using the first two meters depth, inserting plants into the strand of the rope, and harvesting is more productive if made at intervals not longer than 15 days, although its economically viability is pending on further studies.

ADDITIONAL INDEX WORDS: *Vertical cultivation, integrated cultivation, agarophyte.*

INTRODUCTION

Gracilaria species are utilized as human food in Japan (OHMI, 1958), Hawaii (HOYLE, 1978; ABBOTT, 1988) and in the Philippines, and it is also used to feed marine cultivated animals (*e.g.* abalone) in Taiwan (CHIANG, 1981) and Israel (NEORI *et al.*, 1998). However, its major economical importance is as a source of agar (Oliveira *et al.*, 2000). As the natural beds of *Gracilaria* and other commercial agarophytes, namely *Pterocladiella*, *Pterocladia* and *Gelidium* are limited and in some cases overexploited, cultivation is the only alternative to supply the growing demand and with sustainable production (OLIVEIRA *et al.*, 2000).

Integrated cultivation of macroalgae and mollusks, fishes or shrimps is being attempted in several places (BUSCHMANN *et al.*, 1996; CHOPIN *et al.*, 1999; CHOW *et al.*, 2001; ELLNER *et al.*, 1996; KROM *et al.*, 1995; NEORI, 1996; NEORI *et al.*, 1996; NEORI *et al.*, 1998; QIAN *et al.*, 1996). In these systems, excess feed animal excrements produce a great quantity of dissolved nutrients that can cause eutrophization and degradation of the environment. It is well known that macroalgae act as biofilters, absorbing nitrogen and phosphorous compounds, and improving the water quality. On the other hand, because of the high nutrients availability in animal cultivation grounds, macroalgae present high growth rates.

The farming of the mussel *Perna perna* reached a significant scale in the Santa Catarina State, amounting to about US$ 1,5 millions per year (POLI & LITTLEPAGE, 1998). Mussel farming is also very important socially in Santa Catarina, where over than 700 farms are in activity having produced 8.641 ton in 2002 (G. MANZONI, pers. comm., 2003). However, this accelerated growth has been a reason of concerns, once there are indications that the production has exceeded the carrying capacity in some areas (SUPLICY, 2000). Thus, the integrated cultivation of the macroalgae *Gracilaria domingensis* in mussel farms could represent a potential alternative to the expansion and diversification in the mariculture activities in the Santa Catarina State. Besides improving the water quality, it could yield an additional cash crop to the fishermen engaged in mariculture.

The aim of this work was to adapt cultivation techniques that make viable the cultivation of the agar producing macroalga *Gracilaria domingensis* integrated to mussel mariculture in the Santa Catarina State.

METHODS

The experiments were done in the Armação do Itapocoroy Bay, Penha, Santa Catarina State, Brazil (26°47'S - 48°37'W), where there is a large cultivation park of the mussel *Perna perna*.

The algae were collected at rocky shores near the mussel cultivation grounds and cleaned up from epiphytes. The algae were cut in pieces of about 2 g, labeled, weighted and inserted into the cultivation ropes 20 cm from each other. The ropes were placed in two different sites: inside the bay (near to intensive mussel cultivation) and outside the bay (far from intensive mussel cultivation). The ropes were placed vertically, so the plants were kept at depths ranging from 20 to 200 cm.

Growth rates were weekly evaluated during four weeks, based on variation in the fresh weight of each plant. Twenty-four ropes were placed in each site and at the end of every week, six ropes were collected from the inside site and six from the outside site. Although the individual growth rates were obtained, for the purpose of comparison, thalli placed from 20 to 100 cm depth were grouped into the first meter (1 m) whereas those kept from 120 to 200 cm depth were grouped into the second meter (2 m).

To test the best way to attach the algae to cultivation ropes, four types of attachment were tested: 1) inserted into the strands of the rope (IS); 2) tied using hard plastic strings (HP); 3) tied using soft plastic bag strings (SP); and 4) tied using cotton strings (CO). The algae were attached to the cultivation ropes 20 cm from each other (six replicates each treatment) and cultivated at depths ranging from 20 to 200 cm (grouped into 1 m and 2 m cultivated plants, as explained above), outside the bay, during 1 week.

Relative Growth Rate (RGR, % day^{-1}) of each plant was estimated to thalli that presented increase of biomass during cultivation time, according to linear equation of growth (DAWES, 1998):

RGR= (((Wf - Wi)/ Wi)/ t)*100 (Equation 1)

Where: Wi= initial fresh weight (g), Wf= final fresh weight (g), t= cultivation time (days).

The total increase of biomass, at the end of each cultivation period was estimated for each rope, at each depth, represented as percentage of initial biomass (averaged for all replicates in each treatment). Negative yield values were obtained when many plants got lost.

The relative growth rates and biomass yield inside and outside the bay were compared at 1 m and 2 m depth, different cultivation time (one to four weeks) and different types of attachment to the ropes (IS, HP, SP, CO) using ANOVA and the posteriori test of Tukey HDS.

RESULTS

The averaged growth rates observed inside the bay (from 7.84 to 11.34 % day^{-1}; Tables 1 and 2) were always more than twice the values observed outside the bay (from 2.48 to 3.71 % day^{-1}; Tables 3 and 4).

Comparing both sites in one-week cultivation, the plants cultivated inside the bay presented growth rates significantly higher than plants cultivated outside the bay for first meter as well as for the second meter depth ($p<0.01$ for both comparisons).

At the end of the second week of cultivation the growth rates of plants cultivated inside the bay were similar to outside ($p>0.08$) for both, 1 and 2 m depth. However, the total yield per rope was much smaller at outside site because many thalli were lost, despite high growth rates were obtained (Tables 1 to 4).

If one compares the number of losses in the two sites, with one week of cultivation, losses of plants were not a great problem in this site, even in longer periods of cultivation (only one plant was lost for each depth inside the bay; Tables 1 and 2). On the other hand, during the first cultivation week, more than a half of the plants were lost outside the bay, and these numbers sharply increased with cultivation time, resulting in the lost of all plants in the fourth week (Tables 3 and 4).

Plants cultivated inside the bay also could be kept into sea and even grown for longer periods (4 weeks; Tables 1 and 2) than plants cultivated outside the bay (2 weeks; Tables 3 and 4).

Biomass yields measured inside the bay were always positive (Tables 1 and 2), meaning that there was higher biomass production than losses. This pattern was not observed outside the bay (Tables 3 and 4), where there were negative biomass yields values in all the cultivation times and depths tested.

In the first meter inside the bay, there was an increase in the biomass yields with cultivation time but it was proportionally higher in the first and second cultivation weeks. In the second meter at this site, the biomass yields were lower than in the first meter, but the same pattern to the first meter was observed, with proportionally higher biomass yields in the first two cultivation weeks.

Table 1. *Growth rates of plants cultivated inside the bay, at 1 m depth in each cultivation time. NI: initial number of plants; NL: number of lost plants during the interval; biomass yield is the average of earnings (+) or losses (-) of biomass during the interval; and growth rate as average and standard deviation.*

Time	NI	NL	Biomass yield during interval (%)	Growth rate % day^{-1}
1 week	30	1	+ 40.36	9.12 ± 4.74
2 weeks	30	9	+ 73.95	10.54 ± 6.75
3 weeks	30	9	+ 90.55	8.54 ± 7.40
4 weeks	30	12	+ 121.39	11.34 ± 13.82

Table 2. *Growth rates of plants cultivated inside the bay, at 2 m depth in each cultivation time. See codes in table 1.*

Time	NI	NL	Biomass yield during interval (%)	Growth rate % day^{-1}
1 week	30	1	+ 36.46	7.84 ± 4.06
2 weeks	30	9	+ 45.50	8.12 ± 4.90
3 weeks	30	11	+ 73.61	9.87 ± 7.07
4 weeks	30	14	+ 73.25	9.53 ± 9.50

The biomass yields outside the bay were always negative, showing that the losses were greater than the biomass production. The biomass losses increased with cultivation time and were higher in the second meter than in the first.

The comparison of the attaching techniques of the thalli to the ropes showed that the tied plants presented higher averaged growth rates than the inserted ones, but these differences were not significant (Tables 5 and 6). The only exception was for the algae tied by cotton strings, where first meter presented growth rates significantly higher than in the second meter (Table 5 and 6).

As occurred in the later experiment, the growth rates decreased with depths (Tables 5 and 6) and these differences were not significant either.

Tied plants also presented lower number of losses than the inserted plants and the number of losses usually was higher in the first meter than in the second depth for all treatments tested (Tables 5 and 6).

The biomass yield in this experiment showed no significant differences among the treatments to plants cultivated at 1 m depth. However, the biomass yields of plants inserted into the ropes in the second meter depth were significantly lower than the biomass yields of plants tied with soft plastic bag strings ($p= 0.03$).

DISCUSSIONS

Despite the variability of the results, it is shown that *Gracilaria domingensis* growth rates as well as biomass yields were high enough to become economically interesting. Biomass yields of about 40% in one week of cultivation are very attractive, indicating good perspectives to cultivate *G. domingensis* at this site. The observed values are even more attractive when compared to other studies and species of *Gracilaria* (Table 7).

Table 3. *Growth rates of plants cultivated outside the bay, at 1 m depth in each cultivation time. See codes in table 1.*

Time	NI	NL	Biomass yield during interval (%)	Growth rate % day^{-1}
1 week	30	16	- 7.70	3.71 ± 2.84
2 weeks	30	26	- 23.16	2.48 ± 1.69
3 weeks	30	30	- 86.96	-
4 weeks	30	30	- 98.47	-

Table 4. *Growth rates of plants cultivated outside the bay, at 2 m depth in each cultivation time. See codes in table 1.*

Time	NI	NL	Biomass yield during interval (%)	Growth rate % day^{-1}
1 week	30	17	- 23.16	3.01 ± 2.31
2 weeks	30	25	- 38.00	2.97 ± 2.67
3 weeks	30	29	- 78.27	-
4 weeks	30	30	- 94.61	-

Table 5. *Growth rates of plants cultivated outside the bay, at 1 m depth in each attachment type. NI: initial number of plants; NL: number of lost plants during the interval; growth rate. IS: inserted into strand; HP: tied with hard plastic strings; SP: tied with soft plastic bag strings; CO: tied with cotton strings.*

Attachment type	NI	NL	Biomass yield during interval (%)	Growth rate % day^{-1}
IS	40	8	+ 40.13	8.26 ± 5.12
HP	30	2	+ 56.56	9.38 ± 5.05
SP	30	3	+ 55.87	10.17 ± 4.81
CO	30	3	+ 57.08	10.63 ± 4.20

Table 6. *Growth rates of plants cultivated outside the bay, at 2 m depth in each attachment type. See code in table 5.*

Attachment type	NI	NL	Biomass yield during interval (%)	Growth rate % day^{-1}
IS	40	11	+ 17.37	6.22 ± 4.52
HP	30	3	+ 46.92	8.31 ± 4.60
SP	30	3	+ 49.19	8.96 ± 4.02
CO	30	5	+ 29.65	7.18 ± 3.45

Table 7. *Comparative table of averaged growth rates to different species cultivated under different techniques.*

Species	Cultivation Technique	Country	RGR (% day^{-1})
G. domingensis[1]	Vertical	Brazil	2.48 to 10.63
G. gracilis[2]	Vertical	South Africa	1 to 10
G. gracilis[3]	Horizontal	Namibia	0.84 to 4.15
G. cornea[4]	Horizontal	Venezuela	1.4 to 2.5

[1]Present work; [2]ANDERSON *et al*., 2001; [3]DAWES, 1995; [4]RINCONES LEON, 1989.

The low biomass yields achieved outside the bay compared to that observed inside the bay in the cultivation depth and time experiments, was probably a consequence of surge storms that occurred just after the beginning of the experiments. The area inside the bay is more protected and even during the storms, the loss of plants was not a problem. As the area outside the bay is much more exposed to waves, during the storms the algae could be broken and lost, which resulted in negative biomass yields in the experiments of cultivation depth and time. Despite this, very high biomass yields were attained in this same site in the attachment types experiment, in which no surge storms occurred and biomass yields reached 57% in one week. Growth rates in this experiment were also very high, ranging from 6.2 to 10.6 % day^{-1}. These results indicated very good potential for cultivation of *G. domingensis* inside the Armação do Itapocoroy Bay, as well as outside the bay, although in the latter site storms could became a problem.

The influence of surge storms was also decisive to select the cultivation time. After 1 week of cultivation, the probability of breaks or losses of the plants strongly increased. In all experiments comparing cultivation time, best biomass yields could be achieved if the algae were planted and harvested every week. An increase in harvesting frequency also improves the yield by decreasing the probability of thallus fragmentation.

The comparison of the different ways tested to attach the thalli to cultivation ropes showed some variability, but the differences were not significant, at least for the first meter depth. Inserting plants into ropes are faster compared to other ways to attach the plants, which is an advantage because it requires less labor work and no expenses with strings. Another advantage is that because it is faster, plants suffer less desiccation stress.

Seaweed cultivation around the world is usually made by attaching the thalli to horizontal ropes near the sea surface (*e.g.* Dawes, 1995; Rincones Leon, 1989). In the case of *Gracilaria* species, as they can tolerate a large range of irradiances (Dawes, 1995) it is possible to cultivate it in vertical ropes what allows a better exploitation of the water column and higher yield per area. This is confirmed by our experiment that shows high growth rate up to 2 m depth, giving good yields.

Similarly to our results for *G. domingensis*, Anderson *et al*. (2001) found values for *G. gracilis* ranging from 6 to 7 % day^{-1} at 1 m depths and from 4.5 to 6 % day^{-1} at 2 m depths in an upwelling site at South Africa. Comparison of these results evidences the good perspectives to *G. domingensis* cultivation in the Santa Catarina State.

CONCLUSIONS

The long line system used in *Perna perna* cultivation seems to work well also to cultivate *Gracilaria domingensis*. This is highly advantageous once the structures already exist and the integrated cultivation of algae could represent a good alternative to increase the fishermen earnings.

We have shown that the cultivation of *G. domingensis* is technically feasible in the mussel cultivation grounds inside and outside the Armação do Itapocoroy Bay, using the first two meters depth, inserting plants into the strand of the rope, and harvests should be made every week.

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