Available online at www.sciencedirect.com
SCIENCE DIRECT®
Aquaculture
Aquaculture 240 (2004) 201–209
www.elsevier.com/locate/aqua-online

Agricultural fertilizers as alternative culture media for biomass production of *Chondracanthus squarrulosus* (Rhodophyta, Gigartinales) under semi-controlled conditions

I. Pacheco-Ruíz*, J.A. Zertuche-González, E. Arroyo-Ortega, E. Valenzuela-Espinoza

Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Ensenada Baja California, México

Received 21 February 2004; received in revised form 24 May 2004; accepted 26 May 2004

Abstract

The red alga *Chondracanthus squarrulosus* was cultured under semi-controlled conditions to evaluate growth (biomass production) with agricultural fertilizers (ammonium nitrate, ammonium sulphate and urea) vs. analytical grade inorganic salts; sodium nitrate (analytical grade) and seawater were used as controls. The concentration of each agricultural fertilizer used was 1.95 mg l^{-1} $week^{-1}$. Growth of *C. squarrulosus* with agricultural fertilizers and sodium nitrate as control showed no significant differences ($P<0.05$). A maximum growth of 5.4% per day and thallus nitrogen content of 3.4% dry weight were obtained with ammonium sulphate. The results suggest that commercial agricultural fertilizers can be substituted for analytical grade nutrients with no significant effect on the culture.

Keywords: Biomass production; Agricultural fertilizers; *Chondracanthus squarrulosus*; Nutrients

* Corresponding author. Tel./fax: +52 646 1745451.
E-mail address: isai@uabc.mx (I. Pacheco-Ruíz).

0044-8486/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2004.05.044

1. Introduction

Seawater and analytical grade inorganic salts constitute the base for the preparation of culture media (BoId and Wynne, 1978). Regardless of the complexity and effectiveness of the media commonly used in the cultivation of algae, production costs are often prohibitive for commercial cultivation, particularly in seaweed cultivation where large volumes are required (Craigie and Shacklock, 1989; Bidwell et al., 1985). For the commercial cultivation of seaweed, it is necessary to consider low-cost alternatives.

The most important nitrogen sources for macroalgae are ammonium (NH_4^+) and nitrate (NO_3^-), though not all macroalgae grow equally with these sources. In addition, growth rates are not always as significant as those obtained with other nitrogen sources (Hanisak, 1983; Hargreaves, 1988).

Due to the high cost of analytical grade inorganic salts, research has been conducted on possible alternatives that would reduce production costs. Pacheco-Ruíz et al. (1987) cultured *Chondracanthus canaliculatus* (Harvey) Guiry using aerobic digested cow manure. The medium was effective, inexpensive and a good nutrient source for this species, and the results were similar to those obtained with other traditional media. Another alternative is to replace analytical grade inorganic salts by agricultural fertilizers as nutrients. They are inexpensive and have been used in microalgal cultures, demonstrating their nutritional efficiency with respect to the f/2 medium (Granados-Machuca and Buckle, 1984; Valenzuela-Espinoza et al., 1999).

Chondracanthus squarrulosus (S. and G.) Hughey, P.C. Silva et Hommersand (Rhodophyta, Gigartinales) (previously reported as *Gigartina canaliculata* and *C. canaliculatus*) is an endemic carrageenophyte from the Gulf of California. Its high carrageenan content (López-Acuña et al., 2002), along with high in situ growth rates, has motivated several studies to evaluate its potential for culturing (Pacheco-Ruíz et al., 1992; Pacheco-Ruíz, 1999).

This study considers the hypothesis that agricultural fertilizers, like traditional analytical quality nutrients, serve as nitrogen and phosphorous sources for the culture of *C. squarrulosus*, and aims to determine the most appropriate agricultural fertilizer (ammonium nitrate, ammonium sulphate or urea) for the culture of this species under semi-controlled conditions.

2. Materials and methods

C. squarrulosus biomass was collected from Bahía de los Ángeles, in the Gulf of California (Mexico). Prior to the experiment, thalli were allowed to grow under semi-controlled conditions for 2 weeks without the addition of fertilizers, in culture tanks with open seawater flux. The experiments were performed using a closed system of culture tanks with continuous air bubbling, and the seawater was changed weekly. The culture tanks have been described in detail by González-Gómez et al. (1992).

The experiment was done in winter. Fifteen 100-l tanks were used, each containing 500 g of wet thallus under a nitrogen concentration of 1.95 mg N l^{-1} $week^{-1}$ regardless of the nitrogen source used. The agricultural fertilizers tested were: ammonium nitrate

(NH_4NO_3), ammonium sulphate ($(NH_4)_2$ SO_4) and urea (H_2NCONH_2), as nitrogen sources, and phosphorous pentaoxide, as phosphorous source. Sodium nitrate ($NaNO_3$) and disodic phosphate (Na_2HPO_4) were used as control (analytical grade inorganic salts) at a concentration of 10:1 (N/P). Pulse fertilization with interrupted water flow was done twice a week, re-initiating water flow after 3 h. Each treatment was done in triplicate.

Plants were maintained under culture for 4 weeks. A weekly manual harvest was done removing "old" parts of the plant until reaching the initial biomass (500 g per tank). Each tank contained 800 l of culture media. Wet weights of samples were taken with a dynamometer (±10 g) and the growth was calculated by the difference between initial and final weight. Specific growth rates were determined by the formula:

$$\mathrm{SGR} = [(\ln W_t - \ln W_\mathrm{i})/t]100$$

where SGR is the specific growth percentage, W_i is the initial weight, and W_t is the weight at time t (DeBöer et al., 1978). Harvested material was dried in an oven at 60 °C until constant weight was achieved. Total nitrogen analysis of the thallus was done by the modified Kjeldahl method (Strickland and Parsons, 1972).

The irradiance level was regulated using plastic nets over the tanks and measured daily, during 1 month, at the surface and inside the tanks at the time of maximum irradiance (1300 h), using a PAR scalar irradiance meter (Model QSL-100, 4π sensor, Biospherical Instruments). Temperature (maximum and minimum) and salinity of the culture were recorded. As the cultures did not have an inorganic carbon supply, the pH was not controlled.

The results of the culture growth in the tanks and nitrogen content were tested for normality and homogeneity of variances, and a one-way ANOVA was applied. Finally, a Tukey test was performed to compare differences in the growth and in the thallus nitrogen concentration by using different nitrogen sources (Zar, 1997).

Table 1
Tukey test for growth values of *C. squarrulosus*, cultured under semi-controlled conditions with different agricultural fertilizers

Nitrogen source	Calculated value	Critical value	Significance
	Q	q	
NH_4NO_3 vs. $(NH_4)_2S0_4$	0.404	3.993	NS
NH_4NO_3 vs. urea	0.236	3.993	NS
NH_4NO_3 vs. $NaNO_3$	0.067	3.993	NS
NH_4NO_3 vs. seawater	4.848	3.993	*
$(NH_4)_2S0_4$ vs. urea	0.640	3.993	NS
$(NH_4)_2S0_4$ vs. $NaNO_3$	0.337	3.993	NS
$(NH_4)_2S0_4$ vs. seawater	5.252	3.993	*
Urea vs. $NaNO_3$	0.303	3.993	NS
Urea vs. seawater	4.612	3.993	*
Na NO_3 vs. seawater	4.915	3.993	*

NH_4NO_3=$(NH_4)_2S0_4$=urea=Na NO_3≠seawater.
NS=Not significant.
*=Significant.

3. Results

Growth results of *C. squarrulosus* indicate that all the nitrogen sources were capable of sustaining growth, and the Tukey test showed no significant differences in growth among the sources used (Table 1). However, the control was significantly different to all (Table 1). Regardless of the nitrogen source used, growth of *C. squarrulosus* was above 4% per day after 14 days of culture, but growth in the control (seawater) was only 3% per day and at the fourth week of culture, growth ceased and the thallus turned yellow and fragmented. Maximum growth of 5.5% per day was obtained using ammonium sulphate, while the minimum was 0.4% per day in the control (Fig. 1).

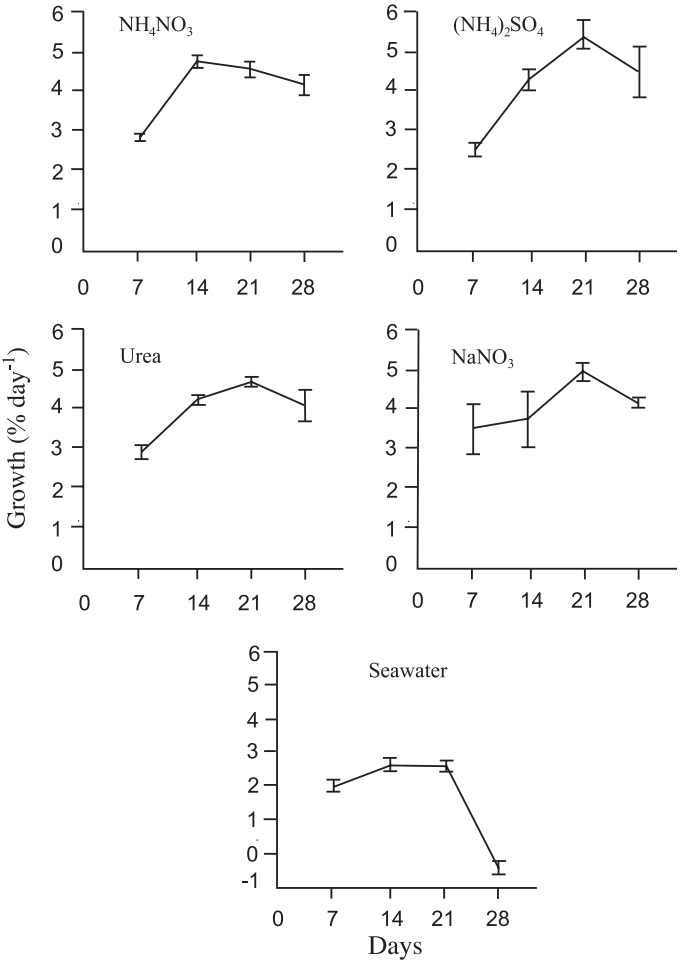

Fig. 1. Growth of *C. squarrulosus* cultured under semi-controlled conditions with different agricultural fertilizers (±=S.E.; *n*=3).

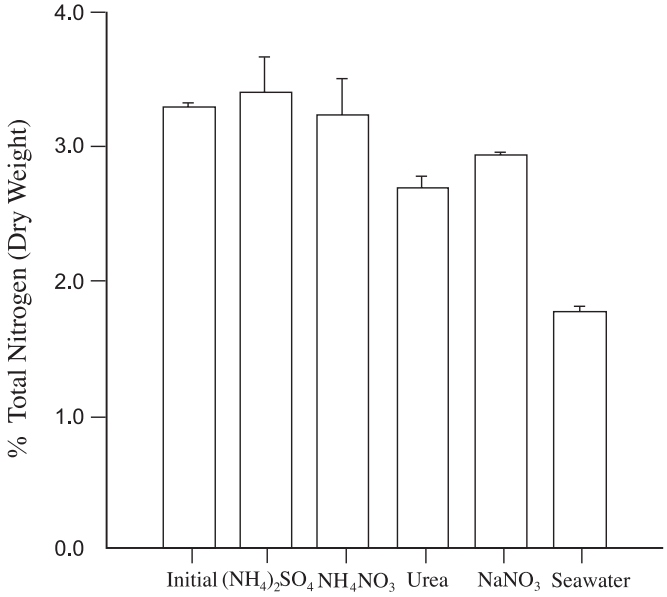

Fig. 2. Total content of nitrogen in *C. squarrulosus* tissue cultured under semi-controlled conditions with different agricultural fertilizers (±=S.E.; n=3).

Tissue nitrogen indicated that cultures with urea and sodium nitrate were not significantly different than cultures using unfertilized seawater. In other words, these two sources of nitrogen were not as efficient as ammonium sulphate and ammonium nitrate in maintaining tissue nitrogen reserves. The highest content of nitrogen in the thallus was obtained with ammonium sulphate (3.4% dry weight) and the lowest with seawater (1.7% dry weight) (Fig. 2). Nitrogen content in thallus cultured in seawater was significantly different to the initial ammonium sulphate and ammonium nitrate (Table 2).

During the experiment temperature varied from 8.4 to 27.8 °C, with an average of 17.9 °C (Fig. 3A). Maximum incident ambient irradiance at noon ranged from 400 to 3700 μmol quanta m^{-2} s^{-1}. Inside the tanks, a maximum of 700 μmol quanta m^{-2} s^{-1} and a minimum of 100 μmol quanta m^{-2} s^{-1} were recorded (Fig. 3B).

The initial pH value in the control and fertilizer media was 8.2 and the final value was 9.2 before the water change; no significant differences in pH were found among treatments (P=0.6643). Salinity remained at 34‰.

4. Discussion

The results of this study indicate that *C. squarrulosus* can be cultured using any nitrogen source and that commercial fertilizer formulations can be as effective as analytical grade reagent sources of nitrogen. Growth of *C. squarrulosus* did not exhibit significant differences among the nitrogen sources tested. The agricultural fertilizers used as nitrogen source were equally effective as the analytical reagent sources. Maximum growth occurred

Table 2
Tukey test for total nitrogen content in tissue values of *C. squarrulosus*, cultured under semi-controlled conditions with different agricultural fertilizers

Treatment	Calculated value	Critical value	Significance
	Q	*q*	
Initial vs. $(NH_4)_2$ SO_4	0.424	4.751	NS
Initial vs. NH_4NO_3	0.271	4.751	NS
Initial vs. urea	2.212	4.751	NS
Initial vs. $NaNO_3$	2.012	4.751	NS
Initial vs. seawater	5.506	4.751	*
$(NH_4)_2SO_4$ vs. NH_4NO_3	0.694	4.751	NS
$(NH_4)_2SO_4$ vs. urea	2.635	4.751	NS
$(NH_4)_2SO_4$ vs. $NaNO_3$	2.435	4.751	NS
$(NH_4)_2SO_4$ vs. seawater	5.930	4.751	*
NH_4NO_3 vs. urea	1.941	4.751	NS
NH_4NO_3 vs. $NaNO_3$	1.741	4.751	NS
NH_4NO_3 vs. seawater	5.236	4.751	*
Urea vs. $NaNO_3$	0.200	4.751	NS
Urea vs. seawater	3.294	4.751	NS
$NaNO_3$ vs. seawater	3.494	4.751	NS

Initial=$(NH_4)_2SO_4$=NH_4NO_3=urea=$NaNO_3$.
Initial=$(NH_4)_2SO_4$=NH_4NO_3≠seawater.
Urea=$NaNO_3$=seawater.
NS=Not significant.
*=Significant.

with ammonium sulphate (Fig. 1), which also enhanced pigmentation and rigidity of the thallus. In addition, we observed that total nitrogen content (Fig. 2) was higher with this compound than with other nitrogen sources. Similar results were found by Bird (1982) for *Gracilaria tickvahiae*.

Both sodium nitrate and urea were equally effective stimulating the growth of *C. squarrulosus*, but these agricultural fertilizers yielded lower growth per day than ammonium sulphate. The difference in growth may be attributed to nitrogen availability. Reduced sources of nitrogen like ammonium frequently produce high growth rates.

Algae in natural environments are exposed to different sources of nitrogen. Ammonium is available from remineralization processes. Nitrate can be received from coastal upwelling and urea is a major excretory product of zooplankton (Parsons and Harrison, 1983). Species may show preferences for one of these sources. It has been well demonstrated that some species of alga grow better on nitrate as nitrogen source. Such is the case of *Gelidium amansii* Lmx (Yamada, 1961, 1972) and Palmaria palmata Lin. (Morgan and Simpson, 1981). Urea is considered a good nitrogen source but studies have shown that it only contributes ≈50% of the nitrogen demand for *Chondrus crispus* Stackhouse (Neish et al., 1977), *Gelidiella acerosa* (Forssk.) Feldman and Hamel (Probyn, 1981) and *Giffordia michelliae* (herv.) Hamel (Lobban et al., 1985). However, some species have been found to grow in the same way using ammonium or any other nitrogen source, such as *Porphyra tenera* Kjellman (Iwasaki, 1967), *Ulva fasciata* Del. (Mohensen et al., 1974) and *Codium fragile* (Suring.) Hariot (Hanisak, 1979). This is the case of *C.*

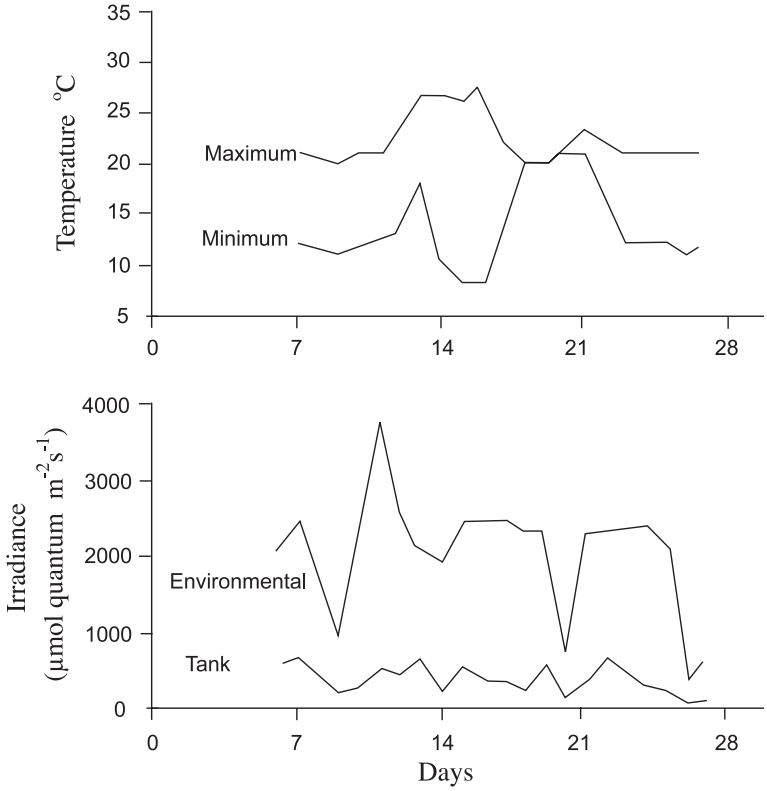

Fig. 3. Temperature and irradiance values during the culture of *C. squarrulosus* under semi-controlled conditions with different agricultural fertilizers.

squarrulosus, the results of this research showing that this alga can be cultured using any nitrogen source.

Of the nitrogen sources tested in this study, we recommend the use of ammonium sulphate because the resulting colour and rigidity characteristics of the plants are the same as those collected in situ. Morgan and Simpson (1981) demonstrated that the addition of nutrients (especially nitrogen) enhances growth and pigmentation. This fact was better observed using ammonium sulphate, which also indicated the nitrogen condition of the thallus (Bird, 1982). The highest concentration of nitrogen found with ammonium sulphate showed that *C. squarrulosus* not only grows well with ammonium sulphate, but also stores it. Similar results were observed for Gracilaria tikvahiae. This species uptakes nitrogen from the environment and stores it for posterior growth (Bird, 1982; Dortch and Conway, 1984).

The irradiance and temperature variations were within the natural conditions for growth of *C. squarrulosus* (Pacheco-Ruíz et al., 1992). Therefore, differences in growth among the different treatments tested in this study are attributed to differences in the nitrogen source used.

Temperatures higher than 30 °C cause the death of the plant. In this study, the highest temperature recorded was 27 °C, the lowest was 8 °C and the average was around 18 °C, which is the optimum temperature for the growth of *C. squarrulosus* (Pacheco-Ruíz et al., 1992; Pacheco-Ruíz, 1999); therefore, the culture was not affected by the temperature.

5. Conclusion

This study demonstrates that commercial agricultural fertilizers can be substituted for analytical grade inorganic salts as a source of nitrogen with no significant effect on the culture of *C. squarrulosus*. The use of agricultural fertilizers constitutes a viable alternative to reduce costs and this biotechnology is a feasible alternative method in the commercial aquaculture of macroalgae.

Acknowledgements

This research was sponsored by CONACYT and Universidad Autónoma de Baja California (UABC). We thank Alberto Gálvez for his assistance and Christine Harris for correcting the English.

References

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

Bird, K.T., 1982. Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). J. Phycol. 18, 344–348.

BoId, H.C., Wynne, M.J., 1978. Introduction to the Algae, Structure and Reproduction. Prentice-Hall, Englewood Cliffs, New Jersey. 710 pp.

Craigie, J.S., Shacklock, P.F., 1989. Culture of Irish Moss. In: Boghen, A.D. (Ed.), Cold Water Aquaculture in Atlantic Canada. The Canadian Institute for Research on Regional Development, Moncton, Canada, pp. 241–270.

DeBöer, J.A., Guigli, H.J., Israel, T.L., D'Eiia, C.F., 1978. Nutritional studies of two red algae: 1 Growth rate as a function of nitrogen source and concentration. J. Phycol. 14, 261–266.

Dortch, H.L., Conway, K., 1984. Interactions between nitrate and ammonium uptake: variation with growth rate, nitrogen source and species. Mar. Biol. 185, 137–158.

González-Gómez, M.A., Zertuche-González, J.A., Pacheco-Ruíz, I., 1992. Nitrogen effect on growth and carrageenan content in *Gigartina canaliculata* Harv. (Rhodophyta Gigartinales), in outdoor tank culturea. Cienc. Mar. 18, 75–83.

Granados-Machuca, C., Buckle, L.F., 1984. Cultivo de las microalgas *Monochrysis lutheri* y *Skeletonema costatum* con nutrientes producidos por estiércol digerido. Inst. Cienc. Mar. Limnol. 1, 242–256.

Hanisak, M.D., 1979. Growth pattern of *Codium fragile* in response to temperature, irradiance, salinity and nitrogen source. Mar. Biol. 50, 319–332.

Hanisak, D.M., 1983. The nitrogen relationships of marine macroalgae. In: Carpenter, E.J., Capone, D.G. (Eds.), Nitrogen in the Environment. Academic Press, New York, pp. 699–732.

Hargreaves, J.A., 1988. Nitrogen biochemistry of aquaculture ponds. Aquaculture 166, 181–212.

Iwasaki, H., 1967. Nutritional studies of the seaweed *Porphyra tenera*: II. nutritional of *Conchocelis*. J. Phycol. 3, 30–34.

Lobban, C.S., Harrison, P.J., Duncan, M.J., 1985. The Physiological Ecology of Seaweeds. Cambridge Univ. Press, New York. 242 pp.

López-Acuña, L.M., Pacheco-Ruíz, I., Hernández-Garibay, E., Zertuche-González, J.A., 2002. Characterization of the carrageenan of *Chondracanthus pectinatus* (Rhodophyta: Gigartinales). Cienc. Mar. 28, 311–318.

Mohensen, A.F., Khaleafa, A.F., Hashem, M.A., Metwalli, A., 1974. Effect of the different nitrogen source on growth, reproduction, amino acid, fat and sugar contents in *Ulva fasciata* Delile. Bot. Mar. 17, 218–222.

Morgan, K.C., Simpson, F.J., 1981. Cultivation of *Palmaria palmata* (Rhodymenia): effect of high concentration of nitrate and ammonium on growth and nitrogen uptake. Aquat. Bot. 11, 167–171.

Neish, A.C., Shacklock, P.F., Fox, C.H., 1977. The cultivation of *Chondrus crispus*. Factors affecting growth under greenhouse conditions. Can. J. Bot. 55, 2263–2271.

Pacheco-Ruíz, I., 1999. Historia de vida de *Chondracanthus squarrulosus* (Gigartinales, Rhodophyta) en la costa noroeste del Golfo de California. PhD Thesis. Universidad Autónoma de Baja California, Ensenada, Baja California, México, 123 pp.

Pacheco-Ruíz, I., García-Esquivel, Z., Aguilar-Rosas, L.E., 1987. Culture of *Gigartina canaliculata* Harv. juvenile germlings by means of media enriched with digested cow manure. Cienc. Mar. 13, 51–58.

Pacheco-Ruíz, I., Zertuche-González, J.A., Cabello-Pasini, A., Brinkhuis, B.H., 1992. Growth strategies and seasonal variation of photosynthesis, growth and biomass of *Gigartina pectinata* Rhodophyta in the Gulf of California. J. Exp. Mar. Biol. Ecol. 157, 263–274.

Parsons, T.R., Harrison, P.J., 1983. Nutrient cycling in marine ecosystems. In: Pirson, A., Zimmerman, M.H. (Eds.), Physiological Plant Ecology IV, Encyclop. Plant Physiol., vol. 12D. Springer-Verlag, New York, pp. 77–105.

Probyn, T.A., 1981. Aspects of the light and nitrogenous nutrient requirement for growth of *Chordaria flageliformis* (O.F Mull) C. Ag. Proc. Int. Seaweed Symp. 10, 334–339.

Strickland, J.D., Parsons, T.R., 1972. A Practical Handbook of Seawater Analysis. Fisheries Reasearch Board of Canada, Ottawa. 310 pp.

Valenzuela-Espinoza, E., Gendrop-Funes, V., Pérez-Castañeda, R., Wilburn-González, J.G., 1999. Larval survival of *Litopenaeus vannamei* (Boone) fed *Cheatoceros muelleri* produced with agricultural fertilizer. Cienc. Mar. 25, 423–427.

Yamada, N., 1961. Studies on the manure for seaweed: 1. On the change of nitrogenous component of *Gelidium amansii* Lmx cultured with different nitrogen sources. Bull. Jpn. Soc. Sci. Fish 27, 953–957.

Yamada, N., 1972. Manuring for *Gelidium* sp. Proc. Int. Seaweed Symp. 7, 385–390.

Zar, J.H., 1997. Biostatistical Analysis. Prentice-Hall, New Jersey. 718 pp.