

Influence of the Macronutrients N, P and K on the Agarophyte *Alsidium triquetrum* (S. G. Gmelin) Trevisan, during Experimental Culture

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Abstract

The knowledge of the nutritional requirements and their relation to the physiology of marine algae growth is key to incorporate new species into aquaculture, whose dynamics tend to be largely unknown. The use of *Alsidium triquetrum* in the pharmacological industry depends on its availability in the natural environment, occasionally scarce. As macroalgae cultivation gains momentum worldwide, it is important to know how the effects of nutrients are modulated in the thallus during cultivation. The linking of the relative growth rates (RGR) of *A. triquetrum* and their relation with the macronutrients N (nitrogen), P (phosphorus) and K (potassium) at the tissue level under culture conditions constitutes the main contribution of this article. P levels tend to decrease as the plant completes its development. Both the concentration of N and P are higher in the stipe for the month of July, N (25.31 ± 0.26) vs P (0.846 ± 0.02) period when the highest vegetative development is reached. The N and P modulate the patterns of the species' development over the annual cycle, unlike K, which is not considered a limiting factor. When the temperature and lighting are not favorable for growth, the plant simply accumulates these compounds. As environmental conditions change, these stored compounds are actively used in their growth. The specimens with an initial weight of 50 g present the best accumulated biomass (RGR) throughout the annual cycle.

Keywords

Alsidium triquetrum, Culture, Phosphorus, Macronutrients, Nitrogen,

1. Introduction

Nutrient requirements of marine autotrophs are divided into three categories: macronutrients, micronutrients and vitamins. Most of these growth elements are found in relatively low concentrations in seawater relative to their concentration in the macrophyte tissue. For example, N and P are concentrated approximately 100,000 times in seaweed, while K is concentrated approximately 10,000 times over environmental seawater concentrations [1].

The definition of “limiting nutrients” goes back more than 100 years to Liebig’s research [2]. This law establishes that the nutrient that is available in the smallest amount with respect to the plant’s other nutrient requirements limits the growth rate, assuming that all other factors are optimal. The concentration of nutrients in seawater is determined by the balance between the rate of nutrient supply (mixing in the water columns, nutrient regeneration, among others) and the demand of nutrients (absorption) by algae [3]. This means that a very low concentration of nutrients in seawater will not indicate whether the algae are slightly, moderately, or severely limited. In addition, natural algal populations may obtain nutrients from sources other than the water column, such as particulate matter on their surface [4], or animal excretion [5] [6].

In autonomous systems, nitrogen is the nutrient that most commonly limits the growth of seaweeds, phosphorus being the second. Nitrogen is available in inorganic forms such as nitrate (NO_3^-) and ammonium (NH_4^+) and in the form of organic urea. Nitrate-based growth is called “new production” because the NO_3^- is supplied externally. For example, from below the thermocline or from the rising current [6]. Primary production based on NH_4^+ and urea is called “recycled production” because invertebrates and fish associated with seaweed regenerate them internally within the system [5].

In production processes on experimental scale, periods of nitrogen-limited growth can be overcome by the addition of fertilizer, joining porous pots containing slow-release fertilizer to the lines (see [8]). These N-subsidy practices under experimental conditions have had undesirable synergistic consequences such as triggering phosphorus absorption [9]. A current trend in aquaculture practices has been to mix organisms of different trophic levels, which does not always deliver desired effects [10] [11]. The development of different crop techniques to grow seaweed is not only an economic option but is also an ecologically viable alternative to conserve natural resources [12].

Agar is the phycocolloid with the longest history of use. Agar was discovered in 1658 in Japan, 200 years before it was introduced into Western countries. This polysaccharide, of high economic value, is used today as a gelling agent [13]. However, not all agarophytes have quality agar that merits cultivation. *Alsidium tri-*

quetrum whose main applications are focused on the pharmaceutical industry [14] [15] [16] [17] [18], does not have quality agar.

The experiences in the management of support structures in cultivars [19], in the detection of the main abiotic factors that influence their growth [20], and on the succession dynamics of their accompanying fauna [21] undoubtedly make the species *Alsidium triquetrum* a good candidate for mariculture. This article's objective is to report for the first time the levels of accumulation of macronutrients N, K, P and their relation to the growth rates of *A. triquetrum* under mariculture conditions.

2. Materials and Methods

2.1. Study Area and Experimental Period

All seaweed beds examined were located between depths of 0.5 to 3 m. Some of the beds were occasionally sampled for specific purposes such as those of “Rincon de Guanabo” [23°10'30"N, 82°05'48"W], which was used for collection of specimens, and to evaluate seasonal variations in composition (Figure 1(E)). Only those located in the vicinity of “Playa Viriato” were studied on a regular basis [23°6'0"N y 82°28'0"W] where the crop trials were additionally evaluated (Figure 1(B)). This locality was chosen for its preferable logistical facilities, the knowledge

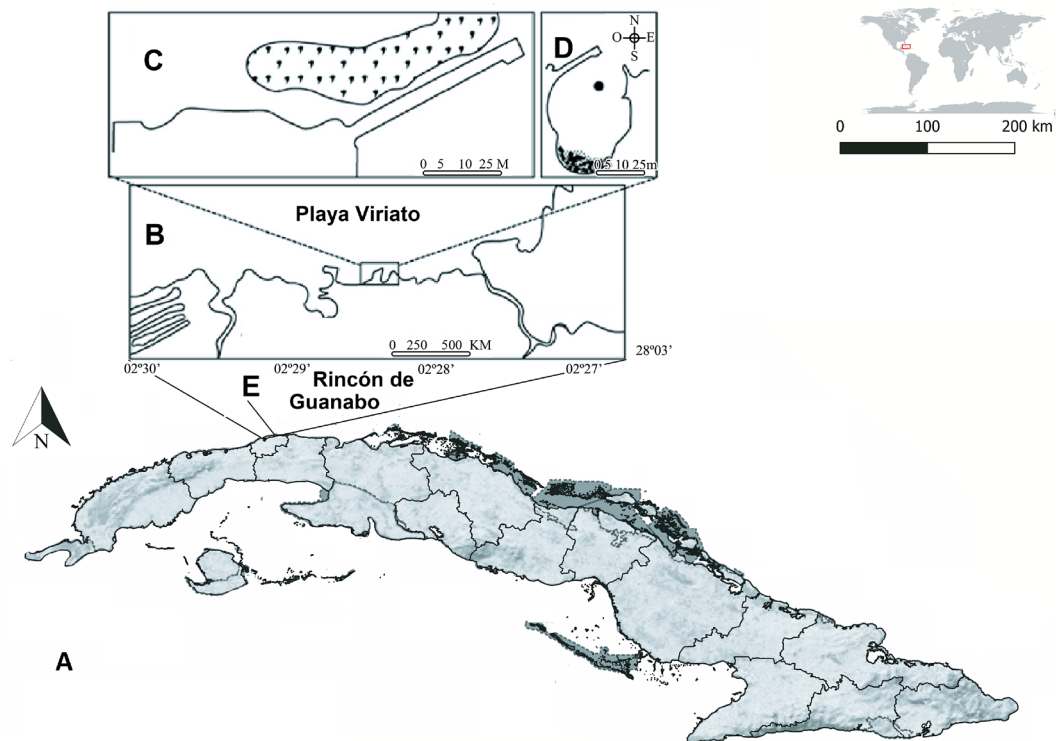


Figure 1. (A) Geographical location of the Cuba Island; (B) Schematic of the experimental site and location of a wild bed of *Alsidium triquetrum* in Playa Viriato; (C) Approximate location of a rocky substrate where wild *A. triquetrum* was collected; (D) Inner section of the roadstead with the black dot showing the approximate location of the experimental farm; (E) Rincón de Guanabo station where some specimens of *A. triquetrum* are collected.

of its hydrodynamic regime and the presence of extensive beds of *Alsidium triquetrum* in its vicinity.

The collection of biomasses for the various purposes was made either in snorkeling or through SCUBA diving. The recording of seasonal variation of biochemical indicators was carried out on a monthly basis to complete the annual cycle. The cuttings were always made manually and were carried out in pre-selected sectors of the bed. (Figure 1(C)).

To assess daily growth, treated in the present study as relative growth rate (RGR), was expressed in grams (g) and based on the accumulation of macronutrients designed to be measured every 60 days (Figure 1(D)), in the two most significant periods of the year (winter-summer).

2.2. Experiments with Cultivated Macroalgae

Mesh bags were used attached to a fixing structure and suspended in the water column with specimens of 25 and 50 g initial weight. Every ten days, five bags were removed from each initial weight, in order to be processed.

Its completion allowed us to quantify the growth and to control it with the variations of the biogenetic elements of the species during its period of the greatest vegetative development.

2.3. Data Collection and Processing

The concentration of phosphorus in the thallus, in the form of P_2O_5 , was estimated using the technique of the yellow color of the vanado-molybdenum phosphoric complex [22]. The Kjeldahl method [23] was used for nitrogen analysis. To quantify nitrogen and total phosphorus, techniques proposed by [24] were applied after oxidation of the sample with potassium sulphate in alkaline solution.

For the analysis of K at tissue level, the material was crushed and ground in a rotating mortar of agate balls, sieved through a plastic mesh of 0.6 - 0.66 μ , four grams of samples processed. Measurements were based on the direct reading method on a Pye Unicam SP-9-800 direct absorption spectrophotometer with air-acetylene flame. A concentrated mixture of HCl:HNO₃:H₂O₂ in ratio 1:2:3.

For weighing, technical scales were used with an error of ± 0.1 g, or when necessary, analytical scales of 0.0001 g precision.

2.4. Statistical Treatment

Results were generally expressed in tables and graphs. Arithmetic means are presented with their extreme values or by ± 1 SD. When variability was considered, it was quantified by mean deviation (MD), standard deviation (SD), or corrected coefficient of variation for bias [25].

The small similarity between the coefficients of variation of many experimental treatments, determined that non-parametric tests would be used in the analysis. For the contrast of two samples, Mann-Whitney Statistic U and the Fisher

Median and Exact Probability tests were used, and for the verification of K independent samples, the analysis of variance of a Kruskal-Wallis range classification [26] was used.

3. Results

The high concentration of K in the younger parts of the thallus causes a noticeable decrease of the element when, for some reason, the branches fall off (Figure 2). Although branches and apices did not vary in a conspicuous way, during the seasonal cycle, the high concentration of K in them allowed the K to be directly and significantly associated with the rate of propagation of the plant, (Figure 3; Kruskal-Wallis, $p = 0.043$), since as K increased, so did its concentration in young tissues.

The P, on the other hand, tends to decrease when RGR increases (Figure 3, Kruskal-Wallis, $p = 0.029$) and the ratio between the oldest parts of the alga and

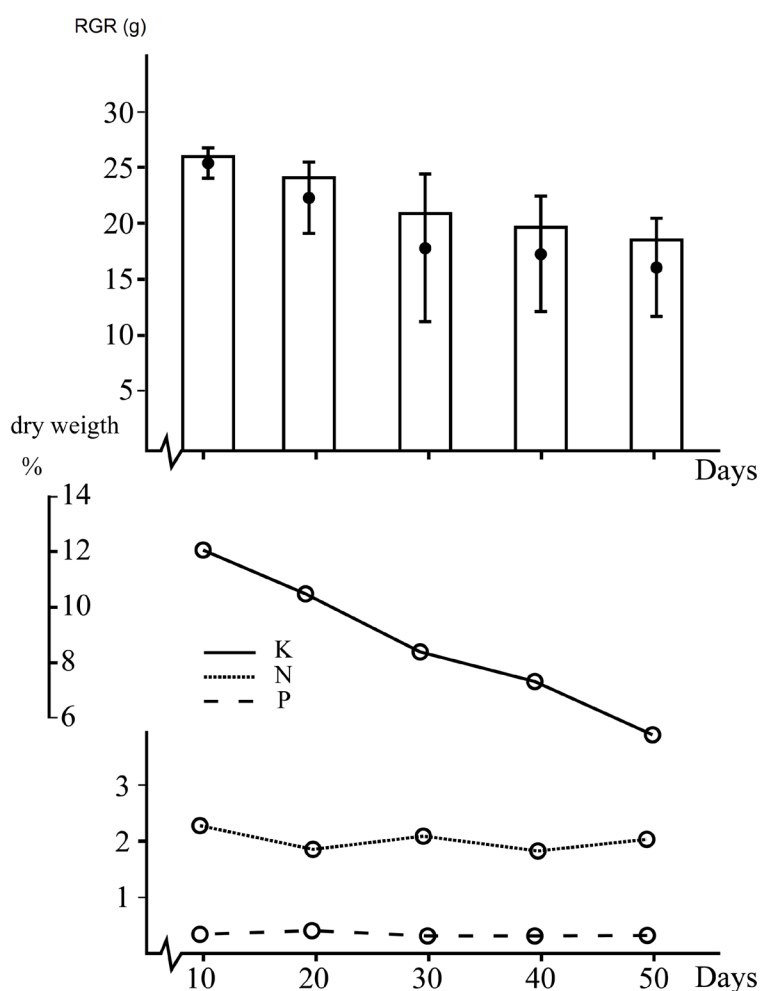


Figure 2. Variation in mean tissue concentration of nitrogen (N), phosphorus (P), potassium (K) in relation to Relative Growth Rate (RGR) change experienced by *A. triquetrum* specimens propagated in bags. Winter. Content expressed in % with respect to dry weight ($10 \times \% = \mu\text{g}\cdot\text{g}^{-1}$). Individuals with 25 g initial weight. Heights referred to extreme values.

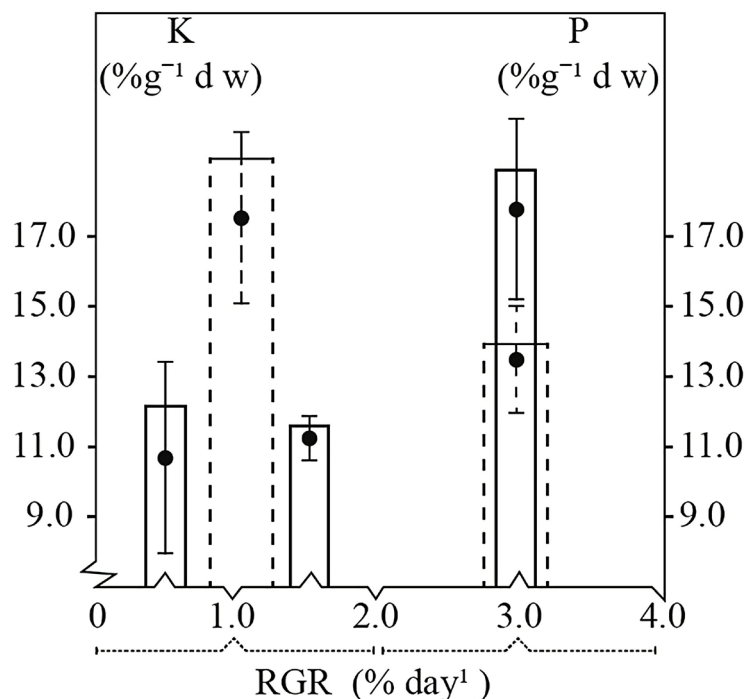


Figure 3. Mean concentration of P and K on the heel of *A. triquetrum* according to growth rate (≤ 1.0 , $\geq 1.0 \leq 2.0 \geq 4.0$, for K (—); ≤ 2.0 , and $\geq 2.0 \leq 4.0$, for P (- - -). Culture in mesh bags for 30 days. Specimens with 50 g initial weight. Vertical heights defined by extreme values.

those of recent formation, which is lower in concentration (Table 1). Together with K, it was a biogenetic element with a high environmental connection, in correlation with all the physical variables evaluated and, like the Mg, significant under the RGR (Table 2).

Under natural conditions, no direct relationship was observed between the tissue concentration of N and the RGR of the algae. In addition, the algae do not accumulate it differentially in the tissue (Table 1), which limits its eventual mobilization to other parts of the thallus. However, the seasonal change of both the concentration and the stoichiometric ratio between the element mentioned and the tissue P is an indirect manifestation of this dependence. The N is gradually decreased from the months of February and March until between June and September ($1.32 \pm 0.22\%$ vs 0.35% d. w (dry weight); U, $\alpha = 0.001$). Unlike P, the seasonal variations in its content in different parts of the thallus were minimal (Table 1).

The relative proportion of N to P changes over the annual cycle from a minimum mean value of the quotient N: P in spring. The proportion of N to P becomes stabilized around 24:1. In the period between the end of the spring and autumn (Figure 4).

The bed of *A. triquetrum* in “Rincon de Guanabo” is located in a shallow and relatively protected area, where the periodic influence of soil runoff decreases the acute limiting effects caused by a prolonged absence of nutrients.

Table 1. Distribution of N and P in two parts of the thallus of *A. triquetrum* during July (J), month in which the species showed its maximum RGR, and April (A), last month of the dry period, when the N a P content in seawater was lowest in the annual cycle (mean DS values \pm referring to three replicates).

ELEMENT	Concentration (mg·g ⁻¹ d. w)	
	Stipe and basal branches	Apice
N	17.83 \pm 0.50 (A)	15.76 \pm 0.60 (A)
	25.31 0.26 (J)	21.95 0.62 (J)
P	0.152 \pm 0.002 (A)	0.074 \pm 0.001(A)
	0.846 0.02 (J)	0.539 0.02 (J)

Table 2. Significant correlations between five centesimal components of *A. triquetrum* branches, their relative growth rate and the value of three hydroclimatic factors during the months of greatest growth rate.

MACROELEMENT	K	Na	Mg	N	P
K (% d. w.)	-				
Na	0.970***	-			
Mg			-		
N				-	
P	-0.925***	-0.867***			-
Abiotic factors					
IT	-0.887***	-0.769***		0.662**	0.880***
AST (°C)	0.845***		0.530*		-0.965***
MRA mm	530*			-0.920***	-0.597**
Metabolic balance					
RGR (% day ⁻¹)			730***		-0.603**

IT = inferred Turbulence; AST = average surface temperature; MRA = monthly rain accumulation. *p \leq 0.10; p \leq 0.05; ***p \leq 0.01.

This fact determined that in relation to the specimens coming from “Playa Vi-riato”, the average content of N was significantly higher in them (1.82% vs 1.43% d. w; U, p = 0.02).

4. Discussion

In some beds of *Alsidium triquetrum*, the maximum value of the ratio N:P never exceeded the ratio 24:1. This fact, together with the decrease of the tissue concentration of phosphorus (P) during the first two summer months (Figure 4), is a demonstration of the nutritional limitations imposed on this species by the almost always oligotrophic environment in which it grows. Although some Rhodophyceae such as *Laurencia intricata* J. V. Lamouroux are able to store P in low-availability environments as secondary adaptation [27], this ability does not appear to occur in *Alsidium triquetrum*.

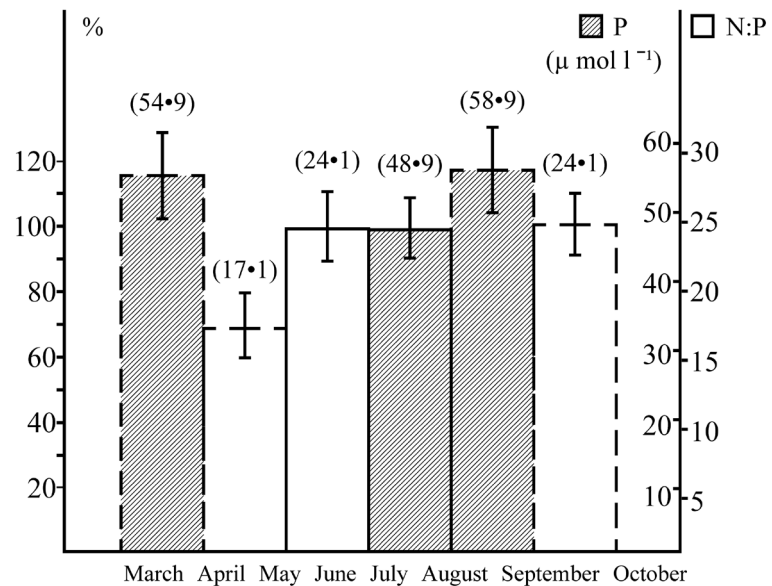


Figure 4. Tissue concentration behavior of P and the ratio N:P in specimens of *A. triquetrum* during the three months before and after June and July, stage in which the species showed its highest RGR (median for each stage is represented between parentheses. Measured average values ± 1 SD).

Nevertheless, emphasized the existence of a general coupling between physiological and morphological properties in algae.

These authors also consider that the growth in macroalgae can be decoupled from accumulation to optimize your available internal concentration.

The oscillation over the annual cycle of the ratio N:P suggests the combination of different limiting factors, responses are different and specific to each season. Thus, the symptoms of limitation of N show its maximum expression during the spring. Similar results were presented by [28], and confirmed the observations of other authors on the link between the internal pattern of variation of N, and its seasonal availability in the field, accordingly [29] [30].

The lack of phosphorus is accentuated in the stage of rapid multiplication. The mean element concentration value was significantly higher than the magnitude reported by [31]. The tropical macroalgae typical of environments with high concentrations of carbonate in sediments (0.07% vs 0.16% d. w). Its decrease in *Alsidium* for basal branches and apex increases RGR, possibly reflecting a low external availability. Phosphorus may be considered less limiting than N in the marine environment [7] [32].

It can be immobilized in carbonate in sediments, when in them, the heterotrophic processes are not significant [33] [34].

This phenomenon also causes manifestations of deficiency in epilithic macroalgae [31].

Temperature or lighting limitations are capable of uncoupling the tissue concentration of some nutrients from the growth rate [35] [36]. This phenomenon is capable of restricting net algal productivity [31].

Probably, the slowdown of growth and the rapid senescence observed at mid-summer in specimens of some beds, is induced by this nutritional deficit and catalyzed by the increase of respiratory processes as the temperature increases. These events seem to correspond with what was observed by [37].

The 25 g specimens presented a greater amount of senescent tissue and did not grow favorably under the culture conditions (Figure 2) regardless of the culture site.

The balance between the limiting effects caused by N and P will depend on the persistence of the algal bed throughout the annual cycle and the more or less episodic nature of the growth of the specimens that integrate the bed. In depleted areas, with very low nutrient levels, the transition between vegetative phases is evident, and, in extreme conditions, after a winter recovery, senescence begins rapidly during the spring. When the degree of beneficial environmental conditions is greater, as it happens in the beds located in “Playa Viriato” where the vegetative development is dramatic until the end of the summer and the period of senescence, shortens between this time and winter. When the temperature and light is not favorable for growth, the plant simply accumulates these compounds. As environmental conditions change, these compounds are actively used in the growth of the algae. Our results are similar to those indicated by [36] for dilution of these compounds at the tissue level.

When the environment is favorable, such as at Playa Viriato, *Alsidium* is present almost all year round with robust and vigorous specimens (large amounts of biomass in young branches and apices). Only at very specific moments in the annual cycle, environmental severity is reflected. In this event, the senescent forms are not evident. They are characterized by having small young biomass, thorn branches and withered basal parts. A diametrically opposite case happens with the populations of the Rincón de Guanabo, where environmental conditions are extreme and the plants end up completely disappearing for months from the natural environment.

In shallow areas, there are greater variations in temperature. This allows the accumulation of N [36] [38]. This balances internal availability [29].

The hydrodynamic also increases the efficiency of the removal of sediments, with the consequent mobilization of interstitial water towards the water column and a greater constancy in the nutrient pulses [39]. Under these mild conditions, changes can be restricted only to variations in the proportion of population morphotypes e.g. Playa Viriato.

The minimum concentration of some biochemical components was associated in species with transition stages between opposite seasonal phases.

During the course of these seasonal phases, the combined action of temperature and nutrient level in the medium is completed and helps relieve the depressive effect that any of these factors may induce by themselves [40].

Without limiting physical factors acting at some point in the annual cycle, seasonal variations in biochemical composition will reflect, to a greater extent, the nutritional status of the plant. As the water or the climate becomes more fa-

avorable, as occurs in the beds of “Rincon de Guanabo”, the extreme variations of the composition tissue will be less accentuated, masking the seasonal pattern.

The oscillations in the components N, P and K around the central values tend, however, to become more frequent. Similarly, the respective CV (coefficients of variation) will become higher.

The trend towards an increase in the proportion of organic Carbon when the plant is N-deficient has been well documented at various scales [41].

At the level of the organism, due to the hyperbolic character of the protein vs carbohydrate, with respect to the amount of N in the medium [42]. At the metabolic level, this has been confirmed by the increase in the percentage of carrageenan in some species kept in a poor environment of N [43] or after receiving a moderate pulse of the element N [44].

Modern agricultural practices have shown that it is only through rigorous phytosanitary control, use of selected varieties and efficient agro-technology that algal yields are substantially increased [21].

The profitability of the process will depend on the balance between operational requirements, survival and productivity, factors all intimately related to the applied propagation technique.

This last aspect becomes very important when marine plants are grown in the natural environment.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Harrison, P.J. and Hurd, C.L. (2001) Nutrient Physiology of Seaweeds: Application of Concepts to Aquaculture. *Cahiers de Biologie Marine*, **42**, 71-82.
- [2] Morris, S.J. and Blackwood, C.B. (2007) The Ecology of Soil Organisms. In: Eldor, A.P., Ed., *Soil Microbiology, Ecology and Biochemistry*, 3rd Edition, Academic Press, Cambridge, 195-229. <https://doi.org/10.1016/B978-0-08-047514-1.50012-3>
- [3] Roleda, M.J. and Hurd, C.L. (2019) Seaweed Nutrient Physiology: Application of Concepts to Aquaculture and Bioremediation. *Phycologia*, **58**, 552-562. <https://doi.org/10.1080/00318884.2019.1622920>
- [4] Schaffelke, B. (1999) Particulate Organic Matter as a Novel Nutrient Source for Tropical Macroalgae. *Journal of Phycology*, **35**, 1150-1157.
- [5] Taylor, R.B. and Rees, T.A.V. (1998) Excretory Products of Mobile Epifauna as a Nitrogen Source for Seaweeds. *Limnology and Oceanography*, **43**, 600-606. <https://doi.org/10.4319/lo.1998.43.4.0600>
- [6] Hepburn, C.D., Frew, R.D. and Hurd, C.L. (2012) Uptake and Transport of Nitrogen Derived from Sessile Epifauna in the Giant Kelp *Macrocystis pyrifera*. *Aquatic*

- Biology*, **14**, 121-128. <https://doi.org/10.3354/ab00382>
- [7] Boyd, P.W. and Hurd, C.L. (2009) Ocean Nutrients. In: Le Quéré, C. and Saltzman, E.S., Eds., *Surface Ocean: Lower Atmosphere Processes*, American Geophysical Union, Washington DC, 36-97. <https://doi.org/10.1029/2008GM000844>
- [8] Neushul, M., Benson, J., Harger, B.W.W. and Charters, A.C. (1992) Macroalgal Farming in the Sea: Water Motion and Nitrate Uptake. *Journal of Applied Phycology*, **4**, 255-265. <https://doi.org/10.1007/BF02161211>
- [9] Perini, V. and Bracken, M.E.S. (2014) Nitrogen Availability Limits Phosphorus Uptake in an Intertidal Macroalga. *Oecologia*, **175**, 667-676. <https://doi.org/10.1007/s00442-014-2914-x>
- [10] Buck, B.H., Nevejan, N., Wille, M., Chambers, M.D. and Chopin, T. (2017) Offshore and Multi-Use Aquaculture with Extractive Species: Seaweeds and Bivalves. In: Buck, B. and Langan, R., Eds., *Aquaculture Perspective of Multi-Use Sites in the Open Ocean*, Springer, Cham, 23-69. https://doi.org/10.1007/978-3-319-51159-7_2
- [11] Stévant, P., Rebours, C. and Chapman, A. (2017) Seaweed Aquaculture in Norway: Recent Industrial Developments and Future Perspective. *Aquaculture International*, **25**, 1373-1390. <https://doi.org/10.1007/s10499-017-0120-7>
- [12] Bezerra, A.F. and Marinho-Soriano, E. (2010) Cultivation of the Red Seaweed *Gracilaria birdiae* (Gracilariales, Rhodophyta) in Tropical Waters of Northeast Brazil. *Biomass and Bioenergy*, **34**, 1813-1817. <https://doi.org/10.1016/j.biombioe.2010.07.016>
- [13] Areces, A.J. (1989) Fisionomía del agar y su industria. Ed. Academia, La Habana.
- [14] Ainouz, L., Sampaio, A.H., Freitas, A.L.P., Benevides, N.M.B. and Mapurunga, S. (1995) Comparative Study on Hemagglutinins from the Red Algae *Bryothamnion seaforthii* and *Bryothamnion triquetrum*. *Revista Brasileira de Fisiologia Vegetal*, **7**, 15-19.
- [15] Calvete, J.J., Costa, F.H.F. and Saker-Sampaio, S. (2000) The Amino Acid Sequence of the Agglutinin Isolated from the Red Marine Alga *Bryothamnion triquetrum* Defines a Novel Lectin Structure. *Cellular and Molecular Life Sciences*, **57**, 343-350. <https://doi.org/10.1007/PL00000696>
- [16] Viana, G.S., Freitas, A.L., Lima, M.M., Vieira, L.A., Andrade, M.C. and Benevides, N.M. (2002) Ant Nociceptive Activity of Sulfated Carbohydrates from the Red Algae *Bryothamnion seaforthii* (Turner) Kütz. and *B. triquetrum* (S.G. Gmel.) M. Howe. *Brazilian Journal of Medical and Biological Research*, **35**, 713-722. <https://doi.org/10.1590/S0100-879X2002000600012>
- [17] Pinto, V.P., Debray, H., Dus, D., Teixeira, E.H., de Oliveira, T.M., Carneiro, V.A., Teixeira, Filho, G.C., Nagano, C.S., Nascimento, K.S., Sampaio, A.H. and Cavada, B.S. (2009) Lectins from the Red Marine Algal Species *Bryothamnion seaforthii* and *Bryothamnion triquetrum* as Tools to Differentiate Human Colon Carcinoma Cells. *Advances in Pharmacological and Pharmaceutical Sciences*, **2009**, Article ID: 862162. <https://doi.org/10.1155/2009/862162>
- [18] do Nascimento-Neto, L.G., Carneiro, R.F., da Silva, S.R., da Silva, B.R., Vassiliepe, S. A.F., Carneiro, V.A., do Nascimento, K.S., Saker-Sampaio, S., da Silva Jr., V.A., Porto, A.L., Cavada, B.S., Sampaio, A.H., Teixeira, E.H. and Nagano, C.S. (2012) Characterization of Isoforms of the Lectin Isolated from the Red Algae *Bryothamnion seaforthii* and Its Pro-Healing Effect. *Marine Drugs*, **10**, 1936-1954. <https://doi.org/10.3390/md10091936>
- [19] Areces, A.J. and Soberats, L.R. (1992) Optimización del cultivo *in situ* de *Bryothamnion triquetrum* (Gmelin) Howe mediante evaluación de diversos sistemas de

- sujeción. *Ciencias Biológicas*, **18**, 65-76. <https://doi.org/10.7773/cm.v18i2.892>
- [20] Areces, A.J. and Araujo, M. (1996) Influencia de la salinidad y la temperatura sobre el crecimiento de *Bryothamnion triquetrum* (Gmelin) Howe (Rhodophyta: Rhodomeleaceae) *Revista Biología Tropical Costa Rica*, **44**, 449-454.
- [21] Areces, A.J., Cabrera, R. and Díaz-Larrea, J. (2020) Biotecnología de agarófitas del género *Alsidium* C. Agardh. Editorial Académica Española, 1-127.
- [22] Jackson, M.I. (1970) Determinaciones del fósforo en los suelos. In: *Análisis químico de los suelos*, Ed. Revolucionaria, La Habana, 213-216.
- [23] AOAC (Association of Official Agricultural Chemists) (1984) Official Methods of Analysis. Kjeldahl Method (2.062). 14th Edition, Washington DC.
- [24] COI/UNESCO (1983) Chemical Methods for Use in Marine Environmental Monitoring. Manual and Guides 12, 53 p.
- [25] Sokal, R.R. and Rohlf, F.J. (1981) Biometry. 2nd Edition, W.H. Freeman, New York.
- [26] Zar, J.H. (1999) Biostatistical Analysis. 4th Edition, Prentice Hall, Englewood Cliffs.
- [27] Reef, R., Pandolfi, J.M. and Lovelock, C.E. (2012) The Effect of Nutrient Enrichment on the Growth, Nucleic Acid Concentrations, and Elemental Stoichiometry of Coral Reef Macroalgae. *Ecology and Evolution*, **2**, 1985-1995. <https://doi.org/10.1002/ece3.330>
- [28] Hein, M., Folager-Pedersen, M. and Sand-Jensen, K. (1995) Size-Dependent Nitrogen Uptake in Micro- and Macroalgae. *Marine Ecology Progress Series*, **118**, 247-253. <https://doi.org/10.3354/meps118247>
- [29] Phillips, J.C. and Hurd, C.L. (2003) Nitrogen Ecophysiology of Intertidal Seaweeds from New Zealand: N Uptake, Storage and Utilization in Relation to Shore Position and Season. *Marine Ecology Progress Series*, **264**, 31-48. <https://doi.org/10.3354/meps264031>
- [30] Young, E.B., Dring, M.J., Savidge, G., Birkett, D.A. and Berge, J.A. (2007) Seasonal Variations in Nitrate Reductase Activity and Internal N Pools in Intertidal Brown Algae Are Correlated with Ambient Nitrate Concentrations. *Plant Cell and Environment*, **30**, 764-774. <https://doi.org/10.1111/j.1365-3040.2007.01666.x>
- [31] Fernández, P.A., Gaitán-Espitia, J.D., Leal, P.P., Schmid, M., Revill, A.T. and Hurd, C.L. (2020) Nitrogen Sufficiency Enhances Thermal Tolerance in Habitat-Forming Kelp: Implications for Acclimation under Thermal Stress. *Scientific Reports*, **10**, Article No. 3186. <https://doi.org/10.1038/s41598-020-60104-4>
- [32] Lapointe B.E., Littler, M.M. and Littler, D.S. (1992) Nutrient Availability to Marine Macroalgae in Siliciclastic versus Carbonate-Rich Coastal Waters. *Estuaries and Coasts*, **15**, 75-82. <https://doi.org/10.2307/1352712>
- [33] Lobban, C. S., Harrison, P.J. and Ducan, M. J. (1985) Nutrients. In: *The Physiological Ecology of Seaweeds*, Cambridge University Press, Cambridge, 75-110.
- [34] Søndergaard, M., Jensen, J.P. and Jeppesen, E. (2003) Role of Sediment and Internal Loading of Phosphorus in Shallow Lakes. *Hydrobiologia*, **506-509**, 135-145. <https://doi.org/10.1023/B:HYDR.0000008611.12704.dd>
- [35] Lapointe, B.E. and Tenore, K.R. (1981) Experimental Outdoor Studies with *Ulva fasciata* Delile. I. Interaction of Light and Nitrogen on Nutrient Uptake, Growth, and Biochemical Composition. *Journal of Experimental Marine Biology and Ecology*, **53**, 135-152. [https://doi.org/10.1016/0022-0981\(81\)90015-0](https://doi.org/10.1016/0022-0981(81)90015-0)
- [36] Duke, C.S., Litaker, W. and Ramus, J. (1989) Effects of Temperature Nitrogen Supply, and Tissue Nitrogen on Ammonium Uptake Rates of the Chlorophyte Seaweeds *Ulva curvata* and *Codium divaricatum*. *Journal of Phycology*, **25**, 113-123.

- <https://doi.org/10.1111/j.0022-3646.1989.00113.x>
- [37] Piñeiro-Corbeira, C., Barreiro, R, Cremades, J. and Arenas, F. (2018) Seaweed Assemblages under a Climate Change Scenario: Functional Responses to Temperature of Eight Intertidal Seaweeds Match Recent Abundance Shifts. *Scientific Reports*, **8**, Article No. 12978. <https://doi.org/10.1038/s41598-018-31357-x>
- [38] Raven, J.A. and Hurd, C.J. (2012) Ecophysiology of Photosynthesis in Macroalgae. *Photosynthesis Research*, **113**, 105-125. <https://doi.org/10.1007/s11120-012-9768-z>
- [39] Hurd, C.L. (2017) Shaken and Stirred: The Fundamental Role of Water Motion in Resource Acquisition and Seaweed Productivity. *Perspective in Phycology*, **4**, 73-81. <https://doi.org/10.1127/pip/2017/0072>
- [40] Boderskov, T., Schmedes, P.S., Bruhn, A., Rasmussen, M.B., Nielsen, M.M. and Pedersen, M.F. (2016) The Effect of Light and Nutrient Availability on Growth, Nitrogen, and Pigment Contents of *Saccharina latissima* (Phaeophyceae) Grown in Outdoor Tanks, under Natural Variation of Sunlight and Temperature, during Autumn and Early Winter in Denmark. *Journal of Applied Phycology*, **28**, 1153-1165. <https://doi.org/10.1007/s10811-015-0673-7>
- [41] Bird, K.T. (1987) Seasonal Variation in Protein: Carbohydrate Ratios in a Subtropical Estuarine Alga, *Gracilaria verrucosa*, and the Determination of Nitrogen Limitation Status Using These Ratios. *Botanica Marina*, **27**, 111-115. <https://doi.org/10.1515/botm.1984.27.3.111>
- [42] Andria, J.R., Vergara, J.J. and Pérez-Llorens, J.L. (1999) Biochemical Responses and Photosynthetic Performance of *Gracilaria* sp. (Rhodophyta) from Cádiz, Spain, Cultured under Different Inorganic Carbon and Nitrogen Levels. *European Journal of Phycology*, **34**, 497-504. <https://doi.org/10.1080/09541449910001718851>
- [43] Neish, A.C. and Shacklock, P.F. (1971) Greenhouse Experiments on the Propagation of Strain T4 of Irish Moss. Atlantic. Reg. Tech. Report. Serial No. 14, 25 p.
- [44] Deборе, J.A. (1979) Effects of Nitrogen Enrichment on Growth Rates and Phycocolloid Content in *Gracilaria foliifera* and *Neoagardhiella bailey* (Florideophyceae). *Proceedings of the International Symposium*, **9**, 263-273.