Population Dynamics of Colonizing Fauna and Its Effect on Growth Rates of the Farmed Red Alga *Alsidium triquetrum* (S. G. Gmelin) Trevisan

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ABSTRACT

Herbivores can drastically alter the morphology of macroalgae by directly consuming tissue and by inflicting structural wounds. Macroalgae host abundant and diverse epibiont communities, the dynamics of which tend to be mostly unknown in space and time. As the cultivation of macroalgae gains momentum worldwide, it is key to measure how epibionts could affect algal performance. We examined the epibiont community associated with farmed *Alsidium triquetrum*, a red macroalga with growing pharmacological interest. Measurements were conducted over two independent 60-day periods, one in summer and one in winter. Epibionts showed different patterns of succession in both seasons. Crustaceans, mainly amphipods, showed the highest overall density, with deleterious effects on daily growth rates of *A. triquetrum* in winter. Adverse effects as a function of epibionts were not detected in summer. *A. triquetrum* is a perennial alga. However, its performance as a crop in the nearshore can be significantly affected by the epibiont community structure that persists in winter. Amphipods and ascoglossan molluscs were risk factors in the mariculture of this agarophyte. In winter, they can destroy plants when they reach more than five individuals per gram of fresh biomass. Results highlight that commercial farming of *A. triquetrum* would be successful if grown throughout the summer.

1. INTRODUCTION

The increasing demand for macroalgal biomass has stimulated a global expansion of macroalgal farming [1]. It has also encouraged research on species that are not yet domesticated but contain components of interest, for the biomedical and pharmacological industry [2, 3]. This is the case of *A. triquetrum* that its distribution enables the renewal and supply of new seedlings in cultivation systems, if production processes are implemented [4]. This is favored by the availability of N and P, whose dynamics guarantee the permanence of the species throughout the annual cycle [5]. While the exploitation of macroalgae gains momentum, fundamental knowledge gaps related to ecological interactions emerge. One example is the limited information regarding epibiotic interactions between farmed macroalgae and macroinvertebrates.

Studies dealing with epibionts associated with macroalgae are scarce, probably due to the considerable time and effort required to sort and identify relatively small macroinvertebrate specimens [6]. However, faunistic information is essential for both mariculture [7, 8] and ecological and biogeographic studies [9].

In coastal areas, macroalgae facilitate survival and successful recruitment needs for a variety of organisms. For instance, macroalgae provide protection against predators [10], tidal currents and waves [11], increase the availability of oxygen in the water [12], and provide a substrate for growth [13]. Similar to wild-standing populations, epibiotic on macroalgal farms could have the attributes of a true community, structured from a set of habitats with staggering subordination levels [14]. Epibionts could also experience typical seasonal variations [15, 16] and be positively or negatively influenced by the metabolic activity of algal tissue [17], all of which could ultimately have synergistic effects on the performance of an algal crop.

The effect that epibionts could have on algal biomass may differ. On the one hand, epibionts could contribute to increased macroalgal growth, for instance, by providing a provision of ammonium [18]. On the other, their presence could reduce yields, tissue quality, or even result in the entire devastation of a farm [19, 20]. These contrasting outcomes highlight the relevance of understanding the dynamics of the epibiont community facilitated by the macroalgal farms as a factor to predict the stability and yields of proposed farm sites and macroalgal species of interest. In this study, we characterized the seasonal population dynamics of epibionts associated with an experimental farm of the red macroalga *Alsidium triquetrum* (S. G. Gmelin) Trevisan. Characterizations were conducted over two distinct climatic seasons. We further determined the relationship between the epibiont communities with growth rates exhibited by *A. triquetrum* during different cultivation periods.

2. MATERIALS AND METHODS

2.1. Experimental Period and Study Site

This study was conducted in Havana, Cuba, over two independent periods of 60 consecutive days. The first assessments were conducted during the dry season (December-February; hereafter referred to as winter), the second, during the rainy season (June-August; hereafter referred to as summer) on an experimental farm installed within a breakwater, approximately 1 km W from the mouths of the Quibú River. The farm consisted of a flexible structure assembled with four corner buoys, each one separated from the other by 10 m but connected using polypropylene rope (8 mm diameter) to form a square. Each buoy was secured to independent moorings made of steel and concrete, sitting on the substrate at no more than 0.50 - 0.75 m from the surface and between 2.5 to 3.0 from the bottom (**Figure 1(a)**). Given the sheltered environmental conditions at the site, no further structural reinforcement was required to hold the farm in place.

2.2. Experimental Macroalgae

The red macroalga *Alsidium triquetrum* is the most robust and ubiquitous agarophyte along the breakwater of Havana. It forms dense mats that persist throughout the year, withstanding extreme salinity shifts caused by freshwater input during the rainy season (summer). Several studies have assessed the

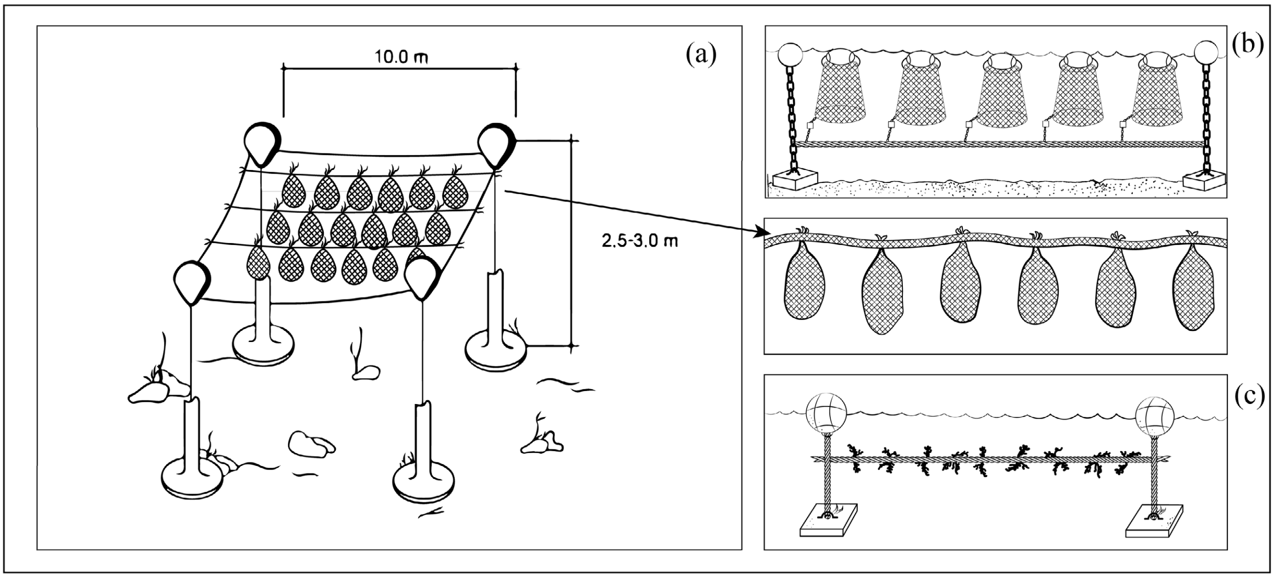

Figure 1. (a) Support structure used to hold the ropes with different planting treatments, (b) bags with 6 cm holes, the arrow indicates (close up)., suspended baskets and attached to supports, (c) direct attachment to the rope.

neuroprotective, antioxidant, and anti-inflammatory potential of *A. triquetrum* [3, 21], raising interest in domesticating the species to extract high-value compounds. Given that *A. triquetrum* could be farmed year-round, it is ideal to evaluate seasonal changes in the dynamics of epibionts on farmed biomass and assess whether epibionts have any effect on growth rates along farming periods.

2.3. Experimental Setup

Thalli were collected from a bed from the vicinity of the experimental site a few hours before deployment at the farm. Each thallus was cleaned vigorously using filtered seawater to remove particles and fauna. Then, 30 thalli were sectioned into 25 and 50 g (wet weight; ww) fragments (n = 15 each) and cleaned once more to ensure samples were free from associated organisms.

The use of two different weights allows to identify how the initial biomass influences growth. By using 50 g samples, the development of epibiosis can be better measured, since there are larger growth areas, and therefore more niches or shelters available. Inverse to that expressed by 25 g specimens, where the availability of effective habitats is limited.

Once cleaned, all fragments were placed individually inside mesh bags (33 × 33 cm with 6 cm mesh openings). In order to arrive at the adequate opening diameter of 6 cm, the intervals (3, 6 and 9 cm were previously checked) according to previous methods [22]. Furthermore, it was verified that the plants grew better with the 6 cm opening of light, because those with 3 cm impeded the passage of light and the 9 cm ones were affected by juvenile fish (**Figure 1(a)**, see detail).

Bags were then installed randomly onto three 10.5 m polypropylene ropes in such a way that each rope held 10 bags separated one from the next by approximately 90 cm. Once assembled onto the ropes, these were installed on the farm by securing their ends to opposite sides of the flexible structure. The newly installed ropes were distanced at 3 m. intervals.

Every ten days, three bags per initial weight (*i.e.*, 25 or 50 g) were randomly selected and removed from the farm to measure thalli growth using dry weight as a proxy and evaluate the colonization process by quantifying, identifying, and measuring the biomass of the associated epibionts. Before detaching the selected mesh bags from their holding rope, they were introduced gently into a bigger polyethylene envelope that was then sealed to avoid escapees of the dwelling animal. Once collected, envelopes were placed inside a cooler and transported to the laboratory for processing. Upon arrival, samples were preserved by adding 10% neutralized formalin with sodium tetraborate. The same process was repeated over the two independent growing seasons until all bags were removed from the farm.

Since little is known about the behaviour of the species under culture conditions; if compared to other species. Establishing experimental arrangements that could presuppose an arbitrary or *a priori* definition of the most incident variables was avoided. Therefore, we opted to carry out a number of short-term experiments that would allow the step-by-step analysis of each factor chosen [*i.e.*, in this case, the growth of *Alsidium*], in rectangular boxes or direct support (**Figure 1(b)** and **Figure 1(c)**).

2.4. Data Acquisition and Processing

Preserved samples were poured onto a 0.25 mm sieve and rinsed with running water to detach all organisms. Organisms collected were then placed on holding containers. Separation and identification of all epibionts were made from small aliquots poured into a Bogoroff chamber. In this study, the identification of epibionts was limited to invertebrates. Their taxonomic classification was conducted to the level of order to determine the dominant groups throughout the experimental period.

The classification of fauna in major taxonomic categories [23, 24] or in subset related to shape, size, or color attributes [25] allow bypassing systematic specialization and facilitates rapid aggregation of sample to groups and classes to calculate various indices [26, 27] or evaluate changes in the community.

Once classified and identified, epibionts were dried in a stove with forced air circulation at about 60˚C for 24 hours. Each group was then weighed three times on an analytical balance 50/200g 0.01/0.1mg. Serie 5035 to obtain the average biomass based on dry weight. The community's equitability was assessed using an asymmetry index expressed as:

$$AI = [\sum (Ni/Nt - 1/k)^2]½$$

where Nt is the total number of organisms, Ni is the number of organisms per group, and k is the number of groups. The index ranges from 0 to 1, indicating a complete evenness or maximum asymmetry, respectively, within the community.

The relative variability in the number of individuals per sample was obtained using the variation coefficient (% V) corrected for bias following [28].

The daily growth rate of *A. triquetrum* was calculated as:

$$DGR\ [\text{daily growth rate}] = [(\ln Wt/InWi)\ 1/t - 1] \times 100$$

where ln = natural logarithm, Wt and Wi are the wet weights of each thallus (g) at time one and time zero, and (t) is the time in days [29]. Wet weight of each thallus was obtained without epibionts.

Data analysis to assess differences in the density, biomass, and asymmetry of epibionts and differences between thalli of 25 and 50 g across seasons was conducted using the Mann-Whitney U test [30] with a level of significance of 0.05. The effect of epibiont density and *A. triquetrum* biomass was explored using linear regressions.

3. RESULTS

The overall epibiont community associated with *A. triquetrum* was divided into 21 taxonomic groups, with only four of those groups showing unique to either summer or winter season (**Table 1**). The overall density of epibionts was always higher in winter compared to summer (**Figure 2**) both in larger (*i.e.*, Wi = 50 g) and smaller (*i.e.*, Wi = 25 g) thalli ($U_\varphi = 0.01$). Crustaceans showed the most diverse group, with ten genera represented in both seasons (**Figure 3**). This group, particularly individuals within the class Malacostraca, showed the highest percentage of organisms (*i.e.*, 93.2% in summer and 91.0% in winter; **Figure 3**). Annelids, mainly polychaetes, were the second-largest group representing 5.7% of the total epibionts during summer and 8.2% in winter. None of the other groups presented more than 1% of the total density of epibionts.

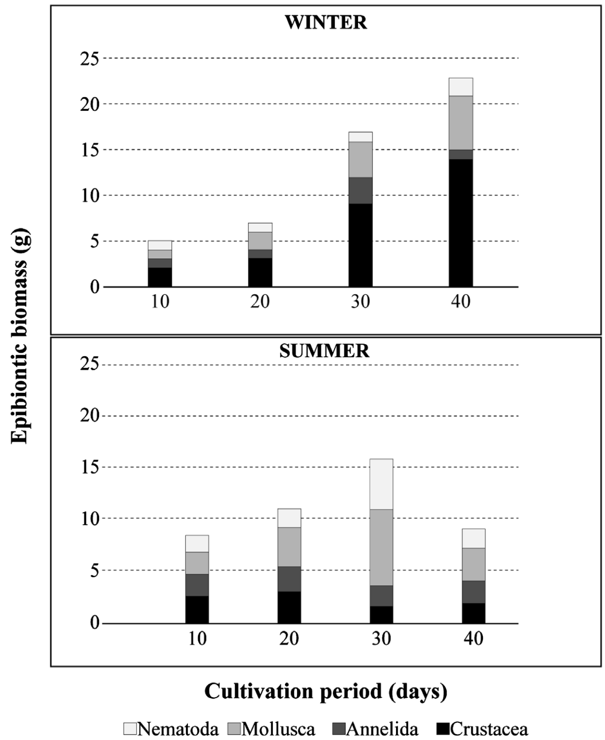

Figure 2. Biomass (dw) distribution of the major groups of epibionts associated with *A. triquetrum* quantified in thalli samples with an initial weight of 50 g (dw = dry weight).

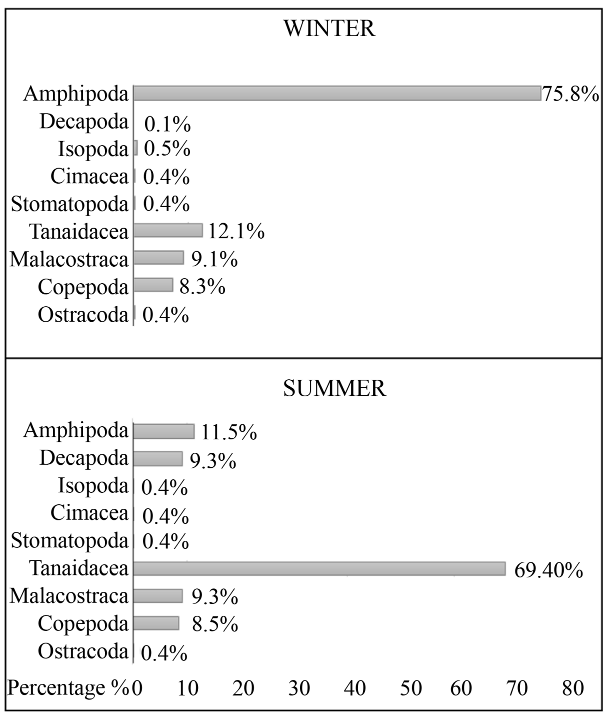

Figure 3. Percentage of crustaceans of the class Ostracoda, Copepoda, and Malacostraca, as well percentage of individuals within orders integrating Malacostraca quantified in thalli samples with an initial weight of 50 g.

Table 1. **Taxonomical composition of epibionts associated with farmed *Alsidium triquetrum* on an experimental farm in the Caribbean Sea off Havana during winter and summer season.**

Taxa	Experimental period	
	Summer	Winter
NEMATODA	x	x
ANNELIDA		
Polychaeta	x	x
Oligochaeta	x	x
MOLLUSCA		
Bivalvia	x	
Gastropoda	x	x
Nudibranchia	x	x
Polyplacophora		x
ARTHROPODA		
CRUSTACEAE		
Nebaliaceae		x
Ostracoda	x	x
Copepoda	x	x
Stomatopoda	x	
Cumacea	x	x
Tanaidaceae	x	x
Amphipoda	x	x
Brachyura	x	x
Penaeidea	x	x
Caridea	x	x
ECHINODERMATA		
Echinoidea	x	x
Ophiuroidea	x	x
Holothuroidea	x	x
CHORDATA		
Ascidiacea	x	x

Despite the similarities of the taxa present in both seasons, colonization and succession patterns of the overall epibiont community differed between winter and summer (**Figure 2** and **Figure 3**). In terms of biomass, crustaceans dominated throughout winter, driving an increment in epibiont biomass for the first 40 days of cultivation (**Figure 2**). On the other hand, opisthobranchs dominated epibiont biomass in summer (**Figure 4(b)**). Contrary to the overall pattern in epibiont biomass observed in winter, biomass decreased 30 days after deployment of *A. triquetrum* in summer (**Figure 2**). Still, epibiont biomass measured in summer was higher than in winter ($U_{\varphi} = 0.01$).

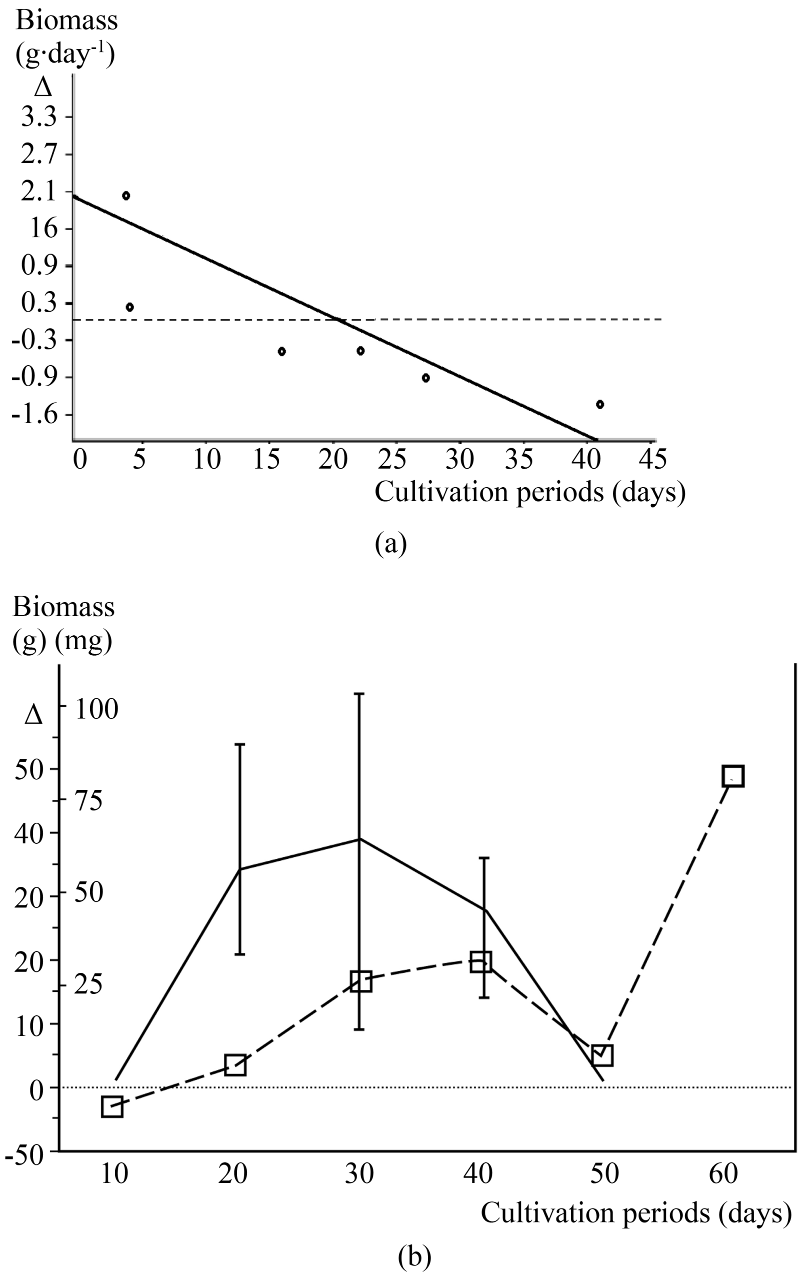

Figure 4. (a) Linear regression of average thalli biomass (ww) and amphipod density in winter ($R^2 = -0.83$; p = 0.02), (b) Evolution of opistobranquia [----] mean biomass (dw) in relation with mean increment in weight (ww) of cultivated *A. triquetrum* [—]. Summer. Individual with initial weight = 50 g of initial weight. (Vertical bars referred to extreme values) (ww = wet weight).

Clear patterns of succession were detected particularly in winter cultivation, with copepods dominating the epibiont community for the first 10 to 20 days after deployment.

Copepods were then replaced by amphipods, which remained abundant throughout the rest of the experimental period (Figure 4(a)). Amphipods, which constituted 75.8% of all quantified organisms in winter, decreased to 11.5% during summers as opposed to tanaidaceans, predominant in summer (69.4%) and underrepresented in winter (12.8%; Figure 3).

Differences in the dominance of species in winter were negatively correlated with changes in the DGR of *A. triquetrum*, shifting from positive to negative as amphipods increased their abundance per gram of tissue (Figure 5(b) and Figure 6). In contrast, DGR of *A. triquetrum* farmed in summer showed a positive trend throughout the entire 60-day period, reaching a maximum DGR 40 days after deployment (Figure 5). The asymmetry index shows a greater asymmetry within the epibiont community 40 days after out planting for winter and summer seasons. Results also show that the asymmetry is greater in larger than in smaller thalli (Figure 7).

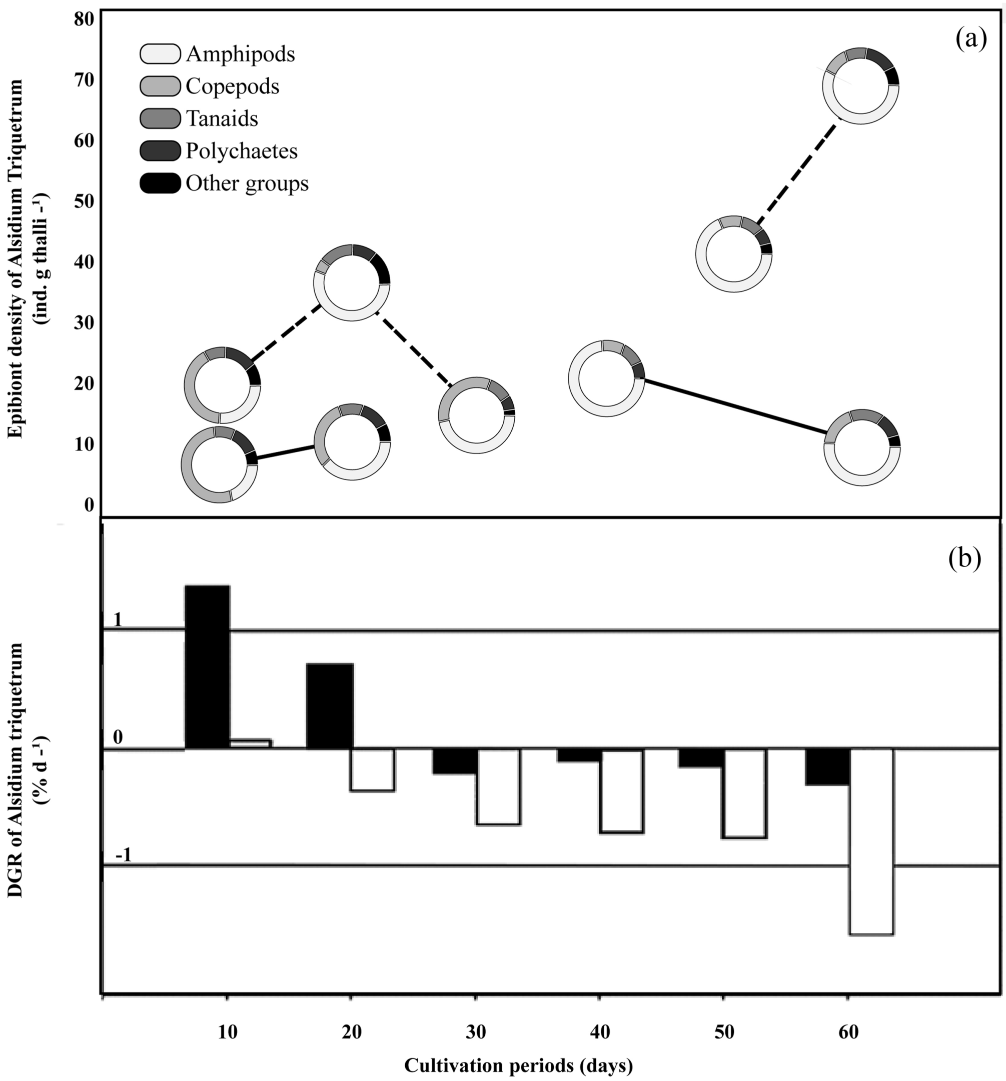

Figure 5. (a) Total density of epibionts per gram of *A. triquetrum* and (b) DGR [Daily Growth Rate] of *A. triquetrum* in relation to changes in the epibionts community throughout a 60-day cultivation period in winter. Dashed lines track thalli changes with an initial weight of 25 g (□) and solid lines of thalli with an initial weight of 50 g (■).

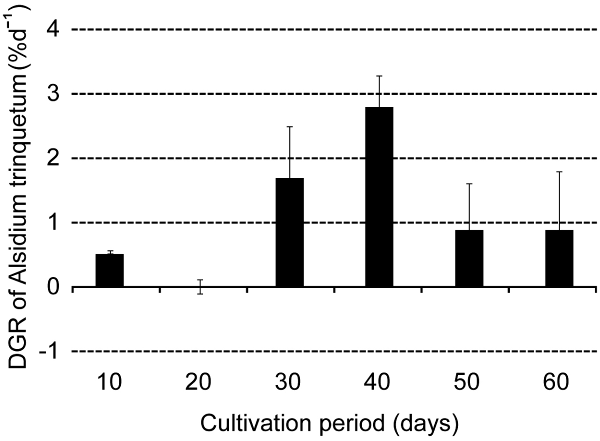

Figure 6. Average DGR of *A. triquetrum* (initial weight = 50 g) throughout a 60-day cultivation period in summer.

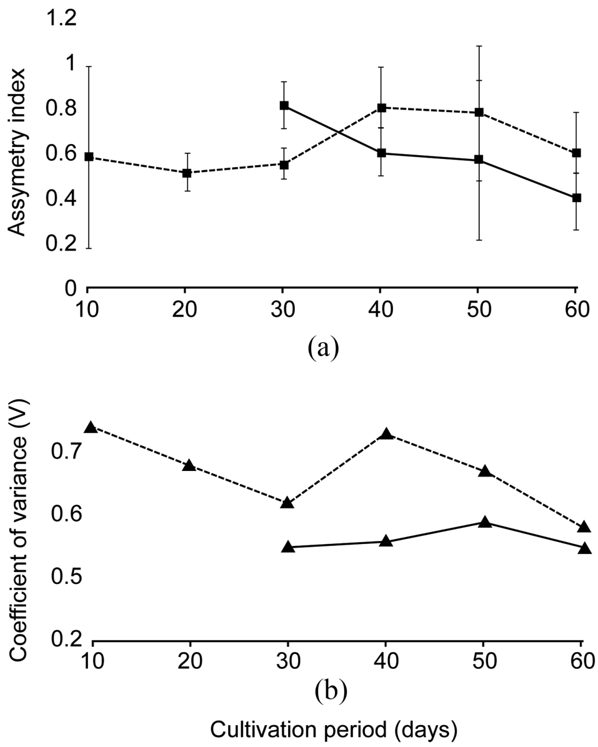

Figure 7. (a) Asymmetry index (circles) and (b) coefficient of variation of the abundance of epibionts (triangles) during a 60-day cultivation period in winter (dashed line) and summer (solid line) in thalli with an initial weight of with 50 g. Vertical bars denote extreme values.

4. DISCUSSION

An increasing search of natural origin antioxidants has fueled the interest of biomedical, pharmaceutical, nutraceutical, and cosmetology industries to explore the chemical and structural properties of multiple marine macroalgae, including *Alsidium triquetrum*. This growing interest in high value macroalgal products has motivated efforts in domesticating new species. Mariculture of *A. triquetrum* as a source of agar has already been explored in Cuba, analyzing distribution patterns, growth rates, and tolerance to temperature and salinity changes aimed at informing site selection [4]. Results herein showed that the interaction of *A. triquetrum* and epibionts should also be considered a factor in determining farm sites and growing periods.

Plant-herbivore interactions in the marine environment can often reduce macroalgal biomass [31, 32]. For example, [20] studied the presence of the isopod *Cymodocea japonica* around the farmed kelp *Undaria pinnatifida*. They found that the size-frequency distribution of the isopod changed throughout the farming period and that consumption rates of isopods increased with temperature. These authors highlighted the risks of intense herbivory during cultivation. Similarly, the epibiont colonization process in farmed *A. triquetrum* followed different patterns linked to differences in environmental conditions in winter and summer. Coinciding with [20], we detected an outbreak of herbivory associated with crustaceans that ultimately damaged our algal crop.

In this study, the seasonal variation in biomass and density of epibionts were influenced by multiple factors, including the contribution of molluscs (greater biomass) and decapods (higher density), the stage of succession within the community, environmental conditions, and the functional state of *A. triquetrum*, which served as substrate. It is important to acknowledge that the patterns in the epibiont described here may be linked to the farming conditions in our experimental site and may differ from other potential farm sites. Studies assessing communities associated with wild-standing macroalgal beds report a greater abundance of amphipods during summer [33, 34] or fall [35], contrasting with our findings. The trends summarized by our study may also be influenced by the presence of opportunistic omnivores such as shrimp, capable of consuming amphipods [36], and typically showing higher biomass in summer. Moreover, a long-term (9-year) study showed that the physical differences of water depth and substrate in the intertidal and subtidal sites influence the composition of macroalgal communities [37], which in turn could also affect the presence of different epibionts.

The performance of structural indicators, such as the degree of asymmetry of epibionts, suggests that the community achieved a state of maturity over the relatively short period of cultivation (*i.e.*, 60 days). Studies have demonstrated that the three-dimensional structural complexity of the macroalgae facilitates both the abundance and the richness of associated organisms [38-40] Similarly, in this study, larger thalli fragments showed greater asymmetry in the epibiont community, possibly related to those fragments having a greater number of branches. The asymmetry observed decreased after 40 days of cultivation, consistent with an increase in equitability towards the later stages of succession [41].

Ascoglossos, are part of another group potentially harmful to crops. With numerous species whose populations are seasonal and disruptive, they can cause massive destruction of vegetation cover at high altitudes when the ratio: population biomass *vs.* vegetable biomass expressed in dry weight exceeds 1% [42]. Together with the phytophagic ichthyofauna, they have been considered the main consumers of algae on coral reefs. In them; however, their populations are generally small and aggregated due to their group specialization in habitat selection and the inherent patience of the ecosystem. Even so, it can generate migratory cycles of short duration, colonizing and depressing the activity of the host plant even at levels far from the bottom of more than two meters (**Figure 4**). The highest densities of individuals, as well as the highest diversity of species, have been found in the Caribbean between the mangrove swamp and the coral sand beds [43], a very appropriate transition zone due to its hydrodynamic and trophic nature for *Alsidium* mariculture [44].

In the summer, however, the massive outbreak of opisthobranchs was the most prominent event in the succession experienced by the community.

In **Figure 8**, the variation in biomass is compared with the use of three methods described for the species by [22], (see **Figures 1(a)-(c)**).

The one that uses the suspended baskets, although it presents considerable accumulations of biomass, is not effective; and it was used briefly, due to the cost of manufacturing the artifacts and the dispersion of the values. In the analysis of the plants in bags, and those fixed to ropes, they were the most widespread and the second the most effective for the propagation of the species for productive purposes [4].

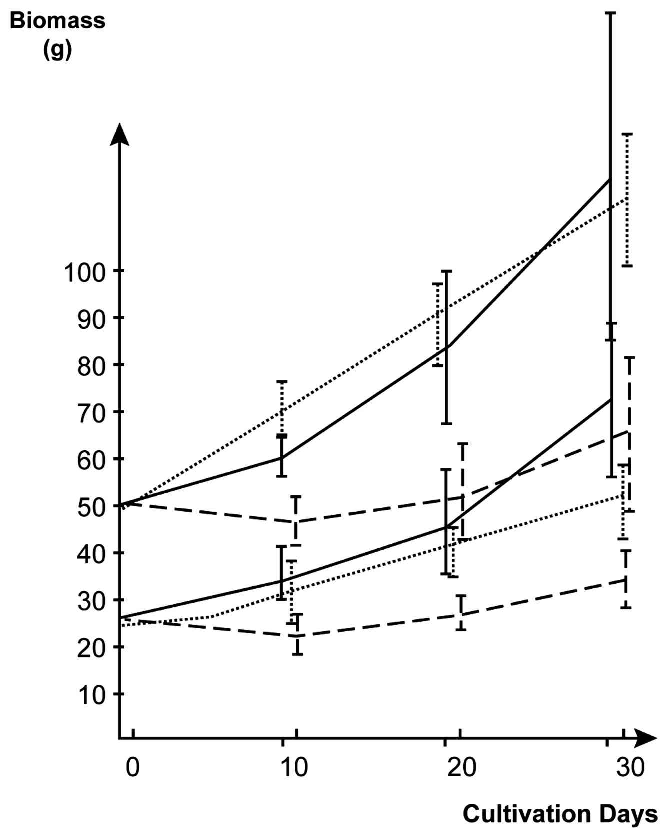

Figure 8. Evolution of the weight of specimens of 25 and 50 g (ww) of *Alsidium triquetrum* during 30 days. In baskets (—), in bags (---), and directly on ropes (…). Each value, bounded ± SD, constitutes the average of five individuals in the summer.

The use of bags made it possible, however, to significantly reduce the loss of cultivated material in winter conditions of turbulence and resuspension of sediments and achieved, during the summer months, increases in biomass of the order of 3.10 $g \cdot m^{-1}$ day. In addition to effectively evaluating the colonizing dynamics of the epifauna, the fundamental objective of this research.

5. CONCLUSION

Although multiple algal species such as *A. triquetrum* are capable of withstanding and thriving despite changes in environmental conditions, they may not prosper in the presence of large densities of epibionts. As shown by our results, the cultivation of *A. triquetrum* in the nearshore must consider the dynamics of epibionts as a crucial factor. This study indicates that site selections for macroalgal farming should also consider the interaction of macroalgae and potential epibionts that may change based on season and cultivation period.

ROLES BY AUTHORS

RC and AA conceived and designed the experiments, analyzed the data, prepared figures, and reviewed all manuscript drafts. JDL prepared figures and worked on conceptualization, data curation, and analysis. SKS contributed to editing and reviewing all drafts of this manuscript. LNG contributed to data analysis, provided reagents, supplies, analysis tools, and reviewed all drafts for this manuscript. JRC-A worked on conceptualization and figure revisions. All authors have read and agreed to the version of the manuscript submitted.

CONFLICTS OF INTEREST

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

REFERENCES

1. Buschmann, A.H., Camus, C., Infante, J., Neori, A., Israel, A., Hernández-González, M.C., Pereda, S.V., Gómez-Pinchetti, J.L., Golberg, A., Tadmor-Shalev, N. and Critchley, A.T. (2017) Seaweed Production: Overview of the Global State of Exploitation, Farming and Emerging Research Activity. *European Journal of Phycology*, **52**, 391-406. https://doi.org/10.1080/09670262.2017.1365175

2. Garbary, D.J. (2017) Harvesting *Ascophyllum nodosum* (Phaeophyceae) Reduces the Abundance of Its Host-Specific Epiphyte *Vertebrata lanosa* (Rhodophyta). *Botanica Marina*, **60**, 297-301. https://doi.org/10.1515/bot-2016-0074

3. Vidal, A., Fallarero, A., Silva, E., De Oliveira, S., De Lima, A., Pavan, R., Vuorela, P. and Mancini-Filho, J. (2006) Chemical Composition and Antioxidant Activity of the Red Algae *Bryothamnion triquetrum* (S. G. Gmelin) Howe). *Brazilian Journal of Pharmaceutical Sciences*, **42**, 589-600. https://doi.org/10.1590/S1516-93322006000400015

4. Areces, A.J., Cabrera, R. and Díaz-Larrea, J. (2020) Biotecnología de agarófitas del género Alsidium C. Agardh. Editorial Académica Española, 1-127.

5. Cabrera, R., Areces, A., Díaz-Larrea, J., García, L. and Cruz-Aviña, J. (2021) Influence of the Macronutrients N, P and K on the Agarophyte *Alsidium triquetrum* (S. G. Gmelin) Trevisan, during Experimental Culture. *American Journal of Plant Sciences*, **12**, 573-585. https://doi.org/10.4236/ajps.2021.124038

6. Sahu, S.K., Ingle, K.N. and Mantri, V.A. (2020) Epiphytism in Seaweed Farming: Causes, Status, and Implications. In: Gothandam, K., Ranjan, S., Dasgupta, N. and Lichtfouse, E., Eds., *Environmental Biotechnology*, Vol. 1, Environmental Chemistry for a Sustainable World, Vol. 44, Springer, Cham, 228-242. https://doi.org/10.1007/978-3-030-38192-9_9

7. Peteiro, C. and Freire, O. (2013) Epiphytism on Blades of the Edible Kelps *Undaria pinnatifida* and *Saccharina latissima* Farmed under Different Abiotic Conditions. *Journal of the World Aquaculture Society*, **44**, 706-715. https://doi.org/10.1111/jwas.12065

8. Ashwini, K., Soundarapandian, P., Jagan, K., Anatharaman, P., Kannan, D., Sanjeev, K., Thirunavukkarasu, P. and Arun, K. (2014) Associated Fauna in Cultured Seaweed *Kappaphycus alvarezii* of Vellar Estuary (South East Coast of India). *International Journal of Marine Science*, **3**, 37-43.

9. Guerra-García, J. M., Cabezas, M.P., Baeza-Rojano, E., Izquierdo, D., Corzo, J., Ros, M., Sánchez, J.A., Dugo-Cota, A., Flores-León, A.M. and Soler-Hurtado, M.M. (2011) Abundance Patterns of Macrofauna Associated to Marine Macroalgae along the Iberian Peninsula. *Zoologica Baetica*, **22**, 3-17.

10. Haavisto, F. (2016) Macroalgal Defenses against Herbivory: Causes and Consequences of Intraspecific Variation. Volume 319 of Turun Yliopiston Julkaisuja. Ser A 2.

11. Denny, M. and Gaylord, B. (2002) The Mechanics of Wave-Swept Algae. *Journal of Experimental Biology*, **205**, 1355-1362. https://doi.org/10.1242/jeb.205.10.1355

12. Stewart, H.L. and Carpenter, R.C. (2003) The Effects of Morphology and Water Flow on Photosynthesis of Marine Macroalgae. *Ecology*, **84**, 2999-3012. https://doi.org/10.1890/02-0092

13. Nieder, C., Liao, C.P., Chen, C.A. and Liu, S.L. (2019) Filamentous Calcareous Alga Provides Substrate for Coral-Competitive Macroalgae in the Degraded Lagoon of Dongsha Atoll, Taiwan. *PLoS ONE*, **14**, e0200864. https://doi.org/10.1371/journal.pone.0200864

14. Aoki, M. and Kikuchi, T. (1990) Habitat Adaptations of Caprellid Amphipods and the Importance of Epiphytic

Secondary Habitats in the *Sargassum patens* Bed in Amakusa, Southern Japan. *Publications from the Amakusa Marine Biological Laboratory, Kyushu University*, **10**, 123-133.

15. Gestoso, I., Olabarria, C. and Troncoso, J.S. (2010) Variability of Epifaunal Assemblages Associated with Native and Invasive Macroalgae. *Marine and Freshwater Research*, **61**, 724-731. https://doi.org/10.1071/MF09251
16. Veiga, P., Rubal, M. and Sousa-Pinto, I. (2014) Structural Complexity of Macroalgae Influences Epifaunal Assemblages Associated with Native and Invasive Species. *Marine Environmental Research*, **101**, 115-123. https://doi.org/10.1016/j.marenvres.2014.09.007
17. Wahl, M., Goecke, F., Labes, A., Dobretsov, S. and Weinberger, F. (2012) The Second Skin: Ecological Role of Epibiotic Biofilms on Marine Organisms. *Frontiers in Microbiology*, **3**, Article No. 292. https://doi.org/10.3389/fmicb.2012.00292
18. Hepburn, C.D. and Hurd, C.L. (2005) A Conditional Mutualism between the Giant Kelp *Macrocystis pyrifera* and Colonial Epifauna. *Marine Ecology Progress Series*, **302**, 37-48. https://doi.org/10.3354/meps302037
19. Newcombe, E. and Taylor, R. (2010) Trophic Cascade in a Seaweed-Epifauna-Fish Food Chain. *Marine Ecology Progress Series*, **408**, 161-167. https://doi.org/10.3354/meps08589
20. Endo, H., Sato, Y., Kaneko, K., Takahashi, D., Nagasawa, K., Okumura, Y. and Agatsuma, Y. (2020) Ocean Warming Combined with Nutrient Enrichment Increases the Risk of Herbivory during Cultivation of the Marine Macroalga *Undaria pinnatifida*. *ICES Journal Marine Science*, **78**, 402-409. https://doi.org/10.1093/icesjms/fsaa069
21. Luiz, H.A.C., Brito da Matta, C.B., de Araújo, M.V., Barbosa-Filho, J.M., de Lira, D.P. and de Oliveira Santos, B.V. (2012) Antinociceptive and Anti-Inflammatory Activities of Crude Methanolic Extract of Red Alga *Bryothamnion triquetrum*. *Marine Drugs*, **10**, 1977-1992. https://doi.org/10.3390/md10091977
22. 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
23. Pielou, E.C. (1975) Ecological Diversity. John Wiley & Sons, New York, viii + 165 p.
24. Schulze, E.D., Beck, E., Buchmann, N., Clemen, S., Müller-Hohenstein, K. and Scherer-Lorenzen, M. (2019) Biodiversity. In: Schulze, E.D., Beck, E., Buchmann, N., Clemen, S., Müller-Hohenstein, K. and Scherer-Lorenzen, M., Eds., *Plant Ecology*, Springer, Berlin, 743-823. https://doi.org/10.1007/978-3-662-56233-8
25. Cairns, J. and Kenneth, D. (1971) A Simple Method for the Biological Assessment of the Effects of Waste Discharges on Aquatic Bottom-Dwelling Organism. *Journal Water Pollution Control Federation*, **5**, 755-772.
26. Patil, G.P. and Taillie, C. (1976) Ecological Diversity: Concepts, Indexes and Applications. *Proceeding of the International Biometric Conference*, Fairland, Maryland, 382-441.
27. Keefe, T.J. and Bergersen, E.P. (1977) A Simple Diversity Index Based on the Theory of Runs. *Water Resources Bulletin*, **11**, 689-691. https://doi.org/10.1016/0043-1354(77)90108-7
28. Sokal, R.R. and Rohlf, F.J. (1981) Biometry. Second Edition, W. H. Freeman, New York.
29. Yong, Y.S., Yong, W.T.L. and Anton, A. (2013) Analysis of Formulae for Determination of Seaweed Growth Rate. *Journal of Applied Phycology*, **25**, 1831-1834. https://doi.org/10.1007/s10811-013-0022-7
30. Zar, J.H. (2010) Biostatistical Analysis. Fourth Edition, Prentice Hall, Hoboken, 944 p.
31. Poore, A.G.B., Campbell, A.H., Coleman, R.A., Edgar, G.J., Jormalainen, V., Reynolds, P.L., Sotka, E.E., Stachowicz, J.J., Taylor, R.B., Vanderklift, M.A. and Duffy, J.E. (2012) Global Patterns in the Impact of Marine Herbivores on Benthic Primary Producers. *Ecology Letters*, **15**, 912-922. https://doi.org/10.1111/j.1461-0248.2012.01804.x
32. Rogers-Bennett, L. and Catton, C.A. (2019) Marine Heat Wave and Multiple Stressors Tip Bull Kelp Forest to

Sea Urchin Barrens. *Scientific Reports*, **9**, Article No. 15050. https://doi.org/10.1038/s41598-019-51114-y

33. Areces, A.J., del Valle, R., Ibarzabal, D., Pol, J. and Hernández, L. (1992) Características generales de la mesofauna asociada a cuatro especies de macroalgas del mesolitoral rocoso cubano. *Ciencias Biológicas*, **25**, 117-131.

34. Válerio-Bernardo, M.T. and Flynn, M.N. (2002) Composition and Seasonality of an Amphipod Community Associated to the Algae *Bryocladia trysigera*. *Brazilian Journal of Biology*, **62**, 735-742. https://doi.org/10.1590/S1519-69842002000400021

35. Duffy, J.E. and Hay, M.E. (2000) Strong Impacts of Grazing Amphipods on the Organization of a Benthic Community. *Ecological Monographs*, **70**, 237-263. https://doi.org/10.1890/0012-9615(2000)070[0237:SIOGAO]2.0.CO;2

36. Nelson, W.G. (1979) Experimental Studies of Selective Predation on Amphipods: Consequences for Amphipod Distribution and Abundance. *Journal of Experimental Marine Biology and Ecology*, **38**, 225-245. https://doi.org/10.1016/0022-0981(79)90069-8

37. Lin, S.M., Tseng, L.C., Ang, P.O., Bolton, J. and Liu, L.C. (2018) Long-Term Study on Seasonal Changes in Floristic Composition and Structure of Marine Macroalgal Communities along the Coast of Northern Taiwan, Southern East China Sea. *Marine Biology*, **165**, 83. https://doi.org/10.1007/s00227-018-3344-9

38. Scrosati, R. and Heaven, C. (2007) Spatial Trends in Community Richness, Diversity, and Evenness Across Rocky Intertidal Environmental Stress Gradients in Eastern Canada. *Marine Ecology Progress Series*, **342**, 1-14. https://doi.org/10.3354/meps342001

39. Thomsen, M.S., Stæhr, P.A., Nejrup, L. and Schiel, D.R. (2013) Effects of the Invasive Macroalgae *Gracilaria vermiculophylla* on Two Co-Occurring Foundation Species and Associated Invertebrates. *Aquatic Invasions*, **8**, 133-145. https://doi.org/10.3391/ai.2013.8.2.02

40. Scrosati, R.A. (2017) Community-Level Facilitation by Macroalgal Foundation Species Peaks at an Intermediate Level of Environmental Stress. *Algae*, **32**, 41-46. https://doi.org/10.4490/algae.2017.32.2.20

41. Odum, E.P. (1969) The Strategy of Ecosystem Development. *Science*, **164**, 262-270. https://doi.org/10.1126/science.164.3877.262

42. Chemello, R. and Milazzo, M. (2002) Effect of Algal Architecture on Associated Fauna: Some Evidence from Phytal Molluscs. *Marine Biology*, **140**, 981-990. https://doi.org/10.1007/s00227-002-0777-x

43. Queiroz, R.N.M. and Días, T.L.P. (2014) Mollusks Associated with Macroalgae of the Genus Gracilaria (Rhodophyta): Importance of Algal Fronds as a Microhabitat in a Hypersaline Mangrove in Northeastern Brazil. *Brazilian Journal of Biology*, **74**, S052-S063. https://doi.org/10.1590/1519-6984.20712

44. Areces, A.J., Cabrera, R. and Díaz-Larrea, J. (2022) Guía ilustrada para el cultivo *in Situ* de *Alsidium triquetrum*. Brazilian Publicações de Periódicos e Editora Ltda. https://doi.org/10.35587/brj.ed.0001359