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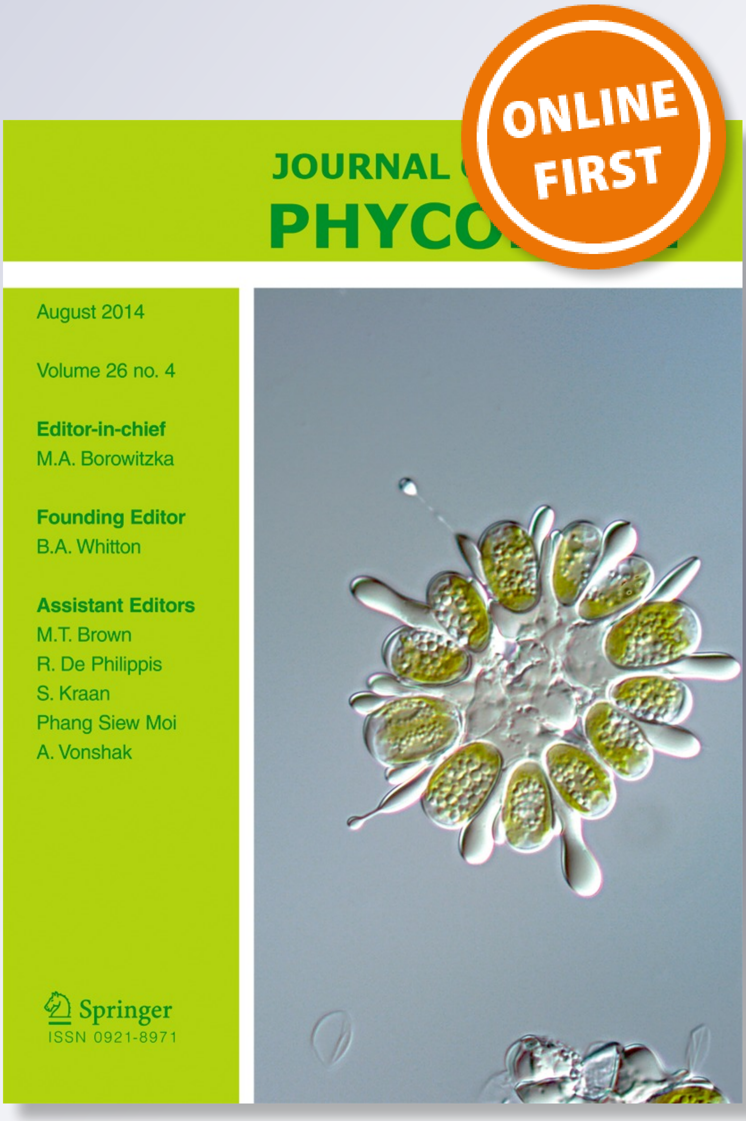

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Abstract The potential risks of cultivating carrageenophyte genera for commercial purposes in the circumtropical belt are debated. However, species introductions of two such genera, *Kappaphycus* Doty and *Eucheuma* J. Agardh, have been reported in 30 different locations in this region over the last 30 years. On several occasions, these introductions did not adequately evaluate potential environmental risks or were conducted without approval from local regulatory bodies. In the present paper, a working protocol is proposed for the quarantine and assessment of the possible effects of the introduction of *Kappaphycus alvarezii* and *Kappaphycus striatus* to shallow marine ecosystems of the tropical Western Atlantic. This protocol is based on field data following from the introduction of eucheumoids onto the Cuban shelf in the early 1990s. It was demonstrated that the propagation of either carrageenophyte in oligotrophic waters of the Cuban Archipelago did not pose a potential risk to the region's biodiversity due to the synergic combination of high herbivory and low rates of growth. Physical features of the substrate and depth were the most important regulators of grazing. These environmental conditions restrict potential cultivation sites in the Cuban Archipelago to a few small regions where nutrient pulses are well established. In these areas, when the canopy of cultivated carrageenophytes is sufficiently high, a significant effect on benthic communities is observed. In consideration of the need to protect places with high intrinsic value, this fact should be considered during site selection.

Keywords Commercial eucheumoids · Ecological hazards · Mariculture · Alien species

Introduction

The deliberate or accidental introduction of exotic species is considered one of the most significant causes of biodiversity loss over the last century. It entails economic costs (Mack et al. 2000) and biogeographic changes, leading to homogenization of the planet's biota (Mooney and Hobbs 2000) and the global loss of allopatric speciation and endemism (Simberloff 2000; Sulu et al. 2004), particularly in terrestrial ecosystems. Since 1971, market forces have introduced the commercial eucheumoids *Kappaphycus alvarezii*, *Kappaphycus striatus* and *Eucheuma denticulatum* into the marine ecosystems of 30 countries for research or commercial production (Ask et al. 2003), although the potential consequences are not considered harmless. Risk factors include the effects of associated biota introduced along with the carrageenophyte biomass, the duration of eucheumoid persistence in the new environment (Sulu et al. 2004) and escape and invasive propagation. These risks have been reported since the late1990s (Lemus Castro 1999; Verlecar and Pereira 2006; Bagla 2008). Case

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studies such as Kaneohe Bay (Rodgers and Cox 1999; Woo et al. 2000), India (Chandrasekaran et al. 2008; Kamalakanna et al. 2010) in the Indo-Pacific region and Venezuela in the western Tropical Atlantic (Lemus-Castro 1999; Barrios 2005) are well documented.

The first introduction of carrageenophytes from the Indo-Pacific into the Neotropical Realm in a natural environment occurred in Guadaloupe, French West Indies, in June 1981. It was conducted by Barbaroux and colleagues (1984) to test the growth of *E. denticulatum*. No subsequent introductions of tropical carrageenophytes in the Atlantic for commercial farming occurred until 1991 (Areces and Céspedes 1992). This delay was mostly likely due to the absence of a tropical based-carrageenan industry in the Philippines and Indonesia until recently and the lack of industrial-scale use of macroalgae in the northwestern Atlantic due to limited biomasses. However, after 1991, carrageenan use increased over the region, and select carrageenophytes were introduced by people aware of their potential benefits in Venezuela (Rincones and Rubio 1999), Brazil (Paula et al. 1998), Mexico (Muñoz et al. 2004), Panama (Batista de Vega et al. 2008; Trespoey et al. 2008) and Colombia (García-Vásquez and Pardo-Casto 2002; Rincones and Gallo 2004). The natural escape of one of these commercial carrageenophytes has been reported in Costa Rica (Rubén Cabrera, unpublished data).

With the exception of the introductions in Colombia, Mexico and Panama, which involved in situ tests with carrageenophytes imported from Cuba, Brazil and Venezuela, respectively, introductions involve carrageenophytes from the Philippines. Only Cuba and Brazil (Serpa-Madrigal and Areces 1995; 1998; Serpa-Madrigal et al. 1997; Paula et al. 2002; Bulboa and Paula 2005; Oliveira 2005; Bulboa et al. 2008; Castelar et al. 2009) have conducted tests to evaluate the potential ecological risks of introducing these taxa. Similarly, few field tests were conducted with biomass previously subjected to a strict quarantine term (Paula et al. 1998; Muñoz et al. 2004), and until recently (Araújo et al. 2013), none of these in situ tests used molecular markers to identify invasive lineages.

Many studies of the ecological impacts of these introductions and quarantine procedures by Cuban researchers remain unpublished. Examples of the planning stages and conceptual foundations would be valuable. This would be accomplished through a brief summary of research results and the development of methodological guidelines, from quarantine procedures to field tests.

Materials and methods

Tests for potential ecological risk assessment were conducted in an approximately 50 ha polygon-shaped area west of the Havana coast, Cuba (23°5′43.45″N; 82°28′31.44″W and 23°5′43.45″N; 82°27′58.65″W; Fig. 1) between January 1991 and September1993. The area and coordinates of this polygon were determined by identifying physical features that constitute coastal reference points and then using GPS and digital cartography on GIS platform (MapInfo v. 6.5). This area was selected for two reasons: the benthic habitats of the archipelago's shelf (eight of 14 zones, considering location; and 20 of 39 habitats, reflecting cover at the macro-scale; Areces 2002) and the many studies of this area of the Cuban shelf, including species inventories and ecological characterizations (Lluis 1974; Vinogradova 1974; Herrera-Moreno and Alcolado 1983; Alcolado and Herrera 1987; Valdés-Muñoz and Garrido 1987; Herrera and Martínez 1987; Lugioyo and Rodríguez 1988; del Valle et al. 1992). Details on the laboratory facilities used for quarantine and testing are provided in the *Protocol for Quarantine and Environmental Risk Assessment* proposed in Annex 1, as are the considerations for ecological risk evaluation.

Apices of up to 5 g each from the thalli of *K. alvarezii*, *E. denticulatum* and *K. striatum* were transported from Bolinao, Luzón, Philippines, for quarantine. Both brown and green morphotypes of *K. alvarezii* and *E. denticulatum* were obtained. Laboratory conditions were observed continuously during the quarantine, and the term of growth was never less than 3 months. The material under quarantine was systematically checked, and any material in poor condition was discarded following the procedure described in Annex 1 (On-line Supplementary Material). When specimens reached 25–30 g, they were transferred to the natural environment. Upon transfer, they were fixed to ropes inside conic baskets 1 m high and with a 6-cm mesh size. They were left here for 6 months, and their responses were evaluated. Every 15 days, the algae were inspected under stereomicroscope for reproductive structures. During this time, efforts were also made to promote growth and avoid dispersion over the area. During the first 2 years of introduction, all tests of potential ecological impacts were made under semi-confined conditions (Fig. 1), and samples were analyzed for reproductive structures at least once a month.

In the field tests of specimens inside baskets or on ropes during the first essays of propagation in semi-confined conditions, *E. denticulatum* did not show stable growth rates and frequently had tissue affected or destroyed by necrosis or "ice–ice", an indication of disease. Therefore, this species was discarded as a farming prospect, and the remaining field trials were conducted with the two *Kappaphycus* species, both of which showed better fitness and growth than *E. denticulatum*.

Figure 2 illustrates the conceptual basis for the field tests. Three levels of analysis were considered with respect to the interaction of the study species with the host ecosystem: (1) as shelter for colonizing epibiosis, (2) as an element in the food web, and (3) as an invasive agent in the ecosystem. When it was established that the rate of grazing pressure could limit the

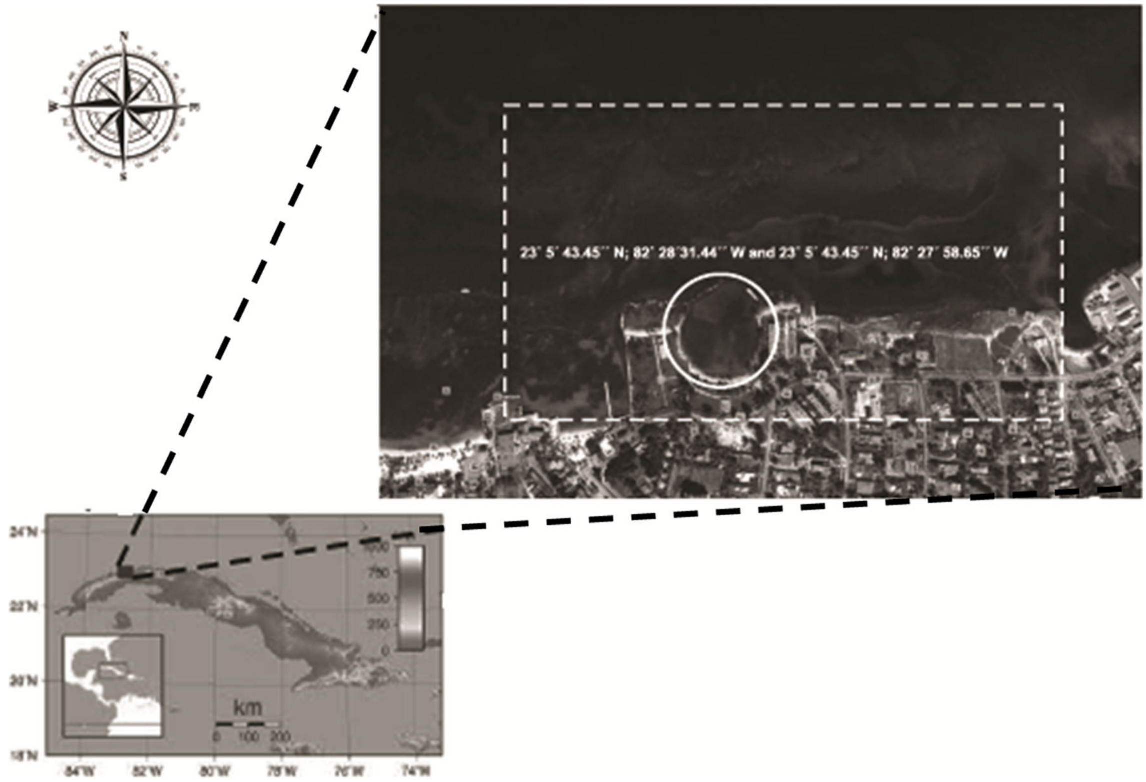
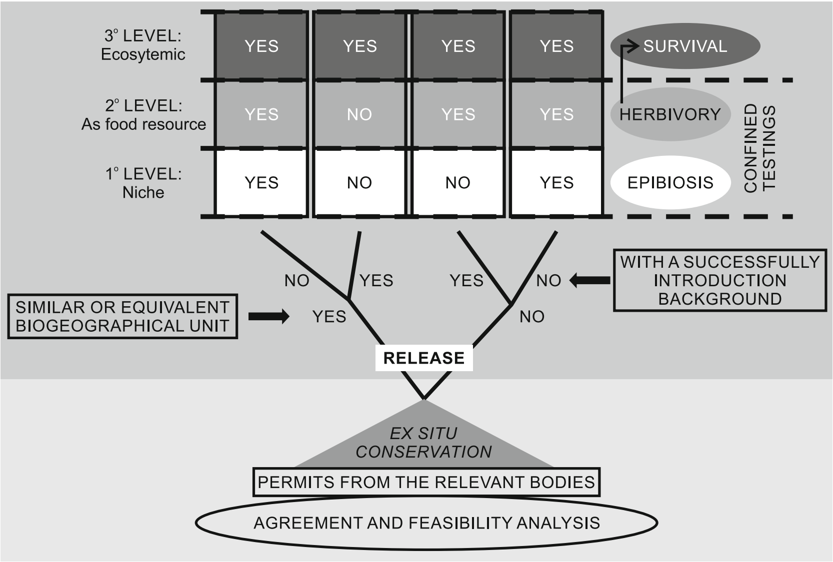


Fig. 1 Study area off Havana's NW coast. The dotted rectangle shows the several reef habitats and *Thalassia*, *Syringodium* and *Halodule* meadows where ecological evaluations were conducted. The *circle* indicates the site of distribution and extensive farming during the first 2 years under semi-confined conditions

dispersal of *Kappaphycus* species in Cuban coastal environments, survival experiments were conducted under total release, and growth tests were conducted at different sites on the island's shelf.

Two "longline" approaches were used for farming with ropes, with ropes placed either at/near the surface or along the bottom. When farming extended to the pilot scale, only rafts were used. Standard approaches were used to fix the specimens to the ropes. Growth rate (TCR) based on initial and final weight (Areces 1995) was formulated according to the equation: TCR=$[\ln(P_f/P_i)/t]\times 100$, where ln is the natural logarithm, P_f is the final weight, P_i is the initial weight and t is

Fig. 2 Operational protocol used for the analysis of potential hazards carrageenophyte introductions from the Indo-Pacific to the Cuban shelf. In this case study, release was done over different biogeographical units in the absence of a successful introduction background



the time of cultivation in days. The specimen material used in the tests ranged between 10 and 200 g, depending on test type, with weight determined to an accuracy of ±0.01 g. Farming durations were 24 h and 10, 15, 30, 44 and >60 days. Grazing pressure on the different biotopes was determined following Hay (1981). For tests that required that the algal biomass be isolated from predators, culture was extended into the interior of baskets with a mesh size of 1 cm. The baskets were cleaned every 2–3 days to prevent clogging by epibiosis. All tests were repeated three to nine times, with a 24-h interval between each test. The spatial heterogeneity coefficient (HE) was calculated using Risk (1972) and Luckhurst (1978) with the equation: $\mathrm{HE}=1-(X_a/X_s)$, where X_a is the linear distance between two points and X_s is the substrate contour between two points. HE was calculated from the average values of X_a and X_s obtained for each of the 2-m perpendicular lines, which formed a cross with equal arm length. Grazing pressure was evaluated among several important biotopes of the reef ecosystem: rock with no vegetation at a depth between 0.5 and 3 m; rock with vegetation at a depth of 2 m; dense sea grass prairie at 2 m of depth, composed of *Thalassia testudinum* and *Syringodium filiforme* of up to 24.3 cm height; coral reef plateau; and slopes of 5, 10 and 15 m depth.

Benthic habitat mapping of the farming area was conducted twice using standard SCUBA equipment, before establishment of the cultivation rafts and 2 years after the start of cultivation on a pilot scale. A 150-m rope marked at 1-m intervals was extended from the entrance of the semi-confined area, along the lateral edge and up to the interior beach. The rope was then moved in parallel at 2-m intervals, recording the type of benthic habitat beneath each mark point.

The epibiosis of the farmed specimens were studied over a 44-day period. After the cultivation period, the samples were transferred in situ to plastic bags with as little disturbance as possible. The bags were then closed tightly and transported to the laboratory. There, the plants were manually cleaned under running water over a sieve of 1- and 0.5-mm mesh to collect the mesofauna. The mesofauna were then fixed in 4 % buffered formalin with sodium tetraborate for subsequent analyses with a stereomicroscope, where the main taxonomic groups were identified and counted, and their densities determined.

Descriptive statistics commonly used in exploratory analysis were calculated, followed by several hypothesis tests. The calculations of experimental biomass were replicated with 3, 5, 10 or 15 subsamples of uniform weight to increase measurement accuracy. The data did not require transformation, and for analysis of the fixed effect of treatment, ANOVA was used after verifying variance normality and homoscedasticity via the Kolmogorov–Smirnov (Siegel 1975) and Brown–Forsythe (Brown and Forsythe 1974) tests, respectively; the LSD multiple range analysis test was used for post hoc comparisons. If the conditions of variance normality and homoscedasticity were not met, the Kruskal–Wallis (Siegel 1975) non-parametric variance analysis test was conducted, and the Student–Newman–Keuls (SNK) test was used for the comparison of multiple means. Data were analyzed using Statgraphics, version 5.0.

Results

Shelter, vegetation and a rough sea bottom significantly affected grazing pressure of carrageenophytes (*K. alvarezii*, both green and brown varieties, and *K. striatum*), mostly from fish of the families Acanthuridae and Scaridae (Figs. 3 and 4). These families are the most important herbivores in the region (Lang 2003). They consumed an average 1.7 g day^{-1} of carrageenophytes, representing 7.2 % of the initial weight of samples. Some daily consumption rates reached 32.3 % of the initial weight and were significantly higher than the average daily growth rate reported in the zone (6 %). Figure 3 shows the significant differences among the zones (ANOVA, p=0.001; LSD, α=0.05). Some zones showed a substantial loss of biomass and others showed biomass increases.

Strong differences in the growth of carrageenophytes among substrates of different complexity were also observed (H-K, α=0.05). The samples that grew on a rocky bottom with a spatial heterogeneity coefficient (HE)=0.36 had an impaired growth rate relative to those growing on sand with rocks with HE=0.12, and the latter had a lower rate than those propagating over sand (Fig. 4).

There were strong differences between the biotopes in shallow areas and those in the outer part of the reef at depths

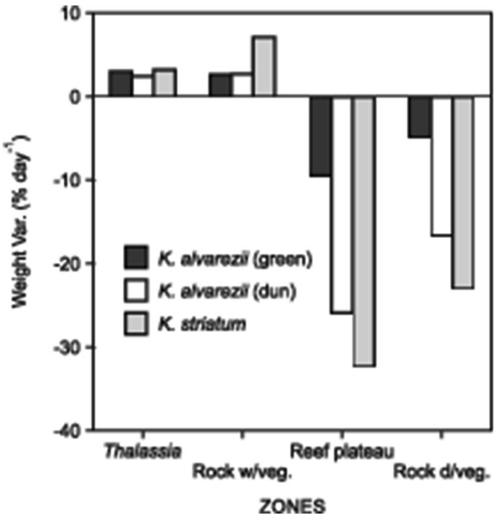


Fig. 3 Average weight variation (% p. h. day^{-1}) per herbivore as a function of initial weight at the time of plantation for two varieties of *K. alvarezii* and specimens of *K. striatus* in four biotopes of the reef ecosystem located 2–3 m deep. *Black bars* represent *K. alvarezii* (*green*), *white bars* represent *K. alvarezii* (*brown*), *grey bars* represent *K. striatus*. *Vertical bars* ±2 standard deviation (*SD*)

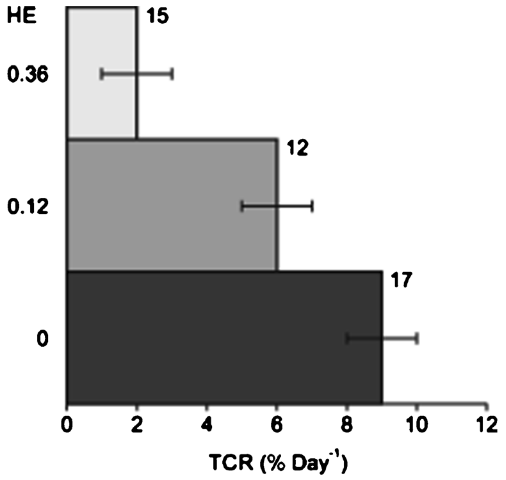


Fig. 4 Effect of bottom structural complexity on the growth of farmed *K. alvarezii* samples. The number of replicates is indicated next to the bars. *Light grey* represents rocks, *black* represents sand and *grey* represents sand with rocks. *Horizontal lines* are equivalent to ±1 SD. *HE* spatial heterogeneity coefficient, *TCR* relative growth rate

between 5 and 15 m. An increase in initial weight was observed only in the samples located in the shallow biotopes. Those located along the reef slope at 10 m depth showed little evidence of weight gain. For the remaining biotopes, there was extensive weight loss, particularly at 5 m depth, where weight loss was complete (Fig. 5). These differences among biotopes may be associated with differences in the distribution of shelters, grazing pressure and specific ecophysiological conditions, which may have been increasingly unfavorable for growth with increasing depth.

Concerning the potential propagation of either eucheumoid by its reproductive structures, these structures were never observed in either the biomass from the field tests or that attached to the cultivated rafts.

The mesofaunal epibiota associated with the carrageenophytes varied in a pattern similar to that observed for them. During the first days of cultivation, after the material was cleaned of epibionts and placed in the culture environment, epibiotic density was significantly higher (H-K, α= 0.05) than in later periods. This trend was evident in both summer and winter, although the introduced carrageenophytes exhibited a smaller number of organisms (Fig. 6).

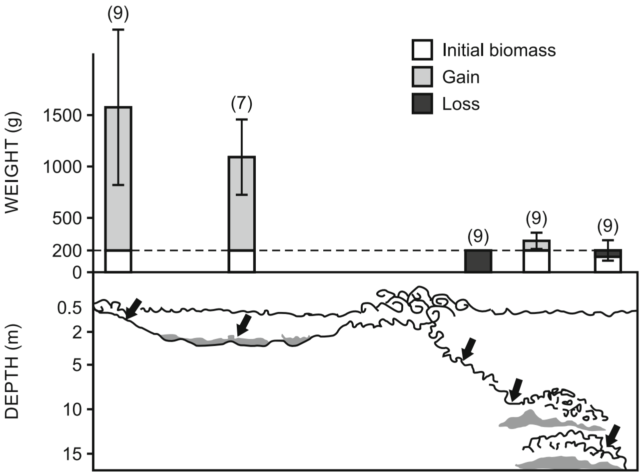


Fig. 5 Mean variation in biomass of *K. alvarezii* v. green in reef biotopes of different depths after 66 days of cultivation. *Vertical bars* ±2 SD (n=9 or n=7)

After a 2-year continued cycle of *Kappaphycus* spp. raft farming, where harvesting and replacement occurred every 45 or 60 days, there was a significant change in the underlying benthic community from the initial plantation densities of 1.2 kg m^{-2} and harvest biomass of 12 kg m^{-2}. The heavily populated *T. testudinum* seagrass bed that had covered the bottom disappeared (Fig. 7). Although changes in the granulometry of the bottom sediments or in the composition and density of meiofauna were not investigated here, such changes in the original habitat may have been relevant. The loss of *T. testudinum* bed may have similarly influenced the grazing pressure on the material that falls off and remains on the bottom.

Discussion

It has been assumed that the most devastating effects of species introductions are those produced by polyphagous or

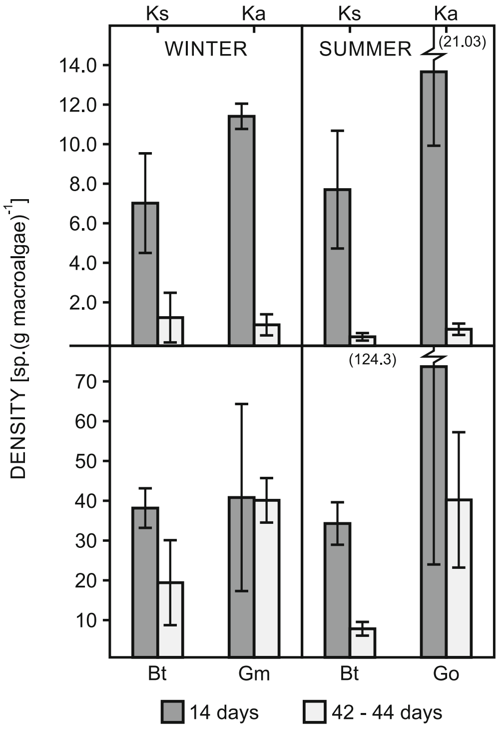


Fig. 6 Variation in mesofauna density [(g macroalgae)$^{-1}$] on five species cultivated during two farming terms (14 and 42–44 days) in extreme seasonal periods. *Ks Kappaphycus striatus*, *Ka Kappaphycus alvarezii*, *Bt Bryothamnion triquetrum*, *Gm Gracilaria mammillaris*, *Go Galaxaura oblongata* (*Tricleocarpa fragilis*). The *vertical bars* are equivalent to ±2 SD

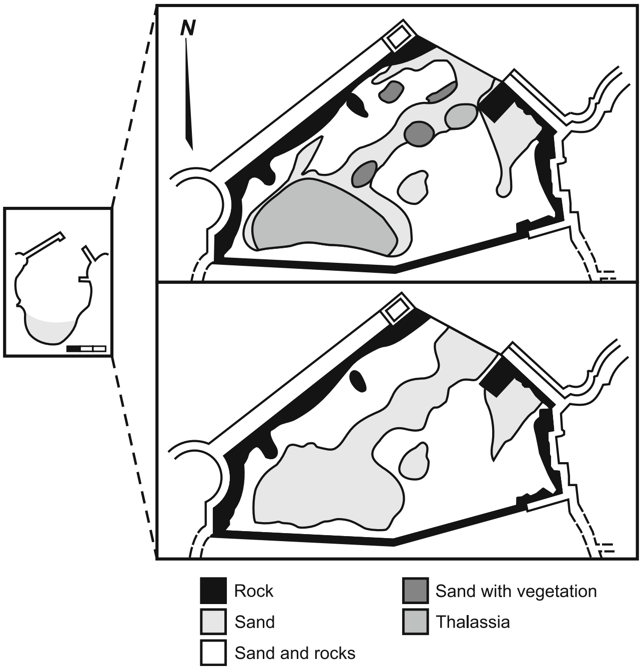


Fig. 7 Change of benthic habitats at the site of *Kappaphycus* spp. raft farming (*shadowed area*) in the semi-confined test area

generalist predators in isolated systems, such as islands. The Caribbean includes several groups of islands and a huge diversity of environments and species, many of them endemic. Similar to terrestrial ecosystems, its marine realm has unique features, leading to increasing concern over the consequences of invasive escapes to the region's ecosystems (Osman and Shirley 2007). Although invasive behavior has been identified in some marine macroalgae (Boudouresque and Verlaque 2002; Wallentinus 2002; Trowbridge 2006) and *Sargassum muticum* (Yendo) Fensholt, *Caulerpa taxifolia* (M Vahl) C. Agardh and *Codium fragile* (Suringar) Hariot subsp. *fragile* (Pickering et al. 2007), there are few documented cases of invasive escapes of commercial eucheumoids, especially considering the numbers of introductions (Ask et al. 2003). However, the effects of competitive exclusion by these species on the algal mat or sessile organisms should not be underestimated. For example, *Kappaphycus* spp. spread rapidly throughout Kaneohe Bay in Hawaii, overgrowing and killing a variety of corals. Two years following the introduction of *Kappaphycus* spp., there were no recognized threats to nearby reefs (Russell 1983); however, 25 years later, the situation was very different. Several surveys demonstrated that these species had spread as far as 6 km from the initial introduction sites, at an estimated rate of 250 m/year (Rodgers and Cox 1999). In addition, its coverage also increased from a maximum abundance of 4.59 % cover to 40 % (±5 SE) cover in a back-reef site (Woo 2000).

Several years of experiments demonstrated that the spread of commercial eucheumoids over the Cuban shelf was unlikely because of several unfavorable abiotic conditions (low nutrient levels, strong irradiance and high water temperature) for growth during most of the year. However, these conditions are not found in the pilot area of the present study, where the evaluation of the potential ecological risks of this introduction was conducted. This pilot area (Fig. 1) was influenced by periodic pulses of organic matter discharged from the nearby Quibú River, with a high degree of eutrophication (Gómez-Quintero and Areces 1976). At this site, the daily growth rates of *K. alvarezii* and *K. striatus* exceeded 6 % (Areces and Céspedes 1992). As these conditions did not provide limitations for growth, they help validate the potential ecological effects of the introduced carrageenophytes. Therefore, the results obtained were highly informative.

Even with such significant growth rates, the effect of fish grazing pressure prevented the establishment of either species almost in all evaluated reef habitats, particularly in areas with scarce vegetation and abundant shelter, such as the reef plateau. *K. alvarezii* and *K. striatus* were only able to survive on substrates in zones with an average depth of 0.5 m or less. It is possible that low tide zones in protected areas with no sea urchins could serve as a zone of spatial escape for the study species when they fall off of farming structures and are freely dispersed. The wave action in such low tide zones might limit fish grazing and enhance survival. Consistent with our findings of limited establishment potential of these carrageenophytes, several surveys of the area years after the completion of the farming experiments corroborated the local extinction of these species.

A comparative analysis between the mesofauna associated with *Kappaphycus* spp. and those associated with other three algae species present in the zone (*Bryothamnion triquetrum*, *Gracilaria mammillaris* and *Tricleocarpa fragilis*) showed much lower densities in the *Kappaphycus* species, in both the winter and summer. This result may be due to several factors; for example, a higher growth rate in the *Kappaphycus* species, which could have increased the biomass and subsequently lowered the density of mesofaunal organisms; or a less compact, less cryptic thalli morphology than that of the local species. It has been shown that thalli morphology affects both the composition and density of the epibiosis associated with intertidal algae (Areces et al. 1992) as well as the physiological condition of the plant. When physiological conditions are poor, the abundance of epibionts increases, particularly of Amphipoda (Areces and Martínez 1992). All of the local species studied here were separated from the substrate and maintained in hanging baskets, potentially contributing to thalli decline, a reduced growth rate and an increase in associated epibiota. Nevertheless, the development of mesofaunal epibiosis follows a similar trend across the five species tested, as shown in Fig. 6. Furthermore, a study of the preference of juvenile spiny lobster *Panulirus argus* Latreille, a main fishery resource in the region, for different macroalgae found no rejection of *Kappaphycus* as shelter (Serpa-Madrigal and

Areces 1995). These findings suggest the possibility for local epifauna to thrive and develop, at least at the niche level, when macroalgal biomass increases due to the propagation of eucheumoids.

The results of the present study also indicate that the escape risk, establishment probability and invasion probability of any commercial eucheumoid in the western Caribbean will depend on several factors (Fig. 8).

Some of these factors are abiotic, such as the intensity of hydrodynamic forces that promote the transportation of fallen biomass over huge distances but also exclude it from subtidal environments via accretion on the shore. The trophic character of water is another relevant factor. It favors significant growth rates for biomass not assimilated by the socio-ecological system, which may become dispersal propagules. Other factors are biotic, such as grazing pressure, which would control the volume of the net or remnant biomass under natural conditions. Finally, there are economic factors involved that favor farming or a "gleaner" activity with continued extraction of biomass and control over the process, as this form of production is effective and profitable. Revenues are focused on the volume of material harvested, whether from cultivation modules or from the remaining biomass on the adjacent bottoms or shores.

Any protocol for evaluating the ecological impacts of eucheumoid farming should consider escape risk. Of those factors that affect the incidence of escapes, two should be quantified at the site of interest: grazing pressure and growth rate (Annex 1). Eucheumoid farming still lacks appropriate dissemination of the few protocols available (Paula et al. 1998; Sulu et al. 2004; MARINALG 2012) concerning quarantine or ecological risk evaluation. Of these protocols, only a few include a comprehensive explanation of the procedures or principles that should be considered in environmental impact assessment. Some proposals focus on monitoring the substrate for colonization by way of spore or propagule fixation (Reis et al. 2007) or on detecting physiognomic changes (Pirani et al. 2008) that, were they to occur, entail environmental impacts that would be difficult to mitigate. In the Cuban case study presented here, no reproductive structures in the thalli of the farmed eucheumoids were observed, which highlights the necessity of complementing ecological risk assessment based on spore fixation with other tests. However, national environment legislation is often not complied with during introductions, which attracts the attention of the media and promotes unfavorable opinions on the suitability and scientific development of mariculture. As with other traditional crops, eucheumoid commercial propagation is likely to have environmental impacts that should be evaluated through a cost–benefit analysis. These impacts imply that farming should take place where there are no risks of usage conflicts or natural heritage deterioration. To carry out this evaluation, assistance from public agencies is needed more than the enthusiasm or the entrepreneurial spirit of any one individual.

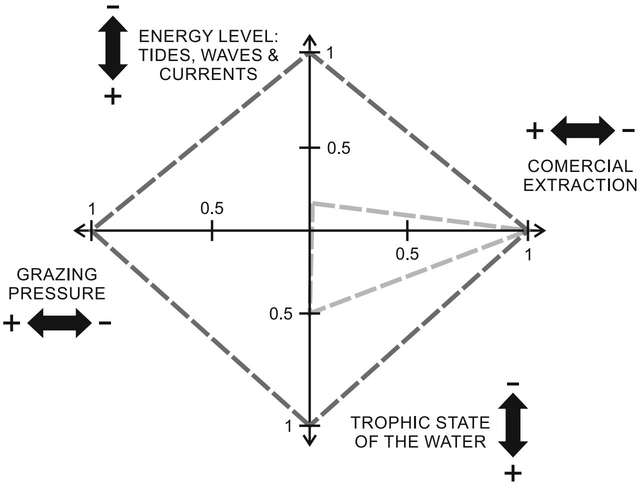


Fig. 8 The probability of invasive escape of *Kappaphycus* spp. into the environment depends on the interaction of four factors. It may be calculated through the area ratio of two polygons: one associated with the environmental conditions most favorable for escape and one derived from local "suboptimal values". "Suboptimal values" are evaluated according to expert criteria or data compared to reference values. The *yellow lines* represent the polygon derived from these "suboptimal values" in the pilot area where farming and evaluation of ecological risks were conducted on the Cuban shelf

Conclusions

1. Invasive behavior of *K. alvarezii* was observed previously in the Peninsula de Araya as well as in Coche and Margarita Island in the eastern Caribbean, and Venezuela banned the propagation of eucheumoids due to violation of environmental legislation, garnering public attention. Despite these efforts, however, the dispersal of eucheumoids over the region developed rapidly during the 1990s, often illegally. As a result, the causes, routes, origins and potentially harmful biological hazards of eucheumoid invasion have been difficult to assess. Such information is essential for risk assessment and management.
2. Grazing pressure became effective in limiting the commercial growth of eucheumoids and their spontaneous dispersal in reef ecosystems in the Cuban archipelago. Together with other limiting environmental factors, large-scale farming seems to be restricted.
3. The evaluation of the ecological risks of such introductions should include assessments of three factors: eucheumoid growth rates, grazing pressure in the main proposed farming habitats and eucheumoid dispersal capability from wave, tidal and current action at these sites.

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