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Research Article

The introduced alga *Kappaphycus alvarezii* (Doty ex P.C. Silva, 1996) in abandoned cultivation sites in Bocas del Toro, Panama

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Abstract

The red alga *Kappaphycus alvarezii* (Doty ex P.C. Silva, 1996) has been intentionally introduced throughout the tropics for mariculture. In some cases, the alga has spread outside cultivation sites and impacts native biota. We conducted surveys of two sites in Bocas del Toro, Panama, where non-native *K. alvarezii* was previously cultivated to determine whether it is established and began to examine potential interactions with native biota. We found that non-native *K. alvarezii* has spread into adjacent seagrass beds, mangroves, and coral patches. The cover of *K. alvarezii* at these sites can be high (>30%), and it appears to smother seagrass, coral, and sponges. However, herbivory by native sea urchins may act as an important agent of biotic resistance, though additional evidence is needed to confirm this.

Key words: red algae, aquaculture, non-native, herbivory, sea urchin, seagrass, coral reef

Introduction

Red algae in the genus *Kappaphycus* have been intentionally introduced throughout the tropics for mariculture (Ask et al. 2003). One such species, *Kappaphycus alvarezii* (Doty ex P.C. Silva, 1996), is native to the Philippines and occurs in shallow reef areas on sandy coral to rocky substrate (Trono 1992). For the past four decades, cultivated varieties of *K. alvarezii* have been introduced to shallow tropical marine habitats around the world, including sites in Africa, South East Asia, Central America, and South America (Ask et al. 2003).

The rapid growth rate, modes of dispersal, and the methods used to cultivate *K. alvarezii* may facilitate its introduction and spread into novel habitats. The species can double its biomass in 15–30 days when cultivated in appropriate sites (Trono 1992), and can spread by means of vegetative fragmentation as well as sexual reproduction by dispersing spores into the water column to be spread by prevailing water currents (Azanza-Corrales et al. 1992). *Kappyphycus* is grown by tying fragments of algae to lines suspended above the sediment (Ask and Azanza 2002), which can facilitate spread to other sites when fragments break free. Cultivation sites also often harbor large aggregations of the alga, and support rapid increases in algal biomass (Trono 1992; Muñoz et al. 2004), providing a constant supply of propagules (fragments).

The establishment of *K. alvarezii* in areas surrounding cultivation sites may impact native communities through habitat alteration or by competition for resources. For example, several species in the genera *Kappaphycus* and *Eucheuma* were introduced into Kane'ohe Bay, Hawai'i in the 1970s (Eldredge 1994). By 1996, the algae had spread to coral reefs up to 6 km away (Rodgers and Cox 1999) where they overgrow live coral (Conklin and Smith 2005). There are similar reports from India (Chandrasekaran et al. 2008) and Venezuela (Barrios et al. 2007).

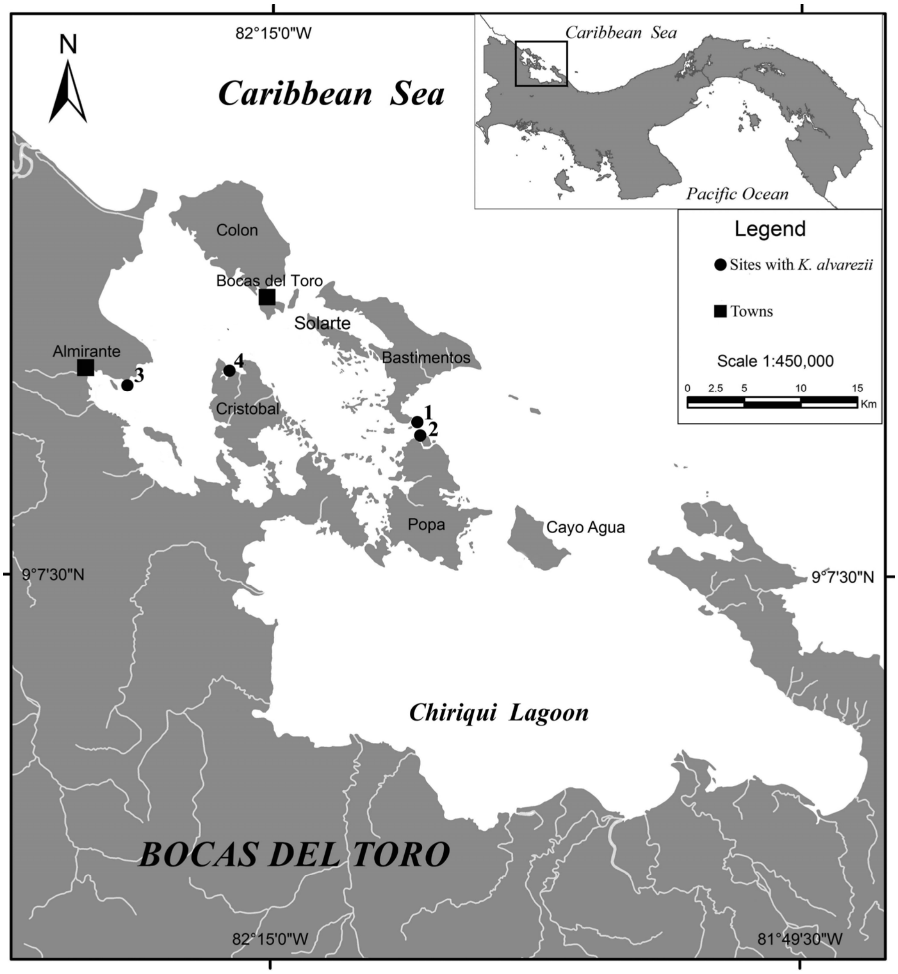

Figure 1. Map of Bocas del Toro showing sampling sites from the present study (3, 4) and sites where loose *K. alvarezii* was reported by G. Jacome and W. Freshwater (1, 2). Numbers refer to site information listed in Appendix 1.

Although the introduction of this species can generate negative ecological impacts, environmental benefits of seaweed farming, such as the removal of nutrients from fish aquaculture effluents (Hayashi et al. 2008), have also been demonstrated (Zertuche-Gonzalez 1998).

In Panama, the first commercial *K. alvarezii* cultivation operations were established in the province of Colon in 2000 with fragments brought to the area by indigenous people who use seaweeds for medicinal purposes (Batista de Vega et al. 2006). In 2002, Gracilarias de Panama established research farms in the Caribbean province of Colon using seedlings that originally came from Venezuela (Trespoey et al. 2006). Although we found records for the first commercial *K. alvarezii* farms in Panama, it is not clear how the species was first introduced into the country. Hurtado et al. (2014) erroneously state that *K. alvarezii* seedlings were introduced by the Smithsonian Tropical Research Institute; however, this statement is inaccurate, and we could not find records of the first introduction of *K. alvarezii* into Panama. Cultivation operations have also begun in another Panamanian province, Bocas del Toro, where at least four growing operations have been established in the past decade (G. Jácome, Smithsonian Tropical Research Institute, Panama, pers. comm.).

In 2013, we encountered two abandoned cultivation sites in Bocas del Toro where *K. alvarezii* was spreading into adjacent coral reefs, seagrass beds, and mangroves. Here we report the occurrence, cover, and habitat associations of *K. alvarezii* at these sites. We also compared sea urchin density and frequency of grazing on *K. alvarezii* to provide a preliminary assessment of the potential role of native species in controlling densities of the introduced alga.

Methods

We surveyed two abandoned cultivation sites in Bocas del Toro, Panama, in October 2013 and March 2014. One site was located in a semi-enclosed bay along the northern coast of Cristobal Island (9°17.31′N; 82°16.20′W; henceforth referred to as 'Cristobal'), while the other was located near the town of Almirante (9°17.35′N; 82°21.32′W; henceforth referred to as 'Almirante') (Figure 1, Appendix 1). The cultivation structures had been left in disrepair; the floats and lines were broken, and *K. alvarezii* was present on the seafloor beneath. Although we do not have specific information on when the farms were abandoned, the fouling communities growing on the lines and floats (Figure 2D) suggest that the structures had been submerged for more than a year. Both sites are adjacent to mangrove cays and characterized by shallow (depth range: 0–3 m) *Thalassia testudinum* (Banks ex König, 1805) beds, interspersed with patches of *Porites* sp. (Link, 1807) and *Millepora alcicornis* (Linnaeus, 1758). The seagrass beds where *K. alvarezii* was present in Cristobal and Almirante had an approximate area of 1000 m^2 and 1200 m^2, respectively.

We confirmed our identification of *K. alvarezii* using molecular techniques. Samples were rinsed with distilled water after collection, stored at -20°C, and then ground in liquid nitrogen. DNA was extracted using a CTAB extraction protocol (Doyle and Dickson 1987). A fragment of the mitochondrial-encoded *cox2-cox3* spacer was PCR-amplified using the primer pairs *cox2*-for and *cox3*-rev (Zuccarello et al.1999) and 1 unit of QTaq (Qiagen) with 1X PCR Buffer, 50 µM each dNTP, 0.1% BSA, 2 pmol of each primer, and 1 µl of DNA extract in 25 µl PCR reactions. Amplification conditions included an initial step at 94˚C for three minutes, followed by 35 cycles of 94˚C for 45 seconds, 50˚C for 45 seconds, and 72˚C for 1 minute 30 seconds with a final extension step of 72˚C for 7 minutes. Amplified products were visualized on an agarose gel and excised bands cleaned using Gelase enzyme. Products were sequenced on an ABI 3100 sequencer with BigDye chemistry (Applied BioSystems). Sequences were cleaned and aligned using Sequencher 5.0 (GeneCodes) and identified using BLAST (NCBI).

We estimated the percent cover of *K. alvarezii* using a 1 m^2 quadrat placed at 46 randomly selected points in Cristobal and 33 points in Almirante. To randomize the location of the sampling points, we created X and Y axes based on our measurements of the length and width for each site. The coordinates (X, Y) of sampling points were randomly determined using a random number generator and corresponded to linear distances from a point of origin. At each point, we also recorded the substratum type, observations of potential interactions between the algae and native biota (e.g. overgrowth, species using the algae as habitat), and noted whether *K. alvarezii* was attached to the substrate.

We observed sea urchins and fishes (Table 1) grazing on *K. alvarezii* during initial surveys; therefore, we also quantified the number of sea urchins (by species) and the frequency of grazing marks on algae inside the quadrats. We confirmed that grazing marks in the field were made by sea urchins by feeding undamaged sections of *K. alvarezii* to *Lytechinus variegatus* (Lamarck, 1816) and *Echinometra lucunter* (Linneaus, 1758) in the laboratory. Fish grazing marks were rarely observed in the field and were inconsistent with sea urchin grazing marks. Fish grazers removed apical tips while urchins scraped tissue away leaving indents along the branches.

Due to violations of assumptions of parametric tests, we used a non-parametric unpaired Wilcoxon's Rank Test corrected for ties to test for differences in *K. alvarezii* percent cover and sea urchin density between the sites and survey dates. The data were analyzed using R version 3.0.2.

Results

We observed loose algal fragments matching the morphology of *K. alvarezii* at both sites (Trono 1992; Figure 2A). DNA sequence analysis confirmed that the alga was *K. alvarezii.* The sequence was identical to *K. alvarezii* isolate E3 (GenBank #AY687427), which was previously isolated in commercially grown algae from Venezuela (Zuccarello et al. 2006).

At both sites, *K. alvarezii* occurred in different sized patches, from small fragments to expansive mats, and in general was more abundant in Almirante. In Cristobal, most *K. alvarezii* patches were no larger than 1 m^2, while in Almirante mats measuring up to 72 m^2 and covered shallow seagrass beds and *Porites* sp. patch reefs (Figure 2B). Percent cover was significantly higher in Almirante than in Cristobal (Figure 3; W = 1266.5, $P < 0.001$). In Cristobal, *K. alvarezii* cover was significantly higher in October 2013 compared to March 2014 ($W = 1097.5$, $P < 0.05$). Cover did not change significantly between the two survey dates in Almirante ($W = 746$, $P = 0.31$).

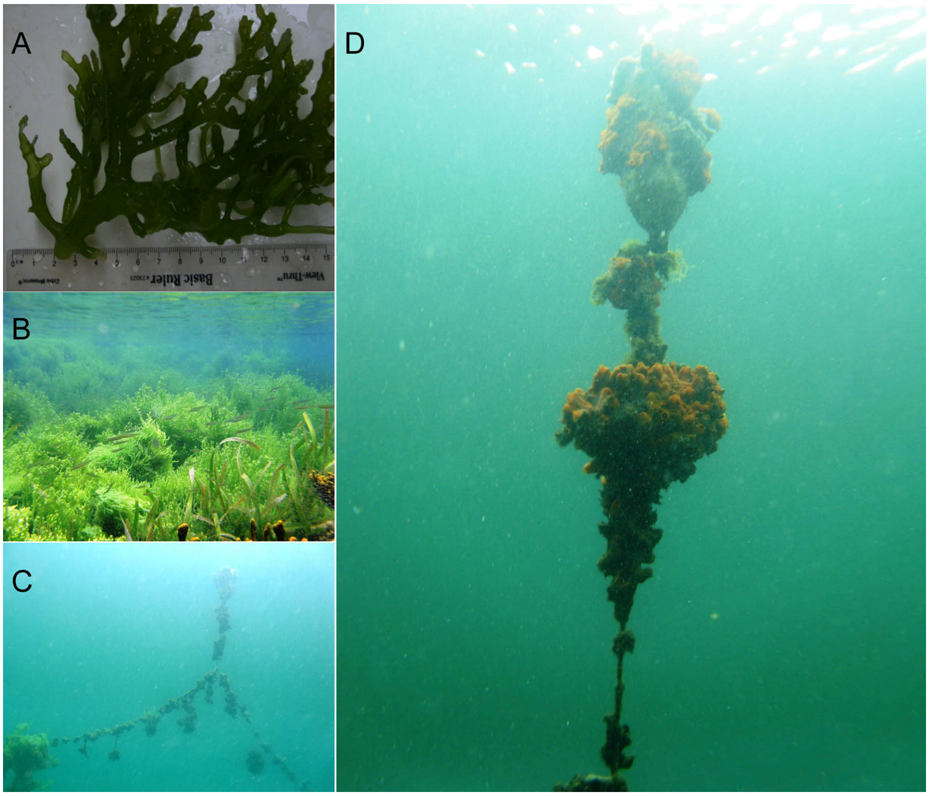

Figure 2. (A) *Kappaphycus alvarezii* collected in Cristobal. (B) Mats of *K. alvarezii* covering a seagrass bed in Almirante. (C) Abandoned cultivation structures. (D) Fouling communities growing on the floats and lines of the farm. Photographs by A. Sellers and G. Jácome.

Table 1. Interactions observed *between Kappaphycus alvarezii* and some native biota.

Smothering/overgrowing	Use as habitat	Herbivory
Thalassia testudinum	*Hypoplectrus* sp. (Gill, 1861)	*Scarus iserti* (Bloch, 1789)
Porites sp.	*Stichodactyla helianthus* (Ellis, 1768)	*Lytechinus variegatus*
Millepora alcicornis	*Ophiothrix suensonii* (Lütken, 1856)	*Echinometra lucunter*
Clathria sp. (Schmidt, 1862)		
Iotrochota sp. (Ridley, 1884)		
Ircinia sp. (Nardo, 1833)		

Percent cover alone may not provide an accurate description of the space used by large macrophyte species, such as *K. alvarezii*. Although we did not systematically measure the canopy height of *K. alvarezii*, the canopy of two of the larger mats present in Almirante had heights of 73 and 82 cm, respectively, while the fragments scattered in Cristobal ranged between 8 and 24 cm tall. At both sites, most of the algae occurred within tens of meters of the cultivation structures, and few fragments had moved > 200 meters from the structures. However, in Almirante, we observed some small fragments entangled in seagrass beds and mangrove roots up to 300 meters away from the structures, likely due to the prevailing southerly current running through the area.

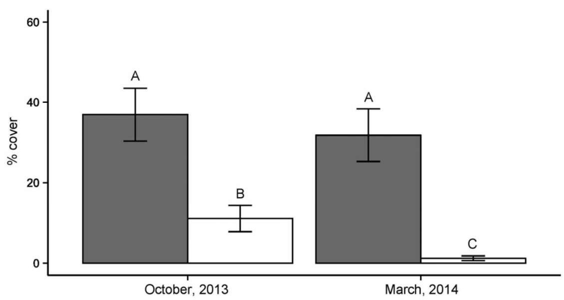

Figure 3. Mean percent cover of *Kappaphycus alvarezii* in sites in Bocas del Toro. Grey bars represent mean percent cover values for Almirante, while white bars represent values for Cristobal. Means with different letters are significantly different ($P < 0.05$). Error bars represent one standard error (SE).

At both Cristobal and Almirante, *K. alvarezii* was found overgrowing native organisms such as seagrasses, corals, and sponges (Table 1). The algae occasionally formed holdfasts and attached to *Porites* sp. coral and *M. alcicornis;* however, the majority of fragments grew in unattached mats. We also observed fish and invertebrates using the algal mats as habitat, as well as sea urchins and fishes grazing on the alga (Table 1). There were significantly higher densities (mean ±SE) of the urchins *E. lucunter* and *L. variegatus* in Cristobal (0.67 ± 0.15 urchins/m^2) than in Almirante (0.15±0.08 urchins/m^2) (W = 549.5, P < 0.01). This difference was largely driven by a significantly higher abundance of *L. variegatus* in Cristobal (W = 499, P < 0.001). In Cristobal, we observed urchin grazing marks on *K. alvarezii* in 80% of the quadrats, while in Almirante grazing marks were present in only 27.6% of the quadrats.

Discussion

The high densities of *K. alvarezii* in farms, and the ease by which fragments can break loose from their growing structures, creates a high potential for spread of this alga outside of cultivation sites. In addition, algal strains and cultivation sites are selected, in part, to maximize the growth rate of algae (Trono 1992; Ask and Azanza 2002), an important invasive trait. Here we have documented the spread of *K. alvarezii* from two abandoned cultivation operations into adjacent marine habitats. Although the algae are freely dispersing, it has not spread far from cultivation structures, and most fragments were found within 200 m of the structures. Furthermore, we did not observe significant changes in *K. alvarezii* cover over a six month period. Rather, we found that cover had decreased in one site where herbivory appeared to be more intense. While our study provides important information regarding the events that take place following the abandonment of a farm, it is not clear when farming operations ceased at our study sites, thus we cannot offer a timeframe for these events. However, the presence of oysters and sponges on the floats and lines indicate that these structures had been submerged for more than a year prior to our first surveys. Future research will be needed to assess the rate at which the species is dispersing and the rate at which herbivores consume the alga.

While it appears that neither the abundance nor spread of *K. alvarezii* increased at our study sites over six months, the establishment of additional farms throughout Bocas del Toro could increase the potential for its spread to other sites. Farms have been established in other areas of the bay, and loose *K. alvarezii* was found along the northern coast of Popa Island (Crawl Cay) in 2009 and identified using molecular techniques (W. Freshwater, University of North Carolina Wilmington, North Carolina, USA, pers. comm.). Farms still operate in that area and *K. alvarezii* has been observed growing on adjacent reefs as recently as July 2014 (G. Jácome, personal communication; Figure 1; Appendix 1). It is unclear whether the algae observed near Popa Island in 2009 has persisted until 2014, or if the algae documented in 2014 recently spread from nearby cultivation sites. Regardless of when it arrived, it is unlikely that the algae present in Popa Island originated in our study sites as these are separated by over 15 km, and we are not aware of any reports of *K. alvarezii* from areas between the sites.

Grazing by native herbivores, such as sea urchins, may reduce the cover of *K. alvarezii*. Significantly higher urchin densities and a higher frequency of urchin grazing marks on algae in Cristobal suggest that herbivory by native sea urchins is stronger there compared to Almirante. Differences in herbivore pressure in Cristobal may explain the differences in *K. alvarezii* cover between the sites, and possibly the reduction in cover we documented in Cristobal over a six month period; however, additional studies are needed to confirm this. Herbivores can indeed become a problem for *K. alvarezii* growers (Ask and Azanza 2002), and urchins in particular have been proposed as effective agents of biocontrol in Hawai'i (Conklin and Smith 2005). If biotic resistance by native sea urchins proves effective at reducing local densities, it may offer potential in controlling populations and mitigating impacts of the introduced alga. Alternatively, differences in the coverage of *K. alvarezii* between sites may also reflect differences in abiotic factors such as nutrient availability and temperature (Ask and Azanza 2002), as well as the age, size, and condition of the farms. The remains of the structures suggest that the farms were approximately the same size; however, this observation must be considered with caution given that sections of both structures had broken off prior to our study. Differences in nutrient availability between the sites may have also led to differences in algal cover. The site near the town of Almirante, where cover was highest, may receive a greater input of nutrients due to its proximity to a river that runs through that town (Figure 1). Manipulative studies are needed to disentangle the effects of herbivores from other factors that may also explain differences in algal cover.

The impact of introduced *K. alvarezii* in Bocas del Toro will depend in large part on its ability to establish and spread to new sites; however, we currently lack enough information to predict the fate of this introduction and its invasive potential. Our observations provide basic insights into the potential impacts of *K. alvarezii* on native ecosystems. The mats that cover *T. testudinum* beds and patches of *Porites* sp. coral in Almirante could reduce the amount of light available to these foundation species, which could lead to reductions in their cover and indirectly affect associated species. In Kane'ohe Bay, Hawai'i, where introduced species of *Kappaphycus/ Eucheuma* have established, the algae overgrow coral colonies, and compete for light with native organisms (Conklin and Smith 2005). However, the *K. alvarezii* introduced to Hawai'i is genetically distinct from cultivated lines grown around the world (including Panama) which may influence its invasive potential (Zuccarello et al. 2006).

Ask et al. (2003) outline strategies to prevent the environmental risk associated with introducing *K. alvarezii* for cultivation, including quarantine, assuring financing for farms, identifying a commercial market for the product, and employing experienced farmers. The first commercial farms in Panama were established in the year 2000 (Batista de Vega et al. 2006); however, it is not clear when or how the alga was initially introduced or whether quarantine practices were followed (G. Batista de Vega, Smithsonian Tropical Research Institute, Panama, pers. comm.). The farms in Bocas del Toro are small operations managed by families from nearby villages (AJS, pers. observation). While we do not have specific information regarding the farmer's expertise in cultivating introduced species, the abundance of loose algae left to disperse suggests little concern for the environmental issues associated with this practice. While commercial production of this species has been carried out in an environmentally sustainable manner in a number of countries (Ask et al. 2003), our findings indicate that these control procedures are not being followed in Bocas del Toro.

Our study demonstrates the consequences of not following environmentally responsible practices when cultivating non-native species. Based on our results, we suggest that local government agencies and farmers develop better strategies to avoid the negative environmental repercussions of this practice. Local farmers could reduce the potential for establishment and spread of *K. alvarezii* by preventing fragments from escaping the farms, and by removing loose algae from areas surrounding their farms. Farmers should also ensure that all introduced algae are removed from a cultivation site after farming operations cease and ensure the alga is not spreading to adjacent native communities. Furthermore, growers must consider the environmental issues associated with cultivating non-native species. Considering the impacts that *K. alvarezii* and related species have generated on coral reefs in other locations where they have been introduced (Conklin and Smith 2005), and the establishment of additional farms in Bocas del Toro, it is critical to continue monitoring the spread of the species from abandoned and active cultivation sites in Panama. If future surveys reveal an increase in the abundance or distribution of the species, it will be important to assess its impacts on native communities, and develop strategies to mitigate those impacts. One potential strategy is the use of native herbivorous urchins as control agents, however, additional studies are needed to determine if herbivores are effective agents of biotic resistance.

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Appendix 1. Locations in Bocas del Toro, Panama where loose *Kappaphycus alvarezii* has been observed invading native habitats. The location of each site is shown in Figure 1.

Site Number	Location	Record Coordinates	Year of Record	Reference
1	Popa Island	9°14.77′N; 82°07.92′W	2009	W. Freshwater (pers. comm)
2	Popa Island	9°14.27′N; 82°07.27′W	2014	G. Jácome (pers. comm)
3	Almirante	9°17.35′N; 82°21.32′W	2013/2014	Present study
4	Cristobal Island	9°17.31′N; 82°16.20′W	2013/2014	Present study