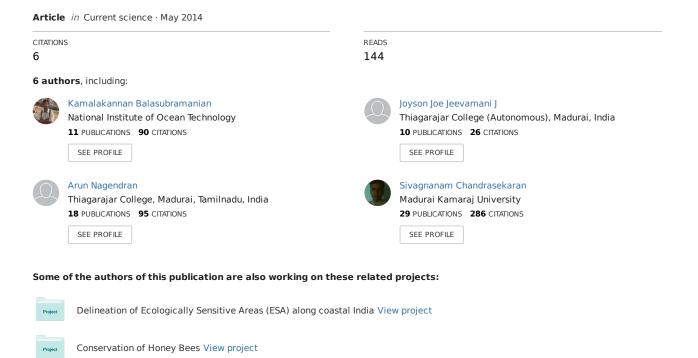
Impact of removal of invasive species Kappaphycus alvarezii from coral reef ecosystem in Gulf of Mannar, India



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Kappaphycus alvarezii is a commercially important red alga being intentionally introduced in marine waters worldwide for the production of kappa carrageenan. Its introduction into the Gulf of Mannar Biosphere Reserve during the 1990s and its subsequent escape from cultivation sites have paved the way for its invasion into the coral reef ecosystem of Kurusadai Island. Since the report of its invasion in 2008, removal of K. alvarezii from the reefs has been started by means of manual removal (hand plucking). This article details the unsuccessful attempt and negative impact of the eradication programme. Regrowth of K. alvarezii from removal points and drifting broken fragments resulting during removal have led to further establishment in the reef environment. Variation in the morphology of K. alvarezii populations after their removal has been observed. A significant reduction in the cover of coral and native algae due to the increase in abundance of K. alvarezii was evident from the study. The need for immediate scientific control measures to eradicate the invasive alga is discussed.

Keywords: Coral reefs, Gulf of Mannar, invasive species, *Kappaphycus alvarezii*, manual removal.

BIODIVERSITY is affected by the invasion of exotic species in new geographical locations. The introduction and spread of non-native species have significantly altered the ecological functions of marine ecosystems^{1,2}. Exotic marine algae which behave as invasive species have impacted the native coral communities at the sites of incursion^{3–5}. One such alga is *Kappaphycus alvarezii* (Doty) Doty ex. P. Silva (Rhodophyta: Solieriaceae). It is one of many seaweeds being intentionally introduced for the production of kappa carrageenan worldwide⁶. The farming of *K. alvarezii* was initiated in the Philippines during 1960s with local varieties of its wild populations, and it has expanded further to other parts of the world with different cultivation technologies^{7,8}. However,

The commercial cultivation of *K. alvarezii* in India was strongly opposed due to the prediction of its likely invasiveness¹¹, as it is exotic to Indian marine environments. However, field vigilance and environmental impact assessments showed no visible harmful effects from this alga¹² and thus its cultivation continues. However, later studies had shown its smothering effect on live corals in Kurusadai Island in the Gulf of Mannar (GoM)^{13,14}. Incidents of *K. alvarezii* invasion on corals in GoM¹³ were reported^{15,16}. There were immediate remedial responses from the State Government organizations to control/eradicate the alga.

Control of *K. alvarezii* in invaded communities has been carried out by either physical or biological methods ¹⁷, or both. An underwater vacuuming system, Super Sucker, has been used as a physical method to eradicate *K. alvarezii* in Kane'ohe Bay, O'ahu. Also, native collector urchins (*Tripneustes gratilla*) were used to control *K. alvarezii*, which clear them through grazing. However, in Kurusadai Island, the Forest Department decided to use the manual removal method to reduce the impact of *K. alvarezii* on corals. In this article, we report on the consequences of the *K. alvarezii* removal process carried out in GoM.

Materials and methods

Study area

Kurusadai Island (9°15′N; 79°12′E) is a part of Mandapam group islands in GoM biosphere reserve, Tamil Nadu (for a map of the study area see ref. 13). Study sites are part of continuous fringing reefs located on the southern side of the Island which extend up to 500 m with varying depths of 0.5–2.0 m. A survey in 2005 revealed the presence of 54.9% live coral cover in the Island¹⁸. The coral ecosystem has experienced a recent coral–algal phase shift¹⁹ due to coral bleaching in 2010.

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K. alvarezii poses serious threats to native corals through overgrowing and smothering ^{9,10}.

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The present study has continued from 2007 after the preliminary report of K. alvarezii invasion on patches of corals in intertidal region of the Island¹³. To examine the effects of K. alvarezii invasion on native coral reef communities, we carried out subsequent monitoring with the addition of control ecosystems devoid of K. alvarezii invasion. The control ecosystems are situated west of the invaded ecosystems and they are separated by a distance of about 750-1000 m. The invaded and control ecosystems were further divided into two sites, viz. site 1 (50 m from the shore) and site 2 (100 m from the shore). Depth at site 1 varies from 0.5 to 1.0 m and that at site 2 varies from 1.0 to 2.0 m for both ecosystems. Other than depth, all other biotic and abiotic factors are common to both sites of control and invaded ecosystems, except the presence of *K. alvarezii* in the invaded ecosystem.

Benthic community analysis

Estimates of the coral cover, live cover of K. alvarezii on corals, live cover of native algae and sand/rubble (expressed in %) were based on 80 randomly placed, 1 m² quadrats (n = 20 per site per ecosystem). Stratified sampling was adopted to select K. alvarezii-invaded coral colonies in the study sites (sites 1 and 2) of the invaded ecosystems. Changes in abundance of K. alvarezii and native algae were estimated independently using 1 m² quadrats without segregation of sites (n = 20 per ecosystem). Quadrats were located with a GPS (Garmin, Taiwan) and were visited periodically once in a month (between March and August) during 2008-2012. All quadrats were at least 1 m apart from each other. Parameters such as species abundance, species richness and evenness were estimated to study algal dynamics. Species abundance was calculated as the ratio between total number of individuals of a species and the number of quadrats in which the species were present. Species richness (S) was determined for each quadrat as the number of identified algal taxa. Simpson index of diversity (1 - D), which measures the probability of any two individuals randomly drawn from a community belonging to the same species, was used as a measure of species richness and evenness. For each quadrat, it was calculated as

$$1-D=1-\sum_{i=1}^{S}[n(n_i-1)/N(N-1)],$$

where S is the species richness, n_i is the number of individuals in the ith species, and N is the total number of individuals of all species present in a quadrat. The value of 1 - D ranges from 0 to 1 and thus a higher value indicates greater diversity.

Though manual removal of *K. alvarezii* was started from 2009 (discussed below), all quadrats used for benthic community analysis in the invaded ecosystems

were not affected by the manual removal process during 2009–2012. Due to mass eradication in 2012, these quadrats were not monitored further.

Assessment of manual removal impact on K. alvarezii

In early March 2009, the Tamil Nadu Forest Department initiated the removal of K. alvarezii by manual method (hand plucking) from invaded coral colonies at intertidal zone in Kurusadai Island. We did the first survey on the same day (8 March 2009). By visual survey, we selected nine removal points, i.e. reef substrates from which the alga was completely removed. Each removal point was covered by a GPS-marked quadrat (1 m²) to analyse the K. alvarezii regrowth pattern. Prior to marking, we estimated area (cm²/m²) and well-drained fresh biomass (kg/m²) of removed K. alvarezii colonies from the respective quadrats, which were noted as initial measurements. Resurvey was done at marked quadrats in early September 2009 (1 September 2009) spanning an time-interval of 175 days and estimated values were taken as final measurements. Algal colonies were removed during lowtide condition following the method of Conklin and Smith²⁰. Daily growth rate (DGR) was estimated from the collected biomass using the equation given by Rueness et al. 21

Growth rate (% day⁻¹) = 100 ln
$$(W_t/W_0)/t$$
,

where W_0 is initial weight, W_t final weight; t is the time-interval (days).

Qualitative data were taken using photographs. All observations and estimations were done during low-tide conditions.

Statistical analysis

Percentage cover data did not satisfy the assumptions of normal distribution, tested using Shapiro-Wilk's test, even after transformations. Mann-Whitney U-test was applied to test the null hypothesis stating that the percentage cover of each benthic component in control and invaded ecosystems, treating them as independent samples, has the same median. Since the data are non-normal, we used the median measure instead of mean for comparisons. Wilcoxon signed ranks test was used to test whether the median of each benthic variable differs significantly between successive years since 2008. However, colony area and biomass data of K. alvarezii collected for manual removal assessment were normally distributed. Hence, we used paired samples *t*-test to reveal differences in their respective means between pre- and post-removal period. All statistical analyses were performed with IBM SPSS Statistics version 20.0.0.

Results

Benthic community analysis

We found no significant difference in the median of the studied benthic variables between successive years in both sites of control ecosystem (Wilcoxon signed ranks test: P > 0.05, N = 20, in all cases; Table 1). Compared to the control ecosystems, a remarkable decline in coral cover was observed in the K. alvarezii-invaded ecosystems (U = 4276.0, P < 0.01; Figure 1). In K. alvareziiinvaded ecosystems, median of coral cover was reduced from 64.9% in 2008 to 33.4% in 2012 and 45.6% in 2008 to 0% in 2012 at sites 1 and 2 respectively (Figure 1). A reduction in median of coral cover was significant between successive years at both sites of K. alvareziiinvaded ecosystems. K. alvarezii showed an increasing trend in its distribution at both sites of invaded ecosystem (Figure 1). Among the sites of the invaded ecosystem, median of K. alvarezii cover on corals had attained the maximum (97.6%) in site 2 rather than site 1 (60.9%) during 2012. It also showed significant difference in the median of K. alvarezii cover on invaded corals during successive years at both sites (Table 1).

During the study period, native algal species also experienced significant reduction in their cover at the K. alvarezii-invaded ecosystems compared to the control ecosystems (U = 9009.0, P < 0.01). Significant reduction in median of native algal cover was observed from the year 2011 in site 1 and from 2010 in site 2 of K. alvarezii-invaded ecosystem (Table 1). The median number of algal species (S) per quadrat (K. alvarezii not included) was 7 (range: 3-11 species, n = 100) in the control ecosystems and 5 (range: 1-8 species) in the invaded ecosys-

tems. Median of species richness (S) showed significant difference between control and invaded ecosystems (U = 2031.5, P < 0.01). In the invaded ecosystem, drastic decline in species richness (S) was observed during postremoval period (Table 2). Simpson diversity (1 - D) per quadrat (without K. alvarezii) was found to be significantly higher (U = 3457.0, P < 0.01) in control ecosystems (median = 0.8, n = 100) than invaded ecosystems (median = 0.7, n = 100). Further, Simpson diversity in K. alvarezii-invaded quadrats reduced gradually during postremoval period (Table 2). This reduction clearly depicted the increase in dominance of K. alvarezii in the invaded ecosystems. Due to increase in abundance, K. alvarezii had shifted from lower rank (9th) during pre-removal to higher rank (3rd) and subsequently attained top rank (1st) after 2009, i.e. post-removal. Consequently, dominant native species such as Gracilaria sp., Gelidiella sp., Caulerpa sp. and Padina sp. were not recorded from the K. alvarezii-invaded ecosystems and other species such as Sargassum sp., Turbinaria sp., Halimeda sp., Ulva reticulate and Hypnea sp. had declined considerably (Table 2).

Manual removal impacts on K. alvarezii

In the invaded ecosystem, the estimated initial (preremoval) mean colony area of K. alvarezii was $1457.44 \pm 113.11 \text{ cm}^2/\text{m}^2$ (mean \pm SE, n=9). Its biomass was estimated to be $4.52 \pm 0.32 \text{ kg/m}^2$. We observed extensive regrowth of K. alvarezii at the removal points (Figure 2a). After 175 days, the mean colony area and biomass of K. alvarezii were found to be $3382.67 \pm 192.09 \text{ cm}^2/\text{m}^2$ and $12.77 \pm 0.68 \text{ kg/m}^2$ respectively. Paired t-test revealed significant difference between pre- and post-removal

Table 1. Comparison of benthic cover components between successive years in control and *K. alvarezii*-invaded ecosystems of Kurusadai Island, Gulf of Mannar (GoM) during 2008–2012

Ecosystem	Site	Benthic cover	Comparison between successive years (P value)					
			2008 vs 2009	2009 vs 2010	2010 vs 2011	2011 vs 2012		
Control	Site 1	Coral	0.520	0.243	0.601	0.654		
		K. alvarezii on coral	_	_	_	_		
		Native algae	0.845	0.248	0.627	0.136		
		Sand/rubble	0.910	0.913	0.825	0.334		
	Site 2	Coral	0.918	0.165	0.856	0.526		
		K. alvarezii on coral	_	_	_	_		
		Native algae	0.756	0.070	0.376	0.433		
		Sand/rubble	0.609	0.983	0.446	0.765		
Invaded	Site 1	Coral	0.010	0.005	0.013	0.010		
		K. alvarezii on coral	0.001	0.000	0.000	0.000		
		Native algae	0.388	0.062	0.000	0.000		
		Sand/rubble	0.209	0.051	0.008	0.028		
	Site 2	Coral	0.002	0.001	0.002	0.031		
		K. alvarezii on coral	0.001	0.001	0.002	0.006		
		Native algae	0.814	0.001	0.001	0.005		
		Sand/rubble	0.109	0.109	0.109	0.273		

Bold values show significant difference at $\alpha = 0.05$ level. P values are based on Wilcoxon signed ranks test.

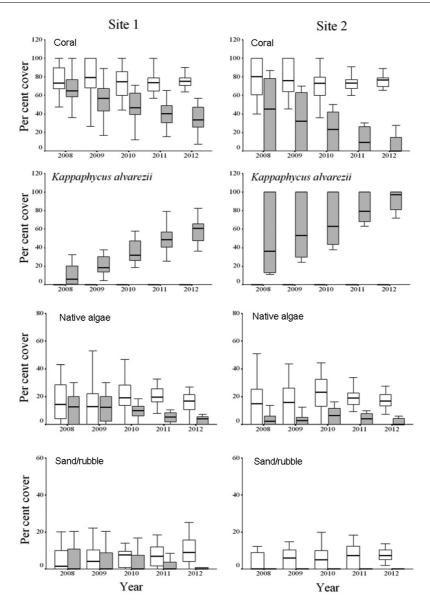


Figure 1. Distribution (% cover) of different benthic components in site 1 (50 m from the shore) and site 2 (100 m from the shore) of both control and *K. alvarezii*-invaded ecosystems in Kurusadai Island, Gulf of Mannar (GoM) during 2008–2012. Box plots show the minimum, 25th percentile, median, 75th percentile and maximum values (outliers not shown). White box represents control ecosystems and filled box represents invaded ecosystems.

groups of K. alvarezii for colony area (t = 23.5, P < 0.01) and biomass (t = 22.1, P < 0.01). After removal, an increase of ca. 132% and 182% was recorded in colony area and biomass of K. alvarezii respectively. Estimated DGR of K. alvarezii for 175 days was 0.6%. Colonies of K. alvarezii fragmented from manual eradication attached themselves to neighbouring healthy coral colonies, and then expanded further (Figure 2 b). Wave action was also responsible for the reintroduction of removed and discarded K. alvarezii colonies from the seashore to uninvaded corals (Figure 2 c). Washed-up fragments of K. alvarezii colonies were observed along the seashore (Figure 2 d). Twelve algal clumps with varying biomass of 150–1125 g

(fresh weight) were collected along the shore of the invaded ecosystem during the study period. In addition to these observations and estimations, we observed a novel adaptation of the species after the interference of manual removal. In 2008, the growth of K. alvarezii was reported to be as a green mat over the top and lateral sides of the corals (Figure 2 e) and the major axis of K. alvarezii closely adhered with the rough surface of the corals in the study region¹³. In contrast, here we noticed unusual dome-like growth of K. alvarezii along the coral landscape (Figure 2 f), particularly on manually damaged K. alvarezii colonies. The dome protruded out of the coral landscape and its height was 10 ± 0.76 cm in site 1 and

Table 2. Species abundance, richness, diversity and evenness of identified algal taxa in control and *K. alvarezii*-invaded ecosystems of Kurusadai Island, GoM during 2008–2012

	Control ecosystem				Invaded ecosystem					
	Pre-removal		Post-removal		Pre-removal		Post-removal			
Algal species	2008	2009	2010	2011	2012	2008	2009	2010	2011	2012
Species abundance ^a										
Sargassum sp.	14.9 (4)	18.9 (3)	23.9(1)	15.3 (2)	19.0(1)	11.9(1)	10.6(1)	4.7(2)	2.4(4)	1.8(2)
Gracilaria sp.	21.8 (2)	22.4(1)	23.7(2)	15.3 (2)	16.2(2)	11.2(2)	5.9(2)	5.5(1)	1.0(8)	0 (6)
Gelideilla sp.	22.4(1)	14.8 (4)	12.8 (4)	14.5 (4)	9.3 (5)	8.6(3)	5.3 (4)	1.4(8)	0 (10)	0 (6)
Turbinaria sp.	17.4 (3)	20.9(2)	20.9(3)	19.1(1)	13.6 (3)	6.3 (4)	5.2 (5)	2.5 (5)	1.2(7)	1.0(4)
Caulerpa racemosa	8.0(6)	3.9 (7)	5.5 (5)	9.5 (5)	5.8 (7)	4.6 (5)	3.5 (7)	2.9(4)	2.6(3)	0 (6)
Caulerpa taxifolia	9.5 (5)	2.3 (9)	2.5(8)	4.0(8)	5.9(6)	4.5 (6)	5.4(3)	0 (10)	2.0(5)	0 (6)
Padina sp.	7.0(8)	3.7(8)	4.6 (6)	8.9 (6)	10.9 (4)	4.3 (7)	1.3 (10)	1.0(9)	1.0(8)	0 (6)
Halimeda sp.	2.6(10)	1.0(10)	1.5 (10)	1.2 (11)	1.9 (9)	0 (10)	3.6 (6)	1.9(6)	0 (10)	1.0(4)
Ulva reticulate	8.0(6)	4.8 (5)	3.7 (7)	4.6 (7)	5.3 (8)	3.1(8)	3.0(8)	1.7(7)	4.1(2)	1.4(3)
Hypnea sp.	7.0(8)	4.2 (6)	1.4 (11)	1.7 (9)	1.0(11)	0 (10)	0 (11)	0 (10)	1.3 (6)	0 (6)
Dictyosphaeria cavernosa	1.1 (11)	1.0 (10)	1.6 (9)	1.3 (10)	1.4(10)	0 (10)	0 (11)	0 (10)	0 (10)	0 (6)
Kappaphycus alvarezii	_	-	-	-	_	2.1 (9)	2.5 (9)	3.5 (3)	4.2 (1)	4.7 (1)
Species richness (S) ^b	5.5 (2.7)	6.0 (2.7)	7.0 (2.0)	7.0 (1.0)	8.0 (1.0)	6.0 (1.7)	5.5 (1.7)	4.5 (2.0)	4.0 (2.0)	2.0 (1.7)
Simpson index of diversity $(1 - D)^b$	0.8 (0.1)	0.7 (0.1)	0.8 (0.1)	0.8 (0.04)	0.8 (0.05)	0.8 (0.1)	0.8 (0.1)	0.7 (0.2)	0.5 (0.6)	0.4 (0.9)

aValues denote abundance (species rank); bValues represent median (interquartile range; n = 20 quadrats for each year); S and 1 - D values of invaded ecosystem devoid of K. alvarezii data.

 18.6 ± 1.56 cm in site 2 (n = 10 per site) of the invaded ecosystem.

During our recent field visit in early March 2013, there appeared a widespread occurrence of K. alvarezii at both study sites of the invaded ecosystem which can be viewed clearly at low tides (Figure 3 a). The number of K. alvarezii colonies was found to be 8.5 and 11.2 (per m²; n = 10 quadrats) in sites 1 and 2 of invaded ecosystem respectively. It is significantly higher than the average number of K. alvarezii colonies observed in the corresponding sites of invaded ecosystem during pre-removal, i.e. 2009 (Table 3). In 2013, estimates of K. alvarezii colony area had ranged from 9.0 to 1716.0 cm² (mean = 502.7 cm^2 , n = 85 colonies) in site 1 and 1.0 to 1722.0 cm^2 (mean = 239.3 cm²; n = 112 colonies) in site 2 of the invaded ecosystems. Compared to mean K. alvarezii colony area during pre-removal, a significant reduction in mean colony area was observed in both sites of invaded ecosystems during post-removal, i.e. 2013 (Table 3; Figure 3 b).

Discussion

The results showed that innate benthos (such as corals, native algae) of Kurusadai Island were not altered much between sites as well as removal periods in the control ecosystems. A minimum level of reduction in coral cover of control ecosystems was observed during the study period (Figure 1). Competition between corals and native algae could be a reason for such reduction. However, such effects are common and fluctuate due to interannual

variations. Absence of the invasive species *K. alvarezii* in the control ecosystems could be a specific reason for such intact ecosystem, whereas innate benthos cover was considerably decreased due to increase in the cover and abundance of *K. alvarezii* in the invaded ecosystems. Failure to restore pre-invasion status and predominant increase in *K. alvarezii* cover at invaded ecosystems could suggest that the removal has been counter-productive. Vigorous regrowth and establishment of *K. alvarezii* population has been observed after the removal process.

The reason for manual removal failure may be due to (i) biology of the invasive species and (ii) inefficacy of the method employed to eradicate K. alvarezii. The alga affects the corals by completely smothering them^{13,14}. Consequently, there could be every possibility for leaving algal fragments within the coral tissues during removal. We observed small fragments of K. alvarezii firmly attached to the corals (Figure 3c), evidently detached during removal. The alga has the ability to coalesce into the tissues of corals, which provides a strong means of attachment⁹. Thus pieces of algal remains inside the coral tissue could facilitate regrowth under ambient natural conditions. Change in the structural form of K. alvarezii (i.e. dome protuberance) was observed only after manual removal. This modification may be due to non-availability of the substrate (i.e. corals) near the replenishing K. alvarezii-invaded coral colonies. As a result, such algal colonies have adapted themselves to extend their colony expansion vertically. Most likely such adaptation still favours its spread by break and drift of protruding branches as fragments due to wave action. Even the smallest

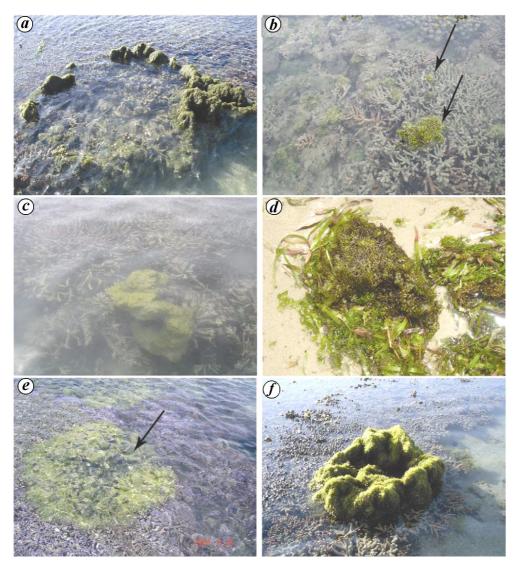


Figure 2. a, Extensive regrowth of K. alvarezii from a removal point; b, Reattachment of drifted algal fragments to live corals; c, Rebound of a discarded massive algal colony into reef substrate through wave action; d, Washed-up algal fragments found along the seashore; e, K. alvarezii smothering corals by growing as elastic rubber sheet (from Chandrasekaran et al.¹³); f, Transition from sheet like appearance to dome-like structure, showing its extraordinary phenotypic plasticity.

fragments of size 0.05 g will be able to disperse and establish themselves widely if given sufficient time⁹.

The method employed for this alga removal (i.e. hand plucking) was not well planned and was unscientific. Available field experimental data on manual removal of *K. alvarezii* from corals in Hawaii²⁰ have shown that the process is a daunting task. In the Gulf of Mannar National Park, the park managers have attempted eradication operations and they removed *K. alvarezii* attached to coral colonies in a 'pluck and throw' manner with no proper strategy to dispose or prevent spread of broken fragments. We observed several *Acropora* coral fragments attached to *K. alvarezii* both on-site (reef substrates) and off-site (seashore; Figure 3 *d* and *e*). To date, local island managers are collecting the washed-up coral

fragments with *K. alvarezii* and dumping them on the seashore. So far, it is considered that the species could spread or reproduce by vegetative fragmentation because sexual reproduction through spores is rare and not viable^{22,23}. Hence fragmentation resulting from manual removal facilitates dispersal of fragments, and such broken fragments seem to attach and re-establish in the reef ecosystem. Widespread observation of higher number as well as small-sized colonies of *K. alvarezii* in invaded ecosystems during our recent visit in 2013 has supported this claim (Figure 3 b). Strong regeneration ability of this species and unscientific eradication strategies have contributed to significant spread of invasion in the locality. Similar negative feedback has been accounted in physical removal of invasive alga *Sargassum muticum* in England²⁴.

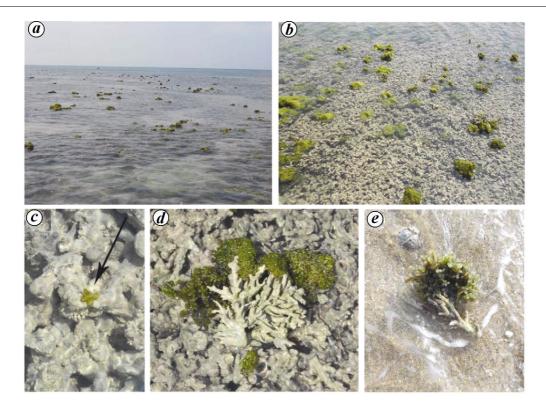


Figure 3. *a*, Photographic view showing *Kappaphycus* algal mounds over corals in March 2013; *b*, Numerous small-sized colonies of *K. alvarezii* resulted from manual eradication; *c*, Small-sized algal piece (1 cm) reattached to corals after removal; *d*, *e*, Drifted coral fragments found on reefs and seashore.

Table 3. Frequency and colony size (cm²/m²) of K. alvarezii in invaded ecosystems of Kurusadai Island, GoM during the years 2009 and 2013

Parameter	K. alvarezii invaded ecosystem	Pre-removal (2009)	Post-removal (2013)	t-test**	P value##
No. of colonies*	Site 1	1.8 ± 0.4	8.5 ± 2.0	14.53	< 0.05
	Site 2	2.2 ± 0.5	11.2 ± 5.0	8.02	< 0.05
Area of colonies#	Site 1	2238.0 ± 1268.5	502.7 ± 389.0	10.77	< 0.05
	Site 2	5855.0 ± 3102.2	239.3 ± 349.9	18.82	< 0.05

Data represent mean \pm SD; *Number per 1 m² quadrat (n = 20 for 2009; n = 10 for 2013); *n = 20 colonies for site 1 and site 2 in 2009; n = 85 colonies for site 1 and n = 112 colonies for site 2 in 2013; **Unequal sample size t-test; **Significance tested at $\alpha = 0.05$ level.

Invasive species as 'ecosystem modifiers' modify, create and maintain new physico-chemical conditions for their comfortable growth and continued expansion²⁵. Their removal should be viewed in the whole ecosystem or community context^{26,27}. Hence unscientific control strategies would exacerbate existing complexities and issues.

Conclusion and implication

This study illustrates the negative effects of the ongoing unscientific manual removal of *K. alvarezii* from invaded coral reef ecosystem in the Gulf of Mannar Marine National Park. If the alga is not controlled properly, there could be chance for it to invade the control ecosystem which is physically separated by considerable distance

from invaded ecosystem and shares similar biogeographic conditions. To eradicate *K. alvarezii* from Kurusadai Island, it is necessary to develop ecologically viable control measures such as use of native herbivores, and mechanical removal by means of sucking pumps which are non-destructive to native communities, especially corals. While it is almost impossible to eliminate established alien species in marine habitats²⁸, it is obvious that prevention would be the option to avoid incidents of invasion.

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