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Growth rates of *Eucheuma denticulatum* and *Kappaphycus alvarezii* (Rhodophyta; Gigartinales) cultured using modified off-bottom and floating raft techniques on the Kenyan coast

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Abstract

The study compared relative growth rates (RGR) of *Eucheuma denticulatum* and *Kappaphycus alvarezii* under modified off-bottom (MB) and floating raft (FR) culture techniques on the southern coast of Kenya. Seasonal variability in RGRs was evident over 10 months in both techniques and sites. RGRs were in the range of $0.9 - 10.2 \% d^{-1}$ for *E. denticulatim* and $0.3 - 5.7 \% d^{-1}$ for *K. alvarezii* at Mkwiro, and $-0.2 - 7.3 \% d^{-1}$ for *E. denticulatum* and $-1.7 - 4 \% d^{-1}$ for *K. alvarezii* at Kibuyuni. The RGR of $4.3 \pm 0.4 \% d^{-1}$ from the FR technique was significantly higher than the $3.2 \pm 0.4 \% d^{-1}$ from the MB technique (P < 0.05). Strong oceanic waves which were accompanied by the loss of seaweed thalli in the MB technique at Mkwiro between April and June led to significantly higher RGR in the FR than in the MB (P < 0.05). The higher percentage herbivory, epiphytes, and susceptibility to 'ice-ice' (white and soft thallus) associated with *K. alvarezii* than *E. denticulatum* in both techniques led to the former having significantly lower RGRs than the latter. Further research on the stability of MB in deep water and accessibility of FR techniques is recommended prior to commercial adoption.

Keywords: Eucheuma denticulatum, culture techniques, Kappaphycus alvarezii, relative growth rate, Mkwiro, Kibuyuni

Introduction

Seaweed biomass is a source of direct human food and phycocolloids which are extensively used in the processing of food additives and nutraceuticals, feeds, fertilizers, biofuels, cosmetics and medicines, among others (Bixler and Porse, 2011). They also act as anti-oxidants and antimicrobial agents (Gupta and Abu-Ghannam, 2011). In particular, the kappa carrageenan (phycocollide) which is predominantly extracted from K. alvarezii (Doty) Doty ex Silva, and iota-carrageenan from E. denticulatum (Collins and Hervey) are used in stabilizing food products, such as frozen desserts, chocolate milk, jellies, and pet foods (Anis et al., 2017). Over the years, the industrial use of seaweed biomass has shifted from exploiting beach-cast seaweeds as fertilizers and a source of potash, to iodine production, and to hydrocolloid extraction (Synytsya et al., 2015). The seaweed industry has demonstrated persistent

relevance to current needs and has a bright future when compared to the past when the industry looked very different (Hafting *et al.*, 2015).

Sources of these seaweeds have been from wild harvesting or artificial cultivation (FAO, 2012). Due to increasing demand in seaweed-based products associated with carrageenan, commercial mariculture of *K. alvarezii* and *E. denticulatum* has been intensified over the last decade in many countries with tropical coastlines (Buschmann *et al.*, 2017). Conventional culture techniques such as floating rafts (FR), long lines, fixed off-bottom lines (FOB), fixed long lines (Hurtado *et al.*, 2008), swing hanging long lines, and single or multiple-raft long lines (Hurtado and Agbayani, 2002) have been used. The culture of eucheumoids using these techniques has spread from Asian countries such as the Philippines and Vietnam to those in the western

Indian Ocean (WIO) such as Mozambique, Madagascar (Mollion and Braud, 1993), Tanzania (Msuya, 2007) and Kenya (Wakibia *et al.*, 2006). Seaweed farming has been widely accepted as an alternative source of livelihood in these countries (Msuya, 2007).

While developing countries continue to use the popular fixed off-bottom techniques in shallow areas of the near shore environment (FAO 2015), developed and developing countries have ventured into deep water cultivation techniques to maximize production of seaweeds. This approach has been triggered by poor production of seaweeds cultivated in shallow water environments associated with perennial infestation by 'ice-ice' syndrome (white and soft thallus), epiphytes, and herbivory. These challenges have been mostly observed in seasons characterized by high water temperature (Msuya et al., 2014). As a result high disparity in production between regions due to diversification of culture techniques has been demonstrated (Buschmann et al., 2017). For instance, in 2014, Indonesia recorded more than ten million tons of carrageenophytes, while in the WIO region, Zanzibar had the highest production of a mere one hundred thousand tons (Buschmann et al., 2017). This scenario suggests that the farming techniques adopted has a direct impact on seaweed production. The deep water culture techniques require sophisticated materials such as motorised boats to access the farms in relatively deeper, stable and productive environments, as opposed to shallow water cultivation where farmers

with low economic power access farms through walking at low tides. Although production statistics from other seaweed cultivating countries in Africa are scarce, limited production of carrageenophytes in the WIO region exists (Msuya *et al.*, 2014). The latest report has revealed that even with the diversification of culture techniques in developed countries, the present sources of cultivated eucheumoids have not met the volume demands of the existing seaweed processing industry (Buschmann *et al.*, 2017).

Recent studies have shown that relocation of seaweed farms from shallow water to deeper water environments reduced the risk of 'ice-ice' syndrome on these seaweeds thus improving their growth rate and biomass (Msuya et al., 2014). However, the studies have not focused on identifying the best culture technique for adoption in deep water environments. This aspect forms part of the background of this study. Recognizing the fact that seaweed biomass production could also be affected by other factors such as the wind patterns (Hurtado et al., 2001) and water quality (Msuya et al., 2014), the present study determined the growth rates of E. denticulatum and K. alvarezii cultured using two deep water culture techniques (FR and MB) on the southern coast of Kenya. The impact of these factors on farmed seaweeds was also investigated. The results from this study are critical in enhancing seaweed production strategies with a focus on formulating a sustainable national seaweed policy for Kenya to contribute to greater economic growth.



Figure 1. Map of the Kenyan coast showing the two study locations on the southern coast.

Materials and methods

Study sites

The study was conducted for ten months in the intertidal areas near Kibuyuni (4.38S, 39.20E) and Mkwiro villages (4.40S, 39.23E) on the southern coast of Kenya (Fig. 1). The two sites were sheltered from strong wave action and tidal currents by a fringing reef. The sandy substratum was colonized by seagrass species, *Thalosodendron ciliatum*, *Thalassia hemprichii*, *Syringodium isoetifolium*, and seaweeds, Glacilaria spp, *Turbinaria* spp and *Sargassum spp*. Echinoderms such as *Tripneustus gratila* and *Echinometra mathei* foraged within the seagrass beds and patchy coral heads. The two sites had been identified as suitable seaweed faming sites (Wakibia *et al.*, 2006).

Pilot scale commercial farming of E. denticulatum and K. alvarezii existed in these areas which were promoted by the World Bank-funded Kenya Coastal Development Project (KCDP). The climate on the southern coast of Kenya is influenced by northeast monsoon (NEM) winds which blow from the northeast between December and March (kaskazi) and southeast monsoon (SEM) winds blowing from the southeast between May and October (kusi). The SEM is characterized by strong winds that are accompanied by low air and water temperatures and solar radiation, with the lowest tides being measured at night. Conversely, the lowest tides of the NEM are measured during the day, the winds are relatively weaker, air and water temperatures are higher and rainfall is generally low. There is a one to two month transition period between the NEM and SEM characterized by variable and weaker winds. The seasons significantly affect the chemical and physical conditions of coastal waters (McClanahan, 1988).

Materials, cultivation techniques and growth experiments

Being a quantitative study, a completely randomized design (CRD) was used to assign stocked ropes for each of the two culture techniques at each site. Thirteen polypropylene ropes were used in each of the culture techniques; five ropes for each seaweed species, and three ropes as controls (without seaweed cutting). Fresh, young and clean seedlings of *E. denticulatum* and *K. alvarezii* were obtained from pre-existing seaweed farms at Kibuyuni and Mkwiro. To compare growth rates of the two eucheumoids, the seaweeds were cultured at these locations using FR and MB techniques, as illustrated in Figures 2 - 6. The MB technique (Figure 4) was a hybrid of fixed off-bottom (Figure 7) and FR techniques (Figure 8).

At each site the FR and the MB techniques were deployed at 5m and 1m water depths respectively, at low tide. The FR site was accessed by use of a motorised boat while the MB site was accessed by foot. The distance between the FR and the MB at Kibuyuni was about 30 m, and 5 m at Mkwiro. The FR and MB techniques used in the present study were modified from those described by Lirasan and Twide (1993). The FR technique (Figure 2) consisted of a floating bamboo raft (4 x 5 m²) anchored to the bottom of a deep lagoon (5 m at low tide) by a polypropylene rope (10 mm diameter) and a 100 kg weight. Similar ropes (6 mm diameter and 5 m long) were stocked with 25 seaweed cuttings of 50-80 g seed densities. Each seaweed cutting was attached to the rope using 'tie-tie' (soft, thin, tying material). Five ropes representing replicates of each species were used to test the daily growth performance of E. denticulatum and K. alvarezii at Kibuyuni and Mkwiro. Each of the stocked ropes was weighed and then stretched randomly along the length of the bamboo raft at 15-20 cm intervals. The same design was repeated in the MB technique at each site.

The MB technique (Figure 4) constituted mangrove poles of 6 cm diameter and 7 m length driven into the seabed and held upright at 6 m above ground. Similar polypropylene ropes as those in the FR technique also stocked with 25 seaweed cuttings were used in the MB technique. However, the seaweed cuttings in the MB technique were suspended by a floating mechanism that was designed to ensure that the seaweed cuttings remained immersed and close to the surface of the water. The design consisted of a two metal rings locked around the length of each pole at 20 cm above the seabed and on the uppermost part of the pole by a small wooden block nailed to the pole. These rings connected the polypropylene ropes from one pole to the other. Three 11 empty plastic bottles were attached to each of the stocked ropes to enhance buoyancy. Field assistance provided by two young men and two ladies selected from the local communities enabled a complete set up of the experiments within the limited duration of the spring tide. However, due to ease of access at low tide, the MB technique was attended actively by both men and women thus enhancing the speed of the experimental set up. Only men with good swimming ability participated actively in the deep water deployed FR technique.

After a culture period of thirty days, the number of missing seaweed cuttings in each monoline displaying 'ice-ice' syndrome were recorded. The number of







Figure 2. The floating raft (FR) technique deployed in the sea.Figure 3. One-month seaweeds being harvested from the floating raft at Mkwiro.Figure 4. The field experimental set up modified off bottom technique.



Figure 5. The layout of modified off-bottom (MB) technique in the sea at high tide. **Figure 6.** Monitoring growth of seaweeds cultured under the modified off-bottom technique at low tide. **Figure 7.** Fixed off-bottom line seaweed culture technique. Figure 1. Map of the Kenyan coast showing the two study locations on the southern coast.

grazed and macro epiphyte-infested thalli was also visually counted before they were weighed using a spring balance (Satorius Model, Germany). The number of cuttings on the stocked rope which displayed 'ice-ice' syndrome or were grazed or infested with macro epiphytes was computed as a percentage of the original number of cuttings on the entire rope. Young healthy thalli from every harvest were then selected as the stocks for the next growing cycle. These procedures were repeated from September 2015 to June 2016 for the two culture techniques. The mean RGR expressed as percent increase in wet weight (wt) per day was calculated according to the formula by Wakibia *et al.* (2006):

RGR =
$$[(w_{1}/w_{2})^{1/t} - 1] \times 100 \%$$

where, w_0 = average wet weight of seaweed cutting at day 0; w_t = average seaweed cutting wet wt at time t and t = time intervals (days).

Plant tissue analysis

After the final growth of seaweeds was measured for each month of culture, approximately 300 g of wet seaweed was harvested from the thalli of the cultivated species. This biomass was then cleaned with seawater, packed in labelled plastic bags, and stored in a cooler box at 4°C before being transported to the Kenya Marine and Fisheries Institute (KMFRI) laboratory for further processing. From each of the 300 g wet seaweed samples a total of 200 g of the seaweed thalli was accurately measured, sun dried for one day and then oven dried at 40°C to a constant weight for the determination of total nitrogen (N) and phosphorus (P) content. Total N and P content was determined using the National Council for Air and Stream Improvement (NCASI) method TNTP-W10900 (NCASI, 2000). This method converted ammonia, organic and inorganic (excluding N_a) nitrogen to nitrate, and organic and inorganic forms of phosphorus to orthophosphate by means of an alkaline acid digestion, without affecting the native nitrate. The digestion was accomplished by heating acidified unfiltered samples in the presence of a persulfate (strong oxidizer) at 120°C and 15 psi for 1.5 hours using an autoclave. Following digestion, the samples were cooled and filtered using GFC (45 nm) micro filters. Using the same dilution factor, the samples were then diluted and their aliquots were calorimetrically analyzed using a QUAATRO autoanalyzer (UK).

Environmental parameters

Air and water temperature, salinity, water motion (diffusion factor), water nitrate and phosphates were

determined at both cultivation sites. Water and air temperatures were recorded every two days at midday and the data was used to compute monthly temperature. A maximum/minimum thermometer (TFA model, Germany) was also deployed at the sites during each culture cycle. Seawater salinity was measured at each site fortnightly using a refractometer (Atago model, Japan). Water motion (diffusion factor) was also measured fortnightly by the rate of dissolution of clod cards made from Plaster of Paris (POP) using plastic balls as molds according to a modified method of Doty (1971). Three replicates of POP balls were left in the water column at sites of each culture technique and retrieved after 24 hours. Upon retrieval, the remaining POP balls were rinsed with fresh water, dried in the oven at 60°C, and weighed to a constant weight. To determine the diffusion factor, the average final weight (dry wt of POP balls in the field) was compared with the average final dry weight of POP balls suspended in a bucket of motionless seawater of equal salinity placed in the laboratory for 24 hours. Water samples for the determination of nitrate and phosphate levels were collected 20 cm below the surface of seawater at each site every fortnight using five 125 ml high density polyethylene bottles. The samples were fixed immediately with mercuric chloride, labeled, and stored in a cooler box at 4°C before being transported to the laboratory for analysis using the Technicon Auto Analyzer II system as described by Parsons et al., (1984).

The macro epiphytes were identified in the field using a field guide to seaweeds and seagrasses (Oliveira *et al.*, 2005), while the herbivorous fish were monitored by swimming and were visually identified using the field identification guide to the living marine resources of Kenya (Anam and Mostarda, 2012).

Data analysis

All data were analyzed using Microsoft Excel and Minitab 17 Statistical Software (2010) for tabular and graphical presentations. Significant differences in relative growth rates between species, sites and culture techniques were determined using t-tests. The Pearson's product-moment correlation test was used to determine the relationships between relative growth rates of cultured *E. denticulatum*, *K. alvarezii* for the two culture techniques, and environmental factors (P < 0.05).

Results

Seaweed and environmental variables

Statistical analysis of the seaweed and environmental parameters measured between sites, species, and The minimum water temperature ranged from 27 to 30°C while maximum ranged from 29 to 33 °C. Air temperature had a range of 26 to 29.5°C and a mean of 27.3 \pm 0.1°C. The minimum and maximum water temperatures varied greatly between months, with higher values being observed in January and February, and lowest values recorded in June. According to Table 1, there was no significant difference in minimum and maximum water temperatures between sites (P < 0.05).

E. denticulatum and K. alvarezii are presented in Table 4.

During the study period the diffusion factor (water motion) was in the range of 1.5 - 5.4 with a mean of 3.6 ± 0.9 on the southern coast of Kenya. The means were highly varied over the ten months with the highest (4.2 ± 0.5) being recorded in March, and lowest in January (3.2 ± 0.3) and February (3.2 ± 0.4). On average water motion of 3.63 ± 0.1 was observed at Mkwiro and 3.51 ± 0.2 at Kibuyuni, 4.04 ± 0.14 and 4.03 ± 0.14 in the FR and MB technique, respectively. Statistical analysis of the mentioned variables in Tables 1 and 3 did not show any significantl difference between sites or culture techniques (P < 0.05). A salinity range of 35 - 35.4 ‰ was observed during the study with an average of 35.4 ± 0.1 ‰ at Kibuyuni and 35.2 ± 0.0 ‰ at Mkwiro.

Table 1. Seweed and environmental factors measured in seaweeds cultured at Kibuyuni and Mkwiro on the southern coast of Kenya (Mean \pm SE,N = 141-285)

Variable	Kibuyuni	Mkwiro	P-value
Thallus N (%)	1.43 ± 0.01	1.44 ± 0.01	0.564
Thallus P (%)	0.04 ± 0.01	0.04 ± 0.01	0.752
'Ice-ice' syndrome (%)	19.2 ± 1.6	7.9 ± 1.5	0.000
Herbivory (%)	22.2 ± 1.8	12.1 ± 1.1	0.000
Epiphytic load (%)	24.5 ± 2.5	15.2 ± 1.1	0.001
Diffusion factor	3.51 ± 0.2	3.63 ± 0.1	0.549
Salinity (‰)	35.4 ± 0.1	35.2 ± 0.0	0.074
Minimum water temperature (°C)	27.8 ± 0.3	28.0 ± 0.2	0.611
Maximum water temperature (°C)	30.5 ± 0.4	30.2 ± 0.3	0.584
Air temperature (°C)	26.8 ± 0.3	27.8 ± 0.3	0.040
Nitrates (µmoles ⁻ l)	1.31 ± 0.2	1.2 ± 0.1	0.722
Phosphates (µmoles ⁻ l)	0.746 ± 0.1	0.624 ± 0.1	0.518
Plant loss (%)	15.3 ± 1.1	11.7 ± 1.4	0.040

Means are significant at P < 0.05

Table 2. Thallus N (%) and P (%) and biotic factors affecting the growth of *E. denticulatum* and *K. alvarezii* on the southern coast of Kenya (Mean \pm SE, N = 141-285)

Variable	E. denticulatum	K. alvarezii	P-value
Thallus N (%)	1.44 ± 0.01	1.46 ± 0.01	0.340
Thallus P (%)	0.04 ± 0.01	0.04 ± 0.01	0.918
'Ice-ice' syndrome (%)	2.2 ± 0.1	9.2 ± 1.4	0.001
Herbivory (%)	6.2 ± 0.9	11.4 ± 0.9	0.001
Epiphytic load (%)	4.6 ± 0.1	10.9 ± 1.0	0.001

Means are significant at P < 0.05

The average thalli N was 1.44 \pm 0.02 % and ranged between 1.35 - 1.55 %, with the highest value being obtained in October and the lowest in April. Thalli P had an average of 0.035 \pm 0.002 % and ranged between 0.031 - 0.045 % with the highest and lowest being obtained in February and November, respectively. Concentrations of water nitrates and phosphates ranged from 0.034 to 5.35 µmoles⁻¹ and 0.004 to 1.896 µmoles ⁻¹, respectively, with averages of 1.252 \pm 0.1 µmoles⁻¹ and 0.685 \pm 0.06 µmoles⁻¹, respectively. There were no significant differences in thalli N (%) and P (%), nitrates (µmoles ⁻¹) between sites (Table 1), and no significant difference in thalli N (%) and P (%), and nitrates (µmoles ⁻¹) between species (Table 2) and between culture techniques (Table 3).

The percentage 'ice-ice' syndrome, herbivory and epiphytic load were higher at Kibuyuni than at Mkwiro (Table 1), higher in *K. alvarezii* than in *E. denticulatum* (Table 2), and higher in the FR than in the MB culture technique (Table 3). The highest levels of 'ice-ice' syndrome (%), herbivory (%) and epiphytes (%) in the present study coincided with the period of low RGR of eucheumoids. The epiphytes observed included *Ulva spp., Enteromorpha ramulosa, Hypnea musciformis, Padina tetrastromatica, Gracillaria corticata, chaetomorpha indica* and the blue-green alga *Lyngbya majuscula*. These epiphytes mostly occurred in the warmer months as compared to the cooler months.

The main algal grazers observed included the herbivorous fish families Scaridae (Parrotfishes), Siganidae (Rabbitfishes), and the omnivorous Acanthuridae (Surgeonfishes). Grazing damage was mainly found on the thalli of affected seaweeds. Evidence of grazing by the sea-urchin *Tripneustus gratila* was also observed.

Relative growth rates of eucheumoids

The relative growth rates in both culture techniques varied greatly within the 10 months of investigation at the two sites (Figs. 2 and 3). At Mkwiro the two techniques showed a general pattern of high growth rates achieved between October (6.3 % d⁻¹) and December $(6.0 \% d^{-1})$ before decreasing in February $(3.2 \% d^{-1})$. A continuous decrease in relative growth rates appeared in the MB technique for the rest of the period while a sharp increase appeared from February to March in the FR technique. RGRs then decreased continuously to their minimum rates in the later months for both techniques. On the other hand, the growth rates achieved by the two culture techniques at Kibuyuni reveal a similar pattern as that at Mkwiro, but with relatively lower growth rates. The highest RGRs of 5.7 and 3.6 % d -1 observed in FR and MB techniques at Mkwiro in October decreased to 1.7 and 1.4 % d⁻¹ in the FR and MB techniques in December, respectively. The highest relative growth rates of both species occurred in October and lowest in June at Mkwiro, while the highest at Kibuyuni occurred in September, and lowest in February.

The relative growth rates of both species were higher in the FR technique than in the MB technique at both sites (Fig. 10). The figure also shows that the growth rate of *E. denticulatum* was higher than *K. alvarezii* at both sites. When the growth rates achieved by each species cultured under each techniques were compared, it was established that RGR of *E. denticulatum* cultured using the FR technique was not significantly higher than that achieved when cultured under the MB technique at Mkwiro (P = 0.231), but differed significantly at Kibuyuni (P = 0.039). On the other hand, the RGR achieved by *K. alvarezii* cultured under the

Table 3. Thallus N (%) and P (%) of seaweeds, diffussion factor and biotic factors measured in the Modified off-bottom (MB) and the Floating raft (FR) culture techniques on the southern coast of Kenya (Mean ± SE, N = 141-285).

Variable	Modified off-bottom	Floating raft (FR)	P-value
Thallus N (%)	1.49 ± 0.04	1.42 ± 0.05	0.228
Thallus P (%)	0.04 ± 0.00	0.04 ± 0.00	0.992
Diffusion factor	4.04 ± 0.14	4.03 ± 0.14	0.960
'Ice-ice'syndrome (%)	8.13 ± 2.5	10.4 ± 5.3	0.696
Herbivory (%)	3.13 ± 1.51	3.57 ± 2.00	0.857
Epiphytic load (%)	17.3 ± 1.8	22.6 ± 2.1	0.060
Plant loss (%)	20.3 ± 1.1	11.7 ± 1.4	0.026

Means are significant at P < 0.05





Figure 8. Monthly trends of growth rates achieved by the modified off-bottom (triangle) and floating raft (rhombus) techniques at Mkwiro.



Figure 9. Monthly trends of growth rates achieved by the modified off-bottom (triangle) and floating raft (rhombus) techniques at Kibuyuni.

FR technique was not significantly different from that achieved when cultured under the MB technique at Mkwiro (P = 0.504) and Kibuyuni (P = 0.130).

Data subjected to the t-test analysis revealed that, regardless of the species cultured and the site selected, the RGR of 4.3 \pm 0.4 % d ⁻¹ achieved by the FR technique was significantly higher than the 3.2 \pm 0.4 % d ⁻¹ achieved by the MB technique (P < 0.05). It was also established that irrespective of the culture technique used and the species, the RGR of 4.7 \pm 0.2 % d ⁻¹ at Mkwiro was significantly higher than the 2.7 \pm 0.1 % d ⁻¹ at Kibuyuni (P < 0.001). With regard to species, the same test analysis showed that, irrespective of the culture technique and site, *E. denticulatum* performed significantly better than *K. alvarezii* on the south coast of Kenya with RGRs of 4.9 \pm 0.2 % d ⁻¹ and 2.5 \pm 0.1 % d ⁻¹, respectively (P < 0.001).

Correlation coefficients of the RGR of the two seaweed species with environmental and seaweed parameters are presented in Table 4. Several of the correlation coefficients were statistically significant at P < 0.05 and at P < 0.01.

There was a positive correlation of RGRs of both eucheumoids with diffusion factor and water nitrates, and negative significant correlation with maximum water temperature, % plant loss, % 'ice-ice' syndrome, % epiphytic load and % plant loss. No correlation was found between RGRs of both seaweeds with thallus N (%). However, there was a negative significant correlation of RGR of *K. alvarezii* with % thallus P, and negative significant correlation of the RGR of *E. denticulatum* with salinity. Percentage herbivory showed significant negative correlation only with the RGR of *K. alvarezii*. Apart from the strong positive correlation



Figure 10. The relative growth rates of eucheumoids achieved under the two culture techniques at Mkwiro and Kibuyuni.

found between the RGR of *E. deticulatum* with the diffusion factor, all the other significant correlations were weak.

Discussion

The monthly trends in seaweed growth rates observed in this study, in which high growth rates occurred in September at Kibuyuni and October at Mkwiro, and the lowest between December and February, are typical of seasonal variations of eucheumoid growth rates observed in previous studies. For instance, in Igang Guimaras, Philipines, the growth rate of *E. denticulatum* was found to increase during the cool months of April and May (Ponce, 1992). Mollion and Braud (1993) found increased growth rate of *E. denticulatum* between April and February before decreasing in March due to grazing and 'ice-ice' attack in Madagascar. Recently in India, at Vizhinjam village, Kerala, high growth of *E. denticulatum* occurred in March and May (Bindu, 2011). Orbita (2013) reported highest growth rate of *K. alvarezii* during the cold period which occurred between June and September, and lowest during the

	RGR of Eucheuma denticulatum		RGR of Kappaphycus alvarezii	
Variable	R	P-value	R	P-value
Diffusion factor	0.597	0.001	0.267	0.012
Minimum temperature (°C)	-0.127	0.231	-0.040	0.705
Maximum temperature (°C)	-0.405	0.001	-0.208	0.047
Salinity (‰)	-0.368	0.001	-0.091	0.390
Herbivory (%)	-0.143	0.177	-0.317	0.002
'Ice-ice' syndrome (%)	-0.258	0.014	-0.645	0.001
Epiphytic load (%)	-0.239	0.023	-0.525	0.001
Plant loss (%)	-0.232	0.027	-0.393	0.001
Nitrate (µM)	0.424	0.001	0.315	0.002
Phosphate (µM)	-0.249	0.017	-0.101	0.338
Thalli N (%)	0.147	0.164	0.179	0.089
Thalli P (%)	0.028	0.793	-0.366	0.001

Table 4. Correlation coefficients of eucheumoids relative growth rates and environmental parameters on the southern coast of Kenya.

R is significant at P < 0.05

warmest period of the year between October and May (Ohno *et al.*, 1996). A 63 % decrease in growth rate of *K. alvarezii* reported in Vietnam also coincided with the warmest month (August) of the year (Ohno *et al.*, 1996; Saleh *et al.*, 2016). High growth rates of *K. alvarezii* was also reported to occur between the southeast wind blowing period (April to December) in Fiji, while low growth rate was observed from January to March (Prakash, 1990). On the southern coast of Kenya, Wakibia *et al.* (2006) observed the highest growth rates of *K. alvarezii* during the cooler months (August and September), a period that usually occurs during SEM, and lowest in the hottest months (January and February), usually occurring during the NEM.

The present results coupled with the above references suggest a relationship between air temperature and seawater quality parameters for suitable seaweed farming. The absorption of high air temperature by the sea during hot periods appears to raise the average temperature in equal margin and consequently compromises the water quality parameters, as manifested by emergence of 'ice-ice' syndrome at this time. Seaweed farmers and investors should be weary of these dynamics to avoid sudden suffering from economic shock and also to enable them to make the necessary mitigation interventions in order to save the seaweed seeds from complete loss during the unconducive seasons.

RGRs at the two sites increased from February to March. RGR at Kibuyuni was relatively stable between March and June, but showed a decreasing trend at Mkwiro in the same period. The decrease at Mkwiro was attributed to massive plant loss caused by interference of the MB culture experiment by strong oceanic waves associated with the SEM. Such a scenario was not observed at Kibuyuni. Despite the big loss of plants from the MB culture technique, the RGRs of both species were significantly higher at Mkwiro than at Kibuyuni, because the loss was compensated by the high growth rates achieved by plants in the FR technique at that period. These results therefore suggested that if the structural set up of the MB technique could be improved to overcome the overwhelming sea waves between March and June, then Mkwiro could be a more suitable site for culture of both species as compared to Kibuyuni.

Previous studies have attributed site differences of eucheumoid growth rates to water quality parameters such as water motion (Wakibia *et al.*, 2006). However, the results of the analysis shown in Table 1 indicate that there was no significant difference in diffusion factor between the two sites, but biotic factors including % herbivory, % epiphytes and % 'ice-ice' syndrome were significantly higher at Kibuyuni than at Mkwiro. These factors were therefore presumed to have fundamentally influenced variation in RGRs between Mkwiro and Kibuyuni. The negative effects of biotic factors on seaweed growth rate have been cited in previous studies (Msuya *et al.*, 2014; Hurtado *et al.*, 2014).

The maximum growth rate of 5.7 % d⁻¹ for K. alvarezii obtained under the FR culture technique in the present study was comparatively higher than the 5.2 % d⁻¹ observed in India (Kavale et al., 2016), 4.3 - 5.1 % day-1 in Brazil (Hayashi et al., 2010), 2 - 8 % d-1 in Mexico (Munoz et al., 2004), and the 5.0 % d⁻¹ reported in Zanzibar (Msuya et al., 2014). However it was lower than the 7 - 9 % d-1 reported by Ohno et al. (1996) in Vietnam, and the 10.7 % d⁻¹ reported by Paula et al. (2002), and the 4.4-8.9 % d-1 in Zanzibar, Tanzania (Dawes et al., 1994). Most importantly, the growth rate of E. denticulatum was above the recommended commercial rate of 3.5 % d⁻¹ set by Doty (1987), and below this for K. alvarezii. The high variation in RGR between the species could have resulted from the difference in sensitivities to water temperature. The fact that K. alvarezii registered encouraging growth only during the cold period while the growth of E. denticulatum was relatively stable throughout the year under the two culture techniques could justify this explanation. However, several explanations have been made for these variations including difference in species' capabilities of tolerating wide range of ecological factors (Glenn and Doty, 1992), response to water motion (Wakibia et al., 2006), their morphological variability (Doty, 1987), and the underlying cell physiology and cell wall responses to the surrounding environment (Hurtado et al., 2014). The production of $H_{0}O_{0}$ by *E. denticulatum* as an oxidative burst was suspected to be part of its chemical defense mechanisms against epiphytic attack (Collen et al. (1994). Since this study never investigated the production of H₂O₂ by both eucheumoids, our explanation could only be limited to the analysis conducted.

The negative correlation between epiphytes (%) and RGR of eucheumoids revealed a scenario of exploitative competition for nutrient resources in which eucheumoids were outcompeted. Chaipart and Lewmanomont (2004) and Hanisak (1987) associated growth fluctuation of *Gracilaria fisheri with competitive growth of epiphytes. In the WIO region*, the negative effects of epiphytes on seaweed cultivation have been Although this study observed significantly lower levels of herbivory, epiphytes and 'ice-ice' syndrome in E. denticulatum than in K. alvarezii, it was not possible to accurately establish the cause of these variations since studies on the defense mechanisms of these species was not part of our investigation. Furthermore, the negative correlations between the RGRs of both species with maximum water temperature, water phosphate, 'ice-ice' syndrome (%), herbivory (%) and epiphytic load (%), were significant, but weak, suggesting that other confounding factors could be contributing to the species difference. Future research should therefore be focused on investigating possible distinguishing physiological and morphological characteristics in E. denticulatum and K. alvarezii that could be influencing resilience to extreme ecological conditions.

Based on the RGR of 4.5 % d⁻¹ and range of 0.3 - 9.2 % d $^{-1}$ observed in the FR, and RGR of 3.2 % d $^{-1}$ and range of -1.7 - 10.7 % d⁻¹ observed in the MB techniques, this study concludes that the two culture techniques are suitable for improving the growth rate of K. alvarezii and E. denticulatum in Kenya, thus improving their biomass. However due to seasonal environmental changes that affect the growth of seaweeds, variation in growth rate may occur between the species and techniques. New seaweed farmers and investors should be well sensitized to the seasonal dynamics of seaweed production in order to maximize production during the conducive seasons and to be ready to accept low production during unfavourable seasons. This could be achieved by developing a sense of optimism in the enterprise and exercising sobriety and patience during the various stages of production.

The advantage of higher impact of water velocity accorded to the seaweeds under the FR and the MB techniques coupled with lower grazing pressure and lower incidence of 'ice-ice syndrome explains the suitability of these culture techniques in improving the biomass of cultured seaweeds. However, mechanisms that ensure stability of the MB technique and accessibility of the FR technique by individuals with limited swimming capacities should be considered. This approach would address the plight of non-swimmers, particularly women, who constitute the majority of seaweed farmers in Kenya. To determine the economic viability, a comprehensive economic analysis of the techniques should be conducted prior to commercial up-scaling. Further studies should be conducted to: 1) investigate possible distinguishing physiological and morphological characteristics in *E. denticulatum* and *K. alvarezii* that could be influencing resilience to extreme ecological conditions; and 2) monitor the trends in herbivory attraction by the different techniques.

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References

- Anam R, Mostarda E (2012) Field identification guide to the living marine resources of Kenya. FAO Species Identification Guide for Fishery Purposes. FAO, Rome
- Anis M, Ahmed S, Hasan M (2017) Algae as nutrition, medicine and cosmetic: the forgotten history, present status and future trend. World Journal of Pharmacy and Pharmaceutical Sciences 6: 1934-1959
- Bindu MS (2011) Empowerment of coastal communities in cultivation and processing of *Kappaphycus alvarezii* – a case study at Vizhinjan village, Kerala. Journal of Applied Phycology 23:157-163
- Bixler H J, Porse H (2011) A decade of change in the seaweed hydrocolloids industry. Journal of Applied Phycology 3: 321-335
- Buschmann AH, Camus C, Infante J, Neori A, Israel A, Hernández-González MC, Pereda SV, Gomez-Pinchetti JL, Golberg A, Tadmor-Shalev N, Critchley AT (2017) Seaweed production: Overview of the global state of exploitation, farming and emerging research activity. European Journal of Phycology 52: 391-406
- Chirapart A, Lewmanomont K (2004) Growth and production of Thai agarophyte cultured in natural pond using the effluent seawater from shrimp culture. *Hydrobiologia* **512**: 117-126
- Collén J, Mtolera M, Abrahamsson K, Semesi A, Pedersén M (1995) Farming and physiology of the red algae *Eucheuma*: Growing commercial importance in East Africa. Ambio 24: 497-501

- Dawes CJ, Luisma AO, Trono GC (1994) Laboratory and field growth studies of commercial strains of *Eucheuma denticulatum* and *Kappaphycus alvarezii* in the Philippines. Journal of Applied Phycology 6: 21-24
- Doty MS (1971) Measurement of water movement in reference to benthic algal growth. Botanica Marina 14: 32-35
- Doty MS (1987) The production and use of *Eucheuma*. FAO Fisheries Technical Paper No. 281, Rome. pp 123-164
- FAO (2012) The state of world fisheries and aquaculture [http://www.fao.org/3/a-i2727e.pdf]
- FAO (2015) Global-seaweed-aquaculture-prodution-1950-2014 [https://www.researchgate.net...]
- Fujita RM (1985) The role of nitrogen status in regulating transient ammonium uptake and Nitrogen storage by macroalgae. Journal of Experimental Marine Biology and Ecology 99: 283-301
- Glenn E P, Doty MS (1992) Water motion affects the growth rates of *Kappaphycus alvarezii* and related seaweeds. Aquaculture 108: 233-246
- Gupta S, Abu-ghannam N (2011) Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. Innovative Food Science and Emerging Technologies 12: 600-609
- Hafting JJT, Craigie JS, Stengel DBD, Loureiro RR, Buschmann AH, Yarish C, Edwards MD, Critchley AT (2015) Prospects and challenges for industrial production of seaweed bioactives. Journal of Phycology 51: 821-837
- Hanisak MD (1987) Cultivation of *Gracilaria* and other macroalgae in Florida for energy production. In: Bird KT, Benson PH (eds) Seaweed cultivation for renewable resources. Elsevier, Amsterdam. pp 191-218
- Hayashi L, Hurtado AQ, Msuya FE, Bleicher-Lhonneur G, Critchley AT (2010) A review of *Kappaphycus* farming: prospects and constraints. In: Israel A, Einav R, Seckbach J (eds) Seaweeds and their role in globally changing environments. Springer Science. pp 255-283
- Hurtado AQ, Agbayani RF, Sanares R, Castro_Mallare MTR (2001) The seasonality and economic feasibility of cultivating *Kappaphycus alvarezii* in Paganatan Cays, Caluya, Antique Philipines. Aquaculture 199: 295-310
- Hurtado AQ, Agbayani RF (2002) Deep-sea farming of *Kappaphycus alcvarezii* using the multiple raft, long-line method. Botanica Marina 45: 438-444
- Hurtado AQ, Critchley AT, Trespoey A, Bleicher-Lhonneur G (2008) Growth and carrageenan quality of

Kappaphycus striatum var. *Sacol* grown at different stocking densities, duration of culture and depth. Journal of Applied Phycology 20: 551-555

- Kavale MG, Sreenadhan N, Singh VV (2016) Cultivation of Kappaphycus alvarezii (Doty) Doty ex P. C. Silva along the coast of Palshet, Guhagar, Maharashtra. Indian Journal of Geo-Marine Science 45 (5): 666-670
- Lirasan T, Twide P (1993) Farming *Eucheuma* in Zanzibar, Tanzania. Hydrobiologia 260: 353-355
- McClanahan TR (1988) Seasonality in East Africa's coastal waters. Marine Ecology Progress Series 44: 191-199
- Mollion J, Braud JP (1993) A Eucheuma (Solieriaceae, Rhodophyta) cultivation test on the south-west coast of Madagascar. Hydrobiologia 260/261: 373-378
- Msuya FE (2007) Combating *Kappaphycus* die-offs in Tanzania. Forum Phycologicum, Newsletter of the Phycology Society S. A 66: 2-4
- Msuya FE, Buriyo A, Omar I, Pascal B, Narrain K, Ravina JM, Mrabu E, Wakibia JG (2014) Cultivation and utilisation of red seaweeds in the Western Indian Ocean (WIO) Region. Journal of Applied Phycology 26 (2): 699-705
- Munõz J, Freile-Pelegrín Y, Robledo D (2004) Mariculture of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) color strains in tropical waters of Yucata´n, Mexico. Journal of Aquaculture 239: 161-177
- National Council for Air and Stream Improvement, Inc., NCASI, (2000). Methods Manual - NCASI Method Color 71.01: Color measurement in pulp mill wastewaters by spectrophotometry. Research Triangle Park NC 27709-3318 919: 558-1999
- Ohno M, Nang H, Hirase S (1996) Cultivation, carrageenan yield and quality of *Kappaphycus alvarezii* in the waters of Vietnam. Journal of Applied Phycology 8: 431-437
- Oliveira CE, Osterlund K, Mtolera MSP (2005) Marine plants of Tanzania. A field guide to the seaweeds and seagrasses. Botany Department, Stockholm University, Sweden. 261 pp
- Orbita MLS (2013) Growth rate and carrageenan yield of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) cultivated in Kolambugan, Lanao del Norte, Mindanao, Philippines. AAB Bioflux 5 (2): 128-139
- Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford. 173 pp
- Paula EJ, Pereira RTL, Ohno M (2002) Growth rate of the carrageenophyte *Kappaphycus alvarezii* (Rhodopyta, Gigartinales) introduced in subtropical waters of Sao Paolo State, Brazil. Phycological Research 50: 1-9

- Prakash J (1990) Fiji. In: Proceeding of regional workshop on seaweeds culture and marketing. Adams T, Foscarini R (eds) South Pacific Aquaculture Development Project. Food and Agricultural Organization of the United Nations GCP/RAS/116 JPN, FAO, Rome, Italy. pp 1-9
- Saleh L, Illijas IM,Yusuf A (2016) Different cultivation periods and seedling sources on the growth of *Kappapphycus alvarezii* cultivated in Pangkep waters, south Sulawesi,

Indonesia. Aquacultura Indonesiana 17 (2): 54-57

- Synytsya A., Copikova J, Kim W. J, Park Y. I (2015) Cell wall polysaccharides of marine algae. In: Kim, S. K. (ed) Hand-book of marine biotechnology. Springer, Berlin, Heidelberg, Germany. pp 543-90
- Wakibia JG, Bolton JJ, Keats DW, Raitt LM (2006) Factors influencing the growth rates of three commercial eucheumoids at coastal sites in southern Kenya. Journal of Applied Phycology 18: 565-573