Growth of *Eucheuma denticulatum* (*spinosum*) cultivated with a net bag verticulture method

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Abstract. Challenges encountered in seaweed cultivation in Pahawang Island, Indonesia include grazing by rabbitfish and sea turtles. Strong currents and waves can also cause seaweed crop failures in this region. To address these challenges, a net bag method was designed. Meanwhile, a verticulture method was developed to overcome area limitations and increase efficiency. The objective of this study was to determine the daily growth rate (DGR) and absolute growth rate (AGR) of the seaweed *Eucheuma denticulatum* (trade name *spinosum*) using a bag-net verticulture method with ten planting depth levels (I-X) at 60 cm intervals from 30 to 570 cm below the surface. The research was carried out from September to November 2019. The data were compared using analysis of variance (ANOVA) followed by a post-hoc test ($\alpha = 0.05$). The highest average final weight (Wt) was 323 g, at 30 cm depth; the lowest was 129 g at 450 cm. The net growth was highest (273 g) at 30 cm and lowest (79 g) at 450 cm. The highest DGR was 7.77% day^{-1} (30 cm depth/level I) and the lowest was 3.95% day^{-1} (450 cm depth/level VIII). The ANOVA showed a significant effect of depth on growth. The post-hoc test showed a significant difference in final weight between 30 cm (level I) and all other depths. However, in general the difference in final weight was not significant between neighbouring depth levels.

Key Words: seaweed, *Eucheuma*, net bags, verticulture, Pahawang, Lampung.

Introduction. Lampung Province is a region of Indonesia with extensive coastal waters. Within this Province, the Pesawaran District was recently established through the partition of South Lampung District. One marine and coastal commodity with potential for development in Pesawaran District is maricultured seaweed. Challenges to seaweed farming in this area include strong wave action and currents, as well as grazing (Yudha 2004; Wijayanto et al 2011).

Economically valuable seaweeds in Indonesia include the eucheumatoid seaweeds (genus *Eucheuma* and *Kappaphycus*), and the genera *Gracilaria, Gelidium*, *Sargassum*, and *Turbinaria* (Aslan 1998; Hendri et al 2018; Kordi 2011; Hurtado et al 2019). Seaweeds are widely used as food or food ingredients because of their high nutritional content, and have many other uses in pharmaceutical, cosmetic and other industrial sectors (Neish 2008; Neish et al 2017; Hurtado et al 2019). In the industrial sector, these seaweeds are widely used as sources of carrageenan, alginate and agar/agarose (Aslan 1998; Hendri et al 2018).

Seaweeds of the genera *Eucheuma* and *Kappaphycus* are carrageenan-producing seaweeds which have become established as commodities cultivated by coastal communities using a variety of methods (Nugroho & Endhay 2015; Neish et al 2017; Hendri et al 2018). The methods developed for the cultivation of these seaweeds include the long line method, the floating raft method, and the off-bottom method (Aslan 1998; WWF 2014; Hendri et al 2017, 2018). Each of these methods has advantages and has been applied with success in some conditions. However, they also each have shortcomings. For example, the floating raft method has high production costs and is easily attacked by grazers. In addition, the structures used are in general easily damaged by strong currents and wave action. Meanwhile, in the off-bottom method the seaweed is easily covered by sediment as well as prone to attack by pests and grazers. The most popular method in recent years is the long line method; however one issue is the extensive area required, which can cause disruption to waterborne traffic for locals and shipping lanes. Longline seaweed farms are also prone to pest and grazer attacks, time-consuming and expensive to maintain (due to their large spatial extent) and the structures and/or the thalli attached to the lines can suffer from strong wave and current action (Aslan 1998 ; Hendri et al 2017; Hendri et al 2018).

As pointed out by Hurtado et al (2019), there is a need for innovation in seaweed farming technology. Verticulture, or the planting of seaweed seedlings at multiple depths in the same area, is one potential solution to address spatial challenges and increase operational efficiency; however research on verticulture methods appears to be limited. Meanwhile, placing cultured seaweeds in net bags (rather than attaching the thalli directly to lines) can reduce the losses from pest and grazer attacks as well as preventing thallus breakage and detachment due to current and wave action.

A verticulture method combining the verticulture concept with the use of net bags was designed to survive in strong currents and high waves, common in the study area. The study aimed to explore the effectiveness of a net bag verticulture method developed based on a previous verticulture method (Hendri et al 2018) by measuring the growth of the seaweed *Eucheuma denticulatum* (trade name *spinosum*) cultivated using this method. If successful, this method should be cost-effective, easy to control, and yield higher production volumes, as well as being more resistant to pest and grazers attacks than the methods commonly in use in the region.

Material and Method

Time and place. This research on net bag verticulture seaweed cultivation took place from September to November 2019 in the waters around Pahawang Island, in Jelarangan Sub-Village, Pesawaran District, Lampung Province, Indonesia (Figure 1). The research site faces the open sea and is exposed to strong currents and large waves.

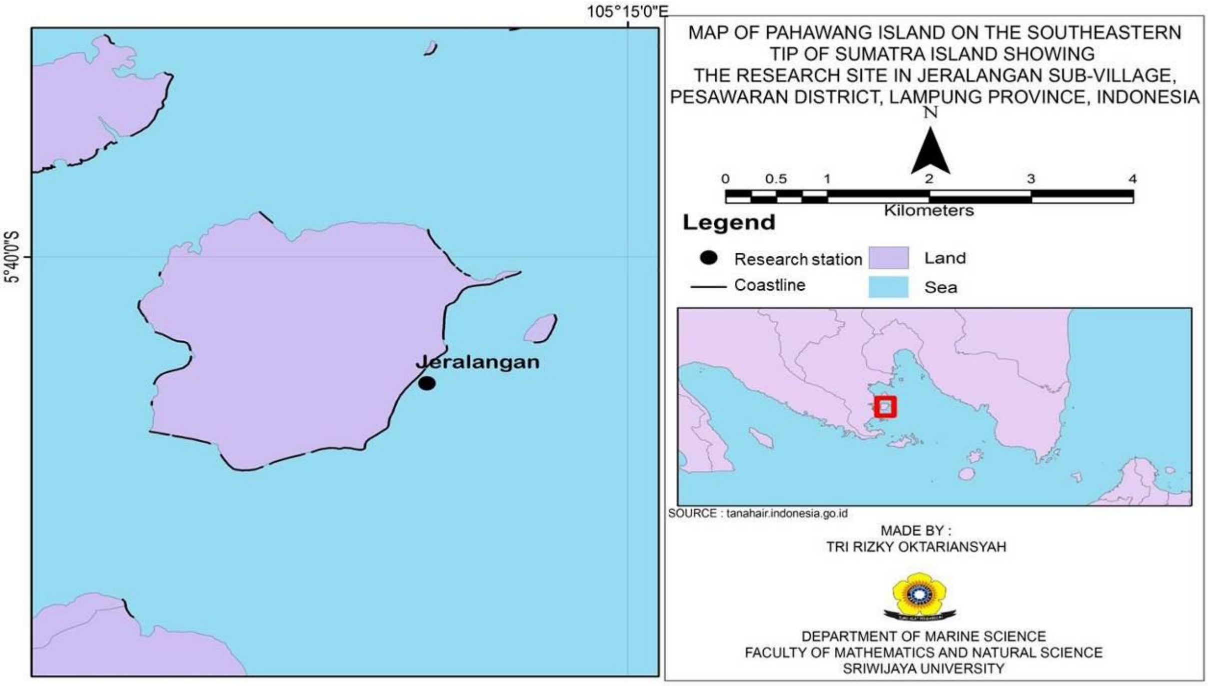

Figure 1. Map of Pahawang Island on the southeastern tip of Sumatra Island showing the research site in Jeralangan sub-village, Pesawaran District, Lampung Province, Indonesia.

Equipment and materials. The research materials used in this study were seeds of the seaweed *E. denticulatum* collected from seaweed farmers in Ketapang, South Lampung. The seeds were checked for quality and only undamaged, healthy seeds were used. The net bags used were 50 cm long with a diameter of 30 cm and mesh size of 1 cm.

Research method. This research employed a verticulture method with net bags to contain and protect the *E. denticulatum* seeds from pests and grazers as well as strong currents and wave action. The net bags were tied to a raft made from bamboo. The seedlings trialled were placed in net bags and planted out on three (3) vertical ropes (replicates). Each replicate series (rope) comprised 10 net bags making a total 30 net bags. The vertical planting ropes were tied to a polyethylene (PE 8) rope tied to a bamboo raft (Figure 2b).The first bag was attached at 30 cm from the sea surface (level I), the second net bag was planted 90 cm from the surface or about 60 cm from the first net bag (level II), and so on down to level X at 570 cm (5.7 m) below the sea surface.

The construction used for seaweed verticulture in this research can be seen in Figure 2. The method trialled in this research (Figure 2b) differs from a previous verticulture trial (Hendri et al 2018, Figure 2a) in several respects. In particular, a bamboo frame was used (enabling a reduction in anchors and rope used) and weights were not attached to the end of each vertical line.

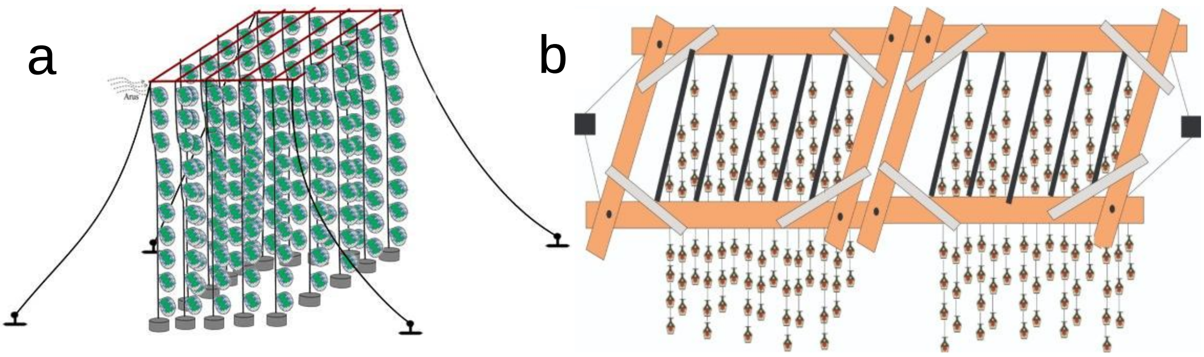

Figure 2. Construction used for seaweed cultivation through verticulture (Hendri et al 2017, 2018) (a), and (b) design used in this research.

The cultivation period during this trial was only 24 days due to the consideration that the nets used were overcrowded with seaweed thalli by this time, so that if they were left longer the net would restrict the seaweed plants and could affect their growth. The initial seed weight (Wo) was 50 g and the seeds were selected from young, fresh and healthy thalli. The planting site faced the open sea on the edge of the reef flat with the depth of 9 meters (sloping downwards), a substrate of broken coral and sand.

Daily growth rate (DGR) and net growth (NG). Daily growth rate (DGR) is a percentage obtained through dividing the difference between final weight and initial weight by the length of the planting period. Net growth at time t (NGt) is obtained by subtracting the initial weight (Wo) from the final weight (Wt). The equations used (Mtolera et al 1995; Gerung & Ohno 1997; Aguirre-von-Wobeser et al 2001; Bulboa et al 2007; Hayashi et al 2007; Hung et al 2009) are shown below:

$$DGR = \left[\left(\frac{Wt}{Wo}\right)^{\frac{1}{T}} - 1\right] x\ 100$$

$$NGt = Wt - Wo$$

Data analysis. The growth data were tabulated and analysed graphically in Microsoft Excel 2016. The data were tested for homogeneity of variance, followed by analysis of variance (ANOVA) and post-hoc LSD test performed in SPSS Statistic 21.0.

Results and Discussion

Water quality parameters. The physical and chemical properties of the ambient seawater play a major role in determining the survival and growth of seaweeds. During this study, the environmental factors measured (Table 1) were within the optimum ranges for eucheumatoid seaweed growth, as defined by Aslan (1998), WWF (2014), and Hendri et al (2018). However, the temperature varied with depth fluctuation. The temperature at the surface was 28^oC, while at 5.1 m below the surface (level IX) the temperature was two degrees lower (26^oC). Another factor that needs to be considered is the current speed. At the research site, the current speed was generally very strong (47-55 cm s^{-1}), exceeding the optimum range (20-40 cm s^{-1}).

Table 1

Water quality parameters measured during the study (mean values)

No	*Parameter*	*Mean values by replicate*			*Optimal range*[a]
		I	*II*	*III*	
1.	Temperature (^oC)[a]	26-28	26-28	26-28	26-32
2.	Salinity (ppt)[a]	31	31	31	27-34
3.	Brightness (%)[b]	100	100	100	100
4.	Depth (m)[b,c]	9	9	9	2-20
5.	Current speed (cm s^{-1})[a,b,c]	47	50	55	20-40
6.	Dissolved oxygen (ppm)[b,c]	7.1	6.8	6.8	≥ 5
7.	pH[a]	7.7	7.8	7.5	7-8.5

[a] WWF (2014), [b] Hendri et al (2018), [c] Aslan (1998).

Current speed is very influential on the flow and distribution of nutrients and thallus condition (Aslan 1998; Hendri et al 2018). Experience has shown that if the *E. denticulatum* seaweed thalli are not protected (in this case by net bags) they will certainly break. In addition, pests and grazers are very common at the study site (Figure 3a). In this trial, the use of net bags (Figure 3b) was effective in preventing attacks by grazers such as sea turtles and rabbitfish.

Figure 3. Protection from grazing. a. Herbivorous fish around the net bags; b. verticulture net bags.

Daily growth rate (DGR). The DGR of *E. denticulatum* over the 24 day study period is shown in Table 2. The DGR was similar for the three replicates, as shown by the low standard deviation (SD) values. There is no DGR for seedlings planted at level IX (510 cm depth) and level X (570 cm depth) as they did not survive.

Table 2

Daily growth rate (DGR) of *E. denticulatum* over the 24 day study period in %/day

Depth (cm) (level)	*DGR (% day^{-1}) by replicate*			*Mean DGR*	*Standard deviation*
	1	*2*	*3*		
I (30 cm)	7.71	7.85	7.76	7.77	0.07
II (90 cm)	7.30	7.27	7.32	7.30	0.03
III (150 cm)	7.03	7.10	7.06	7.06	0.04
IV (210 cm)	6.48	6.54	6.55	6.52	0.04
V (270 cm)	4.79	5.34	4.85	4.99	0.30
VI (330 cm)	4.71	4.66	4.69	4.69	0.03
VII (390 cm)	4.23	4.41	4.26	4.30	0.10
VIII (450 cm)	3.78	3.98	4.08	3.95	0.15

The strong correlation between depth and DGR shown in Figure 4 indicates that around 98% of the variation in DGR can be explained by depth.

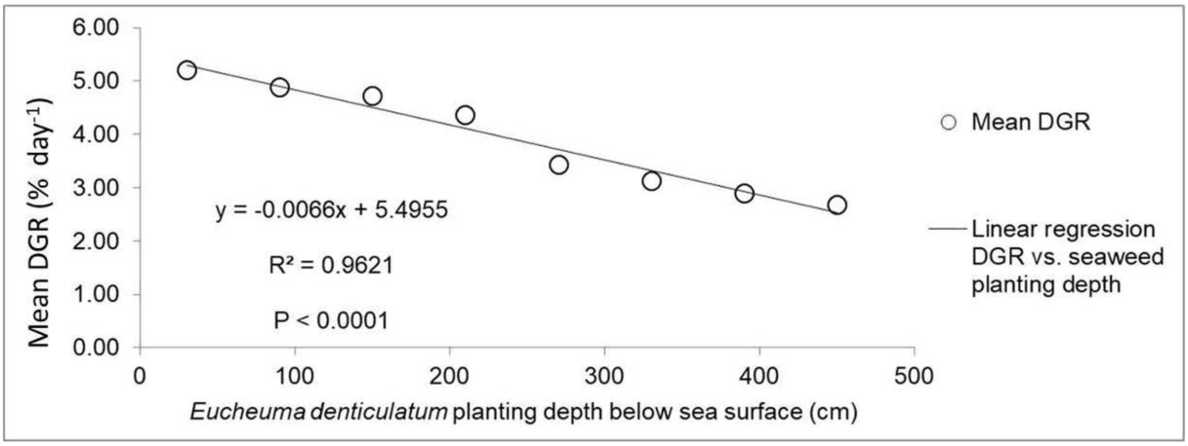

Figure 4. Correlation between mean daily growth rate (DGR) of *E. denticulatum* seaweed (in % growth per day) and depth below the sea surface (in cm) over the 24 day verticulture net bag trial.

***Eucheuma denticulatum* final weight**. The growth of *E. denticulatum* over 24 days was fair to very good at levels I to VIII (30 cm to 450 cm below the sea surface) as indicated by the final weights of the thalli (Table 3). However there were no final weights for level IX (510 cm depth) and level X (570 cm depth) as the seedlings planted at these depths did not survive.

Table 3

Final weight (g) of net bag verticultured *E. denticulatum* (day 25)

Depth (level) (cm)	*Weight (g) by replicate*			*Mean weight by depth*	*Standard deviation*
	1	*2*	*3*		
I (30 cm)	318	329	322	323.0	5.57
II (90 cm)	288	286	290	288.0	2.00
III (150 cm)	270	275	272	272.3	2.52
IV (210 cm)	237	240	241	239.3	2.08
V (270 cm)	158	180	160	166,0	12.17
VI (330 cm)	155	153	154	154,0	1.00
VII (390 cm)	138	144	139	140.3	3.21
VIII (450 cm)	124	130	133	129,0	4.58
Mean weight (all depths)	211.0	217.1	213.9	214.0	3.07

With initial weights of 50 g on Day 1, the final weights of the seaweed thalli on Day 24 ranged from 124 g at the deepest level VIII (450 cm planting depth) to the highest final weight of 329 g for level I, closest to the surface (30 cm planting depth). The mean final weight was also highest at level I and lowest at level VIII. The highest final weight of each series (repetition) is consistently on the surface or level I. Furthermore, the lowest final weight is at 450 cm from the surface (level VIII).

These results are similar to those from previous research on *E. denticulatum* using a verticulture method (Figure 2a) in the waters around Kelagian Island in Lampung Bay (Hendri et al 2017, 2018). In these studies, the final weight was also highest close to the surface and lowest at the deepest planting depth (level 10, 630 cm below the surface). The high final weight close to the sea surface is considered to be due to environmental factors such as temperature, salinity and sunlight penetration, especially the latter which is attenuated with depth in addition to shading by the thalli above.

Net growth (NGt). Net growth NGt (Table 4), defined as the final weight of *E. denticulatum* thalli on day 24 after subtracting the initial seed weight (Wo = 50 g), varied with depth in a similar manner to final weight (Table 3). The standard deviation for each depth was generally low, indicating a high consistency of results and thus the effect of depth on seaweed growth over the study period.

Table 4

Net growth (NGt) over 24 days of net bag verticultured *E. denticulatum*

Depth level (cm)	*NGt by replicate (g)*			*Mean net growth*		*Standard deviation (%)*
	1	*2*	*3*	*(g)*	*(%)*	
I (30cm)	268	279	272	273	446	2.04
II (90cm)	238	236	240	238	376	0.84
III (150cm)	220	225	222	222.3	344.7	1.13
IV (210cm)	187	190	191	189.3	278.7	1.10
V (270cm)	108	130	110	143	186	8.51
VI (330cm)	105	103	104	104	108	0.96
VII (390cm)	88	94	89	90.3	80.7	3.56
VIII (450cm)	74	80	83	79	58	5.80

The linear regression of NGt expressed as a percentage of initial seed weight against depth (Figure 5) indicates that growth would become negative at a depth of around 5 m. This is consonant with the observed mortality of the seedlings at 5.1 and 5.7 m below the sea surface (levels IX and X) during the trial.

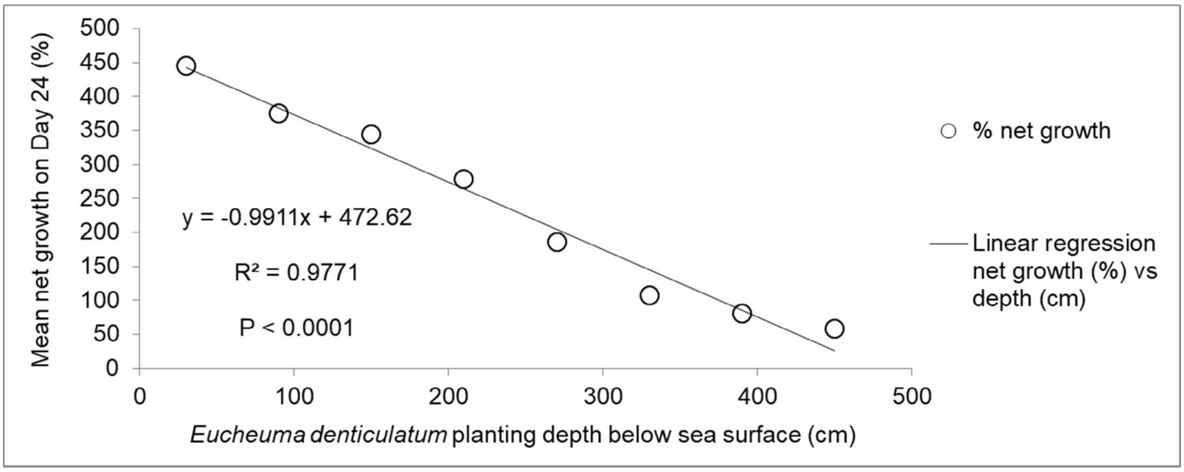

Figure 5. Mean net growth of net bag verticultured *E. denticulatum* seedlings (expressed as a percentage of initial seed weight) by planting depth (cm) after 24 days.

The overall trend and very strong (negative) correlation between depth and weight gain were similar to those observed for the seaweed *Kappaphycus alvarezii* (formerly *Eucheuma cottonii*) and *Gracilaria* sp. cultivated using a similar verticulture method (Hendri et al 2017, 2018). However, the results of this trial differ from those of the previous verticulture research (Hendri et al 2017, 2018), in which seaweed seeds planted at depths down to 6.3 m below the water surface survived with positive weight gain.

The trial results indicate a limit for seaweed survival of around 5 m and likely maximum depths of 2-3 m for viable yields when using net bag verticulture during the rainy season. Seaweed mortality at the two deepest depth levels in this research may have been due to many reasons.

One factor that should be considered is the intensity of insolation due to seasonal variations in sunshine hours and intensity. This trial was conducted at the beginning of the wet season, so that cloud cover often decreased the intensity of insolation entering the water column. Low light penetration can adversely affect seaweed photosynthesis, and thus growth, condition and survival (Neish 2008; Hung et al 2009; Borlongan et al 2017; Largo et al 2017).

Another factor that may have contributed to seaweed mortality at the deeper depths is temperature. At the sea surface, the temperature ranged between 28 and 30^{o}C, while the temperature sometimes reached a minimum of 25^{o}C at depths below 5 m. Sudden temperature changes can trigger stress and cause disruption of the metabolism in the seaweed thallus (Mtolera et al 1995; Gerung & Ohno 1997; Neish 2008; Kim et al 2016; Hendri et al 2018).

***Statistical analyses**.* The test of homogeneity gave a Levene statistic significance value of 0.000. It can therefore be concluded that the data is homogenous. The analysis of variance (ANOVA) showed a highly significant ($p < 0.0001$) difference between the depth groups for all seaweed growth parameters measured. The post-hoc LSD test showed significant differences ($p < 0.05$) for most depth pairs. This can be seen in Table 5 which displays the paired LSD results for final weight (Wt). Results for DGR and NGt were similar. There was no significant difference between the three deepest levels (below 3 m) with surviving thalli.

Table 5

Significance of the differences in final *E. denticulatum* thallus weight after 24 days of net bag verticulture

Level	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VII*
I (30cm)	-	ns	**	***	***	***	***	***
II (90cm)	-	-	ns	*	***	***	***	***
III (150cm)	-	-	-	ns	***	***	***	***
IV (210cm)	-	-	-	-	*	***	***	***
V (270cm)	-	-	-	-	-	*	**	**
VI (330cm)	-	-	-	-	-	-	ns	ns
VII (390cm)	-	-	-	-	-	-	-	ns
VIII (450cm)	-	-	-	-	-	-	-	-

(*** = p < 0.0001; ** = p < 0.01; * = p < 0.05; ns = p > 0.05).

Conclusions. Based on the results of bag net verticulture trials for the cultivation of the *Eucheuma denticulatum* over 24 days, several conclusions can be drawn. Firstly, the growth of the seaweed varied with depth; the highest net growth (323 g) and daily growth rate (DGR of 7.77% day^{-1}) were obtained at a planting depth of 30 cm below the sea surface and the lowest (129 g and 3.95% day^{-1}, respectively) at a depth of 450 cm. Below 5 m depth the seaweeds experienced negative growth with 100% mortality by the end of the trial. Statistical analyses indicate a linear (negative) correlation of growth with depth; however the difference in growth between neighbouring depths was mostly not significant. In particular, the difference in final weight between 30 cm depth (level I) and

90 cm depth (level 2) was not significant. These results indicate that, at least during the rainy season, verticulture at this site should be restricted to shallower maximum depths than in this study. Recommendations based on the results of this research include increased frequency of maintenance and monitoring to improve seaweed growth; and measurements of sea water quality (physical and chemical) parameters at depths down to and deeper than the maximum proposed planting depth before and during the cultivation of eucheumatoid seaweeds using verticulture methods.

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