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Growth of *Kappaphcus alvarezii* in vertical method of multi-trophic system based on feeding rate

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ABSTRACT

There is a need for knowledge to clarify the consistency of the complexity of feeding rates when applied to the multi-trophic aquaculture system. For this reason, three levels of feeding rates in rabbit fish as fed species were investigated to determine the growth performance of *Kappaphycus alvarezii* as nutrient absorber seaweed. Four plastic tanks were arranged in a graded position with a closed recirculation system, each for seaweed, rabbit fish, shells and sea urchins. *Kappaphcus alvarezii* was stocked vertically in the amount of three columns on four hanging ropes. Three levels of rabbit fish feeding rate were tested in this study, namely 5%, 7.5%, and 10% of fish wet weight with a frequency of four times a day. Every two hours after feeding, water was flowed into each tank for two hours. After 42 days of rearing, the results showed that growth performance of *K. alvaresi* varied among the three feeding rate levels. The average body weight gain (AWG), specific growth rate (SGR), daily body weight gain (DWG), and biomass of *K. alvarezii* in columns 1, 2, and 3 at the same feeding rate did not show significant differences ($P>0.05$). However, at different feeding rates, AWG, SGR, DWG, and biomass of *K. alvarezii* increased linearly and showed significant differences for each feeding rate ($P<0.05$). The implication of feeding rate does not seem to be a problem in multi-tropic system, at least up to the level of 10%. This study indicates that the 10% feeding rate is not optimal for the growth of *K. alvarezii*, so it is still possible to increase it to achieve higher growth of *K. alvarezii*.

INTRODUCTION

Nutrient waste in aquaculture operations is a necessity and has been become an issue in the discussion of sustainable aquaculture. Feed is the most significant source of aquaculture effluents **(Boyd & McNevin, 2015; Alves *et al.*, 2018** and **Dauda *et al.*, 2019)**. Fed fish aquaculture removes waste nutrients from fish feces, unconsumed feed, and excretion, which remain in the water bodies in particulate and dissolved form **(Boyd *et al.*, 2020** and **Camelo-Guarín *et al.*, 2021)**. The review of **Nederlof *et al*. (2021)**

quantified that 39–63% N, 18–30% P, and 39–70% C in feed are released as inorganic waste; 5–45% N, 42–57% P, and 6–44% C are released as particles organic matter (POM); and an average of 1%–7% N, 2%–8% P and s–6% C become dissolved organic matter (DOM). This is a threat because it can trigger nutrient enrichment in water bodies, with potentially negative impacts on the aquatic ecosystems **(Alves *et al*., 2018** and **Boyd *et al*., 2020)**.

Integrated Multi Trophic Aquaculture (IMTA) involving several species with different trophic levels has been developed to utilize nutrient waste in water bodies. Specifically for seaweed, its involvement in IMTA practices has been extensively investigated and proven to have the ability to absorb excess nutrients in water **(Neori *et al*., 2004; Chopin *et al*., 2012; Zhang *et al*., 2019** and **Kang *et al*., 2021)**. In addition, seaweed has a commercial value that can provide economic benefits **(Chopin & Tacon, 2021)**. Various treatments in the IMTA study have been investigated to determine their effect on seaweed growth. However, no one has investigated the effect of feed amount on fed species on the performance of other species kept together. In fact, this is important in IMTA operations because feed sourced from fed species is the main source of nutrients to be processed for seaweed growth **(Knowler *et al*., 2020; Kang *et al*., 2021** and **Nederlof *et al*., 2021)**. This indicates that the amount of feed to fed species greatly determines the amount of nutrients available to other species, including seaweed.

The amount of feeding which termed of feeding rate is the percentage of daily feeding based on fish biomass **(Trejchel *et al*., 2014)**. The association between the growth and feeding rate in monoculture systems is complex **(Niu *et al*., 2016)**. Applying it in increasing quantities does not mean increasing production because most of the feed becomes waste as mentioned at the beginning of this introduction. It might be expected that a high feeding rate would promote growth, but several studies confirmed that it initially increases with increasing feeding rate, but then decreases growth rate when the feeding rate is more than a certain limit **(Niu *et al*., 2016; Barani *et al*., 2019** and **Kim *et al*., 2021)**. Therefore, there should be an optimal feeding rate in fish farming **(Barani *et al*., 2019)**, otherwise the feed may be insufficient or excessive for the growth of the cultured species **(Niu *et al*., 2016** and **Kim *et al*., 2021)**.

There is a need for research results to clarify the consistency of the complexity of the feeding rate if it is practiced in the IMTA system which is known to recycle feed waste into a source of nutrition for other species **(Chopin *et al*., 2012** and **Zhang *et al*., 2019)**. For this reason, several feeding rates of rabbitfish, *Siganus guttatus* as fed species were investigated under closed recirculating laboratory conditions to determine their effect on growth performance of *Kappaphycus alvarezii*. This research involved the shellfish of *Perna viridis* species and sea urchin of *Diadema setosum* species as extractive species of organic particles. *K. alvarezii* was reared vertically to position it so that nutrients on the surface and water column could be utilized. There is still a lack of information and knowledge available about seaweed cultivation vertical method in multi-trophic system. While monoculture is known to increase the production of *K. alvarezii* while utilizing waste nutrients in the water column **(Hendri *et al*., 2017; Nursidi *et al*., 2017** and **Pong-Masak & Sarira, 2020)**. The findings of this study would be useful for the development of multi-trophic seaweed cultivation which has practical value in ecological and economic aspects.

MATERIALS AND METHODS

Experimental design

The study was conducted on August to November 2021 in the Laboratory of the Balik Diwa Institute of Maritime Technology and Business, Makassar, Indonesia. Four plastic tanks were arranged in a graded position, each for seaweed, rabbit fish, shells, and sea urchins. Seaweed tanks were constructed for vertical rearing methods. Two diagonal stretches are maked for the four hanging ropes, each consisting of three columns of binding points (C-1, C-2, and C-3). The distance of seaweed between the binding points are 10 cm vertically. A closed recirculation system tank was specially designed for this study **(Fig. 1)**.

Rearing management

K. alvarezii, *P. veridis*, and *D. setosum* with initial weights of 19.8 ± 0.2 g/binding point, 5.0 ± 0.5 g, and 20.0 ± 0.5 g, respectively, were obtained from cultivators in Pangkep Regency. Meanwhile, juvenile S. guttatus with initial weight 2.3 ± 0.2 g were obtained from the Research Institute for Coastal Aquaculture and Fisheries Extension, Maros. Organisms were acclimatized to experimental conditions for 5 days. K. alvaresi was stocked vertically in three column binding points at a water level of 35 cm, after S. guttatus was reared for 7 days. Three levels of feeding rates for *S. guttatus* were tested in this experiment, namely 5%, 7.5%, and 10%. Feed was given four times a day (8:00 a.m., 12:00 p.m., 4 p.m., 8:00 p.m.) with commercial feed (40% protein, 6% fat, 3% fiber). Every two hours after feeding, water is flowed into each tank for two hours by utilizing the gravity of the tank position. The circulating water flows using a pipe equipped with a faucet, starting from the S. guttatus tank, then successively to the *P. viridis*, *D. setosum*, and *K. alvarezii* tanks, then back to the rabbit fish tank. The water pump was used specifically to drain water from the seaweed tank to the rabbit fish tank. Light-Emitting Diodes (LED) Premium Lighting 1120-1260 Lumens are used to assist with light availability. In addition, continuous aeration was provided through pipe with attached airstones which placed in the center of each tanks.

Calculation of growth and water quality

The wet weight of *K. alvarezii* was measured every 7 days for 42 days of rearing. The weight (W) in g was measured using digital electronic balance. A total of four of the twelve tie points were taken randomly with the principle of representativeness to be used

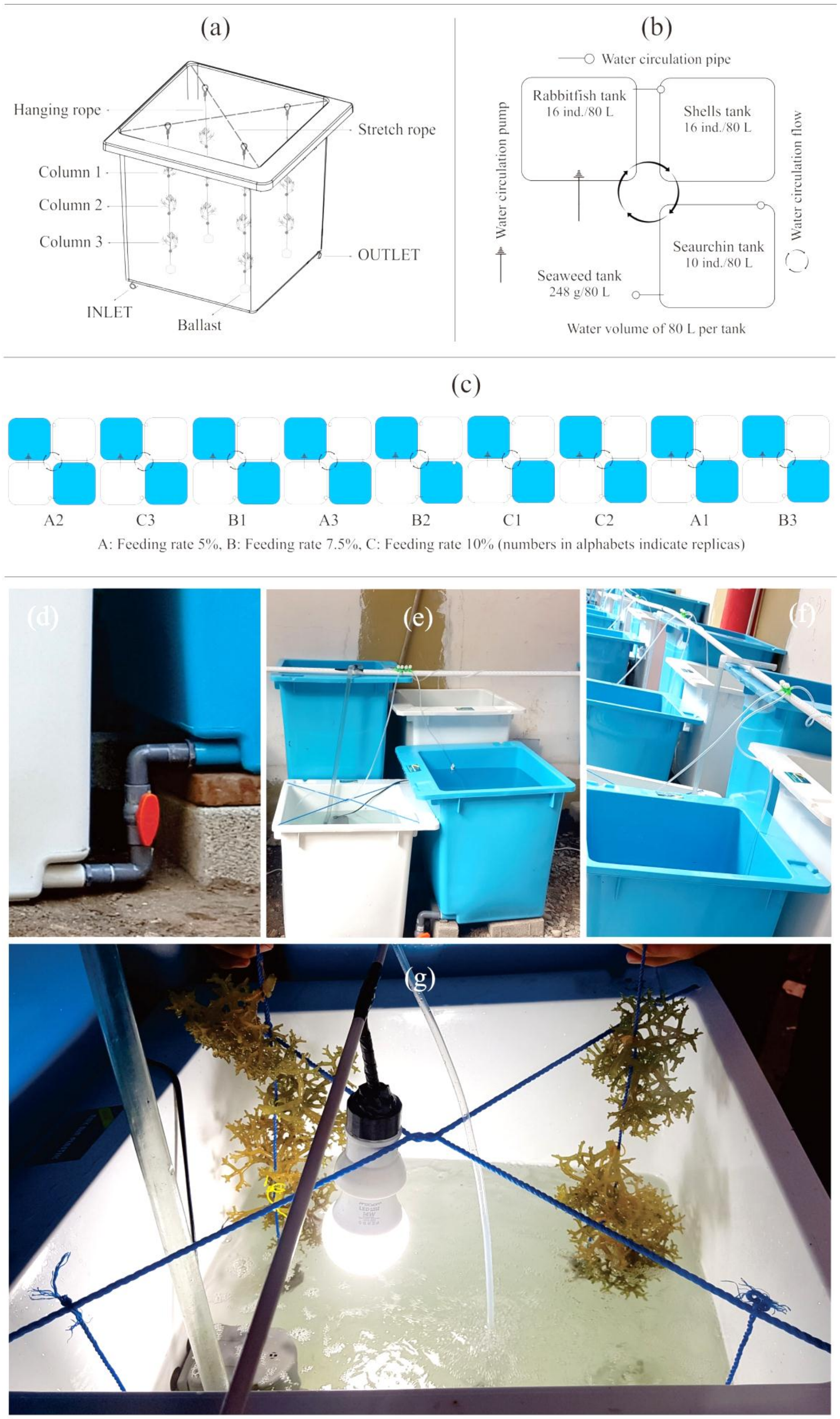


Fig. (1). **Schematic illustration and picture of the study: (a) Setup of seaweed tank, (b,c) Layouts of the experimental system, (d) Setup of recirculation pipa, (e) Graded system recirculation, (f) The rearing units, (g) seaweed tank**

as samples. Growth performance in terms of the average weight gain (AWG, g), specific growth rate (SGR, % day^{-1}), daily weight gain (DWG g day^{-1}) were evaluated following the formula of **Anand *et al*. (2018)** and **Jewel *et al*. (2020)**, while evaluation of seaweed biomass (Y, g) following of **Ganesan *et al*. (2009)**, respectively as follows:

AWG (g) = mean final weight - mean initial weight

SGR (% day^{-1}) = [(ln final weight - ln initial weight) / rearing duration in days] × 100

DWG (g day^{-1}) = (mean final weight - mean initial weight) / rearing duration in days

Y (g) = (total final weight - total initial weight) / rearing area coverage (m^2)

Water quality parameters in *K. alvarezii* tanks, involve temperature (°C), salinity (ppt), pH, dissolved oxygen (mg/L), and total dissolved solid (mg/L) were monitored four times a day (9 a.m., 1 p.m., 5 p.m., 9 p.m.) using a Water Meter 5 in 1 AZ Instrumen 86031. Meanwhile, ammonia (mg/L), nitrate (mg/L), and phosphate (mg/L) were measured at the beginning, middle, and end of the study using a spectrophotometer according to the American Public Health Association **(APHA, 2017)**.

Statistical Analysis

The data of AWG, SGR, DWG, and Y were expressed as mean ± SD. Based on the Shapiro-Wilk Test of Normality and the Test of Homogeneity of Variances, all data were normally distributed and has homogeneity of variance ($P>0.05$). Furthermore, AWG, SGR, and DWG among different feeding rates were analyzed throught t-test and one-way ANOVA. The significance level was determined at the 95% probability level followed by the Tukey's test at 5% level of significance ($P<0.05$). In addition, an independent t-test was used to compare the differences of water quality parameters at the three feeding rates. All of the statistical analyses were performed using the IBM SPSS Statistics Version 25 (SPSS Inc., Chicago, IL, USA).

RESULTS

Growth performance

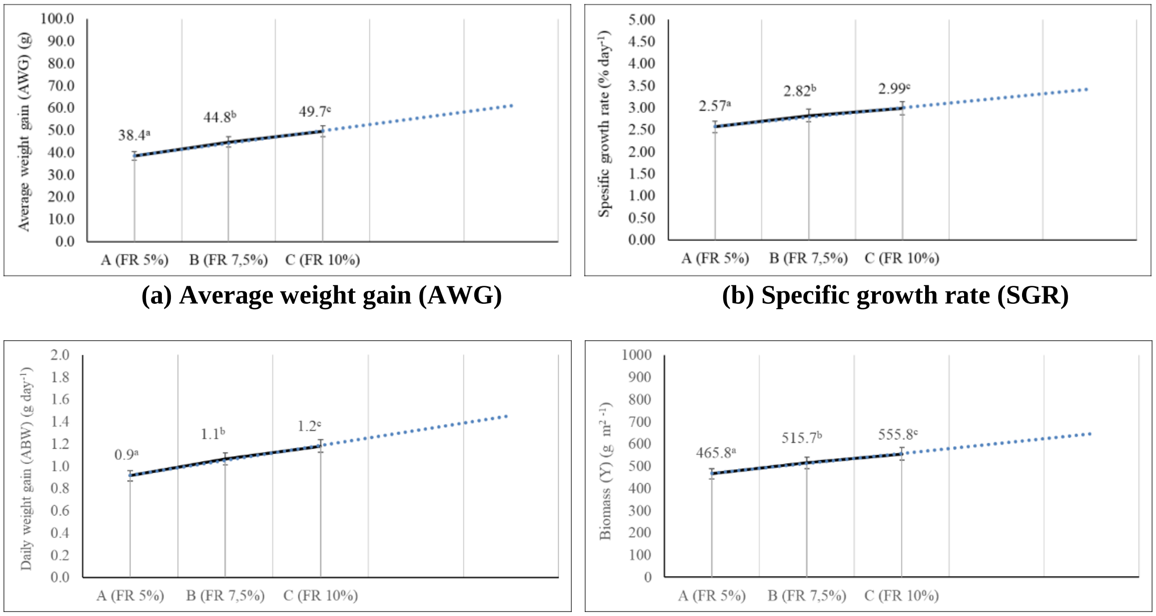
The growth performance of *K. alvarezii* with the vertical method of multi-trophic system in this study was investigated based on the feeding rate of rabbit fish as the fed species. Growth performance expressed by mean values (mean±SD) of average weight gain (AWG, g), specific growth rate (SGR, % per day), daily weight gain (DWG g/day), biomass (Y, g) are presented in **Table (1)** and **Fig. (2)**. Initially *K. alvarezii* was stocked in average weight of 19.77 ± 0.18 g each binding point which did not differ significantly ($P>0.05$) in all experimental units. After 42 days of rearing with the vertical method of multi-trophic system at feeding rates of rabbit fish of 5%, 7.5%, and 10%, all growth performance of *K. alvarezii* varied and significantly different ($P<0.05$) among the three feeding rate levels.

Table 1 presented the all growth performance of *K. alvarezii* in columns 1, 2, and 3 in the same feeding rate, also in the different feeding rates. One-way analysis of variance (ANOVA) showed that all growth performance in terms of ABW, SGR, DWG, and biomass of *K. alvarezii* in columns 1, 2, and 3 at the same feeding rate did not showed significant differences (P>0.05). However, at different feeding rates, the performance of all growth parameters was significantly affected by differences in feeding rates (P<0.05). Tukey's multiple comparisons showed that ABW, SGR, DWG, and biomass of *K. alvarezii* at a feeding rate of 10% were significantly (P<0.05) higher than 7.5% and 5% and all three were significantly different (P<0.05). Regardless of the column point, the overall growth performance of *K. alvarezii* increased significantly with a linear pattern. The trend line by linear forecast indicates that the gain of ABW, SGR, DWG, and biomass of *K. alvarezii* is directly proportional to the feeding rate of rabbit fish **(Fig. 2)**.

Table (1): Final average body weight, specific growth rate, daily weight gain, and biomass of *K. alvarezii* for each column in the vertical method of multi-trophic system over 42 days under closed recirculation conditions

Parameters	Column	Feeding rate (FR)		
		5%	7.5%	10%
Initial mean weight (g)	C-1	19.9±0.36^a	19.8±0.15^a	19.7±0.06^a
	C-2	19.7±0.10^a	19.7±0.06^a	20.1±0.32^a
	C-3	20.1±0.12^a	19.7±0.10^a	19.8±0.09^a
Final mean weight (g)	C-1	58.80±0.95^a	64.20±0.61^b	69.50±0.80^c
	C-2	57.77±2.01^a	64.37±0.90^b	69.00±0.26^c
	C-3	58.10±1.39^a	64.83±0.74^b	69.93±0.21^c
Average weight gain (AWG, g)	C-1	38.90±0.61^a	44.43±0.49^b	49.77±0.75^c
	C-2	38.07±1.91^a	44.70±0.95^b	49.07±0.55^c
	C-3	38.30±1.39^a	45.17±0.76^b	50.13±0.29^c
Spesific growth rate, SGR (% day^{-1})	C-1	2.58±0.01^a	2.80±0.01^b	3.00±0.02^c
	C-2	2.56±0.07^a	2.82±0.04^b	2.96±0.05^c
	C-3	2.56±0.06^a	2.84±0.03^b	3.02±0.02^c
Daily weight gain (DWG g day^{-1})	C-1	0.93±0.01^a	1.06±0.01^b	1.18±0.02^c
	C-2	0.91±0.05^a	1.06±0.02^b	1.17±0.01^c
	C-3	0.91±0.03^a	1.08±0.02^b	1.19±0.01^c
Bomass (Y, g m$^{2\ -1}$)	C-1	470.40± 7.63^a	513.60±4.87^b	556.01±6.40^c
	C-2	462.13±16.11^a	514.93±7.21^b	552.02±2.12^c
	C-3	464.80±11.09^a	518.67±5.90^b	559.47±1.67^c

C-1 = column 1, C-2 = column 2, C-3 = column 3. Values were expressed as mean ± standard deviation (SE) of the three replicate. The mean values with different superscript letters in the same row and column indicate a significant difference (P<0.05).



(a) Average weight gain (AWG) **(b) Specific growth rate (SGR)**

(c) Daily weight gain (DWG) **(d) Biomass (Y)**

Fig. (2). Average and trend line growth performance of *K. alvarezii* reared by vertical method of multi-trophic system over 42 days under closed recirculation conditions

Water Quality Parameters

Water quality parameters recorded during the study were temperature, salinity, dissolved oxygen (DO), pH, and total dissolved solid (TDS. The ranges of all parameter values in *K. alvarezii* tanks during the rearing period are presented in Table 2. The results of the independent t-test showed no significant differences in water quality values between feeding rates and in a suitable range for *K. alvarezii* growth.

Table (2): The range of water quality parameter values for 42 days in *K. alvarezii* tanks reared by the vertical method of multi-trophic system under closed recirculation conditions

Parameters	Feeding rate (FR)			Optimal ranges
	5%	7.5%	10%	
Temperature (°C)	26.1-28.4^a	26.0-28.3^a	26.0-28.6^a	24-31°C [1]
Salinity (ppt)	29.5-30.0^a	29.4-30.0^a	29.4-29.9^a	28-34 ppt [2]
Dissolved oxygen (mg L^{-1})	5.2-6.6^a	5.5-6.9^a	5.8-7.1^a	> 5 mg L^{-1} [3]
pH	7.5-7.9^a	7.6-7.9^a	7.6-8.0^a	8.2-8.7 [4]
Total dissolved solid (mg L^{-1})	15.1-15.9^a	15.8-16.2^a	16.1-16.8^a	*

[1]**Ask & Azanza (2002)**; [2]**Reis *et al.* (2011)**; [3]**Boyd & McNevin (2015)**; [4]**Tee *et al.* (2015)**, * no reference was found on the optimal range for *K. alvarezii*. The range values with the same superscript letters in the same row indicate no significant difference (P>0.05)

DISCUSSION

Growth performance

The general concept and principle of vertical seaweed cultivation is the utilization of nutrients in the water column to a depth that is still accessible to the sunlight needed by seaweed for photosynthesis **(Nursidi *et al*., 2017** and **Pong-Masak & Sarira, 2020)**. Meanwhile, the IMTA system has the concept and principle of utilizing nutrient waste from feed species (fish/shrimp) by particulate organic extractive species (such as shellfish, sea cucumbers, sea urchins, herbivorous fish) and inorganic extractive species (such as seaweed) **(Neori *et al*., 2004; Chopin *et al*., 2012; Buck *et al*., 2018** and **Zhang *et al*., 2019)**. To link these two concepts, two growth performances of *K. alvarezii* as shown in Table 1 are described in this discussion, namely growth at the same feeding rate and growth at different feeding rates.

At the same feeding rate, there was no significant difference of AWG, SGR, DWG, and biomass of *K. alvarezii* in column 1 (depth of 5 cm), column 2 (depth of 15), and column 3 (depth of 25 cm). **Hendri *et al*. (2017)** and **Pong-Masak & Sarira (2020)** reported that SGR and AWG of *K. alvarezii* reared in monoculture in coastal areas were also not significantly different up to depth of 1 m. In this study, light penetration dan recirculation system is the most likely reasons for the growth of *K. alvaresii* between columns not significantly different. These two aspects are known to facilitate the successful culture of *K. alvarezii*, especially in the efficiency of nutrient absorption, besides other aspects **(Hayashi *et al*., 2008** and **Kumar *et al*., 2016)**.

Water level of 35 cm in the *K. alvarezii* tank allows sunlight to penetrated all water columns as an energy supply and photosynthetic activity of seaweed **(Chung *et al*., 2017; Huang *et al*., 2021** and **Kang *et al*., 2021)**. Additional artificial light sources from LED lamps in this study also penetrated to the bottom column of the tank which could have a good effect on photosynthetic activity and seaweed growth performance **(Kim *et al*., 2015** and **Huang *et al*., 2021)**. The Light from these two sources seems to be received in all water columns which results in the growth of seaweed in columns 1, 2, and 3 not being significantly different. This further confirms the ability of seaweed to grow in the deeper water column as long as light is still accessible for photosynthesis **(Nursidi *et al*., 2017** and **Pong-Masak & Sarira, 2020)** and this is the advantage of the vertical method in seaweed cultivation. The recirculation system practices in this study are reasonable to guess that it also contributed to the growth of *K. alvarezii* which was not significantly different in each column. The circulation outlet and inlet are located at the bottom of the tank; thereby the water flow comes from the bottom column of water which is then distributed through the help of aeration to the upper column of water. In such circulation, *K. alvarezii* in each water column gets water mixing and transport flow of dissolved nutrients that can be absorbed for growth **(Hayashi *et al*., 2008** and **Al Azad *et al*., 2017)**.

At different feeding rates, the growth performance of *K. alvarezii*, including AWG, SGR, DWG, and biomass was significantly different and increased linearly following the increase in the feeding rate of rabbit fish. Similar findings were also reported by **(Verdian *et al*., 2020)**, the density of rabbit fish, which has a consequence on the amount of feeding, significantly affected the SGR and biomass of *K. alvarezii* with the same linear relationship as the results obtained in this study. These results indicate a positive contribution of feeding rate in rabbit fish, through feed nutrients, to the growth performance of *K. alvarezii*.

The recirculation design in this study places *K. alvarezii* as the last recipient of the nutrient flow after *P. viridis* and *D. setosum*. However, these three species have different trophic levels with different target nutrients **(Reid *et al*., 2020)**. Shellfish is a suspension feeder that absorbs organic particles suspended in the waters **(Buck *et al*., 2018** and **Park *et al*., 2018)** and sea urchins which are deposit feeder organisms that absorb organic particles deposited on the bottom of the waters **(Buck *et al*., 2018; Zhang *et al*., 2019** and **Reid *et al*., 2020)**. Meanwhile, seaweed is an organism that absorbs inorganic nutrients dissolved in water **(Chopin *et al*., 2012; Buck *et al*., 2018** and **Reid *et al*., 2020)**. Sources of inorganic nutrients absorbed by *K. alvarezii* in this study came from unconsumed feed, feces, as well as excretion and respiration of S. guttatus, P. veridis, and D. setosum, in the form of ammonia (NH_4), nitrate (NO_3), phosphate (PO_4), and carbon dioxide (CO_2) **(Wang *et al*., 2012)**. Based on this nutrient flow, it is logical that the higher the feeding rate, the more inorganic nutrients that flow from the tanks of *S. guttatus*, *P. veridis,* and *D. setosum* that can be absorbed by *K. alvarezii* for growth.

It is important to highlight that the high feeding rate, which is a concern as a source of waste in monoculture cultivation **(Niu *et al*., 2016** and **Kim *et al*., 2021)**, does not appear to be a serious problem in multitropical system cultivation. Several previous studies that reviewed the IMTA system clearly stated that waste from aquaculture, especially unconsumed feed and feces, was mostly utilized by other species based on their trophic level **(Buck *et al*., 2018; Zhang *et al*., 2019; Knowler *et al*., 2020** and **Kang *et al*., 2021)**. Therefore, the findings of this study provide important information that the cultivation of *K. alvarezi*, which mostly practices the longline method and the vertical monoculture method, is very likely to be developed through the practice of vertical cultivation with multitropical systems. If this is implemented, production and productivity will increase because more seaweed is cultivated on the same area of land. In addition, nutrients in the water column can be utilized **(Nursidi *et al*., 2017** and **Pong-Masak & Sarira, 2020)** to reduce aquaculture waste. Moreover, creating species diversification to strengthen economic resilience and sustainable food production systems **(Chopin *et al*., 2012** and **Zhang *et al*., 2019)**.

Water Quality Parameters

Maintenance of optimal water quality in multi-trophic culture is very important to support the growth performance of cultured species. Increasing the feeding rate to increase production has consequences for nutrient loading which will also increase, which can affect a deterioration in several water quality parameters **(Boyd & McNevin, 2015; Niu *et al*., 2016; Jescovitch *et al*., 2018** and **Kim *et al*., 2021)**. However, water quality parameters, including temperature, salinity, dissolved oxygen, and pH recorded in the morning, afternoon, evening, and night in this study did not vary significantly in each feeding rate treatment. Temperature fluctuations between 26.0-28.6°C were quite good for the growth of *K. alvarezii* **(Ask & Azanza, 2002)**. The suitable temperature can improve photosynthetic performance and nutrient absorption to accelerate the growth of *K. alvarezii* **(Kumar *et al*., 2020)**. Salinity fluctuated narrowly in the range of 29.4-30.0 ppt which was conducive to *K. alvarezii* as a stenohaline species **(Reis *et al*., 2011)**. If seaweed lives at the right salinity, then nutrient absorption and growth at the organ, tissue and cell levels will run well **(Lobban & Harrison, 2000** and **Aris *et al*., 2021)**. In addition to the ability of seaweed to produce oxygen through photosynthesis, the aeration factor in this study contributed to the dissolved oxygen **(Kumar *et al*., 2013)**, which was in the range of 5.3-7.5 mg L^{-1}. This is optimal for the life of *K. alvarezii* **(Boyd & McNevin, 2015)** to the process of respiration and decomposition of organic matter **(Ruangdej & Fukami, 2004)**. pH was quite stable during maintenance with fluctuations of 7.5-7.9, slightly lower than the optimal level, but still within the range that *K. alvarezii* could tolerate **(Tee *et al*., 2015)**.

Integrated cultivation systems using the seaweed bioremediation process have been are widely recognized as a method that can reduce excessive nutrient levels **(Chopin *et al*., 2012; Kang *et al*., 2021** and **Nederlof *et al*., 2021)**. This advantage was proven in this study, where the TDS parameter in *K. alvarezii* tanks was relatively low and did not vary significantly in each feeding rate treatment. TDS is an indication of the release of organic waste such as feces and uneaten feed **(Largo *et al*., 2016)**. The low TDS fluctuation between 2.9 and 3.8 mg L^{-1} in this study may be due to the role of P. viridis as a suspension feeder, D. setosum as a deposit feeder, and *K. alvarezii* as an absorber of inorganic nutrients **(Chopin *et al*., 2012** and **Zhang *et al*., 2019)**. Regardless of feeding rate, several types of seaweed, including *K. alvarezii* have been reported to be able to absorb nutrients organic and unorganic and also reduce TDS **(Largo *et al*., 2016; Kambey *et al*., 2020** and **Kang *et al*., 2021)**.

CONCLUSION

Seaweed cultivation with a vertical method of multitrophic system was able to increase the growth performance of *K. alvarezii*. Growth was observed to increase with increasing the feeding rate, thus leading to good utilization of nutrient waste by *K. alvarezii*. The growth trend of *K. alvarezii* is still increasing to the level of 10% but the optimal point remains unknown. Hence, this study provides the basic for further studies to find the optimal feeding rate of feed species for the growth of *K. alvarezii* raised through vertical cultivation with a multi-trophic system, which is then investigated in field conditions. The vertical method of seaweed cultivation with a multi-trophic system has practical value that is beneficial in ecological and economic aspects as well as can strengthen sustainable cultivation.

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