Seaweed (*Eucheuma cottonii*) growth in polyculture application

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Abstract. This study attempts to grow *Eucheuma cottonii* through polyculture with *Gracilaria verrucosa*, shrimp *Penaeus vannamei*, and milkfish *Chanos chanos*. The purposes of this study were to assess the growth rate of *E. cottonii,* to observe the physical and chemical water conditions, and to study the relationship between *E. cottoni*, *G. verrucosa*, *P. vannamei*, and *C. chanos*. Nine units of 5x5 m experimental plots were made with 3 different treatment combinations, 5 kg (A), 4 kg (B), and 3 kg (C) of *E. cottonii,* each of which was reared with 3 kg of *G. verrucosa,* 100 ind. of *P. vannamei,* and 25 ind. of *C. chanos.* Data were directly collected from the field, with 45 days of measurements for *E. cottonii* and 112 days for *P. vannamei*, and *C. chanos*, respectively. Results showed that treatment C yielded the highest mean absolute weight growth of *E. cottonii,* 282.35 g, followed by treatment B, 196.58 g, and then treatment A, 142.44 g. *C. chanos* and *P. vannamei* had good growth but they were not influenced by treatment applications. *G. verrucosa* did not grow well because of competition with *E. cottonii*. Water quality of the pond was still in optimum condition for the growth of the four commodities on study.
Key Words: *E. cottonii,* polyculture, relative growth, water quality.

Introduction. Seaweed, *Eucheuma cottonii*, is one of the major local commodities in Central Sulawesi, but since 2012, its production has drastically declined, especially in the centers of mining exploration, such as the coastal waters of Morowali regency and Palu Bay (Uno 2010). Mining activities, such as nickel in Morowali, gold mining in Poboya and other mining materials in Palu, have led to changes in land function to be mining concession areas (Uno 2010). This condition results in serious worries since mining and seaweed commodities are similarly important regional assets in promoting economy at the local and national level. Mining activities cause the cultivation area of *E. cottonii* decrease in Morowali regency from 78,420 ha in 2011 to 8,410 ha in 2014 (DMAF 2015).

The seaweed farming has been previously done without the use of fertilizers, having technological requirements with minimum capital. The rearing cycle was also short, normally less than two months. It has generated substantial socioeconomic benefits to the marginalized coastal communities in developing countries, most of which have reduced access to alternative economic activities (Arsyad et al 2014). Seaweed production development faces challenge to competition due to several human activities to meet their living needs.

Nevertheless, the presence of many companies in some areas of central Sulawesi has contributed to water pollution, such as nickel mining, makes the landscape of Morowali regency change fairly rapidly. It has been done at the vital points projected as a hotspot of nickel mining in Bahodopito the South of Bungku and gravel mining in Palu Bay. High mining activities along the coast have changed marine water quality that affects *E. cottonii* production as well.

Seaweeds need sunlight to grow and the sunlight is utilized as energy source through photosynthesis by utilizing CO_2 and the sunlight to produce carbohydrates (Foscarini & Prakash 1990). Although the factors required for seaweed growth are relatively simple (nutrients, trace minerals, CO_2 and sunlight) and relatively similar to terrestrial plants, the algae group can utilize them very efficiently resulting in high productivity (Packer 2009). Carbon binding by algae which is a photoautotrophic organism has the potential to reduce the release of CO_2 into the atmosphere and can help reduce the propensity for global warming (Kaladharan 2009). Mixed culture of shrimp, milkfish and seaweed has been done for 30 years and has given good economic contribution to the coastal communities (Suharyanto et al 2010). Murachman et al (2010) found that integrating *E. cottonii* into polyculture activities with black tiger shrimp, *Penaeus monodon* and fish (three commodities) will improve the pond water quality and pollution impact (Neori et al 2004) which in turn will increase the production and the revenue of the farmers. The success of *E. cottonii* culture through recent pond technology is one of the findings described by Troell et al (2003) on the necessity of biological/biochemical process study in closed and opened seaweed culture system.

The target of integrated pond cultivation management can still be improved if the government and the industry together with growers wish to optimize the application of mariculture technology through policy development program, such as pond area expansion, seaweed fronds, fish fry, fertilizer, and integrated milkfish cultivation intensification (Laapo & Howara 2016). There have been several studies that demonstrate the beneficial effect of shrimps cultured with other aquatic species. Shrimps have been mix-cultured with other fish, such as tilapia, several species of macroalgae and shellfish (Akiyama & Anggawati 1999; Bunting 2006; Da Silva-Copertino et al 2009).

This study tries to apply the cultivation of 4 commodities, *E. cottonii*, *Gracilaria verrucosa*, *Penaeus vannamei* and fish *Chanos chanos* in ponds. The objectives of the study were to assess the growth of *E. cottonii*, to observe several water quality parameters, and to study the relationship between *E. cottonii* and *G. verrucosa*, *P. vannamei*, and *C. chanos*.

Material and Method

Study site and experiment setting. This study was carried out in the aquaculture location of Silampayang Village, Kasimbar district, Central Sulawesi province from April to September 2016. The experiments were done in 1 ha of salt water pond, in which the doors were opened to get the seawater in and brackish water pond was added with fresh water until the water level rose to 5 cm then closed to reduce the influx of fresh water. Before culture experiment was done, the pond had been cleaned to remove unwanted juvenile fish and larvae, then SR 55 (Modern Nutricell) was used to stimulate the growth of plankton at a rate of 5 liters per hectare. The pond was left for a week, and then added with organic fertilizer (PT. PKG Indonesia) at a dose of 5 L ha^{-1} in order to grow natural food. Two weeks after the addition of organic fertilizers, water level was increased up to 70 cm deep and kept it until the stocking. The study applied a completely randomized design (CRD) with 3 treatments and 3 replications so that nine experimental units were obtained (Table 1) using 5 x 5 x 1 m net plots (Figure 1).

E. cottonii stocking was carried out in 3 different treatments, 5 kg $unit^{-1}$ (A), 4 kg $unit^{-1}$ (B), and 3 kg $unit^{-1}$ (C). Other commodities reared together with *E. cottonii* used the same weight, 3 kg $unit^{-1}$ for *G. verrucosa*, then 3.1 g of individual weight for *C. chanos*, and 2.1 g of individual weight for *P. vannamei* post larvae, respectively. The fish were obtained from a local milkfish nursery pond operator, while *P. vannamei* post-larvae (2.1 g) were obtained from a commercial shrimp hatchery in Central Sulawesi. *C. chanos* was stocked at a density of 25 ind $unit^{-1}$, while *P. vannamei* was stocked at 100 ind $unit^{-1}$ (Table 1). Both *E. cottonii* and *G. verrucosa* were placed first, while *C. chanos* and *P. vannamei* were stocked in the ponds one week after the seaweed stocking that the seaweed could produce oxygen through photosynthesis. Stocking of *C. chanos* and *P. vannamei* was done in early morning when the air temperature was relatively cool. The fish and shrimp larvae were acclimated in the pond water in which the transport bags were opened and left floating on the pond water about half an hour, then they were released gradually. It was done to let them get used to the pond water temperature and salinity.

Figure 1. Experimental units.

Table 1

Stocking density of *E. cottonii, G. verrucosa, C. chanos,* and *P. vannamei*

No	Biota	Treatment		
		A	B	C
1	*E. cottonii*	5 (kg)	4 (kg)	3 (kg)
2	*G. verrucosa*	3 (kg)	3 (kg)	3 (kg)
3	*P. vannamei* (2.1 g)	100 juveniles	100 juveniles	100 juveniles
4	*C. chanos* (3.1 g)	25 juveniles	25 juveniles	25 juveniles

In day-20 to the seaweed harvest, pond water currents were daily manipulated using a water pump for 1-2 hours to rattle the seaweed and help remove the adhered sand/dirts that could inhibit food absorption process. Initial measurements of water salinity, temperature and dissolved oxygen were done during the stocking. The culture stocks were only fed with the natural food in the ponds during the culture period.

Water sample analysis. Water samples were collected at day 10, 20, 30, 40 and 45 for physical parameters (brightness, temperature, TDS, TSS) and chemical parameters (pH, salinity, nitrate, phosphate) measurements. A secchi disk was used to measure water transparency in bodies of water, while TDS was determined following SNI 06-6989.27-2005 and TSS with SNI 06-6989.3-2004 method. Water temperature measurement used a thermometer and dissolved oxygen was measured using digital DO-meter, whereas salinity measurement used a portable refractometer. Water pH was determined with a digital pH test pen, while nitrate and phosphate were determined following standard procedures of APHA (1989).

Growth measurements. Growth was expressed as absolute growth and relative growth using growth formula of Effendie (2003):

Absolute growth (G) = Wt – Wo (1)

where: G is mean absolute growth (g), W_o is weight of seed at the beginning of the study (g), and W_t is weight of seed at the end of the study (g).

Relative growth was calculated as follows:

$$WG = [(W_1 - W_o) / W_o] \times 100\% \quad (2)$$

$$RGR = [(\ln W_1 - \ln W_o) / (t_1 - t_o)] \times 100\% \quad (3)$$

where: WG is weight gain, RGR is relative growth rate, Wo is initial weight, W_1 is final weight, t_o is initial time, and t_t is final time.

Comparison among treatment effects used ANOVA. It started with homogenity test on growth data following Anderson-Darling, Levenes test for variance equality, and Tukey's test for model additive to meet ANOVA requirements (Montgomery 1991). If there is any significant effect of the treatment on the growth of *E. cottonii*, Fisher's LSD test was applied to assess difference between the treatment effects.

Results

Weight increment of E. cottonii. Polyculture trials of *E. cottoni* + *G. verrucosa* + *P. vannamei* + *C. chanos* (Figure 2) showed very significant weight increment (%) of *E. cottonii* at day-45. Weight measurements were done every 10 days to observe the seaweed growth. At day-45, the growth increment of *E. cottonii* ranged from 512-1,142%, 11 times the initial weight (50 g). The success of *E. cottonii* farming business is largely determined by site suitability as a culture medium.

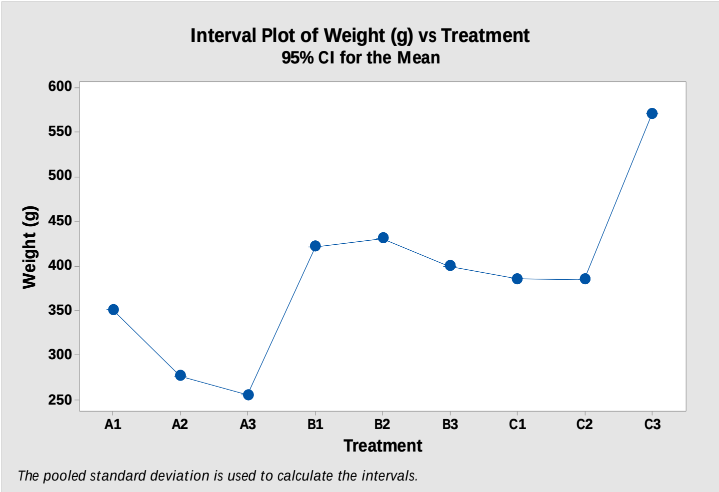

Figure 2. Weight increment (%) of *E. cottonii* at day 45.

This study found that mean absolute weight growth of *E. cottonii* was 282.35 g in treatment C, 196.58 g in treatment B, and 142.44 g in treatment A, respectively. Mean absolute weight growth of *G. verrucosa* was 4.35 g in treatment A, 4.52 g in treatment B, and 4.72 g in treatment C, respectively. Mean absolute weight increment of *C. chanos* was 32.55 g in treatment B followed by 31.79 g in treatment A, and then treatment C, 29.79 g, respectively. Mean absolute weight increment of *P. vannamei* was 22.01 g in treatment A, 23.16 g in treatment B, and 26.90 g in treatment C, respectively. Nevertheless, ANOVA showed that polyculture of *E. cottonii*, *G. verrucosa*, *C. chanos*, and *P. vannamei* only gave significant effect ($p < 0.01$) on the absolute growth of *E. cottonii.* LSD test also reflected that treatment C had highly significantly different effect on the absolute weight increment of *E. cottonii* from that of A and B ($p < 0.01$), but treatment A and B did not have significantly different effect ($p > 0.05$).

Figure 3 demonstrates the relative growth range from 3.8 to 5.41%, reflecting a very good seaweed growth, since daily weight gain of 3% is considered to be quite advantageous. Growth is also one of the biological aspects needed to consider. The size or weight of seaweed seedlings greatly affects the growth rate of the seaweed. The seedling thallus originating from the edge of the plant gives higher growth rate than that of the seed that comes from the base. Seaweed growth is categorized as somatic growth and growth physiology. Somatic growth is growth as measured by weight gain or thallus length, while the growth is seen on reproductive physiology and colloidal content.

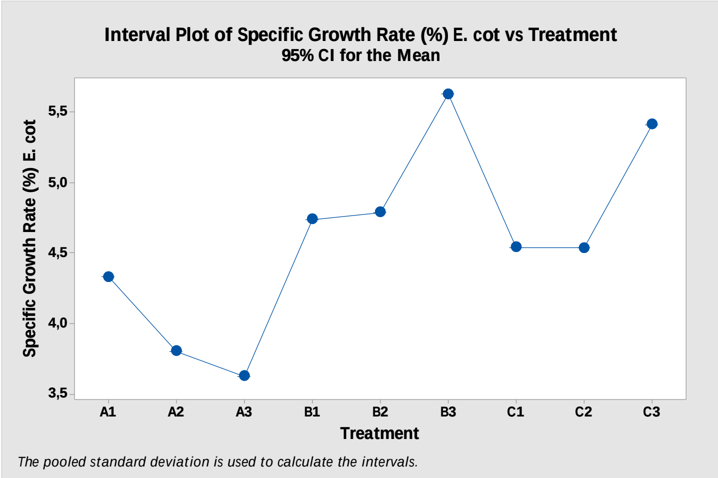

Figure 3. Relative growth rate (%).

Water quality parameters. During the culture period, the water quality parameters obtained in this study were still in the optimum range, except that nitrate and phosphate, which were lower than the optimum range. The measurement data are presented in Table 2.

Table 2

The range of water quality parameters during the study

Water quality	*10 Days*	*20 Days*	*30 Days*	*40 Days*	*45 Days*
Brightness (cm)	60	80	80	80	75
Temperature (°C)	34.4	31	30	30	31.35
TDS (ppm)	32.9	34.8	32.7	35.1	33.9
TSS (ppm)	11.7	12.6	11.1	12.2	11.9
pH	7.2	7.6	7.1	7.4	7.3
Salinity (ppt)	31	30	25	30	29
DO (ppm)	4.2	7.4	7.3	6.7	6.4
Nitrate (ppm)	0.006	0.005	0.005	0.004	0.005
Total Phosphate/P (ppm)	0.009	0.008	0.007	0.007	0.008

Discussion. Polyculture with three commodities (fish, shrimp, seaweed) has been carefully studied in Indonesia (Suharyanto et al 2010; Murachman et al 2010; Pantjara et al 2011; Mangampa & Burhanuddin 2014) and is feasible to be developed in traditional ponds as it has added value. Other study on a polyculture system used 4 commodities reared in the fish pond as well, *C. chanos*, *E. cottonii*, *G. verrucosa* and *P. monodon* and the same stocking time of shrimp and fish, one week after seaweed planting (Guido et al 2014). However, they found slow growth and high mortality of *P. monodon* at day-1 to day-40 due to very low water exchange at low tide where seawater could not reach the water inlet. During the cultivation, *E. cottonii* had also mortality at day-7 due to low pond water salinity, 13-15 ppt, causing thallus turn to white color and release mucus. The growth of *G. verrucosa* after 40 days old seemed to be not good and started getting damaged due to low water brightness, 26-30 cm, no rain, and strong wind. *C. chanos* and other fish in the pond contributed also to *G. verrucosa* growth inhibition through predation because of natural feed depletion and artificial feeding cessation. The shoots of *G. verrucosa* could hardly grow because they had been grazed by *C. chanos* and other fish. Besides, the wind-shaken muddy pond bottom was also other factor inhibiting the shoot growth of *G. verrucosa* seedling that caused mortality.

Our present study found that polyculture application on *E. cottonii* with *G. verrucosa, C. chanos*, and *P. vannamei* gave good growth of *E. cottonii.* Higher *E. cottonii* growth of treatment C may result from wider space availability to grow as a result of lower number utilization of *E. cottonii* than that of two other treatments. *G. verrucosa* had very low mean absolute growth, while growth of *C. chanos and P. vannamei* was not affected by treatment applications. It could result from nutrient competition between both seaweeds, in which *E. cottonii* seemed to be more efficient to use the nutrient in the pond, besides *G. verrucosa* has living environment in brackish water with low salinity level and *E. cottonii* lives in higher water salinity. Previous studies found that suitable water salinity for seaweed growth ranged from 31 to 35 ppt (Naryo Sadhori 1989) and 30 to 35 ppt for *Eucheuma* sp. (Soegiarto et al 1978; Dawes 1981), and 28-32 ppt (Yala 2011) meaning that the water salinity range recorded in this study (25-31 ppt) supported *E. cottonii* growth better than *G. verrucosa*. These levels were similar to those recorded in the mixed-culture of white shrimp *Penaeus merguensis* and *C. chanos* (Jaspe et al 2011).

In bottom soil processing, the water inlet was closed from freshwater input after the pond had been drained. During the culture period, only seawater has entered to maintain the stability of the salinity level. In rainy situation, the water was put out immediately since salinity decline could inhibit the growth of *E. cottonii,* and when the rain stopped seawater was immediately introduced to replace the pond water removal. Seawater intake control and the use of water pump in this study could, in fact, maintain the pond water salinity at the range of 25 to 31 ppt. This activity was continuously done during the culture period that the salinity could be optimal for *E. cottonii,* 28-32 ppt.

Water current manipulation contributes to the growth of *E. cottonii* that can only live in the sea with water currents. The water mass was flowed in the left and right sides of the culture pond under assumption that the use of water pump could replace the sea water current. This manipulation helps maintaining the water quality condition be in optimum range for *E. cottonii* growth, *C. chanos*, and *P. vannamei*.

Water brightness level ranged from 0.6 to 0.8 meters, which enabled the seaweed to photosynthesize up to day-45. This brightness level is higher than that of Guido et al (2014), and thus yields better *E. cottonii* growth. Water transparency is a condition indicating the ability of light to penetrate the water column at the certain depths. Water brightness is very important because it is closely related to photosynthetic activity. Photosynthesis needs light and when the photosynthetic activity is disrupted, the production of dissolved oxygen and chlorophyll-*a* as an indicator of waters fertility will decrease. This brightness level almost reaches the depth range of the culture pond, 0.7-1 m. Good water depth for the cultivation of *G. verrucosa* is 0.5-1.0 m at the lowest tide (fast-toned area) for freelance bottom method, 2-15 m for floating raft method, and 5-20 m for long line method and system paths. This condition is set to avoid the drought and optimize the sunlight acquisition (DMAF 1997). It means that photosynthesis could occur in nearly all water columns to stimulate the seaweed growth. Turbid water (usually containing sludge) can block the sunlight penetration into the water and cause the photosynthesis be disturbed. In addition, dirts can cover the surface of the thallus and cause it rot and broken. As a result, this condition will interfere with the seaweed growth (WWF-Indonesia 2014a).

Water temperature ranged from 30 to 34.4^{o}C. These approached to the upper limit, but still within the optimal range for *E. cottonii,* 26-32^{o}C (WWF-Indonesia 2014b). High seawater temperature could result from global warming that affects species distribution range (Takao et al 2015), including cultured seaweed, *E. cottonii.* It could result from low absorption of light penetration by dissolved substances. This circumstance results in the pond water column to absorb and release the heat more slowly. It is enough to affect the bioecological processes that are closely related to the growth of *E. cottonii*.

Total dissolved solid (TDS) usually consists of organic substances, inorganic salts and gas. TDS content of pond water ranged from 32.7 to 35.1 ppm. This range is still in optimal condition/still safe for fishery activity when referring to Water Quality Criteria based on Decree of Environmental Minister numbered 51/2004 (MLE 2004) that is < 80 ppm.

Total suspended solid (TSS) is one of the important parameters in water caused by the presence of sludge, microorganisms and fine sand all of which are < 1 μm in size. TSS can cause siltation in the body of water and stimulate growth of certain aquatic plants and be toxic to other living things (Asmadi & Suharno 2012). According to Kristanto (2013), suspended solids in water will block the entry of sunlight into the water layer. Photosynthetic process cannot take place without sunlight and can reduce the oxygen production by plants. As a result, the life of microorganisms is disturbed. TSS of pond waters is 11.1-11.9 ppm, and the value is optimal referred to DMAF (2005), TSS content < 25 ppm. Suspended solid is the venue for heterogeneous reactions and serves as a precursor for early precipitation and may inhibit the ability to produce organic substances in the water (Sastrawijaya 2000). If these cannot properly be dissolved, they will settle down on the bottom; nevertheless, before settling down on the substrate, they will float in the water and block the sunlight penetration (Wardhana 2004).

Water pH ranged from 7.1 to 7.6. Water pH has a considerable influence on the seaweed culture and water condition with a neutral or slightly alkaline pH is ideal for the growth of marine organisms. In general, low pH found in the culture ponds could result from rare calcification during the culture period (Boyd 1990). According to Arthur et al (2013), seaweed culture is most effective at neutral water pH, and thus, pH of the pond water is still in the optimum range for *E. cottonii* to grow.

Based on the measurements, dissolved oxygen ranged from 4.2 to 7.4 ppt, and these met the requirement of dissolved oxygen concentrations suitable for *E. cottonii* cultivation, 3-8 ppm, as recommended by DMAF (2008). The main source of oxygen is from the atmosphere and the photosynthesis of green plants. Regular influx of freshwater and seawater to the shallow water, stirring and mixing by wind could contribute to the availability of dissolved oxygen in the water column. Life in the water column can stand if at least 4 ppm of dissolved oxygen is available, and the rest is dependent upon the resistance of organism, the presence of contaminants and the water temperature (Sastrawijaya 2000). According to Effendie (2003), dissolved oxygen levels can fluctuate daily (diurnal) and seasonally depending on the water mass mixing and movement (turbulance), the photosynthetic activity, respiration, and number of wastes entering the water body.

Nitrate concentration ranged from 0.004 to 0.006 ppm. The optimum range of nitrate for *E cottonii* is 1-3 ppm (WWF-Indonesia 2014b). N and P concentration in the water occur in very small amount, but they are highly needed. Mean nitrate concentration in the seawater is 0.5 ppm and phosphorus concentration is even less. Phosphate concentration ranged from 0.007 to 0.009, while the optimum range is 0.01-0.021 ppm (WWF-Indonesia 2014b). Phosphate is a nutrient essential for plant cell metabolism. The presence of phosphate in the water also poses no direct adverse effects to aquatic organisms. Orthophosphate affects water fertility. In natural waters, the dissolved phosphorus is less than 0.1 ppm, and suitable concentration for seaweed cultivation ranges from 0.02 to 1 ppm (Sulistijo 1996). Both compounds can exceed the limit in the water surface (Romimohtarto & Juwana 2007). This compound can be derived from domestic wastes, plant residues, organic compounds or industrial wastes, and the availability of nitrogen in nitrate form can be derived from agricultural wastes, ammonia, human and animal feces or natural processes like lightning (Moos 1986; Ahmad 2004). Denitrification causes N not to accumulate in the sediments (Wetzel 1983).

Therefore, rearing seaweed with herbivores, omnivores, and detritivores could be an environmental friendly culture system, could use relatively little natural resources and yield very low pollution. Polyculture could save energy and feed cost, follow the environmental regulations, and prevent fish infection as well. Species could be selected based on their ecological roles and economic potential. It could also reduce high waste processing cost beyond the herbivore culture to produce income.

Conclusions. Polyculture application on *E. cottonii* with *G. verrucosa, C. chanos,* and *P. vannamei* yielded good growth of *E. cottonii,* and did not influence the growth of *P. vannamei* and *C. chanos*. However, *E. cottonii* cannot be cultivated with *G. verrucosa* due to nutrient competition for growth. Water current manipulation is important to do through periodic mixture of seawater and freshwater in order to create and maintain optimum water quality condition for the growth of the organisms on study.

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