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By Andi Rahmad Rahim

OPTIMIZATION QUALITY OF *AGAR Gracilaria verrucosa* SEAWEED WITH DIFFERENT DENSITY IN EXTENSIVE POLYCULTURE SYSTEM

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ABSTRACT

The polyculture system was used to increase the productivity of extensive brackishwater ponds to produce optimal agar with varying densities of three commodities: milkfish, *Vannamei* shrimp, and *Gracilaria verrucosa*. This study aims to obtain the optimal density of the three commodities in extensive brackishwater ponds with polyculture systems to produce the best *agar* quality for *G. verrucosa*. The research was conducted in the expanse of the Polyculture System Extensive brackishwater Pond in Lamongan Regency. The study used a Completely Randomized Design (CRD) with 3 density treatments (milkfish m^{-2} : *Vannamei* shrimp m^{-2} : *G. verrucosa* g m^{-2}) and 3 replicates: A (10 : 10 : 250), B (20 : 20 : 500), and C (30 : 30 : 1000). Statistical analysis uses one way ANOVA (Analysis of Variance), while Tukey's HSD (Honestly Significant Difference) and Path Analysis use Pearson Correlation. The results showed that the best density obtained in treatment A gave a significant difference from treatments B and C in producing Specific Growth Rate, Absolute Weight, Absolute Length, Carbon Content, and quality of *agar* rendementing the best of seaweed *G. verrucosa*. From the path analysis, CNP nutrients and the growth of *G. verrucosa* seaweed have a strong and very strong influence to improve the quality of *agar* rendementing *G. verrucosa* seaweed.

Keywords: *Agar*, Extensive, *Gracilaria verrucosa*, Density, Polyculture.

1. INTRODUCTION

Government policy through the aquaculture revitalization program places shrimp, milkfish, and seaweed as superior commodities (Directorate General of Aquaculture, 2018). One system that is expected to increase pond production and revive the production of shrimp, milkfish, and seaweed as superior commodities is polyculture. The polyculture system is a way of cultivating various fish species with different ecological niches, so as to increase the productivity of ponds that are traditionally managed. The advantages of this system are it can minimize the risk of crop failure, improve the growth of cultivated commodities, produce quality seafood products, and provide added value to fish farmers through diversification of aquaculture products (Martínez-Porchas et al., 2010) (Israel et al., 2017) (Pantjara, B., M. Mangampa., 2010).

One of the problems faced in the polyculture system is determining the density of fishery commodities that are most effective in utilizing natural feed available in ponds. To be able to utilize the natural food contained in the pond effectively, of course the combination of commodity species must be able to live together without causing competition for food or space (Kristanto, A., Pantjara, B., Insan, 2013). The right density of 3 commodities-shrimp, milkfish, and seaweed-in polyculture media is needed to produce optimal production. *Gracilaria verrucosa* seaweed utilizes the metabolism of milkfish and *Vannamei* shrimp as a source of nutrients to improve the quality of *agar* seaweed. The polyculture system is a beneficial system for seaweed because the waste and food residues from milkfish and *Vannamei* shrimp in the form of detritus are converted into nutrients through a diffusion process to accelerate the growth of seaweed (Samidjan et al., 2018).

Seaweed *G. verrucosa* is one of the agar producers that has been successfully cultivated in Indonesian ponds (Faturrahman et al., 2011). *G. verrucosa* contains *agar* with good gel strength in abundant quantities (Sornalakshm, 2017). *Agar* is a mixture of polysaccharides mainly found in the matrix and cell walls of red algae and is usually extracted from species of algae belonging to the family Gracilariaceae (Painter, 1983)(Niu et al., 2013). In everyday life, gelatin is used as a food ingredient. *Agar* is a thickening and gelling hydrocolloid that is used as a food additive and the demand for agar is increasing due to the increased consumption of processed foods (Ollando et al., 2019)(Valderrama D, Cai J, 2014). Whereas in industry, *agar* is used as an additive in food canneries, pharmaceuticals, cosmetics, paints, and textiles (Istiqomawati., Kusdarwati, 2010)(Marinho-Soriano, E., Morales, C., Moreira, 2002)(Niu et al., 2013).

Agar quality is one of the important requirements to increase its selling value. Therefore, the factors that affect the *agar* content in Gracilaria really need to be considered so that it is economically feasible (Sornalakshm, 2017). In order to achieve maximum production of seaweed, several important factors are needed, one of which is the density between seaweed, milkfish, and *Vannamei* shrimp. The right density between milkfish, *Vannamei* shrimp, and seaweed in a polyculture system will affect the growth of seaweed, where one of the efforts to improve the quality of seaweed is to increase its growth. Appropriate densities can increase business profits in polyculture systems and achieve sustainable cultivation.

One way to increase production is to adjust the density level (Isroni et al., 2020). However, information about the appropriate density in the implementation of polyculture cultivation of milkfish, *Vannamei* shrimp, and seaweed in ponds is still not widely known by the public. It is hoped that the optimization of the density of the three commodities in the polyculture system can be utilized to effectively utilize the ecological space of pond waters. So, it is necessary to determine the optimal density of the three leading commodities of milkfish, *Vannamei* shrimp, and seaweed in extensive ponds with a polyculture system to improve the quality of *agar G. verrucosa* seaweed.

2. RESEARCH METHODS

2.1. Sampel Collection

Seedlings of *Gracilaria verrucosa* (red algae) from tissue culture were obtained from the Polyculture Pond of Pulokerto Village, Kraton District, Pasuruan Regency, East Java Province, Indonesia. Seaweed seeds are 14 days old, clean, fresh, and free from other types. The selected seaweed seeds were collected as much as 100 kg, packed in alkaline conditions, and avoided from the hot sun. Seaweed seeds were transported by motor boat to the research location with a distance of 125 km. After arriving at the research site, the seedlings were adapted for 48 hours before being stocked into the Research Media.

2.2. Research Place

This research was conducted in the Polyculture Extensive brackishwater Pond of Soko Village, Glagah District, Lamongan Regency, East Java Province, Indonesia, with an area of 21.3 ha. This research was carried out for 42 days in one of the polyculture ponds with an area of 0.5 ha, by installing a culture container made of tarpaulin inside the pond.

Fig. 1. Polyculture System Extensive Pond Expanse.

2.3. Research design

This study used a Completely Randomized Design (CRD) with 3 density treatments (milkfish m^{-2} : *Vannamei* shrimp m^{-2} : *G. verrucosa* g m^{-2}) and 3 replicates, namely Treatment A (10 : 10 : 250), B (20 : 20 : 500), and C (30 : 30 : 1000) with a total of 9 treatments (Fig. 2a).

This study used 9 experimental units of 1 x 1 x 1 m tarpaulin (Fig. 2a), with a water level of 50 cm, and with the initial weight and length of *G. verrucosa* seaweed at 10 g and 8.0

– 11.5 cm (Fig. 2b). The source of brackishwater pond came from the estuary of the Solo River that was a source of brackish water with a salinity level of 10 - 20 g l^{-1}. Shrimp and milkfish seeds came from brackishwater pond in Soko Village, Glagah District, Lamongan Regency. The seeds came from the artificial spawning process and were selected based on similar size, intact body shape, and active swimming. Every 500 seeds were collected in controlled media. The initial weight and length of milkfish and *Vannamei* shrimp stocked in the research media were milkfish 45.8 - 48.8 g and 17.14 – 17.48 cm, *Vannamei* shrimp 6.6 – 10.0 g and 10.4 – 10.6 cm (Fig. 2c and 2d).

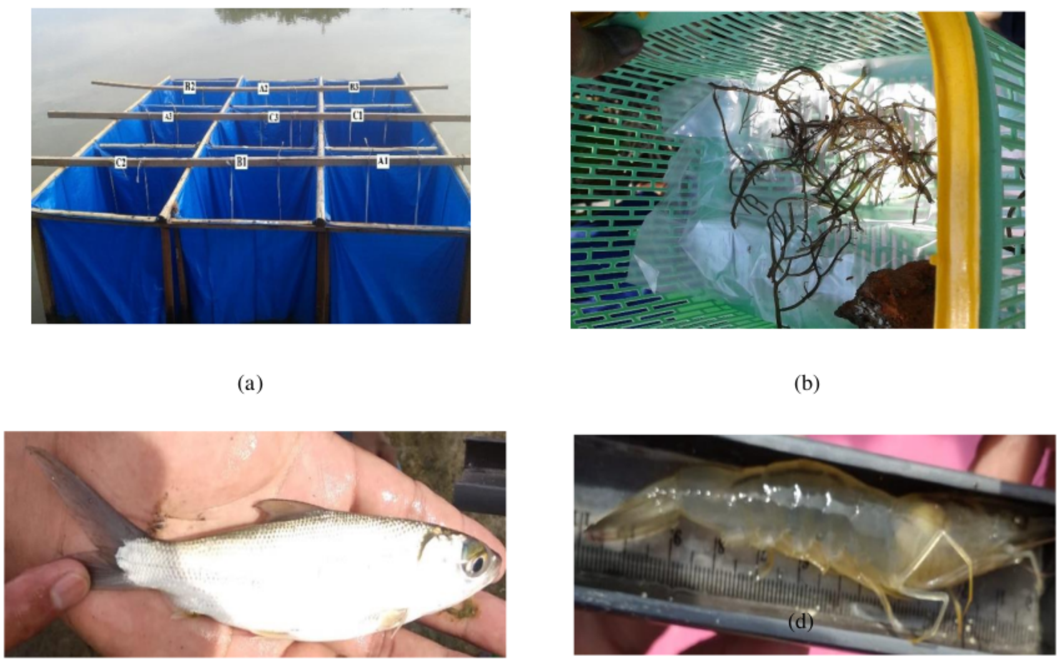


Fig. 2. (a) Research Design (b) Seaweed Seeds of *G. verrucosa* (c) Milkfish (d) *Vannamei* shrimp.

2.4. Seaweed Nutrient Analysis

The measurement of the carbon level of *G. verrucosa* seaweed was using the Gravimetric method with the determination of the ash level and water level converted to carbon level. The nitrogen content of *G. verrucosa* using the Kjeldahl method is destroyed with concentrated sulfuric acid with Zn granules as the catalyst, then collected and titrated with the help of an indicator (Wil liam Horwitz., George W. Latimer, 2006). Phosphorus content of *G.*

verrucosa using the 'UV/Vis spectrophotometry' method uses light that is passed through a container containing a solution, which will produce a spectrum (Lambert Beer's law).

2.5. Quality of Agar Seaweed Analysis

The *agar* level of the *G. verrucosa* seaweed rendement was measured using the weight of the raw material in the form of dry seaweed flour divided by the dry weight of the sample before being made into flour and expressed in a percent; the higher the rendement, the higher the output produced. Viscosity (thickness) of *G. verrucosa* was a processed *agar-agar* powder that had been heated at a temperature of 75^{o}C, then its thickness was measured by using a Brookfield viscosimeter, the unit of viscosity was in the form of centipoises (cps). The gel strength of *G. verrucosa* is the maximum load required to break the polymer matrix in the loaded area, the seaweed gel solid formed from the heating process at the 75^{o}C temperature and allowed to stand for one day until a gel solid is formed, the gel strength measurement is carried out using a Curd meter with units of g/cm^2 (Wil liam Horwitz., George W. Latimer, 2006).

2.6. Growth Analysis

Measurement of the growth of *G. verrucosa* seaweed was carried out every week for 42 days of observation using an analytical balance measuring instrument with an accuracy of 0.0 g and a measuring instrument with an accuracy of 0.0 cm.

2.6.1. Measurement of absolute weight with the formula of (Fortes, 1989)**:**

Absolute Weight (g) = Final Weight of Observation (g) – Initial Weight of Stocking (g).

2.6.2. Specific Growth Rate with the formula of (Dawes, C.J., Lluis, A.O. Trono, 1994)**:**

Specific Growth Rate (% Day^{-1}) =

$$\frac{\text{Final Weight of Observation (g)} - \text{Initial Weight of Stocking (g)}}{\text{Observation Time}} \times 100$$

2.6.3. Absolute Length Growth with the formula (Effendi, 1997)**:**

Absolute Length Increase (cm) = Final Length (cm) – Initial Length (cm).

2.7. Water Quality Analysis

The measurement of prawn farm water quality parameters is carried out by in situ and ex situ bases. In situ refers to temperature (°C) (thermometer), pH (digital pH meter), dissolved oxygen (ppm) (dissolved oxygen meter), salinity (g l^{-1}) (hand refractometer), brightness (cm) (Secchi disk). Ex situ refers to the content of Carbon, Nitrogen, and Phosphorus (ppm) (spectrophotometer with nesslerization method) (Colman, 2010).

2.8. Statistic Analysis

Data analysis of this study used one way ANOVA (Analysis of Variance) to see the significant effect between different density treatments (milkfish m^{-2} : *Vannamei* shrimp m^{-2} : *G. verrucosa* g m^{-2}) on absolute weight (g), specific growth rate (% day^{-1}), absolute length (cm), carbon, nitrogen, and phosphorus content (%), *agar* rendement quality (%), viscosity (cps), gel strength (g cm^{-2}) of *G. verrucosa* seaweed in extensive prawn farms with polyculture systems. If it gave a significant effect ($p < 0.05$), then it was proceeded with the Tukey's HSD test to see significant differences between treatments in each parameter, with a 95% confidence level. Path analysis was used to see how big the correlation between CNP nutrient content parameters and the growth of *G. verrucosa* seaweed with a polyculture system in increasing the rendement of *G. verrucosa* seaweed which is the final product of high-value *agar* products; the model was generated from Pearson analysis (Product Moment Correlation). The correlation value ranges from 0.0 to 1.0; the closer to number one, the stronger the relationship between the observed variables (Sugiyono, 2010).

3. RESULTS AND DISCUSSION

3.1. Growth of Seaweed

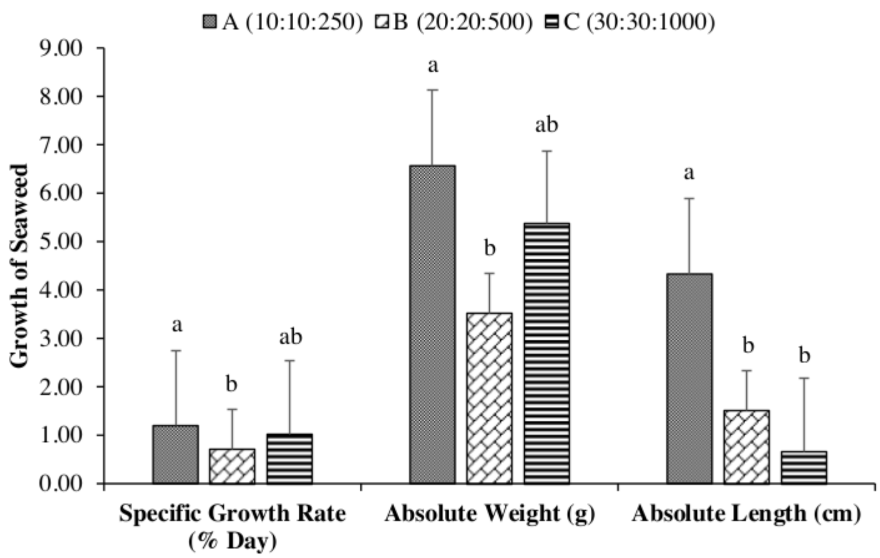


Figure 3. Growth of Seaweed (mean ± SE, n = 9) of *G. verrucosa* for different densities (milkfish m^{-2} : *Vannamei* shrimp m^{-2} : *G. verrucosa* g m^{-2}). Observation parameters were specific growth rate, absolute weight, and absolute length. Different notation/lower case letters indicate statistical significance ($P < 0.05$) between density treatments of culture.

The average growth range of *Gracilaria verrucosa* seaweed was the specific growth rate 0.71 – 1.20 % day^{-1}, absolute weight 3.51 – 6.57 g, and absolute length 0.67 – 4.33 cm. From the Analysis of Variance (ANOVA), the provision of different densities had a significant effect on increasing the specific growth rate, absolute weight, and absolute length of *G. verrucosa* seaweed ($p < 0.05$). According to (Matinfar et al., 2013), the specific growth rate ranges from 3.5 to 3.7 % day^{-1} in *Gracilaria persica*. The density factor in polyculture media also influences the growth of seaweed. Shrimp density has a significant effect on absolute weight and SGR of *Gracilaria corticate* (Fourooghifard et al., 2018). In the results of his research, the SGR range is 0.31 -1.23 % day^{-1} and the absolute weight is 14.92 – 73.67 g. The length of the thalus in *G. verrucosa* (Hudson) Papenfuss can reach 22.33 cm with an absolute weight of 65.91 g (Nana, S.S., 2008). The absolute length of *G. verrucosa* ranges from 2.5 to 3.8 cm (A.R. Rahim et al., 2016). The increase in the length of the thalus can be clearly seen from the shoots that begin to grow at the tip of the thalus, and it can reach an average length of 1.03 - 1.29 cm for 42 days (Muarif, Yala, Z.R., 2017).

3.2. Seaweed Nutrient Content

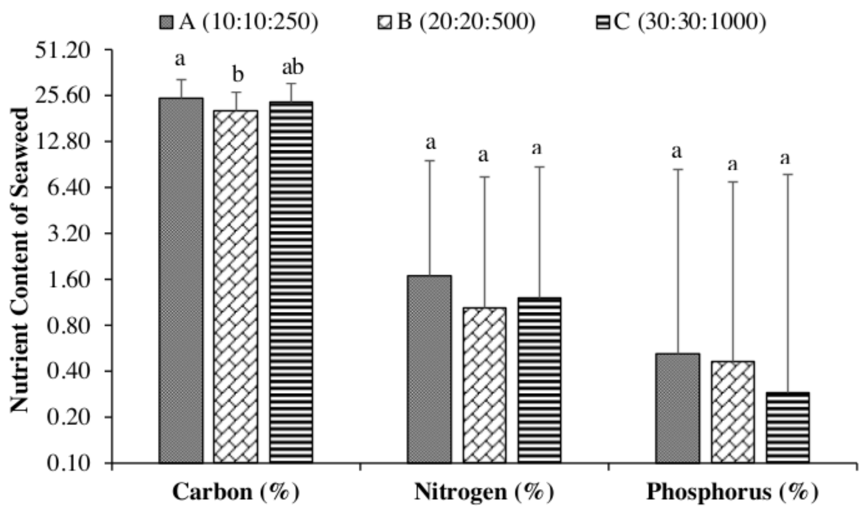


Figure 4. Nutrient Content of Seaweed (mean ± SE, n = 9) of *G. verrucosa* for different densities (milkfish m^{-2} : *Vannamei* shrimp m^{-2} : *G. verrucosa* g m^{-2}). Observation parameters were Carbon, Nitrogen, and Phosphorus. Different notation/lower case letters indicate statistical significance ($P < 0.05$) between density treatments of culture.

The average range of nutrients for *G. verrucosa* seaweed is Carbon 20.26 – 24.60 %, Nitrogen 1.04 – 1.69 %, and Phosphorus 0.29 – 0.52 %. From the Analysis of Variance (ANOVA), the provision of different densities had a significant effect on increasing the carbon content of *G. verrucosa* seaweed ($p < 0.05$). At the same time, the condition of different densities did not significantly affect the nitrogen and phosphorus content of *G. verrucosa* seaweed ($p > 0.05$). The carbon content of *G. verrucosa* is in the range of 23.53 - 29.47 % (A.R. Rahim, 2018b). The carbon content range in *G. verrucosa* cultivated on the coast is 21.38-24.57 % (Erlania et al., 2013). Carbon is the primary nutrient needed by seaweed in the photosynthesis process to produce carbohydrates which are the main components of seaweed

(Stiger-Pouvreau, V., Bourgougnon, N., Deslandes, 2016). (A.R. Rahim, 2018a), the nitrogen range in *G. verrucosa* is between 0.85 – 2.02 %. (Rosyida et al., 2014), the nitrogen content in the thalus tissue of *G. verrucosa* is 0.6 %. High nitrogen content in thalus tissue correlated with the growth of *G. verrucosa* seaweed (Bird et al., 1986)(Rosyida et al., 2014). nitrogen is utilized by seaweed to synthesize amino acids and proteins with the help of the enzyme nitrate reductase, which helps in the growth process (Klionsky et al., 2016). According to (A.R. Rahim, 2018b), the phosphorus content in *G. verrucosa* was 0.20 – 0.26 %. The phosphorus content of seaweed cultivated offshore ranges from 0.06 - 1.07 % (Yuniarsih et al., 2014). the phosphorus content of *G. verrucosa* seaweed on a laboratory scale ranged from 0.03 to 0.10 % (Mulatsih, 2015). High phosphorus levels in brackishwater ponds will support the growth of *Gracilaria* spp (Xu et al., 2008).

3.3. Quality of* Agar *Seaweed

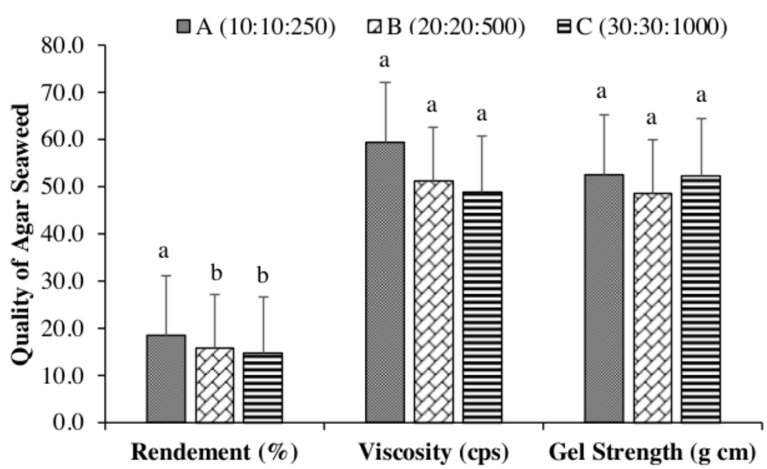


Figure 5. Quality of Agar Seaweed (mean ± SE, n = 9) of *G. verrucosa* for different densities (milkfish m^{-2} : *Vannamei* shrimp m^{-2} : *G. verrucosa* g m^{-2}). Observation parameters were rendement, Viscosity, and gel strength. Different notation/lower case letters indicate showed statistical significance ($P < 0.05$) between density treatments of culture.

The results showed an average range of agar quality for *G. verrucosa* seaweed during this study, the rendement of 14.7 – 18.5 %, Viscosity of 48.8 – 59.4 cps, and gel strength of

48.6 – 52.4 g cm^{-2}. Statistical test ANOVA (Analysis of Variance) giving different densities had a significant effect on improving the quality of agar rendement *G. verrucosa* seaweed ($p < 0.05$). In contrast, the provision of different densities did not significantly increase the quality of agar viscosity and gel strength of *G. verrucosa* seaweed ($p > 0.05$). According to (Mulyaningrum., Suwoyo, 2018), Agar rendements obtained from *G. verucossa* seaweed in brackishwater pond ranged from 10.30 – 27.84 %. The polysaccharide rendement based on the mass of Gracilaria seaweed was 17.0 % (De Castro et al., 2018). The rendement of marine cultured *G. verrucosa* was 8.1-30 % and 14.7 %, respectively (Orosco et al., 1992)(Oyieke, 1993). Agar rendement obtained from *Gracilaria* sp. cultivated in brackishwater pond ranged from 5.768 % to 17.506 % (Yulistiana et al., 2020). Agar rendement from Gracilaria produced in brackishwater pond ranged from 24.6 – 30.6 % (Rahim, A.R., 2021). In the brackishwater pond, Many nutrients are derived from the metabolic activity of polyculture organisms. It forms polysaccharides, such as agarose and agaropectin, acting as primary ingredients for creating agar (Anton, 2017). Gracilaria gel strength ranges from 50 - 300 g cm^{-2} and can reach 500 g cm^{-2} (Myco Supply, 2011). The gel strength of *G. verrucosa* from tissue culture started from 68.2 - 101.8 g cm^{-2} (Rahim, A.R et al., 2016), (Waluyo et al., 2019), the power of the Gracilaria gel in brackishwater pond was 356.76 g cm^{-2}. (Gioele et al., 2017), the gel strength of 3 Gracilaria species was 22.2 – 630 g cm^{-2}. (Rahim, A.R., 2017), the gel strength of *G. verrucosa* was 40.0 – 56.6 g cm^{-2}. This extreme difference in gel strength can be attributed to differences in location and physiological factors (Martín et al., 2013). (Rahim, A.R., 2017), the Viscosity of *G. verrucosa* is 76.67 – 90.0 cps. (Waluyo et al., 2019), the Viscosity of Gracilaria seaweed is 201.6 cps. (Wenno et al., 2012), the nutrients in the waters produced from the cultivation process, the level of nutrients affect the viscosity value of seaweed.

3.4. Water Quality

Table 1. Water Quality Parameters for different densities (Milkfish m^{-2} : *Vannamei* shrimp m^{-2} : Seaweed *G. verrucosa* g m^{-2}) during culture of *G. verrucosa* in Polyculture Brackishwater Pond.

Treatment	Water Quality Day 0							
	Temperature (ºC)	Salinity (g l^{-1})	pH	Dissolved Oxygen (ppm)	Brightness (cm)	Carbon (ppm)	Nitrogen (ppm)	Phosphorus (ppm)
A (10 : 10 : 250)	31.5	15	6.54	5.5	45	4475.32	63.76	25.21
B (20 : 20 : 500)	31.5	15	6.54	5.5	45	4017.09	70.33	21.32
C (30 : 30 : 1000)	31.5	15	6.54	5.5	45	3190.24	65.32	24.51
Treatment	Water Quality Day 42							
	Temperature (ºC)	Salinity (g l^{-1})	pH	Dissolved Oxygen (ppm)	Brightness (cm)	Carbon (ppm)	Nitrogen (ppm)	Phosphorus (ppm)
A (10 : 10 : 250)	31.6	18	6.93	4.2	43	627.43	13.89	18.30
B (20 : 20 : 500)	32.0	18	6.90	3.9	40	624.91	14.63	19.53
C (30 : 30 : 1000)	31.9	18	6.95	3.6	40	552.03	12.21	20.33

The average water quality range during the study was temperature 31.5 – 32.0ºC, salinity 15– 18 g l^{-1}, pH 6.54 – 6.95, dissolved oxygen 3.6 - 5.5 ppm, brightness 40 – 45 cm, carbon 552.03 - 4475.32 ppm, nitrogen 12.21 – 70.33 ppm, and phosphorus 18.30 – 25.21 ppm (Table 1).

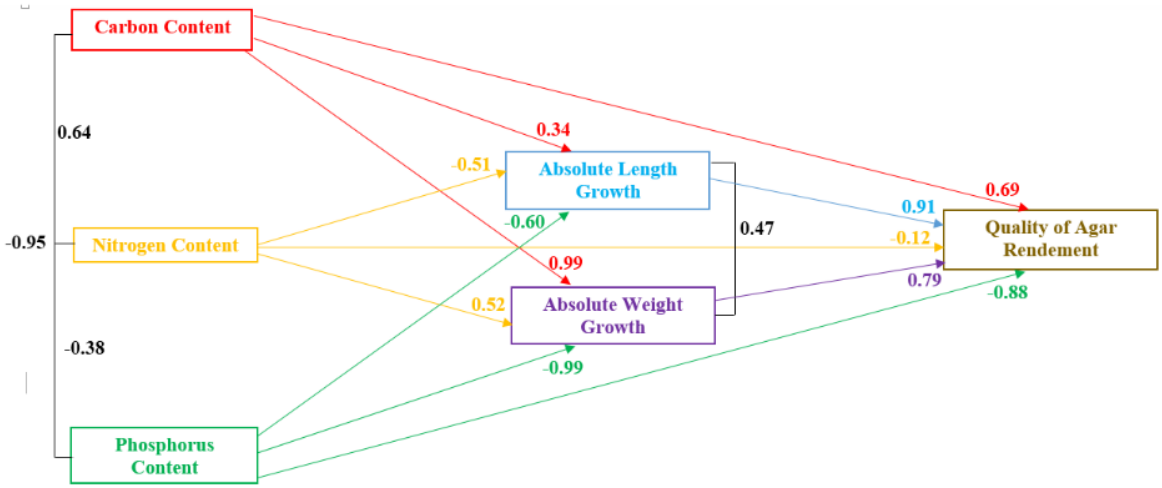
Water quality is one of the most vital factors in seaweed cultivation activities because it can affect the growth and success of seaweed cultivation (Istiqomawati, 2010; Susilowati, T., S. Rejeki, E. N. Dewi., 2012). Water quality parameters determine the development and distribution of macroalgae (Raikar et al., 2001). (Tsai et al., 2005)(Yang et al., 2015), the growth of *Gracilaria coronopifolia* is positively correlated with temperatures between 15 - 35°C and reaches its maximum production level at 30°C. A suitable temperature for the development of *Gracilaria lemaneiformis* is between 12 - 23°C. In contrast to *G. lemaneiformis*, the subtropical species *Gracilaria tenuistipitata* var. liui grows best at 20 - 30°C in brackishwater pond, but its growth rate decreases at temperatures below 15°C or above 32°C (Wu, Chaoyuan, Li, Renzhi, Lin, 1994; Yang et al., 2015). In the study of *Gracilaria fisheri*, the optimum temperature of tropical seaweed in the Caribbean was found between 25 - 30°C (Pakker et al., 1995). Most species of *Gracilaria* sp. grow well at temperatures of 20°C or above (Bird et al., 1986), (Yang et al., 2015). Water temperature controls the growth of seaweed, so it is one of the most important factors. In addition, the temperature can also affect several physiological processes in algae, such as the rate of diffusion and absorption of nutrients (Lapointe, 1984; Yang et al., 2015).

The suitable salinity range for seaweed growth is 33 - 35 g l^{-1} with an optimal 33 g l^{-1}. In the study of *Gracilaria fisheri*, the optimum salinity of seaweed in the Atlantic and Pacific oceans ranged from 15 - 30 g l^{-1} (Bird et al., 1986). (Zhou et al., 2013), studied the effect of salinity on the development and release of *Gracilaria lemaneiformis* carpospora, and found a range of 30 - 35 g l^{-1}. Furthermore, (Choi et al., 2006), the effect of salinity on the growth of *G. verrucosa* and *Gracilaria chorda*, both species grow in a wide salinity range ranging from 5 – 35 g l^{-1}, with an optimum range of 15 – 30 g l^{-1}. Bird and (Bird et al., 1986), *Gracilaria* spp. pale and die when the salinity is less than 15 g l^{-1}, whereas (Kumar et al., 2010), *Gracilaria corticata* at salinity below 15 g l^{-1} causes the thalus to become weak. (Sarkar et al., 2019), *Gracilaria tenuistipitata* cultivation in brackishwater pond, pH 8.02 – 8.05 was obtained. (Fourooghifard et al., 2018), the pH obtained in the cultivation of *Gracilaria corticata* is 7.3 - 8.7. Another study reported that a pH above 8 was optimal for Gracilaria growth (Jayasankar et al., 2006). Alkaline waters with a pH value of 7 - 9 are productive waters (Fourooghifard et al., 2018).

The optimum limit for dissolved oxygen in seaweed cultivation is >4 ppm (Ihsan et al., 2015; Widiastuti, 2011). In line with this, the *Gracilaria tenuistipitata* study in brackishwater pond obtained DO 4.62 – 6.18 ppm (Sarkar et al., 2019). DO in *Gracilaria corticata* cultured with *Vannamei* shrimp ranged from 5.1 to 6.56 ppm (Fourooghifard et al., 2018). All living organisms need to be dissolved oxygen for respiration, metabolic processes, or the exchange of substances, producing energy for growth (Yulius et al., 2019). According to (Amir, 2019), the range of brightness values in seaweed cultivation *Gracilaria* sp. in the brackishwater pond is 40 – 61 cm. The brightness in *Gracilaria* sp. ranged from 50 - 55 cm (Mapparimeng, Liswahyuni, A., Permatasari, A., Fattah, N., 2019). Brightness is a variable related to the amount of light penetration into the waters for the photosynthesis process of seaweed.

(Dickson et al., 2007) reported the range of Carbon in coastal waters is 1900 - 2090 ppm. (A.R. Rahim, 2018b), the range of seaweed carbon in brackishwater pond is 725.78 – 4711.46 ppm. The high carbon content in brackishwater pond waters provides fertilizer, feeding, and metabolic processes. (Takahashi et al., 2006), carbon content is influenced by applying fertilizers and nutrients and carbonate material that enters coastal waters through rivers. (A.R. Rahim, 2018b), the range of brackishwater pond nitrogen is 14.61 – 94.99 ppm. (Fourooghifard et al., 2018) obtained the degree of nitrogen content in seaweed *Gracilaria* sp. of 7.63 – 16.70 ppm. Nitrogen deficiency is characterized by a change in the color of the thalus in red algae to pale (Moore, 1991). The range of phosphorus content obtained in brackishwater pond is 22.02 – 24.22 ppm (A.R. Rahim, 2018a). Meanwhile, it was accepted by (Tarigan., Edward, 2003), phosphorus levels in sea waters ranged from 1.076 to 2.198 ppm. The high content of phosphorus in brackishwater pond is due to the addition of phosphorus fertilizer used to stimulate growth (Anam, 2007).

3.5. Path Analysis



Explanation:

(-) Minus : Observation Parameters Are Inversely Comparable

0.00 – 0.19 : Very Low

0.20 – 0.39 : Low

0.40 – 0.59 : medium

0.60 – 0.79 : Strong

0.80 – 1.00 : Overpowering

Figure 6. Path Analysis Quality of Agar Rendement (%) of *G. verrucosa* for Different Density (Milkfish m^{-2} : *Vannamei* Shrimp m^{-2} : Seaweed *G. verrucosa* g m^{-2}) in Polyculture Brackishwater Pond. Determining the model using correlation analysis, with a range of 0.00 – 1.00, getting closer to 1.00, strengthening the relationship between these parameters in producing a quality of agar rendement *G. verrucosa*.

From Path Analysis, the relationship between Carbon, Nitrogen, and Phosphorus content of *G. verrucosa* seaweed is an independent variable. The growth of absolute weight and absolute length of *G. verrucosa* seaweed is an intermediate variable. Agar quality of *G. verrucosa* seaweed rendement is the dependent variable. The independent and intermediary variables that affect the increase in the dependent variable are the quality of agar rendement *G. verrucosa* seaweed.

The carbon content with a strong category affects the quality of the rendement agar with a positive correlation value of 0.69, which is a 69% increase in the carbon content followed by the rise in the quality of the rendement agar. Carbon is an essential factor in improving the quality of seaweed agar, the final product of red seaweed Gracilaria sp. According to (Diniz et al., 2013), (Chakraborty., Santra, 2008), seaweed needs Carbon to produce carbohydrates in the photosynthesis process. The most abundant substance in seaweed is found in cell walls, such as agarose. Then shallow nitrogen content affects the rendement agar quality with a negative correlation value of 0.12 or 12%, an increase in nitrogen content followed by a decrease in rendement agar quality. Nitrogen [1] content can increase the growth of *G. verrucosa* but has a negative relationship with the formation of agarose (Ak et al., 2011). Phosphorus content strongly affects the rendement agar quality with a negative correlation value of 0.88, namely an 88% increase in phosphorus content followed by a decrease in rendement agar quality. (Briggs., Funge-Smith, 1993) state that phosphorus content is significant, but if the dose is excessive in water, it can inhibit growth. Seaweed growth that is less than perfect indirectly affects the gelatin content of seaweed rendement (Pong-masak et al., 2010).

The growth of absolute length very strongly affects the quality of agar rendement with a positive correlation value of 0.91 or 91%. An increase follows an increase in the whole distance in the quality of agar rendement. (Erlania et al., 2013), the morphology of seaweed *Gracilaria* sp. It has a long thalus; hence, it is more efficient in absorbing sunlight needed in photosynthesis. The process of photosynthesis will produce the final product of seaweed in the form of agarose. As the length of the *thalus* increases, the rate of photosynthesis will increase (Stewart, H.L., Carpenter, 2003). The growth of absolute weight strongly influences the quality of agar rendement; the positive correlation value is 0.79, which is 79% increase in growth of absolute weight followed by an increase in the quality of agar rendement. In the previous study by (Andika Putra Syam, Suardi, 2020), the agar content of seaweed is affected by the weight of the thalus.

The figure showed significant differences in the density of milkfish, *Vannamei* shrimp, and *G. verrucosa* seaweed in Polyculture Extensive brackishwater pond. They could produce nutrient content of Carbon, nitrogen, and phosphorus, which are used to increase absolute weight and absolute length growth in delivering the best final product from seaweed in the form of agar rendement content.

4. CONCLUSION

In conclusion, a significantly different density of milkfish, *Vannamei* shrimp, and *Gracilaria verrucosa* greatly affected the growth, nutrient content, and quality of agar *G. verrucosa* seaweed. Treatment of density A (10 : 10 : 250) was the best density that increased the nutrient content of Carbon, absolute weight, specific growth rate, absolute length, and quality of agar rendement *G. verrucosa* seaweed in brackishwater pond with a polyculture system. While the nutrient content of nitrogen, phosphorus, Viscosity, and gel strength, *G. verrucosa* seaweed did not have a significant effect. The supporting parameters in brackishwater pond water quality are Temperature, pH, DO, Salinity, Brightness, Carbon, Nitrogen, and Phosphorus. They are in the range that can meet the growth and quality of agar-rendement *G. verrucosa* seaweed in extensive brackishwater pond with polyculture systems. The density of the three commodities in an extensive brackishwater pond with the right polyculture system is needed to produce optimal growth and quality of agar seaweed.

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6. CONFLICT OF INTEREST

At the end of the text, under a subheading "conflict of interest steatments". **The authors declare no conflicts of interest.**

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