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1 **QUALITY OF AGAR *Gracilaria verrucosa* SEA WEED WITH**
2 **DIFFERENT DENSITY IN EXTENSIVE POLY CULTURE SYSTEM**

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9 **ABSTRACT**

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

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

1. INTRODUCTION

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

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

48 Seaweed *G. verrucosa* is one of the agar producers that has been successfully cultivated
49 in Indonesian ponds (Faturrahman et al., 2011). *G. verrucosa* contains *agar* with good gel
50 strength in abundant quantities (Sornalakshmi, 2017). *Agar* is a mixture of polysaccharides

51 mainly found in the matrix and cell walls of red algae and is usually extracted from species of
52 algae belonging to the family Gracilariaceae (Painter, 1983)(Niu et al., 2013). In everyday life,
53 gelatin is used as a food ingredient. *Agar* is a thickening and gelling hydrocolloid that is used
54 as a food additive and the demand for agar is increasing due to the increased consumption of
55 processed foods (Ollando et al., 2019)(Valderrama D, Cai J, 2014). Whereas in industry, *agar*
56 is used as an additive in food canneries, pharmaceuticals, cosmetics, paints, and textiles
57 (Istiqomawati., Kusdarwati, 2010)(Marinho-Soriano, E., Morales, C., Moreira, 2002)(Niu et
58 al., 2013).

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

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

75 **2. RESEARCH METHODS**

76 **2.1. Sampel Collection**

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

84 **2.2. Research Place**

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



94 Fig. 1. Polyculture System Extensive Pond Expanse.

95 **2.3. Research design**

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

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

101 – 11.5 cm (Fig. 2b). The source of brackishwater pond came from the estuary of the Solo River
102 that was a source of brackish water with a salinity level of 10 - 20 g l⁻¹. Shrimp and milkfish
103 seeds came from brackishwater pond in Soko Village, Glagah District, Lamongan Regency.
104 The seeds came from the artificial spawning process and were selected based on similar size,
105 intact body shape, and active swimming. Every 500 seeds were collected in controlled media.
106 The initial weight and length of milkfish and *Vannamei* shrimp stocked in the research media
107 were milkfish 45.8 - 48.8 g and 17.14 – 17.48 cm, *Vannamei* shrimp 6.6 – 10.0 g and 10.4 –
108 10.6 cm (Fig. 2c and 2d).



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116 (b)



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120 (d)

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

121 2.4. Seaweed Nutrient Analysis

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

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

129 **2.5. Quality of Agar Seaweed Analysis**

130 The *agar* level of the *G. verrucosa* seaweed rendement was measured using the
131 weight of the raw material in the form of dry seaweed flour divided by the dry weight of the
132 sample before being made into flour and expressed in a percent; the higher the rendement, the
133 higher the output produced. Viscosity (thickness) of *G. verrucosa* was a processed *agar-agar*
134 powder that had been heated at a temperature of 75°C, then its thickness was measured by using
135 a Brookfield viscosimeter, the unit of viscosity was in the form of centipoises (cps). The gel
136 strength of *G. verrucosa* is the maximum load required to break the polymer matrix in the
137 loaded area, the seaweed gel solid formed from the heating process at the 75°C temperature
138 and allowed to stand for one day until a gel solid is formed, the gel strength measurement is
139 carried out using a Curd meter with units of g/cm² (William Horwitz., George W. Latimer,
140 2006).

141 **2.6. Growth Analysis**

142 Measurement of the growth of *G. verrucosa* seaweed was carried out every week for
143 42 days of observation using an analytical balance measuring instrument with an accuracy of
144 0.0 g and a measuring instrument with an accuracy of 0.0 cm.

145 **2.6.1. Measurement of absolute weight with the formula of (Fortes, 1989):**

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

147 **2.6.2. Specific Growth Rate with the formula of (Dawes, C.J., Lluís, A.O. Trono, 1994):**

148 Specific Growth Rate (% Day⁻¹) =

$$149 \frac{\text{Final Weight of Observation (g)} - \text{Initial Weight of Stocking (g)} \times 100}{\text{Observation Time}}$$

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152 **2.6.3. Absolute Length Growth with the formula (Effendi, 1997):**

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

154 **2.7. Water Quality Analysis**

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

160 **2.8. Statistic Analysis**

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

174 **3. RESULTS AND DISCUSSION**

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179 **3.1. Growth of Seaweed**

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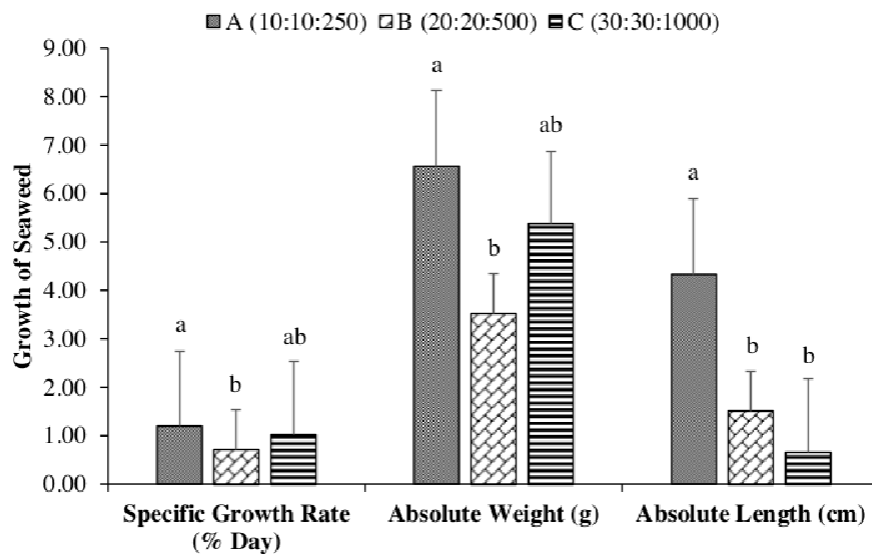
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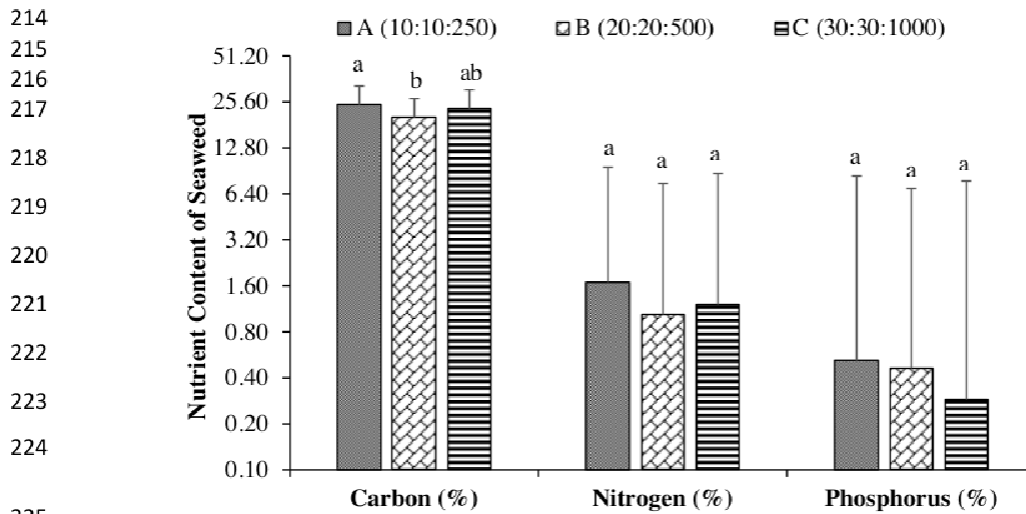
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Figure 3. Growth of Seaweed (mean \pm SE, n = 9) of *G. verrucosa* for different densities (milkfish m^{-2} : *Vannamei* shrimp m^{-2} : *G. verrucosa* $g\ m^{-2}$). Observation parameters were specific growth rate, absolute weight, and absolute length. 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* (Fourouoghifard 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.,

210 2016). The increase in the length of the thalrus can be clearly seen from the shoots that begin to
 211 grow at the tip of the thalrus, and it can reach an average length of 1.03 - 1.29 cm for 42 days
 212 (Muarif, Yala, Z.R., 2017).

213 **3.2. Seaweed Nutrient Content**

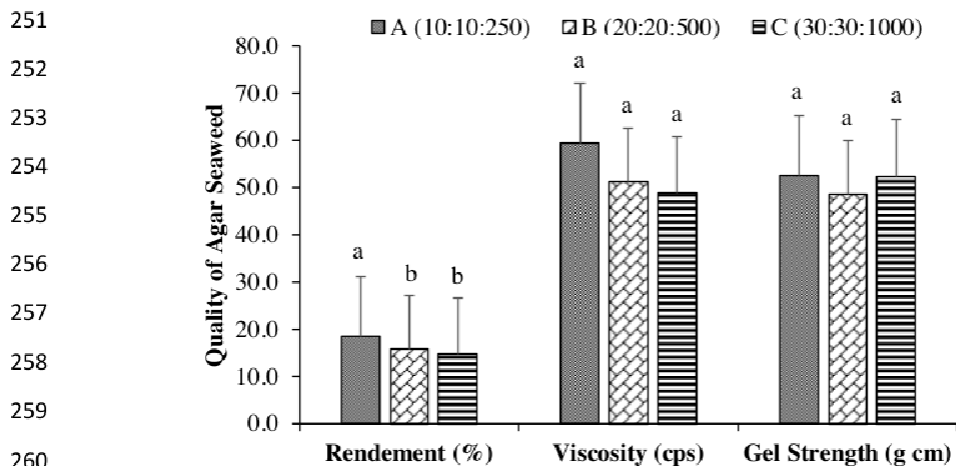


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226 Figure 4. Nutrient Content of Seaweed (mean ± SE, n = 9) of *G. verrucosa* for different densities (milkfish m⁻²:
 227 *Vannamei* shrimp m⁻²: *G. verrucosa* g m⁻²). Observation parameters were Carbon, Nitrogen, and
 228 Phosphorus. Different notation/lower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

230 The average range of nutrients for *G. verrucosa* seaweed is Carbon 20.26 – 24.60 %,
 231 Nitrogen 1.04 – 1.69 %, and Phosphorus 0.29 – 0.52 %. From the Analysis of Variance
 232 (ANOVA), the provision of different densities had a significant effect on increasing the carbon
 233 content of *G. verrucosa* seaweed (p < 0.05). At the same time, the condition of different
 234 densities did not significantly affect the nitrogen and phosphorus content of *G. verrucosa*
 235 seaweed (p > 0.05). The carbon content of *G. verrucosa* is in the range of 23.53 - 29.47 %
 236 (A.R. Rahim, 2018b). The carbon content range in *G. verrucosa* cultivated on the coast is
 237 21.38-24.57 % (Erlania et al., 2013). Carbon is the primary nutrient needed by seaweed in the
 238 photosynthesis process to produce carbohydrates which are the main components of seaweed

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

250 3.3. Quality of Agar Seaweed



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

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

267 48.6 – 52.4 g cm⁻². Statistical test ANOVA (Analysis of Variance) giving different densities
268 had a significant effect on improving the quality of agar rendement *G. verrucosa* seaweed (p <
269 0.05). In contrast, the provision of different densities did not significantly increase the quality
270 of agar viscosity and gel strength of *G. verrucosa* seaweed (p > 0.05). According to
271 (Mulyaningrum., Suwoyo, 2018), Agar rendements obtained from *G. verucossa* seaweed in
272 brackishwater pond ranged from 10.30 – 27.84 %. The polysaccharide rendement based on the
273 mass of Gracilaria seaweed was 17.0 % (De Castro et al., 2018). The rendement ¹ of marine
274 cultured *G. verrucosa* was 8.1-30 % and 14.7 %, respectively (Orosco et al., 1992)(Oyieke,
275 1993). Agar rendement obtained from *Gracilaria* sp. cultivated in brackishwater pond ranged
276 from 5.768 % to 17.506 % (Yulistiana et al., 2020). Agar rendement from Gracilaria produced
277 in brackishwater pond ranged from 24.6 – 30.6 % (Rahim, A.R., 2021). In the brackishwater
278 pond, Many nutrients are derived from the metabolic activity of polyculture organisms. It forms
279 polysaccharides, such as agarose and agarpectin, acting as primary ingredients for creating
280 agar (Anton, 2017). Gracilaria ⁶ gel strength ranges from 50 - 300 g cm⁻² and can reach 500 g
281 cm⁻² (Myco Supply, 2011). The gel strength of *G. verrucosa* from tissue culture started from
282 68.2 - 101.8 g cm⁻² (Rahim, A.R et al., 2016), (Waluyo et al., 2019), the power of the Gracilaria
283 gel in brackishwater pond was 356.76 g cm⁻². (Gioele et al., 2017), the gel strength of 3
284 Gracilaria species was 22.2 – 630 g cm⁻². (Rahim, A.R., 2017), the gel strength of *G. verrucosa*
285 was 40.0 – 56.6 g cm⁻². This extreme difference in gel strength can be attributed to differences
286 in location and physiological factors (Martín et al., 2013). (Rahim, A.R., 2017), the Viscosity
287 of *G. verrucosa* is 76.67 – 90.0 cps. (Waluyo et al., 2019), the Viscosity of Gracilaria seaweed
288 is 201.6 cps. (Wenno et al., 2012), the nutrients in the waters produced from the cultivation
289 process, the level of nutrients affect the viscosity value of seaweed.

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292 **3.4. Water Quality**

293 Table 1. Water Quality Parameters for different densities (Milkfish m² : *Vannamei* shrimp m² : Seaweed *G.*
294 *verrucosa* g m⁻²) during culture of *G. verrucosa* in Polyculture Brackishwater Pond.

Treatment	Water Quality Day 0							
	Temperature (°C)	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 (°C)	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

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

299 Water quality is one of the most vital factors in seaweed cultivation activities because
300 it can affect the growth and success of seaweed cultivation (Istiqomawati, 2010; Susilowati,
301 T., S. Rejeki, E. N. Dewi., 2012). Water quality parameters determine the development and
302 distribution of macroalgae (Raikar et al., 2001). (Tsai et al., 2005)(Yang et al., 2015), the
303 growth of *Gracilaria coronopifolia* is positively correlated with temperatures between 15 -
304 35°C and reaches its maximum production level at 30°C. A suitable temperature for the
305 development of *Gracilaria lemaneiformis* is between 12 - 23°C. In contrast to *G.*
306 *lemaneiformis*, the subtropical species *Gracilaria tenuistipitata* var. *liui* grows best at 20 - 30°C

307 in brackishwater pond, but its growth rate decreases at temperatures below 15°C or above 32°C
308 (Wu, Chaoyuan, Li, Renzhi, Lin, 1994; Yang et al., 2015). In the study of *Gracilaria fisheri*,
309 the optimum temperature of tropical seaweed in the Caribbean was found between 25 - 30°C
310 (Pakker et al., 1995). Most species of *Gracilaria* sp. grow well at temperatures of 20°C or
311 above (Bird et al., 1986), (Yang et al., 2015). Water temperature controls the growth of
312 seaweed, so it is one of the most important factors. In addition, the temperature can also affect
313 several physiological processes in algae, such as the rate of diffusion and absorption of
314 nutrients (Lapointe, 1984; Yang et al., 2015).

315 The suitable salinity range for seaweed growth is 33 - 35 g l⁻¹ with an optimal 33 g l⁻¹
316 ¹. In the study of *Gracilaria fisheri*, the optimum salinity of seaweed in the Atlantic and Pacific
317 oceans ranged from 15 - 30 g l⁻¹ (Bird et al., 1986). (Zhou et al., 2013), studied the effect of
318 salinity on the development and release of *Gracilaria lemaneiformis* carpospora, and found a
319 range of 30 - 35 g l⁻¹. Furthermore, (Choi et al., 2006), the effect of salinity on the growth of
320 *G. verrucosa* and *Gracilaria chorda*, both species grow in a wide salinity range ranging from
321 5 - 35 g l⁻¹, with an optimum range of 15 - 30 g l⁻¹. Bird and (Bird et al., 1986), *Gracilaria*
322 spp. pale and die when the salinity is less than 15 g l⁻¹, whereas (Kumar et al., 2010), *Gracilaria*
323 *corticata* at salinity below 15 g l⁻¹ causes the thalus to become weak. (Sarkar et al., 2019),
324 *Gracilaria tenuistipitata* cultivation in brackishwater pond, pH 8.02 - 8.05 was obtained.
325 (Fouroughifard et al., 2018), the pH obtained in the cultivation of *Gracilaria corticata* is 7.3 -
326 8.7. Another study reported that a pH above 8 was optimal for *Gracilaria* growth (Jayasankar
327 et al., 2006). Alkaline waters with a pH value of 7 - 9 are productive waters (Fouroughifard et
328 al., 2018).

329 The optimum limit for dissolved oxygen in seaweed cultivation is >4 ppm (Ihsan et al.,
330 2015; Widiastuti, 2011). In line with this, the *Gracilaria tenuistipitata* study in brackishwater
331 pond obtained DO 4.62 - 6.18 ppm (Sarkar et al., 2019). DO in *Gracilaria corticata* cultured

332 with *Vannamei* shrimp ranged from 5.1 to 6.56 ppm (Fourooghifard et al., 2018). All living
333 organisms need to be dissolved oxygen for respiration, metabolic processes, or the exchange
334 of substances, producing energy for growth (Yulius et al., 2019). According to (Amir, 2019),
335 the range of brightness values in seaweed cultivation *Gracilaria* sp. in the brackishwater pond
336 is 40 – 61 cm. The brightness in *Gracilaria* sp. ranged from 50 - 55 cm (Mapparineng,
337 Liswahyuni, A., Permatasari, A., Fattah, N., 2019). Brightness is a variable related to the
338 amount of light penetration into the waters for the photosynthesis process of seaweed.

339 (Dickson et al., 2007) reported the range of Carbon in coastal waters is 1900 - 2090
340 ppm. (A.R. Rahim, 2018b), the range of seaweed carbon in brackishwater pond is 725.78 –
341 4711.46 ppm. The high carbon content in brackishwater pond waters provides fertilizer,
342 feeding, and metabolic processes. (Takahashi et al., 2006), carbon content is influenced by
343 applying fertilizers and nutrients and carbonate material that enters coastal waters through
344 rivers. (A.R. Rahim, 2018b), the range of brackishwater pond nitrogen is 14.61 – 94.99 ppm.
345 (Fourooghifard et al., 2018) obtained the degree of nitrogen content in seaweed *Gracilaria* sp.
346 of 7.63 – 16.70 ppm. Nitrogen deficiency is characterized by a change in the color of the thalus
347 in red algae to pale (Moore, 1991). The range of phosphorus content obtained in brackishwater
348 pond is 22.02 – 24.22 ppm (A.R. Rahim, 2018a). Meanwhile, it was accepted by (Tarigan.,
349 Edward, 2003), phosphorus levels in sea waters ranged from 1.076 to 2.198 ppm. The high
350 content of phosphorus in brackishwater pond is due to the addition of phosphorus fertilizer
351 used to stimulate growth (Anam, 2007).

352

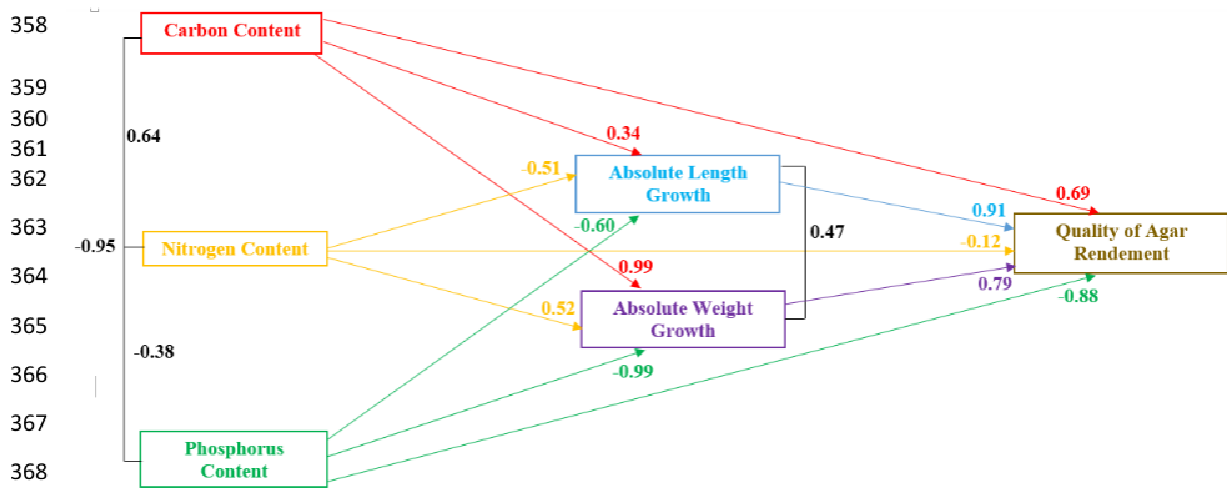
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357 **3.5. Path Analysis**



369 **Explanation:**

- 371 (-) Minus : Observation Parameters Are Inversely Comparable
- 372 0.00 – 0.19 : Very Low
- 373 0.20 – 0.39 : Low
- 374 0.40 – 0.59 : medium
- 375 0.60 – 0.79 : Strong
- 376 0.80 – 1.00 : Overpowering

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

382 From Path Analysis, the relationship between Carbon, Nitrogen, and Phosphorus
 383 content of *G. verrucosa* seaweed is an independent variable. The growth of absolute weight
 384 and absolute length of *G. verrucosa* seaweed is an intermediate variable. Agar quality of *G.*
 385 *verrucosa* seaweed rendement is the dependent variable. The independent and intermediary

386 variables that affect the increase in the dependent variable are the quality of agar rendement *G.*
387 *verrucosa* seaweed.

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

403 The growth of absolute length very strongly affects the quality of agar rendement with
404 a positive correlation value of 0.91 or 91%. An increase follows an increase in the whole
405 distance in the quality of agar rendement. (Erlania et al., 2013), the morphology of seaweed
406 *Gracilaria* sp. It has a long thalus; hence, it is more efficient in absorbing sunlight needed in
407 photosynthesis. The process of photosynthesis will produce the final product of seaweed in the
408 form of agarose. As the length of the *thalus* increases, the rate of photosynthesis will increase
409 (Stewart, H.L., Carpenter, 2003). The growth of absolute weight strongly influences the quality
410 of agar rendement; the positive correlation value is 0.79, which is 79% increase in growth of

411 absolute weight followed by an increase in the quality of agar rendement. In the previous study
412 by (Andika Putra Syam, Suardi, 2020), the agar content of seaweed is affected by the weight
413 of the thalus.

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

419 **4. CONCLUSION**

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

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438 **6. CONFLICT OF INTEREST**

439 At the end of the text, under a subheading “**conflict of interest** statements”. **The authors**
440 **declare no conflicts of interest.**

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