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Increasing The Stocking Density of Grouper Nurseries for Aquabusiness Efficiency in Recirculating Aquaculture System (RAS) with Bioremediation

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ABSTRACT

Water quality improvement was carried out by means of recirculation and bioremediation systems in grouper nurseries with high stocking density, thereby increasing production performance. The goal of this study was to evaluate the effectiveness of aquabusiness by analyzing the rise in grouper nursery stocking density using RAS and bioremediation systems. The test parameters consist of production performance, blood glucose levels, physic-chemistry-biology of water quality, and business efficiency based on productivity and business analysis. Treatment of 1,000 fish/m^3 increased survival, blood glucose levels, total bacteria, *Vibrio* spp., ammonia, nitrite, and all productivity parameters. But it lowers all growth parameters, pH, and dissolved oxygen values. The recirculation system with bioremediation at up to 1,000 fish/m^3 of stocking boosted the profit by 2.64 times. and 14.97% more efficient than 500 fish/m^3 which could not be matched by the high production performance.

1. Introduction

Grouper nurseries are a potential aquabusiness because Indonesia is a supplier of grouper seed for the Asia-Pacific area. Some grouper species have been successfully cultivated in Indonesia: tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*), coral trout grouper (*Plectropomus leopardus*), and hybrid species known in Indonesia as "kerapu cantang" (*E. fuscoguttatus* × *E. lanceolatus*) and "kerapu cantik" (*E. fuscoguttatus* × *E. microdon*). Recently, both hybrids have been popular for cultivated because they are stronger and more resistant to disease (Rimmer and Glamuzina 2017). However, seeds need to be reared at the nursery stage to supply aquaculture requirement (Dennis *et al.* 2020).

Nursery is a transition period to prepare seeds of larger sizes that are more resistant to environmental changes and are more adaptive to aquaculture treatments (Fachry *et al.* 2018). The nursery output is larger seeds that are ready to be stocked in grow-out containers with a higher selling price. Factors that affect the carrying capacity of the nursery include water quality, feed, and fish biomass. Low stocking density leads to lower production costs but a decrease in profitability due to inefficient use of space. High stocking densities can activate stress responses in fish and reduce water quality, ultimately resulting in negative changes in fish production performance (Shao *et al.* 2019).

Reusing water is done through the recirculating aquaculture system (RAS) with a filtration process so that it saves 95% of new water intake (Bregnballe 2015). RAS creates a stable environment and reduces ammonia in the water due to the nitrification process. The RAS filter acts as a place for bacteria to live and filters out solid particles in the water. RAS has been applied in nurseries of dusky grouper (Coelho *et al.* 2021), tiger grouper, and hybrid grouper (Om *et al.* 2020). According to Shao *et al.* (2019), the performance of RAS is decreasing due to the accumulation of metabolic waste that affects the growth and health of fish. Therefore, the improvement of RAS performance is done by addition of water remediation.

Water remediation is carried out by applying microorganisms (bioremediation) to fish rearing

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with RAS (Verma and Gupta 2015). As remediation agents, microorganisms such as *Bacillus* spp., *Paracoccus, Bifidobacterium, Enterococcus, Pediococcus, Saccharomyces, Lactobacillus, Streptococcus, Nitrobacter*, and *Thiobacillus* have been studied in probiotic strains. One type of commercial probiotic EM4 containing *Lactobacillus casei* and *Saccharomyces cerevisiae* can function as water bioremediation to improve water quality and stop the spread of microorganisms that are harmful (Akbar *et al.* 2013). This research analyzes the effectiveness of increasing stocking density for aquabusiness efficiency.

2. Materials and Methods

2.1. Sites for Research

In Kaliasem Village, Banjar District, Buleleng Regency, Bali, the study was carried out in a private business between February and March 2021. Analyses of ammonia, nitrite, total bacteria, and *Vibrio* spp. were performed at the Institute for Mariculture Research and Fisheries Extension, Gondol, Bali.

2.2. Application of RAS and Bioremediation at Different Stocking Densities

The recommended stocking density of grouper seeds in a tank having a total length 2.5-4.0 cm is 1,000-1,500 fish/m^3, 4-5 cm is 750-1,000 fish/m^3, 5-7 cm is 500-750 fish/m^3, and 7-9 cm is 400-500 fish/m^3 (Ismi *et al.* 2012). The research was conducted with four replications and three different stocking density treatments, namely 500 fish/m^3, 750 fish/m^3, and 1,000 fish/m^3 using a completely randomized design (CRD). Cantik grouper seeds were reared for 40 days. Average of starting measurements of 3.6±0.2 cm and 0.92±0.25 g. Three times a day, at satiation, the seeds were fed commercial pellets containing 48–55 percent protein. According to the mouth aperture, the feed comes in sizes of 1.4 mm, 1.5 mm, 2.0 mm, and 3.1 mm.

A concrete tank that was 1 m × 2 m × 0.8 m in size contained 1 m^3 of seawater, with a total of 12 units, was employed for this research. This research used seawater that filtered and stored in a reservoir, then distributed to maintenance tanks. Siphoning is carried out twice a day to remove dirt and leftover feed from the seeds, then water is added back according to the initial volume so that it requires a new water requirement of 25% of the initial volume. Cleaning of rearing tanks and filter components is carried out every ten days. The maintenance tank is equipped with a 1,600 L/hour power-head pump is used to circulate a mixture of bioball, sand, zeolite, charcoal, and artificial foam (dacron), and other materials. Bioremediation containing *Lactobacillus casei* and *Saccharomyces cerevisiae* at 6 ml/m^3 of the dosage refers to the preliminary study of Astari *et al.* (2021).

2.3. Data Collection

Fish are taken as much as 10% of the total population at the beginning (day-0) of rearing and every ten days during rearing to obtain data on body length (BL) and body weight (BW). Equation was used to compute survival rate (%): survival rate = $(N_t / N_0) \times 100$ [N_t = total amount of reared fish (fish); N_0 = beginning fish rearing numbers (fish)]. Absolute length or weight growth rate (cm/day) was calculated by the equation: absolute length or weight growth rate = $(LW_t - LW_0)/t$ [LW_t = average BL or BW at the conclusion of the rearing period (cm); LW_0 = average BL or BW at the start (cm); t = duration of rearing (days)]. Equation was used to determine a specific length or weight growth rate (%): specific length or weight growth rate = $((LW_t / LW_0)1/t - 1) \times 100$ [LW_t = end of the rearing period average BL or BW (cm); LW_0 = average BL or BW at the start (cm); t = duration of rearing (days)]. The equation was used to compute the feed conversion ratio (FCR): FCR = $F / ((B_t + B_d) - B_0)$ [F = feeding volume during rearing (g); B_t = fish biomass at the conclusion of the rearing period (g); B_d = fish mortality rate in terms of biomass (g); B_0 = beginning fish biomass (g)]. The coefficient of variance (CV) of length or weight (%) was calculated using the equation: CV = $(S / Y) \times 100$ [S = the sample's standard deviation for BL or BW at the end of the rearing period (cm or g); Y = average of sample BL or BW values at the conclusion of the rearing period (cm or g)].

Calculation of total bacteria and *Vibrio* spp. carried out according to the research of Roza *et al.* (1996), based on the number of bacteria (CFU/ml) by multiplying the calculated result and the dilution factor.

Physical-chemical measurements of water during rearing consisted of temperature, salinity, pH, and dissolved oxygen (DO) every day. In addition, ammonia and nitrite measurements were carried out every ten days (Table 1).

Blood samples were taken before feeding on day-0 20, and day-40 of rearing. A sample of cantik grouper in each tank was taken blood and tested with a blood glucose test kit (Easy Touch GCU), as modified from Eames *et al.* (2010).

2.4. Aquabusiness Efficiency

Grouper nursery business in experiments carried out for 40 days in one production cycle is assumed to have eight production cycles in one year. The survival rate, final length, amount of feed consumption,

Table 1. Physics-chemistry of nursery media for cantik grouper (*Epinephelus* sp.) as measured during reared for 60 days in concrete tank

Parameters	Unit	Measurement method	Measurement time
Temperature	°C	Thermometer	Every day
Salinity	g/L	ATC refractometer	Every day
pH	-	ATC digital pH meter	Every day
Dissolved oxygen (D.O.)	mg/L	D.O. meter TOX-90i	Every day
Ammonia (NH_3)	mg/L	Indophenol blue spectrophotometrically (SNI 2003)	Day 1 and every ten day
Nitrite (NO_2^-)	mg/L	Sulfanilamide spectrophotometrically (SNI 2003)	Day 1 and every ten day

bioremediatory requirement, and cost are based on the research results. The calculation of aquabusiness efficiency is carried out based on assumptions that are adjusted to the existing capacity at the research site. Production of 40 units with a stocking density of 500, 750, and 1,000 fish/m^3. The seeds that are kept are cantik grouper size 3.6±0.2 cm with a purchase price of IDR 1,500/fish, and a selling price of IDR 500/cm. Bioremediation required in 1 production cycle is 1,200 ml at a price of IDR 40,000 per bottle (1 L). The price of feed for a size of 1.4 mm (2 kg) is IDR 492,000, a size of 1.5 mm (5 kg) is IDR 573,000, a size of 2.0 mm (20 kg) is IDR 1,804,000, and a size of 3.1 mm (20 kg) is IDR 1,510,000.

Assumptions are calculated based on electricity and water needed during the research. The price of kwh of electricity is IDR 1,209/kwh. Discharge of seawater and freshwater pumps in each treatment of 10 m^3/hour requires electricity of 1.84 kwh. The total need for seawater in each treatment per production cycle reaches 600 m^3 consisting of 40 m^3 of initial water filling, 400 m^3 of 25% water change daily, and 160 m^3 of tank cleaning. The total electricity requirement for each treatment per production cycle is 2,027.55 kwh consisting of a seawater pump of 110.40 kwh, a blower of 710.40 kwh, lamps of 165.20 kwh, and a freshwater pump of 81.55 kwh. And a recirculation pump of 960.00 kwh.

Business efficiency calculations are carried out based on productivity (Pristianingrum 2017). Calculation of productivity is an approach to the ratio of output per input that produces three measures of productivity (Sarjono 2001). Single factor productivity was listed in Table 2. Multi-factor productivity was calculated by the equation: multi-factor productivity = (total output (IDR) - (material input (IDR) + energy input (IDR))) / (labor input (IDR) + capital input (IDR)). Total productivity was calculated by the equation: total productivity = total output (IDR) / (labor input (IDR) + material input (IDR) + energy input (IDR) + capital input (IDR).

Table 2. Productivity variable of single factor of cantik grouper nursery business

Single factor	Description
Labor	Output to labor input ratio
Material	Output to material input ratio
Energy	Output to energy input ratio
Capital	Output to capital input ratio

The cost parameters in the business analysis consist of investment costs (IDR) and total costs (IDR). Total costs include fixed costs (IDR) and variable costs (IDR) (Assegaf 2019). The calculation of business efficiency is based on a business analysis. Profit (IDR) was calculated by the equation: profit = total business revenue (IDR) - total cost of production (IDR). Break-even point analysis (fish) was calculated by the equation: break-even point = fixed cost (IDR) / (selling price (IDR) – (variable cost (IDR) / amount of production (fish))). The cost of goods manufactured (IDR/fish) was calculated by the equation: cost of goods manufactured = total cost (IDR) / total production (fish). The equation was used to determine the revenue and cost ratio (R/C ratio): revenue and cost ratio = IDR for total revenue / total cost. Analysis of payback period (year) was calculated by the equation: payback period = investment cost (IDR) / profit (IDR).

2.5. Data Analysis

Analysis of variance (ANOVA) and the Duncan test (= 0.05) were used to compare and check the water for *Vibrio* spp., total bacteria, blood glucose levels, and production performance. The table displayed business efficiency and water quality criteria.

3. Results

The results of the production performance of cantik grouper that were reared for 40 days with different stocking densities are listed in Table 3. Different stocking density treatments significantly affected survival rate (P<0.05). For all treatments, the feed conversion ratio (FCR) results did not differ substantially (P>0.05). The treatment of 1,000 fish/m^3 resulted in the best survival rate and FCR values of 94.7±1.1% and 0.62±0.03.

At days-0 through 40, the total amount of bacteria and *Vibrio* spp. in the water were significantly

Table 3. Production performance of cantik grouper with recirculation and bioremediation systems with different stocking densities after 40 days of rearing

Parameters	Stocking densities (fish/m^{3})*		
	500	750	1,000
Survival rate (%)	89.5±1.2^{b}	93.6±1.4^{a}	94.7±1.1^{a}
Production (fish)	447±6^{c}	702±11^{b}	947±11^{a}
Absolute growth rate for length (cm/day)	0.131±0.005^{a}	0.117±0.005^{b}	0.114±0.007^{b}
Absolute growth rate for weight (g/day)	0.257±0.019^{a}	0.199±0.016^{b}	0.203±0.013^{b}
Specific length growth rate (%)	2.28±0.09^{a}	2.13±0.05^{ab}	2.08±0.09^{b}
Specific weight growth rate (%)	6.33±0.27^{a}	5.88±0.22^{b}	5.91±0.14^{b}
Final length (cm)	8.8±0.2^{a}	8.2±0.3^{b}	8.1±0.5^{b}
Ratio of feed conversion	0.63±0.04^{a}	0.70±0.06^{a}	0.62±0.03^{a}
Amount of feed consumption (g):size 1.4 mm	378.01±18.78^{a}	428.26±3.67^{ab}	444.00±54.28^{b}
size 1.5 mm	716.65±66.79^{a}	950.36±163.60^{a}	1,282.94±138.33^{b}
size 2.0 mm	671.94±91.35^{a}	931.39±79.84^{b}	1,155.52±52.52^{c}
size 3.1 mm	1,178.93±110.17^{a}	1,600.17±118.71^{b}	1,920.49±24.48^{c}
Coefficient of variance of length (%)	5.56±1.07^{a}	6.98±1.10^{a}	6.41±0.84^{a}
Coefficient of variance of weight (%)	17.66±3.26^{a}	19.41±2.67^{a}	16.76±2.92^{a}

*A substantial difference is indicated by a superscript letter following the mean value (or standard deviation) in the same rows ($p<0.05$)

($P<0.05$) impacted by varied stocking densities where cantik grouper were being reared (Table 4.). The maximum ranges of total bacteria and *Vibrio* species were 11,000 CFU/ml to 301,000 CFU/ml and 3,800 to 6,320 CFU/ml in 1,000 fish/m^{3}.

Temperature, salinity, pH, and DO in different stocking density were close to optimal values based on SNI (2014) shown in Table 5. Treatment of 500 fish/m^{3} were able to reach the lowest ammonia and nitrite 1.9098 and 1.7634 mg/L.

The blood glucose levels of cantik grouper for different stocking density ranged from 34 to 73 mg/dl (Figure 1). The range of grouper blood glucose levels tends to increase with the increase in stocking density.

The calculations of the need for sea water and electricity to produce one cantik grouper were based on the business assumptions adjusted to the existing capacity at the research site (Table 6). Significantly ($P<0.05$) different stocking densities affect the requirement for seawater and electricity to produce one cantik grouper. The lowest seawater and electricity requirement was found in the 1,000 fish/m^{3} treatment of 15.8 ± 0.19 L and 0.05±0.0006 kwh to produce one cantik grouper in one production cycle.

The results of the business efficiency of cantik grouper nursery with different stocking density treatments based on productivity are shown in Table 7. The calculations were based on the business assumptions adjusted to the existing capacity at the research site. Significantly different stocking densities ($P<0.05$) on all productivity parameters except material productivity. Treatments of 1,000 fish/m^{3} were able to reach the highest productivity.

The results of the calculation of business efficiency for the nursery of cantik grouper fish in different stocking density treatments based on business analysis are listed in Table 8. The lowest unit break event point shown in 1,000 fish/m^{3} of 36,082 fish. Different stocking densities significantly ($P<0.05$) on the R/C ratio and payback period of the cantik grouper nursery business. The highest R/C ratio value shown in 1,000 fish/m^{3} at 1.57. In other words, every IDR 1.00 spent on expenses will result in IDR 1.57 in revenue, making the company using this strategy profitable. The lowest payback period shown in 1,000 fish/m^{3} of 1.07 years, meaning that the investment costs incurred can be returned in 1 year 25 days or about nine times the production cycle.

Table 4. Total bacteria and *Vibrio* spp. in different stocking density for 40 days of rearing

Stocking density (fish/m^3)	Total bacteria*			Total *Vibrio* spp.*		
	Day-0	Day-20	Day-40	Day-0	Day-20	Day-40
500	17,000^c	90,000^a	159,000^a	3,860^b	2,600^a	3,020^a
750	8,200^a	108,000^b	210,000^b	2,563^a	3,880^b	3,150^a
1,000	11,000^b	110,000^b	301,000^c	3,800^b	6,320^c	3,850^b

*A substantial difference is indicated by a superscript letter following the mean value (or standard deviation) in the same rows ($p<0.05$)

Table 5. The range of physical-chemical parameter values of water for cantik grouper rearing media with different stocking densities for 40 days of rearing

Parameter	Stocking density (fish/m^3)			Optimal value	Source
	500	750	1,000		
Temperature (°C)	27.8-30.3	27.8-30.5	27.8-30.4	28-32	(SNI 2014)
Salinity (g/L)	26-34	26-34	26-34	28-33	
pH	7.0-7.7	6.9-7.8	6.8-7.8	7.5-8.5	
DO (mg/L)	5.38-7.03	5.08-6.88	4.30-6.54	>4	
NH$_3$ (mg/L)	0.4467-1.9097	0.6823-3.3125	0.9764-3.4684	<0.1	(Suwoyo 2011)
NO$_2^-$ (mg/L)	0.1675-1.7634	0.3318-2.0500	0.5465-3.4435	<1	(SNI 2014)

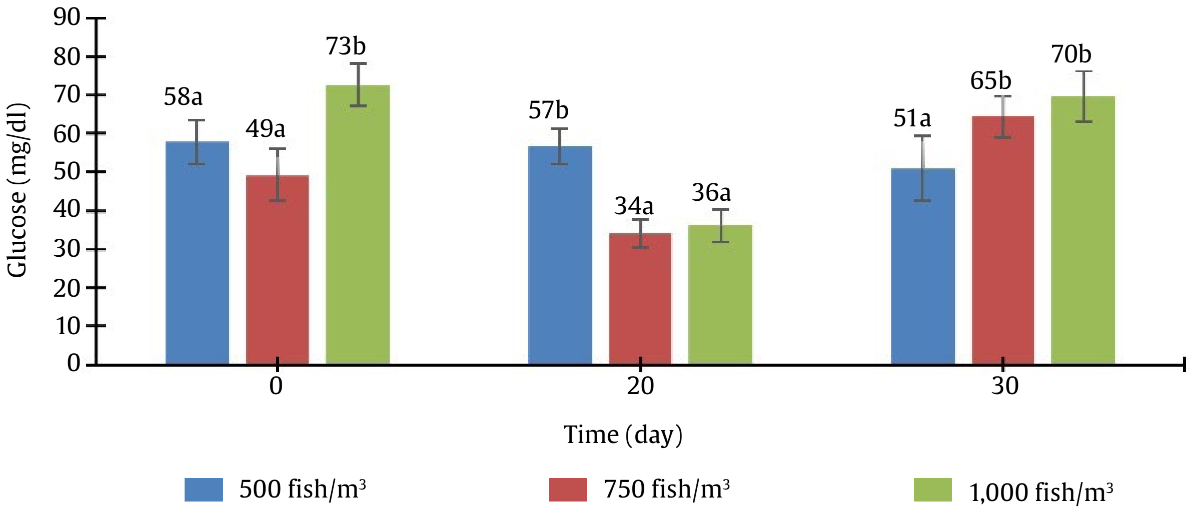

Figure 1. Cantik grouper blood glucose levels after 40 days of rearing in recirculation and bioremediation systems with different stocking densities

Table 6. The need for sea water and electricity to produce one cantik grouper for 40 days of rearing

Parameter	Stocking density (fish/m^3)		
	500	750	1,000
Sea water needs (L)/fish	33.5±0.44^c	21.4±0.34^b	15.8±0.19^a
Electricity needs (kwh)/fish	0.11±0.0015^c	0.07±0.0011^b	0.05±0.0006^a

*A substantial difference is indicated by a superscript letter following the mean value (or standard deviation) in the same rows ($p<0.05$)

Table 7. Productivity in different stocking densities for 40 days of cantik grouper rearing

Parameter	Stocking density (fish/m^3)		
	500	750	1,000
Single factor productivity:			
a. Labor	9.24±0.19^{a}	13.56±0.50^{b}	17.99±0.82^{c}
b. Material	1.71±0.04^{a}	1.74±0.07^{a}	1.78±0.07^{a}
c. Energy	30.53±0.64^{a}	44.81±1.66^{b}	59.44±2.71^{c}
d. Capital	1.35±0.03^{a}	1.98±0.07^{b}	2.62±0.12^{c}
Multy factor productivity	0.45±0.02^{a}	0.70±0.07^{b}	0.96±0.09^{c}
Total productivity	0.68±0.01^{a}	0.85±0.03^{b}	0.98±0.04^{c}

*A substantial difference is indicated by a superscript letter following the mean value (or standard deviation) in the same rows ($p<0.05$)

Table 8. Productivity of cantik grouper nurseries in each treatment with different stocking densities for 40 days of rearing

Parameter	Stocking density (fish/m^3)		
	500	750	1,000
Investment cost (IDR)	444,465,000	444,465,000	444,465,000
Fixed cost (IDR)	63,391,500	63,391,500	63,391,500
Variable cost (IDR)	375,434,962	528,884,777	679,667,697
Total cost (IDR)	438,826,462	592,276,277	743,059,197
Production (fish)	143,120	224,560	303,040
Total revenue (IDR)	598,654,667	878,810,667	1,165,708,000
Profit (IDR)	159,828,205	286,534,390	422,648,803
Break event point unit (IDR)	46,146	38,577	36,082
Cost of goods manufactured (IDR/fish)	3,067	2,638	2,452
Revenue/cost ratio	1.36^{b}	1.48^{a}	1.57^{a}
Payback period (year)	2.80^{c}	1.58^{b}	1.07^{a}

4. Discussion

The aquabusiness efficiency of cantik grouper nursery is closely related to the goal of fish nurseries, namely maximizing business profits through increasing production performance (Tajerin *et al.* 2011). The survival rate (SR) is the main parameter in production performance. Sales in the nursery business are carried out by calculating the number of fish produced. Nursery activities are said to succeed if they have a high SR value. SR of cantik grouper is strongly influenced by cannibalism due to disparity in fish size, lack of food, light intensity, and frequency of encounters between fish (Yang *et al.* 2015).

Regular grading operations to guarantee that each tank is held the same size of fish to suppress cannibals, management of feed to control hunger, and optimum stocking densities of fish size are the methods used to reduce cannibalism in grouper (Ismi *et al.* 2012). The number of fish mortality due to cannibalism at different stocking densities was not significantly different. To improve the sale of the cantik groupers, it is still possible to increase the stocking density by up to 1,000 fish/m^3. In addition to a large number of cantik groupers, fish growth also affects sales.

Sales in the nursery business of cantik grouper are carried out based on the length of cm/fish. The price of cantik grouper seeds is around IDR 500-700/cm. Better fish growth will produce in larger sizes, so the selling price will also increase (Ismi 2019). Because of the increased struggle for space brought on by higher fish stocking densities, which stress grouper development. This can cause slow growth, disturbed space for movement, and competition in taking feed. An increase in stocking density can be done to a certain degree. These restrictions change depending on the species, age, and size of the fish as well as the system or method of fish rearing (Alit 2010).

Cantik grouper growth and survival rates are affected by feeding management. Inadequate feeding will reduce growth, especially at lengths below 4 cm, increasing cannibalism (Alit and Setiawati 2018). The cannibalism nature of grouper can be lost with adequate artificial feeding (Alit and Setiawati 2016). Adequate artificial feeding during research resulted in nursery groupers up to 1,000 fish/m^3 with recirculation and bioremediation systems had no significant effect on FCR values. According to Alit (2010), groupers are reef fish that like to swarm and are always actively looking for food, so an increase in stocking density can be done up to 1,000 fish/m^3. Based on this, the increase in feed cost is also in line with the increase in the production of cantik grouper that will be produced.

Variations in the size of cantik grouper produced uniformly will reduce the competition for feed and fish dominance in obtaining space, feed, and oxygen. The lower the coefficient of variance, the greater the level of fish uniformity (Budiardi *et al.* 2008). The coefficient of variation of length or weight in the differential density between treatments did not differ significantly ($P>0.05$) from one group to the next. The coefficient of variance of length or weight values in all treatments was below 25%, so the size obtained was still considered uniform (Mattjik and Sumertajaya 2013). Therefore, the coefficient of variance value is below 25%, indicating no size disparity. Size disparities can increase competition for fish in containers because relatively large fish are easier to get food, and small fish are less competitive (Harianto *et al.* 2014). An increase in stocking density of up to 1,000 fish/m^3 does not cause size disparities in cantik grouper as long as water quality is maintained and feed is available in sufficient quantities.

According to Mahardika *et al.* (2020), Viral nerve necrosis (VNN) is frequently transmitted in grouper nurseries as a result of increased total bacteria and *Vibrio* spp. *Vibrio* spp. and the overall bacteria were impacted by the stocking density treatment. The low to moderate category made up the majority of the microorganisms collected during stocking density. *Vibrio* spp. obtained in treatments of 500 fish/m^3 were 2,600 to 3,860 CFU/ml, 750 fish/m^3 were 2,563 to 3,880 CFU/ml, and 1,000 fish/m^3 were 3,800 to 6,320 CFU/ml. *Vibrio* spp. obtained during the stocking density experiment was included in the medium level.

Temperature and salinity are a physical factor that affects the growth of fish. According to De *et al.* (2016), higher rearing media temperature, fish metabolic rate will also increase so that fish appetite increases. Increased appetite can further increase growth and survival rates (Harianto *et al.* 2014). Effects of fish metabolism that occur, such as triggering respiratory movements, high levels of oxygen consumption, and high excretion, result in fish requiring more energy. The different stocking densities in the given treatment did not affect the temperature and salinity during maintenance (Hendriansyah *et al.* 2018).

Water chemical factors include pH and dissolved oxygen (DO) that can affect fish metabolism and growth. pH value >8 affects the ammonia content in the water and change in values >0.5 in one day affects the fish's appetite. The denser the stocking, the lower the pH value. DO plays a role in the oxidation of produces energy for fish life and waste materials. The recirculation system requires sufficient DO for the nitrification process (Lu *et al.* 2020). The DO value will likewise drop as stocking density rises.

Ammonia and nitrite are parameters that affect growth because they reduce oxygen consumption due to damage to the gills, use more energy due to stress, and interfere with oxygen binding in the blood, which can ultimately lead to death. Ammonia can be converted into nitrite by bacteria through nitrification (Lu *et al.* 2020). The increasing stocking density will increase the value of ammonia and nitrite. Ammonia and nitrite values that exceed optimal values can trigger stress in cantik groupers. The blood glucose levels of cantik grouper were significantly affected ($P<0.05$) by an increase in stocking density of up to 1,000 fish/m^3. Increased blood glucose levels tend to occur due to increased stocking density. The range of blood glucose levels obtained in each treatment was 500 fish/m^3 of 51 to 58 mg/dl, 750 fish/m^3 of 34 to 65 mg/dl, 1,000 fish/m^3 of 36 to 73 mg/dl. Normal blood glucose levels in grouper ranged from 28.8 to 34.2 (Porchas *et al.* 2009). The hypothalamus is stimulated to release corticotropin-releasing factor in response to the stress response in the agricultural environment (CRF). Adrenocorticotropin hormone is released by the anterior pituitary gland in response to CRF (ACTH). ACTH will stimulate internal cells (adrenal medulla) to produce cortisol and catecholamine hormones such as epinephrine. These hormones stimulate the process of glycogen deposition in the liver and muscles, thereby increasing blood glucose levels (Samsisko *et al.* 2014).

The production obtained is close to the maximum potential from applying the best technology in a similar ecosystem. It can be said that the fish farming carried out has managed the cultivation business with business efficiency (Badiola *et al.* 2018). The total seawater requirement at each different stocking density per production cycle reaches 600 m^3 consisting of 40 m^3 of initial water filling, water changes from siphoning (25% of the initial volume) every day to about 400 m^3, and 160 m^3 of cleaning rearing tanks. An increase in stocking density of up to 1,000 fish/m^3 in a recirculation system with bioremediation reduced the need for seawater to 15.8±0.19 L to produce one cantik grouper in one production cycle. The total electricity requirement for each treatment at different stocking densities per production cycle is 2,027 kwh consisting of a seawater pump of 110 kwh, a blower of 710 kwh, a lamp of 165 kwh, and a freshwater pump of 81 kwh, and a recirculation pump of 960 kwh. The increase in stocking density of up to 1,000 fish/m^3 in the recirculation system with bioremediation was also able to reduce the electricity demand to 0.0535±0.0006 kwh to produce one cantik grouper in one production cycle.

Cantik grouper aquabusiness can be more efficient because the amount of output is increased followed by a decrease in the share of input costs to total production costs (Tajerin *et al.* 2011). An increase in the input and the resulting output is related to productivity. According to Sarjono (2001), the increase in productivity can be divided into four group calculations. With fewer resources, the first group still produced the same amount. The resources of the second group are less. More productive outcomes are achieved. With the same resources, more production results can be attained in the third category. The fourth group has larger energy resources and produces a lot more. An increase in stocking density of up to 1,000 fish/m^3 implies productivity in the fourth calculation group, i.e., greater resources lead to significantly higher output results. The maximum yield obtained from applying the best technology in a similar ecosystem can be said that the fish farmer has managed his cultivation business with high efficiency (Tajerin and Noor 2005).

According to Tajerin *et al.* (2011), factors that affect grouper aquaculture inputs consist of seed prices, feed prices, and labor prices. Investment costs and fixed costs showed the same results in each treatment. Variable costs increase as the stocking density of cantik groupers increases. The increase in variable costs occurred due to the addition of the number of seeds and the amount of feed needed during the maintenance of cantik grouper. Costs as a whole rise as variable costs do as well. The increase in total costs was accompanied by an increase in output in the form of total grouper production.

In conclusion, based on the different density experiments, the recirculation and bioremediation system can be carried out with an increase in stocking density of up to 1,000 fish/m^3 to maximize the profits obtained so that there is an increase in the aquabusiness efficiency of cantik grouper nursery. Stocking density of 1,000 fish/m^3 were 14.97% more efficient and profit were 2.64 times higher than 500 fish/m^3. However, there is an increase in stress seen in blood glucose levels and a decrease in growth parameters and water quality.

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