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# Production and Business Performance Enhancement of the Elver Eel (Anguilla bicolor bicolor) Nursery with Different Stocking Densities in a Recirculating Aquaculture System

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#### ABSTRACT

This study aimed to evaluate the effect of nursery for elver eels (Anguilla bicolor bicolor) with different stocking densities in a recirculation aquaculture system on production and business performance. The nursery was conducted in the eel cultivation business unit located in Bogor Regency, West Java, Indonesia. The nursery used the non-equivalent control group design method. Treatments consisted of nurseries with different stocking densities, consisting of one control group (4kg/m<sup>3</sup>) and two treatment groups (5 and 6kg/ m<sup>3</sup>). Each group was repeated four times. The eel (A. bicolor bicolor) tested weighed 9,65±0,80g/ eel. The nursery was carried out for 60 days of maintenance, moreover it used 12 units of a circular fiberglass tank (0,5m<sup>3</sup> of water) with a recirculation system. Commercial feed (50% protein) in the form of a paste was given twice a day at a feeding rate of 2,0-2,8%/day. The final average weight of eels in the 5kg/ m3 treatment (19,71±0,13 g) was greater than in the 4kg/  $m^3$  (18,75±1,04g) and 6kg/  $m^3$  (16,37±1,66g). The best productivity (10,14±0,08g/ L), absolute growth rate (0,17±0,00g/ day), specific growth rate  $(1,19\pm0,01\%/\text{ day})$ , and biomass absolute growth rate  $(42,83\pm0,67g/day)$  were obtained in the 5kg/m<sup>3</sup> treatment. The coefficient of weight variation of the 4 and 5kg/ m<sup>3</sup> treatments was significantly different from the 6kg/m3 treatment. Survival rates and feed conversion ratios did not differ significantly between treatments. The best business performance was obtained in the 5kg/  $m^3$  treatment (payback period = 5.25 years and R/C ratio = 1,25). The best production and business performance enhancement of elver eel (A. bicolor bicolor) nurseries with different stocking densities in a recirculating aquaculture system were obtained in the 5kg /m<sup>3</sup> treatment.

# **INTRODUCTION**

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Eel (*Anguilla* sp.) is a freshwater aquaculture commodity with an economic value adding to being a source of high-quality protein (**Shiraishi & Crook, 2015; Sakurai & Shibusawa, 2021**). Eel trading on the world market reached a value of 503,1 million USD in 2022 (**Tridge, 2024**). The amino acid components and important nutrients contained in eel are needed by the human body to support biological systems (**Gómez-Limia** *et al.*, **2019**). Eel cultivation production is not yet sustainable, because it still relies on the availability of seeds caught from nature (**Emmanuelle** *et al.*, **2020**). The scarcity

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of seeds that occurs in several eel species in the world is caused by overfishing and environmental destruction (Nilsson & Stage, 2017; Aprahamian *et al.*, 2021). The world's potential eel seed resources are in Europe and Asia. The European continent has the *Anguilla Anguilla* species which is distributed along the coastlines of France, Portugal, Spain, England, and other European coasts (FAO, 2024a). Meanwhile, the Asian continent has the *Anguilla japonica* species which lives in China, Japan, South Korea, and Taiwan (FAO, 2024b). Not only *A. japonica*, but the Asian continent has *Anguilla bicolor bicolor* which is widely distributed almost along the coastline of Indonesia (Pike *et al.*, 2020a). *A. Anguilla* and *A. japonica* are commercially valuable eel species that are included in the Critically Endangered and Endangered categories according to the IUCN Red List (Pike *et al.*, 2020b; Pike *et al.*, 2020c). These opportunities need to be optimized so that *A. bicolor bicolor* can become the next prime eel species.

Nursery is a transitional period to produce seeds that are larger and adaptive to fluctuations in the cultivation environment (Fachry *et al.*, 2018). Seeds from nature need to be cultivated and their productivity increased to meet seed demands in the grow-out segment (Dennis *et al.*, 2020). Intensification of aquaculture through increasing stocking density is one solution to increase productivity (Diatin *et al.*, 2015). One of the ways to intensify is by increasing stocking density. Stocking density in aquaculture will affect growth, survival, feed efficiency, and productivity of cultivated commodities (Magondu *et al.*, 2013). Fish cultivation with high stocking densities will encounter critical standing crop (CSC), which is characterized by a decrease in growth rate. Growth will stop completely when it reaches the carrying capacity (CC) point (Hepher & Pruginin, 1981). This condition causes stress to the fish, so the fish can easily get diseased and subsequently dies (Huang *et al.*, 2020).

The issue in sustainable aquaculture is the availability of water and land which competes with increasing population (Feucht & Zander, 2015; Suprianto *et al.*, 2021). One thing that drives the achievement of high productivity is water quality (Diatin *et al.*, 2018) as well as land and water efficiency (Diatin *et al.*, 2020). Water availability is an important factor in supporting sustainable fish farming (Suprianto *et al.*, 2021). According to Davidson *et al.* (2016), a recirculating aquaculture system (RAS) is a solution to the issue of limited water and land. The recirculation system is a closed production system that efficiently controls cultivation water quality (Mongirdas *et al.*, 2017; Fudge *et al.*, 2023), water and energy consumption (Almeida *et al.*, 2020), and commercial-scale land use (Ahmed & Turchini, 2021). The principle of a recirculation system is water management with constant circulation through drainage systems, filtration (physical, chemical, and biological), recirculation pumps, disinfection, and aeration and/or oxygenation (Timmons & Losordo, 1994; Taufik *et al.*, 2023). The recirculation system can produce 1 ton of fish by consuming only 1m<sup>3</sup> of water (Bregnballe, 2015). Increasing aquaculture productivity using a recirculation system is

an efficient production strategy to maximize profits and overcome issues in sustainable aquaculture (Suantika *et al.*, 2018).

Increasing stocking density with a mass-scale recirculation system applied to the hybrid grouper (Epinephelus fuscoguttatus × Epinephelus lanceolatus) (Shao et al., **2019**), Oreochromis niloticus (Manduca et al., 2021), and E. fuscoguttatus  $\times$  E. Microdon (Astari et al., 2023) has been proven to be able to increase productivity and manage the quality of cultivation water. Similar research was also conducted by Tan et al. (2018) which showed that the growth of adult eels A. marmorata was limited when reared at high stocking densities. Meanwhile, nurseries with different stocking densities do not affect the growth of elver A. japonica (Amano et al., 2021). According to Budiardi et al. (2023), increased production performance and best efforts of A. bicolor *bicolor* fingerling stage nurseries with different stocking densities (4, 5, and  $6 \text{ kg/ m}^3$ ) were produced by the highest stocking density treatment. The nursery of elver A. bicolor bicolor implemented by one of the eel farmers in Bogor Regency currently uses a stocking density of 4kg/m<sup>3</sup>. This opens up further research opportunities to improve production and business performance in the nursery of elver A. bicolor bicolor. Research on the optimal stocking density for nursery elver-stage eels in mass-scale recirculation systems is an interesting problem to solve. Moreover, from an economic perspective, optimal stocking density will have a direct impact on the sustainability of aquaculture businesses. This study aimed to evaluate the effect of nursery for the elver eel (Anguilla *bicolor bicolor*) with different stocking densities in a recirculation aquaculture system on production and business performance.

# MATERIALS AND METHODS

# 1. Nursery system

The nursery was carried out in the eel cultivation business unit located in Bogor Regency, West Java, Indonesia. The nursery used a quasi-experimental method, namely non-equivalent control group design which consists of one control group and two treatment groups. Treatment was in the form of a nursery with different stocking densities. The control group was a nursery with a stocking density of  $4\text{kg/m}^3$ , while the two treatment groups were nurseries with a stocking density of 5 and  $6\text{kg/m}^3$ . Each group was repeated four times (12 experimental units). The eel (*A. bicolor bicolor*) tested weighed 9,65±0,80g/ eel. Nursery was carried out for 60 days of maintenance.

**Table 1.** Non-equivalent control group design of elver eel (*A. bicolor bicolor*) nursery with different stocking densities for 60 days culture in a recirculating aquaculture system

uquu	carcare system			
Group	Stocking density	Repitition	Test fish	Nursery period
Control	$4 \text{ kg/m}^3$	4 times	Anguilla bicolor bicolor	
Treatment	5 kg/m <sup>3</sup>	4 times	stage elver	60 days
	6 kg/m <sup>3</sup>	4 times	(9,65±0,80 g/eel)	

Eels were given commercial feed in the form of pasta with a protein content of 50% using a restricted (feeding rate) method. The feeding rate was given as much as 2%/day at the beginning of the nursery period. As weight and appetite increased during nursery, the feeding rate was increased to 2.8%/day. Feeding was done by placing the feed on the feeding tray, then watching for  $\pm 20$  minutes or until the feed runs out. The remaining feed was lifted from the surface of the water to be weighed and recorded. Feed was given twice a day, namely at 08:00 a.m. and 4:00 p.m.

The nursery used three recirculating aquaculture systems, each system consisting of four experimental units and one filtration system unit (Fig. 1). The experimental unit was a circular fiberglass tank with a diameter of 1,3m filled with  $0,5m^3$  of water. Water circulation in the recirculation system was carried out by a submersible pump with Qmax  $6m^3$ / h which was placed in the filtration system. The filtration system consists of physical filters (synthetic cotton and Japanese mattresses), chemical filters (zeolite stones and ginger coral), and biological filter media (biofoam and bioball). The filtration system was equipped with a sand filter tube containing zeolite stones. The aeration installation was supported by a 230-watt air pump which was connected to a pipe to the aerotube in each experimental unit. As many as 60 tropical almond leaves hang on each experimental unit. The filtration system was cleaned every 30 days.





Fig. 1. The recirculating aquaculture system (RAS): A. Experimental design scheme, B. One unit of RAS for elver eel (*A. bicolor bicolor*) nursery with different stocking densities for 60 days of culture

The circular fiberglass tank and recirculation system were disinfected with chlorine at a dose of 10ppm seven days before stocking the test eels. Before the experiment was carried out, eels were adapted to a circular tarpaulin tank measuring 4m in diameter (water volume 7,5m<sup>3</sup>) with a recirculation system with a stocking density of 4kg/m<sup>3</sup>. The eels were fasted for 24 hours before being transferred to the experimental unit. During nursery, probiotics containing *Nitrosomonas* sp. and *Nitrobacter* sp. were given to nursery media at a dose of 20 ppm per day.

### 2. Water quality parameters

The water quality analyzed includes temperature, pH, dissolved oxygen (DO), alkalinity, ammonia, nitrite, and nitrate. Temperature and pH were measured twice a day (08:00 a.m. and 4:00 p.m.) using HANNA Instruments HI98107-pHep<sup>®</sup> (Hanna Instruments Indotama Inc. – Jakarta, Indonesia). Dissolved oxygen was measured using a Lutron DO–5510<sup>®</sup> (Lutron Electronic Enterprise Co., Ltd. – Taiwan) twice a day (08:00 a.m. and 4:00 p.m.). Alkalinity was measured using the titrimetric method, while ammonia, nitrite, and nitrate were measured using an OPTIMA SP-300<sup>®</sup> Spectrophotometer (Optima Inc. – Tokyo, Japan) every 15 days.

#### 3. Production Performance

Biometric data in the form of fish weight and length were measured every 15 days with a sample size of 30 fish per experimental unit (**Jacome** *et al.*, **2012**). Fish sampling was carried out randomly using a scoop net. Biometric measurements used digital scales (HWH DJ-1002C<sup>®</sup> - Japan) with an accuracy of 0,01g and a ruler with an accuracy of 0,1cm. Fish biomass from each experimental unit was weighed on days 0 and 60 using a CAMRY ACS-JC36<sup>®</sup> (Camry Industries Company Ltd. – Kowloon, Hong Kong).

Production performance parameters observed during nursery were calculated using a formula which includes productivity (g/L) = Bt / L [Bt = biomass at the end of the nursery period (kg); L = volume of tank used (L)], survival rate (%) = (Nt / N0) × 100 [Nt = number of eel at the end of the nursery period (eels); N0 = number of eel at the beginning of the nursery period (eels)], absolute growth rate (g/day) = (Wt – W0) / t [Wt = average body weight at the end of nursery (g); W0 = average body weight at the start of nursery (g); t = nursery period (days)], specific growth rate (%/day) = ((ln Wt - ln Wo) / t) × 100 [Wt = average body weight at the end of nursery (g); W0 = average body weight at the start of nursery (g); t = nursery period (days)], biomass absolute growth rate (g/day) = (Bt – B0) / t [Bt = average biomass at the end of nursery (g); B0 = average biomass at the start of nursery (g); t = nursery period (days)], feed conversion ratio = (Pa / (Bt – (Bm + B0))) [Pa = amount of feed given (g); Bt = eel biomass on day t (g); Bm = eel mortality biomass (g); B0 = eel biomass on day 0 (g)], and coefficient of weight variation (%) = (S / Y) × 100 [S = standard deviation (g); Y = sample mean (g)].

### 4. Business performance

Business performance was calculated through business analysis using assumptions based on the results of trial production performance (Table 4). Business analysis is intended for calculations within one year. Business analysis was calculated using the following formulas: total costs (USD) = fixed costs + variable costs, revenue (USD) = production amount × selling price, profit (USD) = revenue – total costs, break-even point (USD) = (fixed costs/(1 – (variable costs/revenue per year))), cost of production (USD/kg) = total costs / production amount, payback period (year) = investment costs / profits, and r/c ratio = revenues / total costs.

#### 5. Statistic analysis

Production performance and business performance parameter data were calculated using Microsoft Excel 365. Production performance parameter data were then processed using SPSS version 26.0 through analysis of variance (One-way ANOVA). The significantly different data were subjected to the Duncan test at a 95% confidence level. OriginPro 2024 was used to present data in graphical form. Water quality parameters were analyzed descriptively through tabular presentation.

### RESULTS

#### 1. Production performance

The production performance of the research results is presented in Table (2). Stocking density treatment influences the production performance of elver eel. Diversity of values was obtained for each parameter and test treatment.

Doromotor	Stocking density treatment (kg/m <sup>3</sup> )			
r di dificici	4	5	6	
Productivity (g/L)	7,70±0,47 <sup>a</sup>	$10,14\pm0,08^{b}$	$10,04{\pm}1,08^{b}$	
SR (%)	99,15±0,83 <sup>a</sup>	99,32±0,58 <sup>a</sup>	$98,47\pm0,89^{a}$	
AGR (g/day)	$0,15\pm0,02^{b}$	$0,17\pm0,00^{b}$	0,11±0,03 <sup>a</sup>	
SGR (%/day)	$1,10{\pm}0,09^{b}$	1,19±0,01 <sup>b</sup>	$0,88\pm0,18^{a}$	
BmAGR (g/day)	$30,83\pm3,93^{a}$	42,83±0,67 <sup>b</sup>	33,63±9,01 <sup>a</sup>	
FCR	$1,72\pm0,18^{a}$	1,59±0,02 <sup>a</sup>	2,05±0,64 <sup>a</sup>	
CWV (%)	31,6±3,11 <sup>b</sup>	$30,02\pm3,50^{b}$	25,40±1,14 <sup>a</sup>	

**Table 2.** Production performance of elver eel (A. bicolor bicolor) nursery with different stocking densities during 60 days of culture in a recirculating aquaculture system

Values in the form of numbers followed by different superscript letters on the same row show significantly different results at the 95% test level (Duncan test); SR = survival rate, AGR = absolute growth rate, SGR = specific growth rate, BmAGR = biomass absolute growth rate, FCR = feed coversion ratio, CWV = coefficient of weight variation.

Productivity values showed significantly different results based on statistical tests (P<0,05), stocking density treatments of 5 and 6kg/ m<sup>3</sup> produced the best productivity (Table 2). Survival rates and feed conversion ratios were not significantly different for all treatments (P>0,05). The parameters of absolute growth rate, specific growth rate, biomass absolute growth rate, and coefficient of weight variation were significantly different (P<0,05) between treatments. The 5kg/ m<sup>3</sup> stocking density treatment obtained the highest values for absolute growth rate, specific growth rate, and biomass absolute growth rate compared to other stocking density treatments. The best coefficient of weight variation was obtained by the stocking density treatment of 6kg/ m<sup>3</sup>.

The highest decrease in survival rate during 60 days of culture occurred in the 6kg/m<sup>3</sup> treatment (Fig. 2). The survival rate of eels in the 5kg/m<sup>3</sup> treatment tended to be more stable from the 15<sup>th</sup> to the 60<sup>th</sup> day. High mortality in the 4kg/m<sup>3</sup> treatment began to be observed on the 30<sup>th</sup> to the 45<sup>th</sup> day. The highest survival rate at the end of the culture period was achieved by 5kg/m<sup>3</sup> stocking density treatment, followed by 4 and 6kg/m<sup>3</sup> treatment.

The average body weight of eels in all stocking density treatments increased during 60 days of culture (Fig. 3). The initial average body weight of eels for all treatments on day 0 was  $9,65\pm0,80$ g/ eel. The largest average body weight at the end of the research period was obtained by eel cultured in the 5kg/m<sup>3</sup> treatment, which was  $19,71\pm0,13$ g, followed by 4kg/m<sup>3</sup> treatment worth  $18,75\pm1,04$ g; the 6kg/m<sup>3</sup> treatment was worth  $16,37\pm1,66$ g.



**Fig. 2.** Survival rate of elver eel (*A. bicolor bicolor*) nursery with different stocking densities during 60 days of culture in a recirculating aquaculture system



**Fig. 3.** Average body weight of elver eel (*A. bicolor bicolor*) nursery with different stocking densities during 60 days of culture in a recirculating aquaculture system



**Fig. 4.** Biomass of elver eel (*A. bicolor bicolor*) nursery with different stocking densities during 60 days of culture in a recirculating aquaculture system

The three treatments had different biomass on the initial day of culture; the highest biomass was  $2,86\pm0,09$ kg (6 kg/m<sup>3</sup>), followed by  $2,45\pm0,16$ kg (5 kg/m<sup>3</sup>) and  $2,13\pm0,18$ kg (4 kg/m<sup>3</sup>) (Fig. 4). The eel biomass in the 5kg/m<sup>3</sup> treatment began to outperform the 6 and 4kg/m<sup>3</sup> treatments on the 15<sup>th</sup> day. The highest biomass on the 60<sup>th</sup> day of the research was obtained by eels which were cultured in the stocking density treatment of 5kg/m<sup>3</sup>, valued at  $6,58\pm1,17$ kg, followed by 6kg/m<sup>3</sup> worth  $5,40\pm0,79$ kg and 4kg/m<sup>3</sup> worth  $4,87\pm0,41$ kg.

Donomatan	Stocking density treatment (kg/m <sup>3</sup> )			Optimal value
Farameter	4	5	6	Optimal value
Temperature	25 27 7	240.282	24 0 20 2	28–33
(°C)	23-27,7	24,9–28,2	24,8–28,2	(Luo et al. 2013)
ъЦ	5671	5575	5178	6,5–9,0
рп	3,0-7,4	5,5-7,5	3,4-7,8	(Boyd 2015)
Dissolved	2264	2165	2064	>5,0
oxygen (mg/L)	2,5-0,4	2,1-0,5	2,0-0,4	(Boyd 2015)
Hardness	26 222	10 226	56 220	75–150
(mg/L)	30-232	40-230	30-220	(Bhatnagar dan Devi 2019)
Alkalinity	A . C.A	4–36	4-84	50-200
(mg/L)	4-04			(Boyd 2015)
Ammonia	0 0 125	0,002–0,127	0,002–0,164	<0,05
(mg/L)	0-0,133			(Boyd 2015)

**Table 3.** Water quality parameters of elver eel (A. bicolor bicolor) nursery with different stocking densities during 60 days of culture in a recirculating aquaculture system

1004		Rahman e	t al., 2025	
Nitrite	0.24.0.76	0 12 0 60	0.12.0.60	<0,66
(mg/L)	0,24-0,76	0,13-0,09	0,13-0,09	(Boyd 2015)
(mg/L)	0,31–1,10	0,31–1,12	0,33–1,14	(Bhatnagar dan Devi 2019)

The water quality values are presented in Table (3). The data obtained for each parameter shows that the quality of the culture water is still within the tolerance range for the survival and growth of eel fish. This is proven by the high survival rate of eels in all treatments (Table 2).

### 2. Business performance

Business performance calculated through business analysis is presented in Table (5). Assumption components are used in analyzing eel nursery businesses with different stocking densities in recirculation systems (Table 4). Stocking density treatment produces different business performance in eel nurseries.

Component	Stocking density treatment (kg/m <sup>3</sup> )			
Component	4	5	6	
Number of fiberglass tank (unit)	12	12	12	
Stocking size (g/eel)	9,65	9,65	9,65	
Harvest size (g/eel)	18,80	19,70	16,40	
Stocking biomass (kg/cycle)	24,00	30,00	36,00	
Harvest biomass (kg/cycle)	46,20	60,48	60,21	
Survival rate (%)	99,15	99,32	98,47	
Feed conversion ratio	1,72	1,59	2,05	
Amount of feed required (kg/cycle)	50,00	60,00	70,00	
Nursery period (month/cycle)	2	2	2	
Number of cycle/year (cycle/year)	6	6	6	

**Table 4.** Assumptions used in the business analysis of elver eel (A. bicolor bicolor) nursery with different stocking densities in a recirculating aquaculture system

The assumption of increasing production is uniform for all treatments. The number of production facilities for each treatment was increased to 12 units from 4 units of circular fiberglass tank in the experiment. The results of production performance, the amount of feed required, and the nursery period were adjusted according to research that has been carried out.

 Table 5. Business analysis of elver eel (A. bicolor bicolor) nursery with different stocking densities in a recirculating aquaculture system

Daramatar	Stocking density treatment (kg/m <sup>3</sup> )			
T arameter	4	5	6	
Investments cost (USD)	19.572	19.572	19.572	

Fixed cost (USD)	2.036	2.036	2.036
Variable cost (USD)	10.961	13.157	15.338
Total cost (USD)	12.997	15.193	17.374
Production (kg)	277,20	365,04	361,26
Price/kg (USD)	51,83	51,83	51,83
Revenue (USD)	14.367	18.920	18.724
Profit (USD)	1.370	3.727	1.350
Break-even point (USD)	8.587	6.684	11.256
Break-even point (kg)	165,68	128,95	217,17
Cost of production (USD)	46,89	41,62	48,09
Payback period (year)	14,28	5,25	14,49
R/C ratio	1,11	1,25	1,08

Note: 1 USD = IDR16.400 (Bank Central Asia, 3 June 2024, 01:02 a.m. UTC)

The eel production business obtained the highest profit during six nursery cycles through rearing with a stocking density of  $5\text{kg/m}^3$  (Table 5). The lowest break-even point for cost and production was obtained by treatment of  $5\text{kg/m}^3$ . The cost of production for the  $5\text{kg/m}^3$  treatment is the lowest compared to other treatments. The payback period for the eel nursery business is shorter through maintenance at a stocking density of  $5\text{kg/m}^3$ . The R/C ratio of the  $5\text{kg/m}^3$  treatment effort was the highest.

### DISCUSSION

Intensification of cultivation by increasing stocking density affects production and business performance in the recirculation system. Increasing stocking density does not always have a positive impact on all aquaculture production performance parameters (**Refaey** *et al.*, **2018**; **Long** *et al.*, **2019**). Increasing stocking density in nurseries can maximize harvest biomass, however at certain stocking density values, fish growth performance tends to be inefficient (**Manduca** *et al.*, **2020**). Factors that limit fish growth performance at high stocking densities include oxygen consumption levels, rearing water quality, fish stress response, and space for nursery containers (**Ardiansyah & Fotedar**, **2016**). The intensity of competition for optimal growth is increasingly intense for each eel. The implication is that cultivation with increased stocking density has the potential to result in slower individual growth (**Moniruzzaman** *et al.*, **2015; Mordenti** *et al.*, **2016**).

The biological productivity of eel can be identified based on the amount of biomass produced by a population that lives in a particular location (**Rasmussen & Pedersen**, **2023**). Harvest biomass is determined by individual weight, number of individuals, and survival rate of fish in a cultivation container. In this research, the fact was obtained that it was not only the highest stocking density ( $6kg/m^3$ ) that obtained the best productivity value. The best productivity was obtained in nurseries with stocking densities of  $5kg/m^3$ 

and  $6\text{kg/m}^3$ , even the  $5\text{kg/m}^3$  treatment produced the highest biomass at the end of the nursery period, to  $6,58\pm1,17$ kg. This result is not similar to research by **Budiardi** *et al.* (2023) which obtained the highest harvest biomass in the highest stocking density treatment ( $6\text{kg/m}^3$ ). This difference is caused by the longer nursery period in this research, namely 60 days. The carrying capacity of the environment decreases as the cultivation period progresses. Moreover, in cultivation with high stocking density, the decline in environmental carrying capacity occurs more quickly, thus affecting cultivation productivity.

Survival rate is one of the main benchmarks for increasing stocking density (Xiaolong *et al.*, 2017). Cultivating eels with high stocking densities cannot be separated from the risk of mortality which threatens the performance of aquaculture production (Mordenti *et al.*, 2014). Mortality in intensive care is caused by complex factors. Increasing stocking density has an impact on competition between individuals to obtain oxygen, space, and food for growth (Lima *et al.*, 2018). The three stocking density treatments achieved a survival rate of >97% (Table 1), while cultivation of the elver eel *A. marmorata* in Vietnam only achieved a survival rate of around 60% (Thuc & Van, 2021). This proves that the recirculating aquaculture system can increase the survival rate of elver eels, even at high stocking densities.

The highest accumulation of eel mortalities from each treatment occurred during the last 14 days of the culture period (Fig. 2). Mortality that occurs during nursery is caused by a decrease in the environmental carrying capacity. According to **Rasmussen** *et al.* (2024), stocking density is one of the causes of annual natural mortality in eels in their habitat. The filtration system reached a saturation point in managing fecal waste and feed residue, which was characterized by high ammonia values (>0.05mg/ L) (Table 2). The depletion of oxygen solubility and the gradual escalation of ammonia compounds in the cultivation media have an impact on decreased fish appetite, stress, and mortality (Wang *et al.*, 2019). Fish populations that experience massive growth in limited space are vulnerable to disruption in physiological processes, health, growth rate, and even survival (Wedemeyer, 1996).

Production performance is simplified as growth that experiences additional weight and volume in a unit of time (**Hartnoll, 1982**). Stocking density treatment affects the growth rate of eel. Variations in the growth rate of eel are relatively high compared to other cultivated commodities (**Hirt-Chabbert** *et al.*, **2014**). Each experimental unit for each treatment (4, 5, and 6kg/ m<sup>3</sup>) contained 207, 259, and 311 eels. The number of individual eels influences the intensity of competition between individuals to obtain food, oxygen, and space to move (**Budiardi** *et al.*, **2022**). The low growth rate in nurseries with high stocking densities is also caused by the environmental carrying capacity decreasing more quickly (**Zaki** *et al.*, **2020**).

Nursery with a stocking density of 6kg/ m<sup>3</sup> limits the growth rate of eels because the number of individual eels is too high. The absolute growth rate and specific growth

rate in treatment 4kg/ m<sup>3</sup> (0,15±0,02 g/day; and 1,10±0,09 %/day) and 5kg/ m<sup>3</sup> (0,17±0,00 g/day; and 1,19±0,01 %/day) had better performance than the 6kg/ m<sup>3</sup> treatment (0,11±0,03 g/day; and 0,88±0,18 %/day). Meanwhile, the specific growth rate of *A. bicolor bicolor*, which was cultured in round HDPE (high-density polyethylene) tanks with a recirculation system with a stocking density of  $5,04\pm0,01$ kg/ m<sup>3</sup>, was 0,50±0,02%/day (**Taufiq-Spj** *et al.,* **2020**). This shows that the results in this study are about two times higher. This proves that the carrying capacity of the recirculation system in this study can support more optimal growth of eels up to a stocking density of 5kg/ m<sup>3</sup>. In addition, the use of feed with higher protein (50%) in this study was a factor in the optimal growth rate.

The biomass absolute growth rate of the eel nursery was more optimal at a stocking density of  $5\text{kg/m}^3$  (Table 2) so the harvest biomass produced in the  $5\text{kg/m}^3$  treatment was higher than in the 4 and  $6\text{kg/m}^3$  treatments. Similar to the results of research on the production of glass eels *A. Anguilla* in seminatural ponds by **Pedersen** *et al.* (2023), the lowest and highest stocking densities produced low biomass, while the highest biomass was obtained at the median stocking density. Maximum biomass production at a stocking density of  $5\text{kg/m}^3$  is caused by the optimal carrying capacity of the recirculating aquaculture system.

Eel cultured at a stocking density of  $5\text{kg}/\text{ m}^3$  were able to utilize feed efficiently (Table 2). Eels behavior, often clustering in cultivation media, influences the appetite of the eels themselves (Harianto *et al.*, 2014). The appetite of eel can be observed through the daily feeding rate. The highest feeding rate was  $2,20\pm0,29\%/\text{day}$  ( $5\text{kg}/\text{ m}^3$ );  $2,18\pm0,28\%/\text{day}$  ( $4\text{kg}/\text{ m}^3$ ), and the lowest  $2,08\pm0,38\%/\text{ day}$  ( $6\text{kg}/\text{ m}^3$ ). Feeding rate correlates with the growth performance of the elver *A. bicolor bicolor*; a low feeding rate has an impact on a low growth rate (**Taufiq-Spj** *et al.*, 2017). The appetite of fish in the  $6\text{kg}/\text{ m}^3$  stocking density treatment began to decrease in the 7<sup>th</sup> week of the nursery period, this was caused by the weakening of the recirculation system carrying capacity. The ammonia waste borne by the recirculation system in the  $6\text{kg}/\text{ m}^3$  treatment (0,164mg/L) was higher than in other treatments (Table 3) because the amount of feed given was correlated with the amount of nursery biomass (Silva *et al.*, 2022).

Eels grow optimally in populations that have uniform individual sizes (**Pedersen** *et al.*, **2017; Budiardi** *et al.*, **2022**). Variations in eel size can be identified through the coefficient of weight variation value. A low coefficient of weight variation value indicates uniform eel size (**Harianto** *et al.*, **2021**). Eel cultivated with a stocking density of  $6 \text{kg}/\text{m}^3$  has the most uniform weight. The large number of eels in the  $6 \text{kg}/\text{m}^3$  treatment triggered more uniform growth. The uniformity of fish size from the start of stocking and the intense intensity of competition to consume feed opens up equal growth opportunities between individuals.

The ammonia waste (NH<sub>3</sub>) excreted by the eel will be converted into nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) through a biological process supported by a recirculation system

(**Campanati** *et al.*, **2021**). The amount of ammonia excreted by fish is influenced by the amount and quality of protein contained in the feed (**Handajani** *et al.*, **2018**). Ammonia and nitrite in each treatment obtained values that were too high than the fish growth tolerance limit. Especially in the highest stocking density treatment (6kg/m<sup>3</sup>), an increase in nitrogen compounds and a decrease in oxygen solubility caused a reduction in fish appetite.

Depletion of dissolved oxygen in recirculation systems occurred because the level of oxygen consumption increased along with biomass growth (**Badiola** *et al.*, **2018**). Not only for eels, oxygen is also consumed by nitrifying bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.) (**Mnyoro** *et al.*, **2022**). Similar to dissolved oxygen, the degree of acidity (pH) decreased over the maintenance period. The decreasing pH value is influenced by the breakdown of organic matter and dissolved CO<sub>2</sub> (**Ramli** *et al.*, **2018**). High stocking density will produce nitrogen compounds (organic material) and high concentrations of dissolved CO<sub>2</sub>. Carbon dioxide reacts with water (H<sub>2</sub>O) to produce carbonic acid (H<sub>2</sub>CO<sub>3</sub>), which turns into bicarbonate ions (HCO<sub>3</sub><sup>-</sup>) releasing H<sup>+</sup> ions. The recirculation system components were equipped with a ginger coral filter containing calcium carbonate (CaCO<sub>3</sub>), which functions to maintain pH stability (**Scabra & Budiardi, 2020**). Calcium carbonate reacts with carbonic acid to form calcium bicarbonate (Ca(HCO<sub>3</sub>)<sub>2</sub>), which is alkaline in water (**Rizki** *et al.*, **2020**).

The alkalinity and hardness values obtained are in the optimal range for freshwater fish cultivation. The hardness value gradually increased, indicating that the ginger coral filter worked through calcium bicarbonate which contains calcium ions (Ca). According to **Lukas** *et al.*, (2017), adding calcium carbonate at the right dose can increase the growth of eel. Apart from the use of ginger coral, the role of alkalinity in maintaining water pH fluctuations is also supported by ketapang leaves in the cultivation media (Neuman *et al.*, 2023).

The nursery carried out inside the room was not optimal enough to maintain the temperature stability of the culture media. However, this was not a factor that reduced the eel's appetite during the nursery. The highest feeding rate (5kg/m<sup>3</sup> treatment) was 2,20 $\pm$ 0,29%/day which was obtained through maintenance at a water temperature of 24,9–2,.2°C. Meanwhile, the maintenance feeding rate of *A. bicolor bicolor* was highest in the research of **Taufiq-Spj** *et al.* (2018), namely 2,12 $\pm$ 0,05%/day with a water temperature of 27,66 $\pm$ 0,05°C. On the other hand, **Luo** *et al.* (2013) obtained the highest feeding rate, of 2,13 $\pm$ 0,03%/day for *A. bicolor pacifica* and 1,82 $\pm$ 0,01%/day for *A. marmorata* at a water temperature of 28°C.

This research shows a difference in financing structure specifically on variable costs. Eel cultivation with a stocking density of  $6 \text{kg}/\text{ m}^3$  has the highest variable cost value (15.338 USD), resulting in a high total cost (17.374 USD). These occurred because the seed and feed input required for nursery in the  $6 \text{kg}/\text{ m}^3$  treatment is higher than in other treatments, namely 36 and 70 kg. The highest profit value and R/C ratio were

obtained in the nursery with a stocking density of  $5\text{kg/m}^3$  (3.727 USD and 1,25). Thus, based on business performance, the elver *A. bicolor bicolor* nursery is more profitable with a stocking density of  $5\text{kg/m}^3$ .

The application of different systems and technologies in fish farming produces different financial values (**Diatin** *et al.*, **2021**), therefore choosing the right technology is an important factor in increasing farmers' profits. Based on **Huang** *et al.* (**2016**), eel farmers of *A. marmorata* in Taiwan who use earthen, concrete, and indoor pond structures earn an average profit of 179, 1.189, and 762 USD respectively. This research proves that cultivation using a recirculation system is more profitable with a profit value of up to 3.727 USD.

# CONCLUSION

Increasing the production performance and nursery business of the elver eel *A*. *bicolor bicolor* in the recirculating aquaculture system recommended in this research is a nursery with a stocking density of  $5\text{kg/m}^3$ . The survival rate and feed conversion ratio between treatments were not influenced by differences of stocking density, however, the best biomass absolute growth rate was obtained in the  $5\text{kg/m}^3$  treatment. The highest harvest biomass in the  $5\text{kg/m}^3$  treatment impacted the best profits and R/C ratio. The  $4\text{kg/m}^3$  treatment had low productivity, so business performance was not optimal. The carrying capacity of the recirculation system began to decrease at  $6\text{kg/m}^3$  treatment, which was characterized by high ammonia values, so production performance was inefficient. Further research regarding the development of recirculating aquaculture systems is needed for elver eel nurseries with high stocking densities.

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