Original article

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# Improved performance of botia fish *Chromobotia macracanthus* with the utilization of blood clam shell in the recirculation system

# Peningkatan kinerja produksi ikan botia *Chromobotia macracanthus* dengan pemanfaatan cangkang kerang darah pada sistem resirkulasi

## Rani Ria Rizki<sup>1</sup>, Iis Diatin<sup>1\*</sup>, Tatag Budiardi<sup>1</sup>, Irzal Effendi<sup>1</sup>

<sup>1</sup>Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java, Indonesia \*Cooresponding author : Iisdiatin@yahoo.co.id

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# ABSTRACT

Intermediate and holding rearing of botia face several problems such as the limited land, water quality, and decreased growth. The application of a recirculation culture system using the blood clam filter is increased to water quality and expected to solve the problems. This study aimed to analyze the production performance of botia fish on intermediate and holding rearing in the recirculation system by utilizing blood clams as the filter. This study used a factorial completely randomized design with two factors; clam particle sizes (1 mm, 2 mm, and 3 mm) and dosages (1.4 g/L, 1.8 g/L, and 2.2 g/L). Every experiment was conducted in three replication. The aquarium used in this study was  $40 \times 40 \times 60$  cm<sup>3</sup>. The size of fish samples was  $3.5 \pm 0.5$  cm with the stocking density (3 fish/L, each test aquarium). The recirculation system was applied seven days before the fish were stocked. Every 15 days, weight and length of fish were measured (for 60 days). The results of physical (temperature) and chemical (pH, dissolved oxygen, ammonia, and nitrite) water quality in the recirculation system using the blood clam filter showed good conditions for botia fish. The stress response of botia blood glucose and TKO fluctuates with environmental changes. Mineral water and fish produced by calcium, magnesium, and phosphorus increase until the end of maintenance. There is an interaction at TKH between particle size and the dose of blood shells, whereas, LMPW, LMPL, and RKP significantly different only the use of dose 2.2 g/L.

Keywords: Clamshells, botia fish, pH value, minerals, recirculation.

# ABSTRAK

Permasalahan pada proses penampungan ikan botia yaitu keterbatasan lahan, kualitas air yang buruk dan pertumbuhan ikan botia yang lambat. Penerapan sistem resirkulasi menggunakan cangkang darah dapat meningkatkan kualitas air dan kinerja produksi. Penelitian ini bertujuan menganalisis kinerja produksi budidaya ikan botia pada sistem resirkulasi dengan pemanfataan cangkang kerang darah sebagai bahan filter. Penelitian ini menggunakan rancangan acak lengkap faktorial dua faktor, yaitu ukuran partikel cangkang kerang darah (1 mm, 2 mm, dan 3 mm) dan dosis cangkang kerang darah (1.4g/L, 1.8g/L, dan 2.2g/L). Setiap perlakuan dilakukan dengan tiga kali ulangan. Akuarium yang digunakan dalam penelitian berukuran 40×40×60 cm<sup>3</sup>. Ikan yang digunakan berukuran  $3.5 \pm 0.5$  cm dengan padat tebar 3 ekor/L. Sistem resirkulasi dioperasikan selama tujuh hari sebelum ikan ditebar. Bobot dan panjang ikan diukur setiap 15 hari selama 60 hari pemeliharaan. Penelitian dalam sistem resirkulasi menggunakan cangkang kerang darah pada media filter menghasilkan kondisi kualitas air suhu, pH, oksigen terlarut, amonia dan nitrit air. Respons stres ikan berupa glukosa darah dan tingkat konsumsi oksigen (TKO) berfluktuasi seiring dengan perubahan lingkungan. Mineral air dan ikan yang dihasilkan meliputi kalsium, magnesium dan fosfos meningkat hingga akhir pemeliharaan. Parameter tingkat kelangsungan hidup (TKH) memiliki interaksi antara ukuran partikel dan dosis cangkang kerang darah, sedangkan untuk laju pertumbuhan bobot mutlak (LPMB), laju pertumbuhan panjang mutlak (LPMP) dan rasio konversi pakan (RKP) berbeda nyata dengan penggunaan dosis 2.2 g/L.

Kata kunci: Cangkang kerang darah, ikan botia, pH, mineral, resirkulasi.

#### **INTRODUCTION**

Botia fish Cromobotia macracanthus is an endemic species of freshwater ornamental fish from Cobitidae family that has limited distribution only around Kalimantan and Sumatera island (Kottelat, 2013). The fishing activity of this species reached around 20-50 million of juvenile per year (Slembrouck et al., 2012), most happened in rainy season along with the increase of water level in the river. In fishing season, the size of fish is around 0,5-6,0 cm. Botia fish that captured in rainy season, is carried and is reared in production center located close to consumers center in several big cities (Permana et al., 2011; EKI, 2017). Yet, LIPI (2014) stated that some big cities have limited land and worst water quality causing limiting factor for botia fish culture.

The optimation of land and water quality improvement could be done through an application of resirculation system for fish culture. This system is reused the water in the container after several physical filtration, chemical filtration, and biological filtration, and is supplied dissolved oxygen in water (Bragnballe, 2015). The study about resirculation system applied for botia fish rearing has been done by Ghofur & Harianto (2018), meanwhile the resirculation system applied for red rainbow fish has been done by Nurhidayat (2012). The production process in botia fish rearing is still suffered from other obstacle, such as the fish growth. Botia fish larvae need six to eight months until it reaches the size of 2-2.5 inch (BRBIH, 2010).

The enhancement of production process by improving the production infrastructure can be done by using blood clam Anadara granosa shell as filtration media to improve water quality (Iriany & Anugerah, 2015; Yusuf et al., 2016). Blood clam Anadara granosa shell is a by-product contained calcium carbonate Jubaedah et al., 2017). Yusuf et al. (2016) stated that chemical compounds in clam shell are chitin, calcium carbonate, calcium hydroxyapatite, and calcium phosphate. The mineral compound such as calcium and phosphor in clam shell could increase growth and could also as an absorbent by absorb heavy metals (Iriany & Anugerah, 2015). Furthermore, blood clam shell contain calcium for bone tissue formation process that can increase fish growth and establish required environmental condition for fish allowing to increase metabolism system and fish growth (Kadarini et al., 2015; Jubaedah et al., 2017). The use of blood clam shell in resirculation

system could increase the environment condition lead to better survival rate of fish and better fish growth due to the availability of sufficient mineral in water. Thereforem blood clam shell can be used as filtration media in resirculation system to improve production performance of botia fish.

#### MATERIALS AND METHODS

This study held for three months started from August to November 2019 in Production Technique and Aquaculture Management Laboratorium, Aquaculture Department, Faculty of Fisheries and Marine Science, IPB University.

# **Experimental design**

This study used factorial complete randomized design. There were two factors, such as particle size (P) of blood clam shell consisted of three treatments (1 mm, 2 mm, and 3 mm), and dosages consisted of three treatments 1,4 g/L, 1,8 g/L, and 2,2 g/L) (Tabel 1). Each treatment was repeated three times. The treatments were according to initial study.

Table 1. The combination treatments of particle size and dosage of blood clam shell

Particle -	Dosage (g/L)				
size (mm)	D1	D2	D3		
P1 (1mm)	(1,4g/L) P1D1	(1,8g/L) P2D1	(2,2g/L) P3D1		
P2 (2 mm)	P1D2	P2D2	P3D2		
P3 (3 mm)	P1D3	P2D3	P3D3		

#### Rearing container and filter media setting

The rearing containers were 27 aquariums and each of them had size of 60 cm×40 cm×40 cm with water level of 20 cm (the total water volume was 48 L). The water quality was maintained by reducing water pH to 4 by using 1% CH<sub>3</sub>COOH solutions.

Blood clam shell as main filter media was dried by using an oven in temperature of 110°C for 24 hours. Then dried blood clam shell was mashed by using feed grinding machine and was sifted out by using a sieve with a mesh size of 6, 8, 16, and 30 until the size particle of 1 mm, 2 mm, and 3 mm are collected (Yusuf *et al.*, 2016). The filtration of dried blood clam shell to collect those different particle sizes were used different mesh. Each size of blood clam shell particle was wrapped up by using asahi cloth.

#### **Resirculation system installation**

Resirculation system was build up by usig PVC pipe with diameter of 3 inch and lenghth of 10 cm. The water pump that used for this resirculation system had 24 W and the aeration was placed in each side of aquarium. PVC pipe was filled with blood clam shell and was performed 50% of blood clam shell changing. In addition, in all treatments, other filter media was added (sintetic cotton sized of 20×5 cm and 15 bioballs) into PVC pipe.

#### **Botia fish rearing**

Botia fish sized of  $3.5 \pm 0.5$  cm were stocked in each aquarium with stockinh density of 2 fish/L (SNI, 2013). Botia fish was reared for 60 days and fed with blood worm as much as three times a day (08.00, 12.00, and 16.00 WIB) in *at satiation*. Then, in every 15 days, from 30% of fish biomass, the fish weight and long were measured.

#### **Parameters of observation**

The measurement of water chemical, physical, and mineral

The measurement of observed parameters were chemical (pH, dissolved oxygen, alkalinity, hardness, ammonia, and nitrite), physical (temperature). The measurement of mineral were water (calcium, magnesium, and phosphor), and fish mineral (calcium and phosphor)

#### The respons of stress

The blood glucose level was measured by examining three fish of each treatments. The measurement of blood glucose level used blood glucose test kit (*Gluco* DR *Auto* AGM 4000). The number showed in the test kit was the result of blood glucose level in mg/dL. The use of glicometer that is usually conducted in human blood sample can also be used for zebra fish blood sample (Eames *et al.*, 2010).

The measurement of oxygen consumption level was done of every 15 days of rearing. Before that, the fish did not fed in a day. Then, a jar with volume of 5 L was filled with water and named with a label of treatment. The 3 L water put into each jar of each treatment; the aeration was installed for 24 hours to maintain maximal dissolved oxygen; the measurement of dissolved oxygen conducted in the beginning of the aeration was turned off; three botia fish was weighed and put into jar; then, the measurement of dissolved oxygen conducted in every hour for four hours, afterward, this measurement result is noted as oxygen consumption level. The oxygen consumption level was calculated according to NRC method (1997) as following:

$$TKO = \frac{V \times (DO_{t0} - DO_{tt})}{W \times t}$$

Notes:

TKO = oxygen consumption level (mg O<sub>2</sub> g/ hour)

V = water volume (L)

- DOt<sub>0</sub> = the concentration of dissolved oxygen in the initial of study (mg/L)
- DOtt = the concentration of dissolved oxygen in the certain time t (mg/L)

W = the fish weight (g)

T = observation period (jam).

#### Fish production performance

The survival rate (SR) is the percentage of the ratio of survived fish in the end of the study with the total fish in the initial of study. The survival rate was calculated by using the following formula (Goddard, 1996):

$$SR = \frac{Nt}{No} \times 100$$

Notes:

SR = The survival rate (%)

Nt = The total survived fish in the end of study

No = The total fish in the initial of study

The growth rate is the performance of average weight of each fish from the initial to an end of study. The growth rate was calculate by using this following formula (Goddard, 1996):

$$GR = \frac{Wt - Wc}{t}$$

Notes:

t

GR = Growth rate (g/fish/day) Wt = Average fish weight in the

Wo = Average fish weight in the initial of study (g)

= Rearing period (days)

Feed conversion ratio was calculated by following formula (Goddard, 1996):

$$FCR = \frac{F}{Wt + Wo - Wd}$$

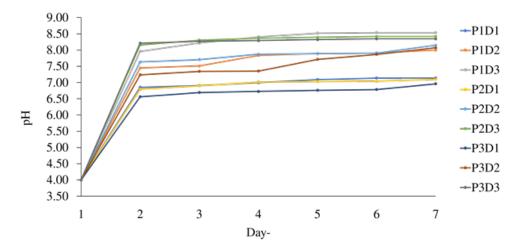


Figure 1. The water pH for seven days before botia fish are stocked. Note: \*P1D1: the particle size was 1 mm and the dose was 1.4 g/L; P1D2: the particle size was 1 mm and the dose was 1.8 g/L; P1D3: the particle size was 1 mm and the dose was 2.2 g/L; P2D1: the particle size was 2 mm and the dose was 1.4 g/L; P2D2: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size

Notes :	
FCR	= Feed conversion ratio
F	= The total by amount of eaten fed
	(kg)
Wt	= Fish biomass in the end of study
	(kg)
Wd	= Dead fish biomass during the study
	(kg)
W0	= Fish biomass in the the initial of
	study(kg)

#### Data analysis

The data obtained from this study was collected for further analysis. The fish production performance (SR, GR, FCR) was analyzed by using two-way ANOVA with confidence level of 95%. If it showed significantly different result, the further analysis is Tukey test. The result of water mineral, fish mineral, water chemical, and water physical data was analyzed descriptively by using table and figure. The data analysis in this study used Microsoft Office Excel 2010 and SPSS 23.0 software.

#### **RESULT AND DISCUSSION**

The performance of blood clam shell as filtration media in botia fish rearing directly affected toward the change of water quality (water chemical quality). The generated environment would affect fish physiological condition (stress response), visual pigments of fish, and production performance (survival rate, growth rate, and feed consumption). The performance of blood clam shell as filtration media showed below:

#### Water pH and alkalinity

Before the botia fish are stocked, the resirculation system should be maintained already for seven days. During the initial study, the water pH was measured everyday for seven days, meanwhile, the water alkalinity was measured at day-1 and day-7 (Figure 1 and 2).

During the initial study for seven days, the alkalinity was 10 mg.L at day-1 and the water pH was 4 in all treatments (Figure 1 and 2), then, the resirculation system was executed for maintaining the environment for botia fish, afterward, the water alkalinity and water pH were improved drastically, yet at day-3, the water pH was remained stable. The value of water alkalinity and water pH was remained stable. The value of water alkalinity and water pH was remained stable. The value of water alkalinity and water pH was remained stable. The value of water alkalinity and water pH improvement were happen due to the CaCO<sub>3</sub> content in blood clam shell. The calcium content in blood clam shell was 43% and the magnesium was 0.30%. Meanwhile, the water alkalinity and water pH during botia fish rearing for 60 days was showed in Table 2 below.

The water alkalinity and water pH when the botia fish were stocked, was already maintained (Figure 1 and 2). The result of water alkalinity and water pH measurements for 60 days botia fish rearing were increased until the end of the study (Table 2), and the water hardness was also increased at the end of the study. All data were showed in Table 2 below.

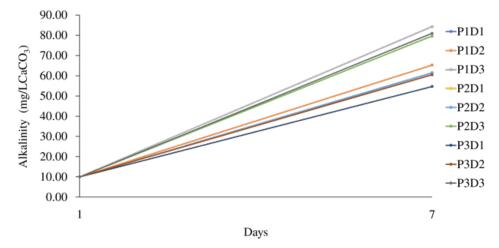


Figure 2. The water pH for seven days before botia fish are stocked. Note: \*P1D1: the particle size was 1 mm and the dose was 1.4 g/L; P1D2: the particle size was 1 mm and the dose was 1.8 g/L; P1D3: the particle size was 1 mm and the dose was 2.2 g/L; P2D1: the particle size was 2 mm and the dose was 1.4 g/L; P2D2: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 3 mm and the dose was 1.8 g/L; P3D2: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size

		pН		Alkaliı	nity (mg/L	CaCO <sub>3</sub> )	Hardne	ess (mg/L C	CaCO <sub>3</sub> )
Treatments	Hari ke								
	0	30	60	0	30	60	0	30	60
P1D1	7.1	5.8	6.2	61.3	26.9	32.5	106.7	174.8	158.8
P1D2	8.0	6.1	6.5	65.3	25.4	25.4	123.3	193.5	196.2
P1D3	8.5	5.8	6.3	84.3	22.6	31.1	151.7	181.5	241.6
P2D1	7.1	6.0	6.4	54.7	25.4	28.3	96.7	173.5	186.9
P2D2	8.1	5.8	6.1	61.5	19.8	28.3	123.3	181.5	206.9
P2D3	8.4	6.0	6.2	79.7	28.3	38.2	140.0	177.5	254.9
P3D1	7.0	5.6	6.0	54.7	25.4	18.4	93.3	133.5	228.2
P3D2	8.1	6.0	5.9	60.5	32.5	24.0	123.3	186.9	249.6
P3D3	8.3	6.2	6.3	82.3	24.0	25.4	140.0	174.8	230.9

Table 2. The water pH, water alkalinity, and water hardness of botia fish rearing

\* Note: P1D1: the particle size was 1 mm and the dose was 1.4 g/L; P1D2: the particle size was 1 mm and the dose was 1.8 g/L; P1D3: the particle size was 1 mm and the dose was 2.2 g/L; P2D1: the particle size was 2 mm and the dose was 1.4 g/L; P2D2: the particle size was 2 mm and the dose was 1.8 g/L; P2D3: the particle size was 2 mm and the dose was 1.8 g/L; P3D1: the particle size was 2 mm and the dose was 1.4 g/L; P3D2: the particle size was 3 mm and the dose was 1.4 g/L; P3D2: the particle size was 3 mm and the dose was 2.2 g/L; P3D1: the particle size was 3 mm and the dose was 2.2 g/L; P3D2: the particle size was 3 mm and the dose was 2.2 g/L; P3D2: the particle size was 3 mm and the dose was 2.2 g/L; P3D2: the particle size was 3 mm and the dose was 2.2 g/L; P3D3: the particle size was 3 mm

According to the value of water pH, water alkalinity, and water hardness for 60 days rearing, the reduction of this parameters were showed along times and after the addition of blood clam shell as filtration media, the parameters were increased. The longer of the botia fish rearing, feses waste would be produced and feed residual would be a sludge and experienced a decomposition. This kind of waste would cause the increasing of  $CO_2$  in water and water pH alteration. Fish respiration process would also affect  $CO_2$  level and water pH (Suwandi *et al.*, 2012). Sarkar *et al.*, (2018) stated

that the water alkalinity level is an important criteria to determine suitability of water sources for fish farming. High alkalinity level can reduce pH fluctuation in water. The alkalinity level in water for best fish growth is between 75–200 mg/L, meanwhile, the alkalinity level in >300 mg/L and <75 mg/L can cause stress in fish. The hardness level in water was keep increased until the end of fish rearing. The increasing of hardness level in water caused by Ca and Mg compound in blood clam shell (Devi *et al.*, 2017), hardness contains Ca<sup>2+</sup> and Mg<sup>2+</sup> cations (mg/L CaCO<sub>3</sub>).

Treatments	Temperature (°C)	Ammonia (mg/L)	Dissolved oxygen (mg/L)	Nitrite (mg/L)	
-	Range of value	Rata-Average value	Average value	Average value	
P1D1	26.6–28.1	0.001	6.9	0.27	
P1D2	26.7–27.9	0.004	6.4	0.23	
P1D3	27.0-28.3	0.014	6.4	0.25	
P2D1	27.0-28.4	0.001	6.9	0.23	
P2D2	26.7–28.3	0.006	6.9	0.34	
P2D3	26.7-28.2	0.006	6.7	0.43	
P3D1	26.5-28.2	0.001	6.5	0.33	
P3D2	26.0-28.2	0.003	6.6	0.28	
P3D3	26.5-28.8	0.007	6.8	0.21	
Reference	24-30*	Max. 1*	>3*	<1**	

Table 3. The value of temperature of water, ammonia, dissolved oxygen, and nitrite during 60 days of botia fish rearing

References: \*SNI (2013); \*Deswati *et al.*, (2018). Note: P1D1: the particle size was 1 mm and the dose was 1.4 g/L; P1D2: the particle size was 1 mm and the dose was 1.8 g/L; P1D3: the particle size was 1 mm and the dose was 2.2 g/L; P2D1: the particle size was 2 mm and the dose was 1.4 g/L; P2D2: the particle size was 2 mm and the dose was 1.8 g/L; P3D1: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 3 mm and the dose was 1.4 g/L; P3D2: the particle size was 3 mm and the dose was 2.2 g/L; P3D3: the particle size was 3 mm and the dose was 2.2 g/L; P3D3: the particle size was 3 mm and the dose was 2.2 g/L; P3D3: the particle size was 3 mm and the dose was 2.2 g/L; P3D3: the particle size was 3 mm and the dose was 2.2 g/L; P3D3: the particle size was 3 mm and the dose was 2.2 g/L; P3D3: the particle size was 3 mm and the dose was 2.2 g/L; P3D3: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle size was 3 mm and the dose was 2.2 g/L

The hardness level in water of 0-10 mg/L or >300 mg/L is unsuitable for the growth of fish larvae and fish juvenile. The temperature of water, ammonia, dissolved oxygen, and nitrite obtained during 60 days of botia fish rearing was found optimum for fish growth and fish survival rate as showed in Table 3 below.

According to the Table 3, the value of water temperature, ammonia, and dissolved oxygen during 60 days of botia fish rearing were in appropriate range (SNI, 2013) for botia fish rearing, therefore, it could support the production process of botia fish. Nitrite value that obtained during 60 days rearing was showed less than 1 mg/L, which as Deswati *et al.*, (2018) stated that this value is still in appropriate range for fish farming. Yet, the value of nitrite would keep increase as the fish rearing get longer.

#### Mineral compound in water and fish

According to mineral data obtained during 60 days of rearing, the calcium, phosphor, and magnesium in water were increased until day-60, so did the calcium and phosphor in fish.

The calcium compound in blood clam shell was amount 43%. According to chart showed in a, c, and e (Figure 3), the calcium, phosphor, and magnesium were increased due to the soluble mineral compound in water. The increasing value of calcium and phosphor was caused by

the availability of mineral produced by blood clam shell, then it can be absorbed through fish respiration process in gill and skin for bone mineralization process (Liang *et al.*, 2018).

#### **Stress response**

The response stress parameters that measured for 15 days rearing were blood glucose evel and oxygen consumption level. The results of these measurement in all treatments were fluctuated and increased at day-30 and day-45, then reduced at day-60

Blood glucose level and oxygen consumption level that obtained during this study was reduced at day-15 and day-45. Polakof et al. (2012) stated that high glucose level can also activate glucosensor mechanism in brain areas that has been related to the control of food intake. The role of environment can also affect stress level since the fish have got stressor from the environment (Ardi et al., 2016). In a stress condition, in order to maintain the homeostatic condition that would enhance metabolic activity, fish blood glucose is increased. Moreover, Polakof et al. (2012); Bartonkova et al. (2016); Aslamyah et al. (2018) stated that fish has different stress response depending on environment condition and metabolic activity, therefore the increasing metabolism can cause available energy for growth getting less, therefore, increased metabolism can also increase oxygen consumption level.

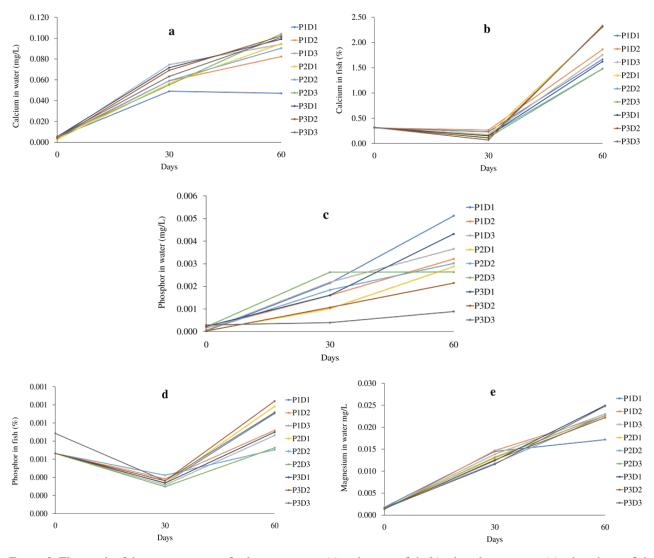


Figure 3. The result of the measurement of calcium in water (a), calcium in fish (b), phosphor in water (c), phosphor in fish (d), and magnesium in water (e) during 60 days rearing. Note: P1D1: the particle size was 1 mm and the dose was 1.4 g/L; P1D2: the particle size was 1 mm and the dose was 1.8 g/L; P1D3: the particle size was 1 mm and the dose was 2.2 g/L; P2D1: the particle size was 2 mm and the dose was 1.4 g/L; P2D2: the particle size was 2 mm and the dose was 1.8 g/L; P3D1: the particle size was 3 mm and the dose was 1.4 g/L; P3D1: the particle size was 3 mm and the dose was 1.4 g/L; P3D2: the particle size was 3 mm and the dose was 1.2 g/L; P3D2: the particle size was 3 mm and the dose was 1.2 g/L; P3D3: the particle size was 3 mm and the dose was 1.2 g/L; P3D3: the particle size was 3 mm and the dose was 1.2 g/L; P3D3: the particle size was 3 mm and the dose was 1.2 g/L; P3D3: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 1.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3 mm and the dose was 2.2 g/L; P3D4: the particle size was 3

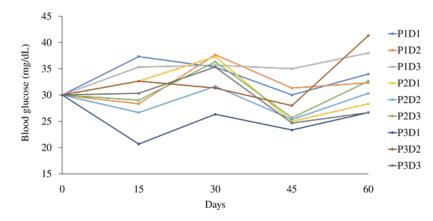


Figure 4. Blood glucose level in fish during 60 days of rearing. Note: P1D1: the particle size was 1 mm and the dose was 1.4 g/L; P1D2: the particle size was 1 mm and the dose was 1.8 g/L; P1D3: the particle size was 1 mm and the dose was 2.2 g/L; P2D1: the particle size was 2 mm and the dose was 1.4 g/L; P2D2: the particle size was 2 mm and the dose was 2.8 g/L; P2D3: the particle size was 2 mm and the dose was 2.2 g/L; P2D3: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 3 mm and the dose was 1.4 g/L; P3D2: the particle size was 3 mm and the dose was 1.4 g/L; P3D3: the particle size was

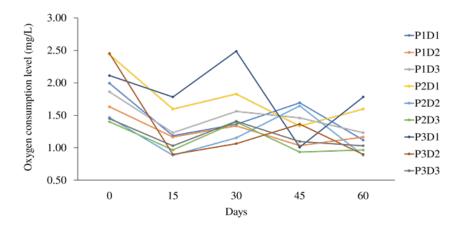


Figure 5. The oxygen consumption level during 60 days of rearing. Note: P1D1: the particle size was 1 mm and the dose was 1.4 g/L; P1D2: the particle size was 1 mm and the dose was 1.8 g/L; P1D3: the particle size was 1 mm and the dose was 2.2 g/L; P2D1: the particle size was 2 mm and the dose was 1.4 g/L; P2D2: the particle size was 2 mm and the dose was 1.8 g/L; P2D3: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 2 mm and the dose was 3 mm and the dose was 1.8 g/L; P3D3: the particle size was 3 mm and the dose was 3 mm and the do

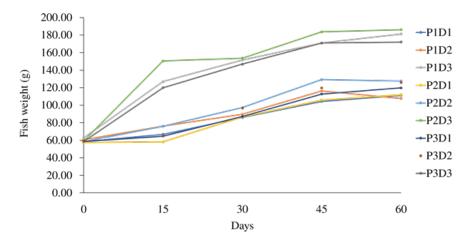


Figure 6. The increased weight during 60 days of botia fish rearing. Note: \* P1D1: the particle size was 1 mm and the dose was 1.4 g/L; P1D2: the particle size was 1 mm and the dose was 1.8 g/L; P1D3: the particle size was 1 mm and the dose was 2.2 g/L; P2D1: the particle size was 2 mm and the dose was 1.4 g/L; P2D2: the particle size was 2 mm and the dose was 2.2 g/L; P2D3: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle siz

#### Fish production performance

Fish production performance during this study showed increased length and weight for 60 days rearing. The data of increased length and weight of botia fish showed in Figure 6 and Figure 7 below.

According to Figure 6 and Figure 7, the fish weight and length were increased until the end of the study. The increased growth of botia fish that was reared in filtration media added with blood clam shell (in certain size and dose) containing CaCO<sub>3</sub>, showed a certain mechanism and reaction in water that capable to establish an environment

for fish growth and fish survival. The mineral contained in blood clam shell can be utilized by fish for bone mineralization process (Liang *et al.*, 2018), and Meilisza *et al.* (2011) stated that fish can absorb directly calcium and magnesium form water or feed.

The production performance of botia fish includes SR, GR, and FCR. In SR parameter, it showed that there was an interaction between the particle size and the dose of blood clam shell. Meanwhile, in GR and FCR parameter were not showed any interaction between the particle size and the dose of blood clam shell. Yet,

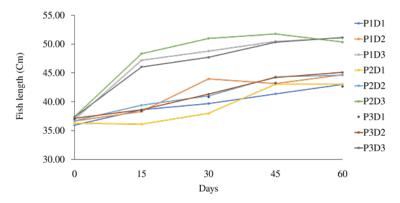


Figure 7. The increased length during 60 days of botia fish rearing. Note: \* P1D1: the particle size was 1 mm and the dose was 1.4 g/L; P1D2: the particle size was 1 mm and the dose was 1.8 g/L; P1D3: the particle size was 1 mm and the dose was 2.2 g/L; P2D1: the particle size was 2 mm and the dose was 1.4 g/L; P2D2: the particle size was 2 mm and the dose was 2.2 g/L; P2D3: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 2 mm and the dose was 2.2 g/L; P3D1: the particle size was 3 mm and the dose was 1.4 g/L; P3D2: the particle size was 3 mm and the dose was 1.8 g/L; P3D3: the particle siz

according to treatment with dose of 2.2 g/L was found significantly different result especially in treatment P1D3, P2D3, and P3D3 as showed in Table 4 below.

The different within SR was caused by the interaction between the particle size and dose of blood clam shell. Furthermore, the SR parameter resulted during botia fish rearing was showed higher value by 84,03–100%. This value was higher than previous study done by Ghofur and Harianto (2018). Yet, the value of GR and FCR parameters was only caused by the different dose of blood clam shell.

Fish growth is affected by internal and external factor. This study indicated the effect of external factor associated with the environment (physical, chemical, and biological) (Hidayat et al., 2013). The external factor resulting from the increased of CaCO<sub>3</sub> compound in water showed significantly different result in chemical quality of water. Furthermore, this result affected fish appetite that could improve fish weight and length. An optimal environment could support metabolism process, therefore, it could maintain fish appetite and fish physiology condition for avoiding stress and increasing fish survival rate. NRC (2011); Liang et al., (2018) stated that fish is capable to absorb the calcium whether from fish feed or from water for their growth.

#### CONCLUSION

The addition of blood clam shell by 2.2 g/L as media filtration affected fish survival rate, fish growth, and feed conversion ratio.

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