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Effect of Stunting Duration to the Physiological Condition of Elver of the Indonesian Shortfin Eel (*Anguilla bicolor bicolor*)

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ABSTRACT

Anguilla bicolor bicolor is a commercially valuable fish species with a high economic significance and serves as an export commodity in the fishery sector. However, the availability of eel stocks remains limited, as the seeds required for rearing are still dependent on natural catches. This study aimed to assess the effects of stunting caused by limiting the amount of feed (2%) and lowering the maintenance media temperature (24°C) on the physiological condition of elvers. The study was conducted over a period of six months (May 2017 to November 2017) at the Physiology Laboratory of Aquatic Animals, FPIK IPB. A completely randomized design was used with three treatment periods: 2, 4, and 6 months, each with three replications. The elvers had an initial body weight of 2-3g per individual and a body length of 9-12cm. The fish were kept in aquariums measuring 60 x 40 x 30cm, with 50 individuals per aquarium, and were fed commercial pellets (1.5mm size, 46% protein content). The results showed that after 2, 4, and 6 months of stunting, the elvers maintained normal physiological conditions, with survival rates above 87%. The specific growth rate of the elvers was close to zero (0.1%), and the coefficient of variation was below 20%. Stunting through feed restriction (2%) and the use of low-temperature media (24°C) did not negatively impact the physiological condition or cause stress. Instead, it effectively controlled growth and reduced individual size variation among the elvers.

INTRODUCTION

Stunting is a condition used to describe fish that exhibit stunted growth, where the body size achieved is below the normal average size observed in other adult specimens of the same species **(Chizinski, 2010)**. Stunting is used to meet the demand for economically valuable fish, particularly ornamental species, that are cultivated to reach a specific size **(Livengood *et al.*, 2009)**. Stunting can occur either naturally **(Byrne *et al.*, 2013)** or artificially **(Lingam *et al.*, 2018)**. In the wild, the fish can experience stunt when there is population density **(Aday *et al.*, 2002; Amundsen *et al.*, 2007; Sarma, 2017)**, food limitations **(Santiago *et al.*, 2004; Fekri *et al.*, 2015)**, space limitation **(Abdel-Tawwab, 2005; Limbu *et al.*, 2015)**, and disease **(Boerlijst & Roos, 2015)**. Artificial method of stunting can be done through feed malnutrition **(Wickins, 1985)** and maintenance media temperature settings **(Fekri *et al.*, 2018; Fekri *et al.*, 2019)**. Stunting can affect the morphology, anatomy, behavior, and physiology of fish **(Chizinski *et al.*, 2010)**. **Chizinski (2007)** states that the effect of stunting population include slower growth, faster reproduction, and smaller size. Environmental factors such as predation abundance and food limitations can cause stunt **(Van *et al.*, 2007)**.

Freshwater eels are important animals because they have a unique catadromous life history and are used as food resources **(Arai, 2014)**. Until now, eel rearing technology is still very minimal, and to meet consumer demand, 99% of eels come from rearing activities **(Arai, 2016)**. In eel rearing, seeds are obtained from the wild. However, the high exploitation of glass eels and elvers in nature has led to a decrease in their populations **(ICES, 2011; Arai, 2014; Solomon, 2016)**. Between 1984 and 2000, the populations of glass eels and elvers decreased by 64% in Japan, 43.5% in Europe, and 8.3% in the United States **(Ringuet *et al.*, 2002)**.

One of the efforts to restore eel populations in nature is restocking eel, using correct elver body weight size ± 2g/ individual, timely stocking and correct stocking location **(Baskoro *et al.*, 2016)**. In the case of eels, the glass eel abundance season coincides with the correct time for stocking in nature. **Harrison *et al*. (2014)** stated that tropical eel seed migration occurs throughout the year, with factors such as rainfall and tides influencing this migration. If the timing of glass eel abundance for capture and rearing (3-4 months) aligns with achieving the correct body weight size (approximately 2g per individual), and stocking is done afterward, the timing may not be ideal. This is because the summer season is approaching, which can result in lower survival chances for the stocked elvers. Moreover, if the elvers are reared to the intended body weight of approximately 2g per individual and continued to be raised until the next rainy season, their body weight may exceed 100g per individual. This size is unsuitable for stocking purposes, as it becomes inefficient **(Baskoro *et al.*, 2016)**.

To ensure that the stocked elvers remain around 2g per individual in body weight at the time of the next rainy season, the elvers should be stunted. Seeds that undergo stunting exhibit compensatory growth, making them suitable for rearing activities. This approach allows eel fish rearing to be programmed year-round **(Bombeo-Tuburan, 1988; Baskoro *et al.*, 2016)**.

Several fish species that have undergone stunting include the bluegill (*Lepomis macrochirus*) **(Aday *et al.*, 2002)**, the Nile tilapia (*Oreochromis niloticus*) **(Bhujel *et al.*, 2007)**, the discus fish (*Symphysodon* spp.) **(Livengood *et al.*, 2009)**, perch (*Perca* *fluviatilis*) **(Gosch *et al.*, 2010)**, rohu (*Labeo rohita*) **(Kumar *et al.*, 2011)**, pike (*Esox lucius*) **(Pullen, 2013)**, and eel (*Anguilla bicolor bicolor*) **(Fekri *et al.*, 2015)**. While stunting is still practiced in ornamental fish, it primarily involves limiting food intake and increasing fish density or space restrictions. However, stunting through environmental temperature regulation to control metabolic rates has not yet been explored. This approach, which affects the metabolism, could influence feed consumption rates and growth **(Fekri *et al.*, 2018)**. This study aimed to examine the effects of stunting through a 2% feed restriction and a reduction in rearing media temperature (24°C) on the physiological condition, growth, and coefficient of variation of elvers.

MATERIALS AND METHODS

The study was conducted over a six-month period, from May 2017 to November 2017, at the Physiology Laboratory of Aquatic Animals, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia. Physical and chemical water analyses were performed at the Aquaculture Laboratory, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia. Blood analyses, including glucose, hemoglobin, and hematocrit measurements, were conducted at the Physiology Laboratory, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, Indonesia. The chemical composition of the body, including water content, protein, fat, carbohydrate, and ash, was analyzed at the Research Center for Biological Resources and Biotechnology Laboratory, Bogor Agricultural University, Bogor, Indonesia.

Experimental fish

The experimental fish used in this study were elvers (*Anguilla bicolor bicolor*) with a body weight of 2-3g per individual and a body length of 9-12cm per individual. These elvers were obtained from rearing at PT Laju Banyu Semesta, Bogor, West Java. The elvers were maintained at a density of 50 fish per aquarium. The fish were fed a commercial powdery feed, which was processed into dry pellets of 1.5 mm and contained 46% protein. The amount of feed provided each day was 2% of the total fish biomass. The feed was given twice daily: ¼ of the daily amount at 08:00 and ¾ at 16:00. Feed was administered daily without interruption, except during total water volume replacement. Cleaning was performed every morning to remove residual feed and feces before feeding. The total water volume was replaced once a month. To stabilize the temperature in the aquarium, a water heater was used, and the room was maintained at a controlled temperature using air conditioning. Throughout the rearing period, physical and chemical water measurements, including dissolved oxygen, pH, and ammonia levels, were taken at the beginning, middle, and end of the study. The water temperature was maintained at 24°C.

Experimental design

The experiment used a complete randomized design with three duration treatment times and each treatment having three replications. The treatments used were:

- Treatment 1: Stunting for two months of rearing.
- Treatment 2: Stunting for four months of rearing.
- Treatment 3: Stunting for six months of rearing.

The six-month treatment period is related to the elvers' needs for the sustainability of the cultivation process.

Trial procedure

The containers used in this study were 21 aquariums with an internal recirculation system. This adhesive serves to separate the filter parts and parts for rearing. The aeration system first deposits the water used in a reservoir for 2-3 days. The aquarium dimension used was 60 × 40 × 30cm^3, with the filter section measuring 10 × 40 × 30cm^3 and the maintenance media part was 50 × 50 × 40cm^3. Water was poured into the aquarium as much as 2/3 from the total aquarium volume.

Observation procedure

Observations in this study included:

- Daily monitoring of the number of dead fish throughout the rearing period.
- Weighing the body weight of all elvers using a digital scale with a precision of 0.01g, conducted at the beginning and end of the experiment.
- Blood measurements: glucose levels were determined using the GOD-PAP (Glucose Oxidase Phenol 4-Aminoantipyrine) method **(Sacks *et al.*, 2011)**, and hematocrit and hemoglobin levels were measured according to the **Practice Guidelines (2015)**. These measurements were taken at the beginning and end of the experiment.
- Measurement of the chemical composition of the elvers' bodies, following the methods of the Association of Official Analytical Chemists **(AOAC, 1980)**. These measurements were made at the beginning and end of the experiment.
- Measurement of physical and chemical water parameters, including daily temperature control, dissolved oxygen, pH, and ammonia levels, which were measured during each total water volume replacement.

Test parameters

The parameters tested in this study were divided into three groups:

- **Biometrics**: survival (S), specific growth rate (SGR), and coefficient of variation (CV).
- **Physiology**: chemical composition of the body (water, protein, fat, carbohydrate, and ash) and blood parameters (glucose, hemoglobin, and hematocrit).
- **Environment**: temperature, dissolved oxygen, pH, and ammonia levels.

Survival (S)

The survival was calculated using the formula of **Goddard (1996):**

$$S = N_d / N_0 \text{ x } 100$$

Where:

S = survival (%)

N_d = total number of die fish (individual)

N_0 = total number of initial fish (individual)

Specific growth rate (SGR)

The specific growth rate was calculated using the formula of **Huisman (1987)**:

$$LPSB = \left[\sqrt[t]{\frac{\hat{w}t}{\hat{w}o}} - 1 \right] x 100$$

Where:

SGR = specific growth rate (% day-1)

$\hat{w}t$ = the average weights of eels at the end of the experiment (g)

$\hat{w}o$ = the average weights of eels at the beginning of the experiment (g)

t = duration of rearing time (days)

Coefficient of diversity (CD)

The coefficient of diversity was calculated using the formula of **Baras *et al.* (2011)**:

$$CD = (s / y) \text{ x } 100$$

Where:

CD = coefficient of diversity

s = standard deviation of eel fish weight

y = average weight of elver

Data analysis

Data obtained during the study were analyzed using Microsoft Excel 2010 and SPSS software version 21.1, which included:

1) Analysis of variance (ANOVA) with F test at 95% confidence interval. This analysis was used to determine whether the treatment had a significant effect parameter on the survival (S), the specific growth rate (SGR), the coefficient of diversity (CD), blood glucose, hemoglobin, hematocrit, chemical composition of the body and if it had a real effect, then Tukey's further test was performed on 5% level **(Steel & Torrie, 1981)** to determine the difference between treatments.
2) The quantitative descriptive analysis was used to explain physical-chemical water environments during rearing.

RESULTS

The results of the measurements and observations during the research, including survival (S), specific growth rate (SGR), and coefficient of variation (CV), are presented in Table (1).

Table 1. Survival, specific growth rates and diversity coefficient in each treatment during rearing

Parameter	Treatment (month)		
	2	**4**	**6**
S (%)	95± 3.94[a]	94 ± 4.08[b]	87 ± 2.06 [b]
SGR (%)	0.031 ± 0.01[a]	0.032± 0.03[a]	0.044 ± 0.01[a]
CD (%)	15.05± 1.26[a]	17.01± 5.09[b]	17.09± 2.83[b]

A numbers on the same line followed by the same letter are not significantly different at the 5% test level (Tukey multiple test tube).

The value of survival (S) on all treatments ranged from 85.0-98.9%, SGR value near 0% (0.03-0.045%), and CD <20% (13.7-19.9%). The blood description of elver after two, four and six-months stunting are presented in Table (2).

Table 2. The blood description of elver (before and at the end of each treatment)

Parameter	Before treatment	Treatment (month)			Normal range	Source
		2	4	6		
Glucose (mg dL^{-1})	77±2.43[a]	63.5± 3.12[a]	60.4± 3.52[b]	52.0± 2.18[c]	47-227	**Arthanari *et al.* (2016)**
					50-246	**Fazio *et al.* (2013)**
					70-110	**Rahardjo *et al.* (2011)**
Hb (g 100 mL^{-1})	5.78±0.52[a]	5.34± 0.77[b]	4.97± 0.82[b]	4.40± 0.45[b]	3.5-5.5	**Hamid *et al.* (2013)**
					4.2-9.4	**Fekri *et al.* (2015)**
Hematocrit (%)	13.0±1.15[a]	11.5± 2.36 [b]	12.5± 1.86 [b]	12.2± 2.11 [c]	6-28	**Fekri *et al.* (2015)**
					9-13	**Diansyah *et al.* (2014)**

A numbers on the same line followed by the same letter are not significantly different at the 5% test level (Tukey multiple test tube).

Glucose, hematocrit, and hemoglobin decreased during the period of stunting but remained in the normal range. Table (3) presents the results of the proximate analysis of Elver after two, four, and six months of stunting.

Table 3. The chemical composition of the body (%) of elver at before and the end of each treatment

Chemical composition of the body	Before treatment	Treatment (month)		
		2	4	6
Water	73.93±0.68[a]	75.78±0.12[a]	76.35±1.10[b]	73.20±0.82[a]
Protein	16.24±0.10[c]	15.63±0.90[b]	15.86±0.53[a]	16.08±0.37[c]
Fat	4.10±0.40[a]	3.89±1.99[c]	3.05±0.78[b]	5.56±0.01[b]
Carbohydrate	2.13±0.31[a]	2.54±1.51[ab]	3.13±0.49[ab]	2.40±0.75[b]
Ash	3.60±0.06[c]	2.16±0.30[bc]	1.61±0.35[a]	2.76±0.32[ab]

A numbers on the same line followed by the same letter are not significantly different at the 5% test level (Tukey multiple test tube).

The statistical tests generally show that the values before and after the two, four, and six-month stunting treatments are not significantly different between treatments. The water content, protein, fat, carbohydrate, and ash levels in elver after treatment exhibited fluctuating values across each treatment. Although different real test values were observed in each treatment, the differences were not extreme.

The results of the physical-chemical parameters of water during the rearing of elver for different durations (two, four, and six months) are presented in Table (4).

Table 4. Range values of physical-chemical parameters of water during rearing

Parameter	Treatment (month)			Optimum range	Source
	2	4	6		
Dissolved oxygen (mg L^{-1})	7,4-7.5	7,5-7.4	5.6-6.7	>2	**EA, 2010**
pH	6.2-6.4	6.3-6.5	6.2-6.6	5,0–8,0	**EA, 2010**
Ammonia (mg L^{-1})	0.00023-0.00026	0.00025-0.00027	0.00029-0.00031	<0,5	**EA, 2010**

The mean values of physical-chemical water parameters of the media during maintenance at different time treatments were in the optimal range for survival and growth of elver. The feed was given in a limited dose, hence it did not produce much waste, especially ammonia.

DISCUSSION

Feed amounts at 2% of maintenance in elver biomass (2-3 g/individual) resulted in a survival rate of 85.0-98.9%, with a specific growth rate (SGR) approaching 0% (0.03-0.045%), and a coefficient of diversity (CD) value of <20% (13.7-19.9%). These survival rate values are consistent with those obtained by **Arief *et al.* (2011)**, who used feed with 42% protein content. In this study, the low SGR of the elver suggests that the feed amount provided was more suitable for maintenance of the body rather than promoting growth. A 2% feed amount is considered a maintenance level, meaning that the food consumed by the fish is primarily used for basal metabolic needs, which prevents growth or body weight decrease.

Fekri *et al.* (2015) found that feeding elver (1-2 g/individual) with 3% maintenance feed resulted in an SGR range of 0.1-0.2%. Similarly, Fatkurrohman (2013) reported that a 3.9% maintenance feed for elver with a body weight of 1-3 g/individual resulted in an SGR of 0%. In contrast, **Bakeer (2006)** conducted experiments on *Anguilla anguilla* weighing 35.30g/ individual, where four different feeding levels (2, 4, 6, and 8% of biomass) produced SGR values of 0.88, 0.94, 1.00, and 1.08%, respectively.

Media temperature (24°C) was able to influence the decrease in elective eel metabolic rate leading to decreased feeding activity which resulted in low elver growth (0.03-0.045%), and the variability coefficient value <20% (13.7-19.9%). The results of this study show a low coefficient of diversity, which can be attributed to several factors: the elver were uniformly stocked by size, the feed percentage was reduced, and as a result, competition between individual elver for food was minimal. Additionally, the use of 24°C as the temperature in the maintenance media led to a low appetite in the elver. **Baras *et al*. (2011)** stated that temperature impacts fish in terms of survival, growth, and size heterogeneity in certain species.

In general, environmental conditions and feeding rates affect the fish blood description. Based on the data in Table (4), it appears that blood glucose, hematocrit, and hemoglobin levels decrease with the duration of rearing, but the value in blood parameters is still on normal limits. The results of blood measurements after each duration of stunting (two, four, and six months) showed glucose levels of 63.5, 60.4, and 52.0mg/ dl, respectively. Normal blood glucose levels for fish range from 40 to 90mg/ dl **(Rahardjo *et al.,* 2011)**. **Wells (2009)**, **Fazio *et al.* (2013)** and **Bianchi *et al.* (2014)** postulated that the value of the blood component shows the genetic variation and physiological conditions of the fish. The description of blood depends on aquatic biota, species of fish, age, sexual maturity and health status of fish **(Radu *et al.,* 2009; Patriche *et al.,* 2011)**. Blood glucose is an important ingredient for the functioning of specific tissues, including brain, gill, erythrocytes, and gonads **(Polakof *et al.,* 2012)**. Blood glucose in the fish body is a major source of energy and fuel supply as well as an essential substrate for cell metabolism, especially brain cells. Fish have a mechanism to maintain a certain level of blood glucose and each fish has different levels of glucose depending on size and activity **(Driedzic *et al.,* 2013; Soengas, 2014)**. According to **Porchas *et al.* (2009)**, the value of blood glucose can be used as a stress indicator in fish, and blood glucose concentrations can express the general health condition of fish **(Bartoňková *et al.,* 2016)**. **Yasemi and Nikoo (2010)** stated that under stress, an increase in glucocorticoids results in an increase in blood glucose levels to address the high energy requirements needed at times of stress. Conversion of glucose into energy to cope with stress can occur if the glucose in the blood is able to quickly enter the cells. Once inside the cells, glucose is metabolized to meet the body's physiological needs and the energy requirements of the cells. After a high intake of glucose, processes such as glycogenesis and lipogenesis will occur **(Polakof *et al.,* 2012; Kamalam *et al.,* 2016)**.

Hemoglobin (Hb) is one of the protein structures that play a role in the transport of blood oxygen in the fish body **(Rummer & Brauner, 2015)**. Measurements of hemoglobin post-stunt (two, four and six months) showed that hemoglobin levels tended to decrease but the decrease was still within the normal limits of eel fish (5.34, 4.97, and 4.40%). **Mazon *et al.* (2002)**, **Ravichandra (2012)**, and **Cho *et al.* (2015)** stated that the change in hemoglobin above normal limits indicates the fish are in a state of stress. The results of **Fekri *et al.* (2015)** also showed the value of elver hemoglobin is 4.2g of 100 mL^{-1}. **Harianto *et al.* (2014)** stated that the best elver rearing yields hemoglobin content of 4.89g/ 100mL. **Radoslav *et al.* (2013)** stated that the increase in hemoglobin indicates that fish are in a state of stress. **Rummer and Brauner (2011)** stated that physiologically, hemoglobin determines the resilience of the fish body due to its very close relationship with the oxygen binding capacity of the blood, the ability of blood to transport oxygen depends on Hb in the blood. **Wells *et al.* (2005)** stated that 1 gram of hemoglobin can bind approximately 1.34ml of oxygen.

The results of the measurement of hematocrit levels from all three treatments also tended to decrease (11.5, 12.5 and 12.2%). According to **Snieszko *et al.* (1960)**, the normal value of hematocrit in the fish seed is 5-60%. **Harianto *et al.* (2014)** stated that the best rearing elver has a hematocrit content of 16.20%.

The feed used is powder-shaped feed which is processed into dry pellets of 1.5mm that are drowning with protein content of 46%. The feed provided is based on optimum protein requirement of eel that is 40-50% from the total feed intake **(Rovara, 2007)**. Feeding with high protein content will lead to good survival and growth for elver eel when given by optimal amount of feed, but with the introduction of the treatment of feeding at the maintenance level and the use of low maintenance media temperature (24°C) low growth rate (close to zero).

The results of proximate analysis post-stunt of elver showed a decrease in protein content caused by increased protein utilization with the of rearing duration, but the decrease was not significant. This means that the utilization of protein by the body is sufficient to cover energy needs with the feed restriction provided and low temperature during the maintenance. In contrast, carbohydrate levels of the body post-stunt increased, this increase is due to the decrease in protein components of the body during maintenance. The decrease in protein levels is suspected that the protein requirement is not fulfilled so that protein in the body is used to maintain a stable condition (homeostasis).

The results of physical-chemical measurements of water show a range that can still be tolerated by elver: media temperature at 24°C, dissolved oxygen at 5.6-7.5mg L^{-1}, pH at 6.2-6.6 and ammonia at 0.00023-0.00031mg L^{-1}. Water temperatures affect the rate of metabolism, growth **(Lefebvre *et al.*, 2011)**, and feeding activity **(Maltez, 2016)**. Water temperature also affects the solubility of oxygen in the waters. The higher the temperature the lower the oxygen solubility rate **(Kale, 2016)**. According to **Bhatnagar and Devi (2013)**, to increase productivity, the dissolved oxygen content in water should still be 5mg L-1. **EFSA (2009)** states that pH eel rearing should be at neutral values in the range of 6.0-8.0. The ammonia value increases with the duration of the stunting treatment, but is still within the tolerable range. **Yudiarto *et al.* (2012)** state that ammonia concentrations between 1.5-2mg L-1 can still be tolerated by elver. The growing tendency of ammonia levels in media with longer periods of stunting suggests that the longer the incarceration period, the greater the energy shortage so that the fish utilizes larger body proteins for their energy sources. Table (3) shows that the chemical composition of protein content decreases with the length of the stunting period. The reduced body protein levels mean that more body proteins are catabolisir to produce energy, and the resulting ammonia in media tends to increase (Table 4). This refers to

Effendie (2007) that the need for protein is affected by several factors such as fish size, water temperature, feeding rate, feed availability and quality, the energy contained in feed and protein quality. In general, the physical-chemical data of water in media with this stunting treatment is still in the optimal range for survival and growth of elver.

CONCLUSION

Stunting of elver with a body weight of 2-3 g/individual and a body length of 9-12cm, achieved through feed restriction (FR 2%) and the use of a low-temperature medium (24°C), did not cause a decrease in physiological condition or stress. This approach successfully controlled growth (SGR <0.044%) and reduced variation in individual elver size (coefficient of diversity <20%).

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