Physiological study of Indonesian shortfin eel *Anguilla bicolor* on different temperature medium using a recirculation system

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Abstract. The purpose of this research was to determine the physiological responses of the Indonesian shortfin eel *Anguilla bicolor*, which is preserved in the optimum salinity and at different temperatures. It will serve as a reference base for the development of the *A. bicolor* cultivation activities in East Nusa Tenggara, given the elevated market value and the nutritional qualities of the Indonesian eel. The study used a complete randomized design consisting of three treatments and three replications. The treatment given is the optimal salinity with different temperatures in the recirculation system: salinity 5 ppt at 28ºC for treatment A, was salinity 5 ppt at 30ºC for treatment B, and salinity 5 ppt at a temperature of 32ºC for treatment C. The characteristics of the used *A. bicolor* seeds were an initial weight of 3-6 grams with a stocking density of 4 g L^{-1}. Sampling was conducted on the 0, 30th and 60th maintenance days. The results showed that a temperature of 32ºC provided the best physiological response compared to other treatments, nevertheless it gave the lowest survival.

Key Words: temperature effect, growth, blood glucose, oxygen consumption level.

Introduction. Indonesian shortfin eel *Anguilla bicolor* is one of the economically important fish because it has a high selling value. The prices of eel in 2016 and 2017 at the Palabuhan Ratu area at the Sukabumi West Java reached 28.2708 up to 35.3385 USD kg^{-1} for 2 or 3 eel. While the size of *A. bicolor* reached 63.6167 up to 84.8223 USD kg^{-1}, the size of glass eel reached 176.71 up to 247.40 USD kg^{-1}. These price fluctuations depend on the season of abundance (Lukas et al 2017).

Eel have a high nutrition especially EPA and DHA, compared to other fish: EPA 742 mg 100 g^{-1}, DHA 1337 mg 100 g^{-1}, vitamin A 4700 IU 100 g^{-1} in eel meat, and 15.000 IU 100 g^{-1} in the liver, plus vitamins B1, B2, D and E (Pratiwi 1998). This is what determines the high selling price of eel.

The development of eel in Indonesia is still limited to the western part. This is due to the myth that developed in eastern Indonesia regarding fish has caused people to be less interested in developing this fish. In addition, there is a lack of knowledge and skills due to the undeveloped eel cultivation technology in eastern Indonesia. East Nusa Tenggara (ENT) is one of province in eastern Indonesia. ENT has the potential to develop eel. Based on observations in the field and interviews with local residents in several rivers in Kupang city and district, it is known that eels are often captured for food. Stadia eel caught has reached the silver eel. This is very unfortunate considering the long reproduction time of eel fish. Therefore, it is necessary to develop eel cultivation in eastern Indonesia, especially in the city of Kupang.

Temperature and salinity greatly affect the growth and viability of eel fish. Changes in water temperature can affect the metabolism and osmoregulation activity of eel fish, which in the end affects the appetite of fish and their survival. Temperature plays a role in the solubility of different types of gases in the water, as well as all biological activities in the water. A 10°C increase of the temperature will raise the metabolic rate by 2 up to 3 times (Howerton 2001). Edeline et al (2005) stated that at the temperature of 18ºC and salinity 34 ppt provides the highest growth of European eel *Anguilla anguilla*, while Kearney et al (2008) stated that a salinity of 17.5 ppt and a temperature of 17.5ºC provide the highest survival in *Anguilla australis* and *Anguilla dieffenbachii*. Temperature and salinity affect the standard metabolism, food intake, conversion of feed and stimulation of hormones (Boeuf et al 2001). Lukas et al (2017) found the value of salinity and optimum calcium levels on the maintenance of the *A. bicolor* stadium glass eel is 8 ppt with a level of Ca 10 up to 30 mg L^{-1} for an improved growth and viability of *A. bicolor*.

A pathway to improve the performance of eel fish production is through the engineering of the cultivation environment. Maintenance of fish in environmental conditions that are not optimal can increase physiological stress in fish and ultimately reduce fish productivity. Energy efficiency can be done by providing an optimal living environment, in terms of temperature and salinity in the cultivation medium. A recirculation system provides the control of optimal environmental parameters influencing fish growth and health, increases bio-securities, mitigates the water use and other environmental impacts (Summerfelt et al 2008). Through this research the expected growth and viability of the *A. bicolor* can be improved through the manipulation of ambient temperature.

The aim of this research is to determine the optimum temperature for the survival rate of *A. bicolor* and the physiological responses at different temperatures of the *A. bicolor* at the elver stadium.

Material and Method

Time and place. This research was conducted for two months in the recirculation laboratory, Faculty of Marine and Fisheries, Nusa Cendana University, Kupang, East Nusa Tenggara.

Experimental design. This research used a salinity of 5 ppt of with different temperatures. The research design used was a complete randomized design (CRD) with three treatments: (A) temperature of 28ºC, (B) temperature of 30ºC, and (C) temperature of 32ºC, and each treatment had three replications. The *A. bicolor* used originated from Cimandiri estuary, Pelabuhan Ratu, Sukabumi West Java, with weight ranging from 3 to 6 g. Glass eel was transported from a catching area using water with a salinity of 5 ppt. Post-transport, *A. bicolor* samples were maintained in a tank with a salinity of 5 ppt and a temperature of 28ºC for seven days. Full aeration was performed on each experimental unit. The stocking density used was 4 g L^{-1}. Temperature adaptation was done gradually, by increasing the temperature by 1ºC every 6 hours. After reaching the highest temperature (32ºC), the *A. bicolor* samples were stocked into each experimental unit according to the treatment provided. The aquarium used was 30x20x20 cm filled with 20 L of water.

Parameters measured.

- The survival rate (SR) was calculated using the following formula:

$$SR(\%) = \frac{Nt}{No} x100$$

Where:
SR= Survival rate (%)
N_t = Final number of experimental fish (fish)
N_0 = Initial number of experimental fish (fish)

- The specific growth rate (SGR) was calculated based on the following equation:

$$SGR = \frac{LnWt - LnW0}{t} x\ 100\%$$

Where:
SGR = specific growth rate (% day^{-1})
W_t = average biomass of fish at time t (g)

W_0 = average biomass of fish at initial time (g)
t = time of sampling (day)

- The oxygen consumption level (OCL) in standard metabolism was calculated using the equation below:

$$OCL = \frac{V \, x \, (DOo - DOt)}{w \, x \, t}$$

Where:
OCL = oxygen consumption level (mg O_2 g^{-1} $hour^{-1}$)
V = water volume in the tank (L)
DO_o = initial dissolved oxygen concentration (mg L^{-1})
DO_t = final dissolved oxygen concentration (mg L^{-1})
w = weight of experimental fish (g)
t = observation period (hour)

- The coefficient of diversity (CD) was calculated using the equation below (Huisman 1987):

$$CD = \frac{\text{Standard deviation}}{\text{Average of sample}} \times 100\%$$

- Blood glucose levels (BGL) were calculated based on the work of Wedemeyer & Yasutake (1977).
- Physical and chemical parameters of water were temperature measured using thermometer, pH measured using pH-meter, DO measured using DO-meter, ammonia measured using spectrophotometer.

Statistical analysis. The data obtained in this study was analyzed using the variance of analysis (ANOVA). If case of significant differences, Duncan's further test was carried out. Water physics and chemistry data was analyzed descriptively and presented in table form.

Results. The results showed that the highest survival rate was shown in treatment A, namely the 5 ppt salinity with a temperature of 28ºC at 94.49±5.79% and the lowest at treatment C, at 5 ppt salinity at 32ºC at 77.45±16, 28%, but statistically there were no significant differences between the treatments (P>0.05). The survival graph for each treatment during the study is shown in Figure 1.

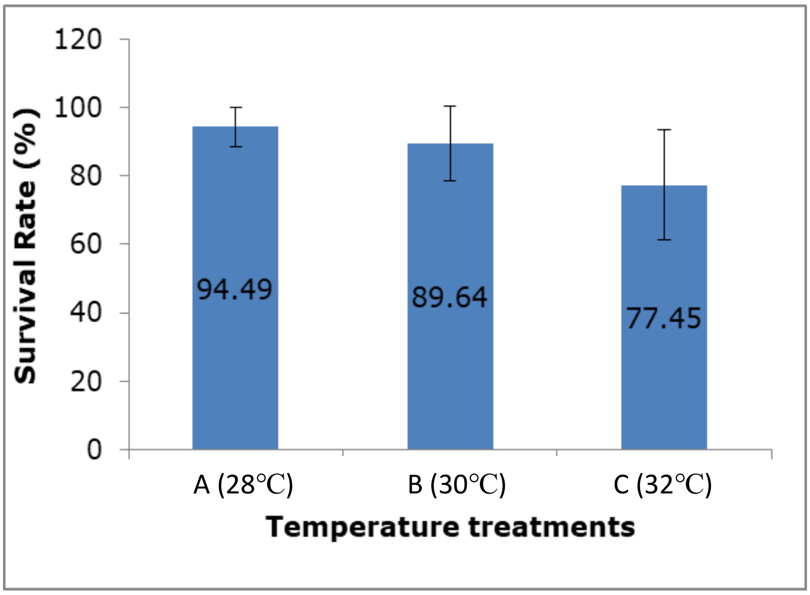

Figure 1. Average survival rate of *Anguilla bicolor* elver for 60 days of maintenance.

The physiological response shown by *A. bicolor* elver in the form of absolute growth (W), blood glucose levels (BGL), coefficient of diversity (CD) and oxygen consumption level (OCL) are presented in Table 1, while the growth pattern for 60 days of maintenance is shown in Figure 2.

Table 1

The physiological response of *Anguilla bicolor* elver at the end of maintenance

Parameters	*Treatments*		
	A (28ºC)	*B (30ºC)*	*C (32ºC)*
W(g)	2.00±0.68[a]	2.49±0.20[bc]	3.83±1.28[c]
BGL (mg dL^{-1})	54.5±4.27[b]	55.00±2.83[b]	42.5±3.53[a]
CD (%)	24.42±3.89[b]	22.12±4.48[a]	21.90±5.63[a]
OCL (mg O_2 g^{-1} $hour^{-1}$)	0.11±0.04[a]	0.09±0.01[a]	0.06±0.01[a]

Statistical analysis showed that absolute growth was significantly different between treatments (P<0.05) with the highest absolute growth shown in treatment C followed by treatment B and the lowest in treatment A. This indicates that the temperature greatly influences the growth rate of *A. bicolor* elver at optimal salinity.

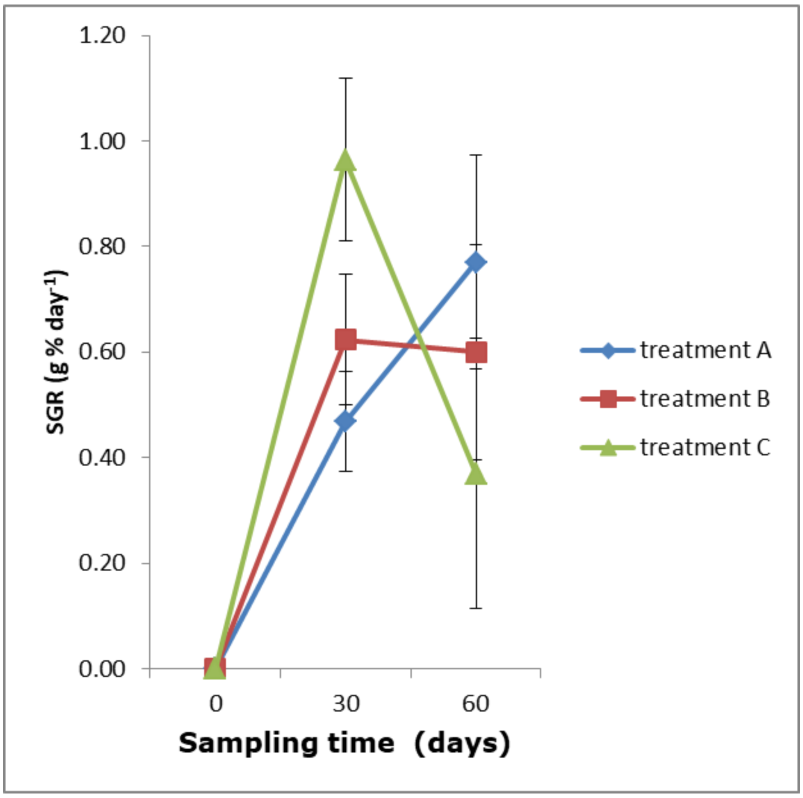

Figure 2. Growth pattern of *Anguilla bicolor* for 60 days of maintenance in each treatment.

Oxygen consumption level (OCL) of *A. bicolor* is showed in Figure 3. Statistical analysis showed that the OCL was not significantly different between the treatments (P<0.05).

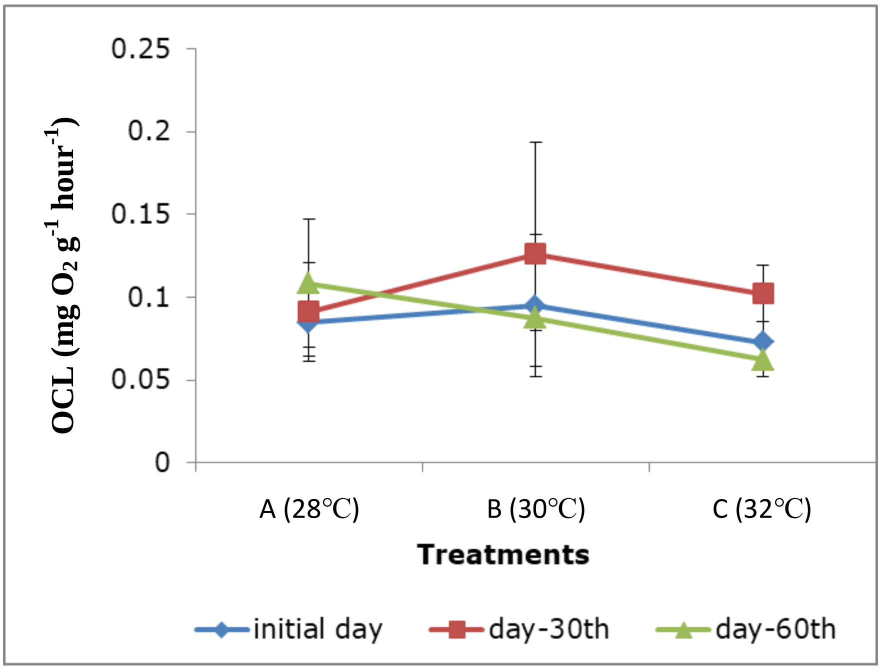

Figure 3. Pattern of *Anguilla bicolor* elver oxygen consumption level during maintenance.

Figure 4 shows a pattern of blood glucose levels (BGL) for 60 days of maintenance. The 30th day of maintenance, the blood glucose level in treatment A was higher than treatment B and C. The overall BGL continued to decline until the 60th day. This is thought to be due to the *A. bicolor* elver adaptation capabilities to the cultivation environment. However, a 32ºC temperature provides the most comfortable environment for *A. bicolor*, thus suppressing the stress level of *A. bicolor* elver in sizes 5 g-12 g.

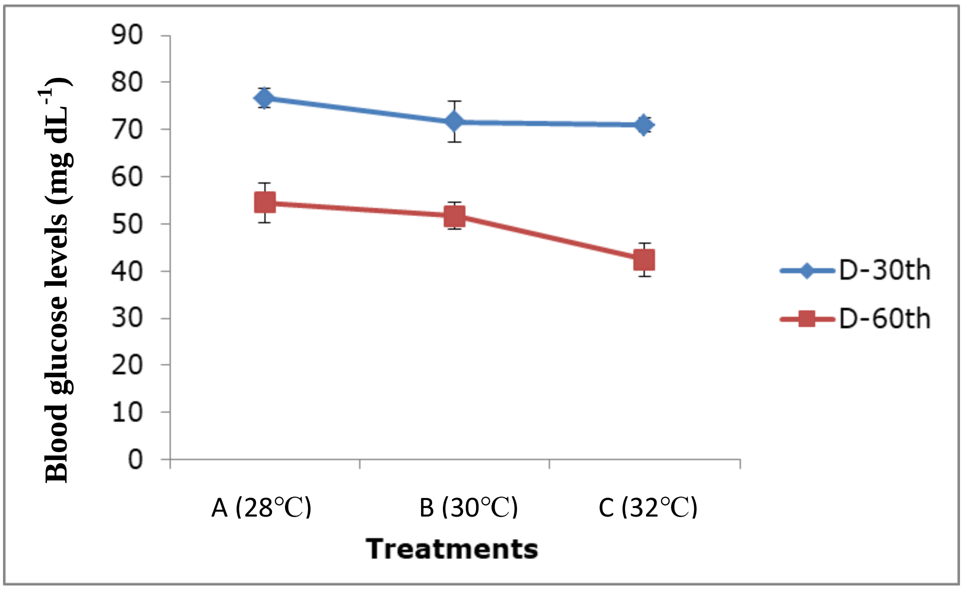

Figure 4. Pattern of *Anguilla bicolor* elver stress response during maintenance through blood glucose levels.

Figure 5 shows a pattern of coefficient of diversity (CD) for 60 days of maintenance. The initial day to 30th day of maintenance in treatment B was lower than treatment A and C, but in the 60th day the CD of treatment B and C were the same. A low value of the coefficient of diversity indicates that fish appetite is almost uniform.

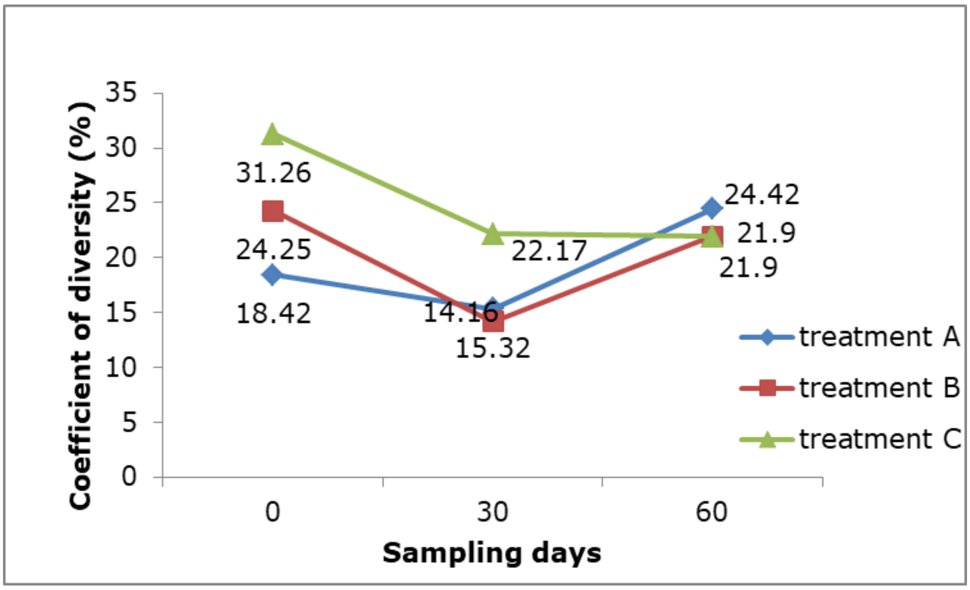

Figure 5. The pattern of *Anguilla bicolor* coefficients of diversity shown for 60 days of maintenance.

The physical chemistry parameters of water in the form of temperature, pH, dissolved oxygen (DO), and ammonia levels during the maintenance period are presented in Table 2.

Table 2

Water quality for 60 days of maintenance

Parameters	*Treatments*			*Optimum range*
	A (28ºC)	*B (30ºC)*	*C (32ºC)*	
Temperature (ºC)	28±0.38	30±0.31	32±0.63	23-32 (Usui 1974)
DO (mg L^{-1})	5.2–6.8	5.9–6.8	6.0–6.6	>3 (Bieniarz et al 2000)
pH	6.7–7.9	6.2–7.9	7.0–8.0	6.0-8.0 (Ritonga 2014)
Ammonia (mg L^{-1})	0.001-0.016	0.002-0.075	0.007-0.057	0.1 (Yamagata & Niwa 1982)

The range of physical chemistry of water shown in Table 2 in the form of temperature, dissolved oxygen, pH, and NH_3 for 60 days of maintenance is still within the range of tolerance for the survival and growth of *A. bicolor*. Feasibility of physical chemical parameters of water during this study caused the survival rate of *A. bicolor* to range between 77.45-94.49%.

Discussion. The mortality that occurred during 60 days of maintenance of *A. bicolor* was thought to be caused by a competition in the use of oxygen and space. The larger the size of the eel, the more oxygen it needs, also requiring more space for movement. Data showed that for 60 days of maintenance there was a change in the size of the *A. bicolor* body weight in each treatment, with the highest growth response occurring in treatment C (Table 1). A faster growth of *A. bicolor* reduces the survival rate in correlation with the movement space and oxygen demand. This is consistent with Hepher & Pruginin (1981), who stated that a lack of oxygen can cause death, especially in small-sized fish. Treatment C gave the highest absolute growth, 3.83 ± 1.28^{c}, but also the lowest survival rate 77.45%. Wedemeyer (1996) stated that increasing body size affects stocking density, which has a negative impact on the fish physiological processes and behavior, like foraging and food intake, affecting their health, growth rate and eventually causing their survival rate decline.

Salinity and temperature affect the survival rate and specific growth rates of *A. australis*, *A. dieffenbachii* and *A. anguilla* (Edeline et al 2005; Kearney et al 2008). Temperature affects nutritional preferences in eel. Sun et al (2014) found that temperature had a significant effect on the feed consumption, faeces production, nitrogen excretion, energy use, growth rate and metabolic rate of Cobia fish (*Rachycentron canadum*). Optimal temperatures accelerated the growth.

The results showed that treatment C, at 32ºC, had the highest physiological impact, influencing the growth, namely absolute weight and coefficient of diversity, and the stress response related to the blood glucose level (BGL) and to the oxygen consumption level (OCL). Although it generated the lowest survival rate, economically it still provides greater benefits compared to other treatments. Viadero (2005) states that water temperature is one of the most important physical factors that affect fish growth and productivity because fish are cold-blooded animals whose body temperature follows the temperature of the environment (poikilothermal). Furthermore, it is explained that temperature deviations from optimal values have a negative impact on growth and productivity as well as on the survival rate of eel.

The growth pattern of *A. bicolor* for 60 days of maintenance showed that on day 0 to day 30 the highest growth rate occurred in treatment C (at 32ºC) and the lowest was in treatment A, at 28ºC. On the contrary for the maintenance between the 30th day to the 60th day, the growth pattern shown was inversely proportional. This is because the increasing bodyweight of *A. bicolor* causes a lower growth rate. Kono & Nose (1971); Gwither & Grove (1981) stated that the smaller the size of the fish is, the faster the appetite increases, due to a higher rate of emptying of the stomach. Fish size affects the frequency of feeding NRC (1977) and Hickling (1971).

The decrease in the level of oxygen consumption due to the increase of the body weight of *A. bicolor* elver explains that the fish are in a comfortable condition stimulating the appetite and maintaining the use of oxygen at a low level. The low value of oxygen consumption of *A. bicolor* elver is an indicator that the metabolic activity of *A. bicolor* runs well. In accordance with Garcia et al (2006), which stated that the metabolic rate of aquatic organisms can be determined from the oxygen consumption level (OCL) of the organism. As a result, the energy is used less for the metabolic activity and more for growth. In accordance with Linder (1991), which stated that energy in feed is physiologically used for metabolism and maintenance of the body, the remainder will be deposited into the body tissues in the process of growth and synthesis of reproductive product.

Caspers (1983) stated that changes in temperature are one of the factors causing stress in fish. The results showed that *A. bicolor* elver was in a comfortable condition, despite an increase in blood glucose levels with increasing body weight, but still within the range of normal blood glucose levels. Increased glucose levels in the blood are caused by an increase in catabolic activity in the body, namely changes in glycogen to glucose as a result of increased metabolic activity of the body. When changes in the environment become uncomfortable conditions, metabolic activity will increase. An indicator of stress in fish is the increase of the blood glucose levels. Stress conditions in fish causes a decrease in blood volume, leukocyte count and liver glycogen and an increase in blood glucose levels (Affandi & Tang 2002).

The coefficient of diversity (CD) is a description of the diversity in a population in an experiment (Hanafiah 2002). The initial maintenance weight size has a diversity coefficient of 18.84±10.69 for treatment A, 24.24±8.19 for treatment B and 31.26±7.14 for treatment C. The high value of the coefficient of diversity at the beginning of maintenance is due to the heterogeneity of the eel seeds used, of natural origins. During the maintenance period, the body size changes both in weight and length of *A. bicolor* elver, which modifies the coefficient of diversity value. The three treatments showed a decrease in the value of the coefficient of diversity on the 30th day, but the lowest decrease in the CD value occurred in treatment A while treatment B and C showed a significant pattern of decreasing CD value. This is probably due to the increase in appetite between the 1st and the 30th day of the treatment B and C caused by the near optimum temperature value of the media the use of the incoming energy for growth than for the metabolism. The increase in CD value occurs on the 30th day until the 60th day of maintenance. This is likely due to an increase in the body weight while the maintenance container is of a fixed size so that it reduces the movement space and the oxygen demand of *A. bicolor* which has an impact on the survival rate. In addition, eel is territorial with large size specimens monopolizing the available space and feed, while the smaller sized *A. bicolor* cannot utilize feed optimally. The results showed an increase in the value of CD which was significant in treatments A and B.

The *A. bicolor* elver used in the research is heterogeneous with seeds collected from their natural environment. According to Hanafiah (2002), the coefficient of diversity is 20-25% in the medium category in heterogeneous conditions. The results showed that the coefficient of diversity at the end of maintenance in all treatments, ranging between 20% and 25%, suggests that the temperature treatment of 28 to 32ºC provides good feed to biomass productivity and efficiency during the maintenance period.

Conclusion. According to our study, the optimal temperature for increasing the survival of *A. bicolor* elver is 28ºC, but it provides the lowest absolute growth and physiological responses. The optimal temperature for the maintenance of *A. bicolor* measuring 3-6 grams is 32ºC providing the best physiological response, followed by the treatments with temperatures of 30ºC and 28ºC, respectively. Increasing the growth of Indonesian shortfin eel *A. bicolor* can be done by manipulating the temperature of the maintenance medium.

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