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To cite this article: N Taufiq-Spj *et al* 2017 *IOP Conf. Ser.: Earth Environ. Sci.* **55** 012015

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# The Use of Water Exchange for Feeding Rate and Growth Promotion of Shortfin Eel *Anguilla bicolor bicolor* In Recirculating Water System

N Taufiq-Spj<sup>1,2,3</sup>, S Sunaryo<sup>1</sup>, A Wirasatriya<sup>1,2</sup>, and D N Sugianto<sup>1,2</sup>.

<sup>1</sup> Fac. of Fisheries & Mar. Scie., Diponegoro Univ. Semarang, Cent. Java, Indonesia.

<sup>2</sup> Member of Center for Coastal Disaster Mitigation and Rehabilitation Studies, Center of an Excellence Research University, Diponegoro Univ. Semarang, Cent. Java, Indonesia.

<sup>3</sup> E-mail: taufiqspj\_1999@yahoo.com

**Abstract.** The shortage of eel's seed *Anguilla* spp. and it's consumption size will induce the hunting of this species. Thus, stocks are critically endangered. Central Java have two indigenous species i.e. *A. marmorata* and *A. bicolor bicolor*. These two species will be endanger if management of stock do not conduct properly. The use of water exchange by replacing water volume for feeding rate and growth promotion of Indonesian shortfin eel *A. bicolor bicolor* in RWS will at least give a basic information in appropriate aquaculture systems and as a part of disaster mitigation of indogenous species extinction. The study used 3 replications and 3 treatments of water exchange, i.e. T<sub>1</sub>: 50, T<sub>2</sub>: 100, and T<sub>3</sub>: 200 % of water removed d<sup>-1</sup>. The fish were fed with approx. 2.2% dry basis of formulated feed. Tanks with volume of 1 m<sup>3</sup> contain a density of approx. 20 kg seed stock of shortfin eel fingerlink size per m<sup>3</sup> water in RSW. The result shows that feeding rate among treatments are significantly different ( $\alpha < 0.001$ ), where T<sub>1</sub>= 1.62±0.23% d<sup>-1</sup>, T<sub>2</sub>= 1.89±0.06% d<sup>-1</sup> and T<sub>3</sub>= 1.99±0.06% d<sup>-1</sup>. Individual and Biomass weight gain increase (IWG: T<sub>1</sub>= 0.039±0.003, T<sub>2</sub>= 0.050±0.001, T<sub>3</sub>= 0.076±0.002 kg and BWG: T<sub>1</sub>= 7.087±0.443, T<sub>2</sub>= 11.051±0.894, T<sub>3</sub>= 19.722±0.831 kg respectively) by increasing water exchange ( $\alpha < 0.001$ ). Meanwhile the absolute growth was found higher in individual (IAG) compare to biomass (BAG) and both of them increase by increasing water exchange ( $\alpha < 0.001$ ). Where for IAG: T<sub>1</sub>= 55.42±3.69, T<sub>2</sub>= 71.02±2.40, T<sub>3</sub>= 106.12±1.98% and BAG: T<sub>1</sub>= 35.11±1.13, T<sub>2</sub>= 52.94±6.17, T<sub>3</sub>= 90.77±3.14% respectively.

**Key words:** Eel, *Anguilla bicolor bicolor*, water exchange, feeding rate, growth promotion, mitigation.

## 1. Introduction

Eels, *Anguilla* sp. is a catadromous species and a popular fish for the worldwide dishes known as *unagi*, *kabayaki* and *unadon* in Japan, jellied eel in England. Some dishes of *sidat* (*panggang* and *bothok*) in Indonesia, dishes of elver or smoked eel also found in some countries of Europe and Australia. European eel *Anguilla anguilla* as well as Canadian/American eel *A. rostrata*, and Japanese eel *A. japonica* stocks are critically endangered [1,2]. This condition will push the eel traders to hunt the seed or consumption size in any places of countries. Whilst the Indonesian eels, *A. marmorata*, *A. celebensis*, *A. ancestralis*, *A. borneensis*, *A. bicolor bicolor*, and *A. bicolor pacifica* [3,4], are in a condition of disaster mitigation especially in stock and their population [5,6].



The shortage of eel’s seed in Japan caused tracking and hunting seed to Canada, US, Indonesia, Phillipines, and Malay. The price of eel’s seed (glass eel) increases very steeply up to US\$ 2,600 per pound of eel and known as “gold rush” [7]. Moreover, the price of Indonesian glass eel in fisherman firsthand just only US\$ 150 to 220 per kilogram (in 2016). However, this condition will also induce big hunting for this species. Hence, the population of these species (Central Java have only two sepecies of *A. marmorata* and *A. bicolor bicolor*) now is decreasing due to rush hunting and improper environment especially at *Bendung* (dam) Gerak Banyumas and *Bendung* (dam) Menganti, Cilacap – Central Java (Muslim, pers. com., 2016). This condition requires mitigation and rehabilitation of the species especially in lower and mid estuaries before extinction disaster occur. The statistical fisheries of Indonesian long and shortfin eels unfortunately were not officially recorded.

From 2009 to 2011 the activity of fisherman in catching glass eel was going down after the application of government regulation (No PER 18/MEN/2009) about the banning of eel seeds export decreased the activity of glass eel hunting during that time. In 2012 there are a few culture in this species and become bigger in the following years. The culture techniques in the society nowadays slightly develop and become a strong desire during 2015. This is due to the increase demand of eels in Indonesian market. Nevertheless, culture systems which is used by society commonly still use traditional earthen impounding water system. For this reasons, the study on manipulating water exchange will hopefully improve the feeding rate of organism as well as weight gain.

Eel farming, which is responsible for over 90% of all *Anguilla* production worldwide, is reliant on growing out wild-caught juvenile eels (glass eels or eel fry). Historically, farming and trade in East Asia involved the Japanese eel *A. japonica*, native to the region; from the 1990s large quantities of European eel *A. anguilla* glass eels were also imported, due to decreasing availability of *A. japonica* (Shiraishi and Crook, 2015). International trade caused *A. Anguilla* to be listed in the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora in 2007. In addition, the European Union banned all trade in *A. anguilla* from the EU in 2010. As a consequence, the Americas and South-East Asia have become increasingly important sources of juvenile eels of other *Anguilla* spp. for farms in East Asia [8]. The aims of this study were to evaluate water exchange for feeding rate and growth performance (i.e. absolute growth and weight gain) as well as to compare effectiveness of water coloumn interm of biomass density.

## 2. Methodology

Big pencil sizes (elver) of eel *A. bicolor bicolor* were obtained from seed collector farm in Purwokerto (Central Java). Biomass of fish seeds (approx. 20 kg) had been distributed to every tank with 1 m<sup>3</sup> water volume, hence various masses will slightly differ among the tanks. Completely random design and analysis co-variance were used with 3 treatments and 3 replications. In this basic recirculating system [9] nine tanks of high density poly ethylene (hdpe), 1 water reservoir, single biofilter and single patch to collect water were used in every treatment. In order to recirculate water, submerged pump was also used in every group of treatment. In term of water exchange from the tank, additional fresh water of 50, 100 and 200 % d<sup>-1</sup> of water replacement was applied in recirculating water system. The fresh water was flown through perforated hose with valve for adjusting water volume. A mixture of commercial feeds (Table 1) was given by 2.2 % d<sup>-1</sup> dry basis and given twice a day in the morning (06.00 a.m) and before dark (05.00 pm), uneaten food was collected and weighted after two hours feeding. This feeding percentage is mainly due to initial study that maximum feeding rate never attain 2.2 % and as a concern to save the environment.

**Table 1.** Feeds proximate analysis given to fish

Feeds	Water (%)	Ash (%)	Protein (%N)	Lipid (%)
1 (50%)	10,51	13,18	39,67	12,17
2 (50%)	9,25	10,98	27,71	12,04

Estimated marginal mean was used in Feeding Rate, while individual and biomass growth were analyzed by using generalized linear model repeated measurement ANOVA and post hoc test using multiple comparison based on observed means. Feeding Rate (%) were calculated by using this formula, i.e. amount of food given per day minus uneaten food divided by fish biomass in every tank. Absolute growth of individual and biomass of fish per tank in group of treatments (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>) was calculated by the difference of weight at “t” time and initial weight divided by initial weight in percent. Water temperature, pH and dissolve oxygen were recorded every second day, while salinity, NH<sub>3</sub>, NO<sub>2</sub><sup>-</sup> and alkalinity measured in every 20 days. As an addition, in order to evaluate water coloum efficiency, biomass density l<sup>-1</sup> was calculated by amount of biomass in the end of measurement divided by water volume in the captive tank.

### 3. Result and Discussion

*Anguilla* sp. is a taft organism, they can survive even captive in a limited water condition. The results of this study show that fish culture was commonly found healthy in every tank of treatment, fish behaviour look active in T<sub>1</sub> (50%) and T<sub>2</sub> (100%), and look very active in T<sub>3</sub> (200% water exchange). Daily feeding, absolute growth both of individual and biomass of *A. bicolor bicolor* generally increase due to increasing water exchange. Water quality seems to show in normal range of temperatures, pH, salinity, and alkalinity, but increased consistenly in NH<sub>3</sub> and NO<sub>2</sub><sup>-</sup> concentration during 100 days culture.

#### 3.1. Feeding Rate

The given paste feed assembled with fish biomass (approx 2.2% of body weight) in the tanks, uneaten food was decreased by increasing water exchange and feeding rate is increased by increasing water exchange. The increase of feed rate shows significant value on the time series of observation ( $\alpha = 0 < 0.01$ ) in 100 days culture (Table 2 and Fig. 1). The influencing factors for feeding rate are food and feeding habit. Concerning food habit, it will be difficult to distinguish the treatments due to the feed given used simply from similar sources, form, and type of food.

Nevertheless, from the factor of feeding habit, differentiation among treatments can be seen from the environmental exchange. These conditions show that increasing the water exchange can reduce uneaten food. Hence water qualities in the rearing tank were better in higher water exchange (Table 3). Eels living in a better environment took more food for their consumption (Table 2). Moreover in a high food consumption, it will also leave uneaten food and faeces, and will induce deterioration of organic matter, especially in NH<sub>3</sub> as a results of protein degradation (Table 3).

Table 2. Mean of Feeding Rate (% d<sup>-1</sup>) of eel during 100 days observation

Treat- ments	Days of observations					Mean	±SD
	01-20	21-40	41-60	61-80	81-100		
T <sub>1</sub>	1.30	1.75	1.54	1.92	1.57	1.62	0.23%
T <sub>2</sub>	1.79	1.90	1.89	1.91	1.96	1.89	0.06%
T <sub>3</sub>	1.93	2.07	1.93	1.98	2.04	1.99	0.06%

Table 2. show that T<sub>3</sub> with 200% of water exchange gave best value of FR (1.99±0.06% d<sup>-1</sup>) and followed by T<sub>2</sub> (1.89±0.06% d<sup>-1</sup>) and T<sub>1</sub> (1.62±0.23% d<sup>-1</sup>). These conditions were also indicated by post hoc test base on observed means, where the mean different of FR are significantly different at 0.05 level or 95% confidence interval. Mean of feeding rate during 100 days observation increased by increasing the water exchange, but as seen from the rhythm for all treatments, the feed rate seems to be fluctuates (Table 2).

This fluctuation of feeding rate may correspond with water quality measured, where at treatment with 50 % water exchange (T<sub>1</sub>), temperature had highest in fluctuation ( $\pm$ SD = 1.06°C), lowest dissolve oxygen, highest in NH<sub>3</sub> and NO<sub>2</sub><sup>-</sup> concentrations compare to T<sub>2</sub> and T<sub>3</sub> (Table 3). In term of water temperature, [10] used different temperatures for racing *A. marmorata* and *A. bicolor pacifica* in recirculating water system. A better FR for *A. bicolor pacifica* found was 2.41 $\pm$ 0.04% day<sup>-1</sup> and for *A. marmorata* (2.23 $\pm$ 0.10% day<sup>-1</sup>) occurred at 33°C, while temperature at 28°C stated have lower FR.

**Table 3.** Water quality (Mean  $\pm$ SD) measured in every 20 days during 100 days culture system.

Treatments		T°C	S‰	pH	O <sub>2</sub>	NH <sub>3</sub>	NO <sub>2</sub> <sup>-</sup>	Alkalinity
T <sub>1</sub>	Mean	28.93	1.27	7.43	3.73	0.0089	0.0078	66.17
	$\pm$ SD	1.06	0.06	0.06	0.15	0.0028	0.0025	6.79
T <sub>2</sub>	Mean	29.53	1.27	7.47	4.40	0.0088	0.0078	70.33
	$\pm$ SD	0.36	0.06	0.06	0.10	0.0016	0.0014	6.43
T <sub>3</sub>	Mean	29.63	1.13	7.64	4.70	0.0071	0.0062	82.00
	$\pm$ SD	0.26	0.06	0.05	0.10	0.0007	0.0006	1.00

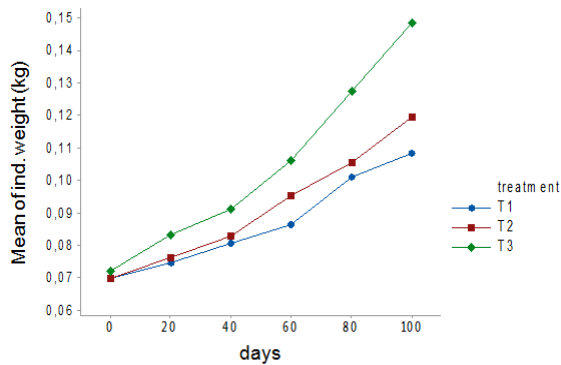
### 3.2. Growth performance

#### 3.2.1. Absolute growth

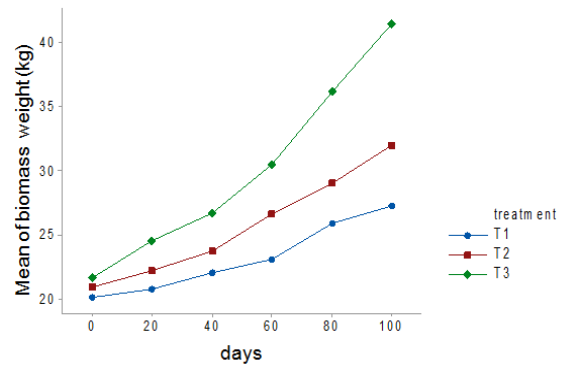
Absolute growth is normally used by farmers in order to get figure whether the culture had been efficiently conducted or not. During 100 days observation the cultured organisms have grown significantly different either individually or biomass by the time. ANOVA analysis of those growth measured during 6 times observation the grown show significantly different by the time ( $\alpha = 0 < 0.01$ ). Figure 2 and 3 show that the treatment 3 (T<sub>3</sub>) gave the best growth either in individual and biomass of fish followed by T<sub>2</sub> and T<sub>1</sub>. The growth pattern of both individual and biomass seems to be similar. When continued to conduct post hoc test, the different of individual and biomass mean weight indicated significantly difference ( $\alpha = 0 < 0.01$ ). These growth performance seems related to the feeding rate above, the highest feeding rate gave the best growth, whilst, the lowest feeding rate followed by low growth of *A. bicolor bicolor*.

Relationship between feeding rate, growth and FCR was described by [11]. They reported that, there is a rapid increase in growth rate when the feeding rate is over maintenance requirement since the energy consumed equals the energy required by fish to maintain itself without mobilizing endogenous energy reserves. Then, growth increases at a decreasing rate to a maximum point of feeding rate. Moreover, FCR decreases as the feeding rate increases towards to the maximum rate. However, this level varies according to the fish species, water temperature and fish size [11].

In holding *A. bicolor bicolor* in this study, the rearing tank with lower water exchange caused rapid exchange of water qualities, hence organism that live in this habitat required a higher adaptation to its environment. Lower water exchange almost provided high NH<sub>3</sub> and NO<sub>2</sub><sup>-</sup> and became consistently bigger during 100 days observation. Alkalinity will be related to hydrogen ion concentration (pH), and the pH will affect to NH<sub>3</sub> and NO<sub>2</sub><sup>-</sup> concentration. The pH concentrations was between 7.43 to 7.64, these correspond with [12] stating that *Nitrosomonas europaea* can grow better in pH between 7 to 8. Nevertheless, increasing NH<sub>3</sub> and NO<sub>2</sub><sup>-</sup> along the culture, can be related to dissolve oxygen which is concerned to have relatively low concentration (3.73 to 4.70 ppm) (Table 3).



**Figure 1.** Growth performance in weight (kg) (individual absolute growth) of fish (*A. bicolor bicolor*) measured in every 20 days during 100 days culture in different volumes of water exchange.



**Figure 2.** Growth performance in weight (kg) (Biomass absolute growth) of fish (*A. bicolor bicolor*) measured in every 20 days during 100 days culture in different volumes of water exchange.

This adaptation processes require high energy of captive fish. Protein is sparing effect of lipid, if energy in the feed for organism is inadequate to be used for environmental adaptation. Hence protein energy will be used for adaptation, not for growth. Hereafter the graph of feeding rate looks fluctuate inconsistently especially for T<sub>1</sub> of 50% water exchange d<sup>-1</sup>(Table 2) compared to growth performance (on Figure 1 & 2).

Low growth in T<sub>1</sub> occurred during the first 40 days culture and start to grow exponentially after 60 days culture (Figure 1 & 2). These correspond to stress during intial culture where the death rate was bigger than T<sub>2</sub> and T<sub>3</sub> and in fact that initial weight was smaller than T<sub>2</sub> and T<sub>3</sub> (Table 3.). The eels seem to face in a new environment and have to adapt with different water exchange, where the previous environment of eels were captive in earthen pond with quite enough running water of water exchange. The initial decrease in weight of *A. anguilla* has previously been observed by [13], and can be regarded as a common phenomenon caused by the time needed for the eels to complete their metamorphosis and to adapt to their new environment. [14] describes an initial reduction in length and weight of European glass eels in aquaria, fed or unfed, while pigmentation advances. These explanation may correspond with decreasing weight in one of replication tank of T<sub>1</sub> and T<sub>2</sub> in the early culture of 20 days. On the other hand, T<sub>1</sub> has water temperature mean of 28.93°C with slightly higher in ±SD (1.06°C) than others (Table 3).

This temperature fluctuation will lead to unstable metabolic rate of aquaculture organisms. The organism will take more energy and oxygen consumption as the water temperature increases [15]. For this reason, mean values of dissolve oxygen in T<sub>1</sub> almost lower compare to T<sub>2</sub> and T<sub>3</sub>. Since *A. marmorata* and *A. bicolor pacifica* are tropical and subtropical species, they can tolerate higher water temperatures [10], while some of Indonesian shortfin eel can tolerate to 41°C temperature [16]. The optimal growth temperatures of the juveniles of *A. bicolor pacifica* and *A. marmorata* are estimated to be 30.1°C and 27.6°C respectively [10]. On the other hand, *A. Anguilla* entered a state of torpor at water temperatures of 1°C and 3°C when fish acclimated at 23°C and 29°C respectively while temperature decreases 1°C every 10 min., *A. rostrata* could still survive when acclimated at ≤ 5°C for more than five weeks though the fish ceased feeding and displayed a dramatic decreased in oxygen consumption rates [17, 18]. In this temperature fluctuation perspective, *A. bicolor bicolor* still has a normal feeding habit while some time protruding their head onto water surface may due to limited dissolve oxygen.

In calculation during 100 days culture, Table 4 shows that individual absolute growth (IAG) consistently higher in every treatment than biomass (BAG), while both of them increase by increasing water exchange. Comparison among treatments have signficantly different either individual or

biomass ( $\alpha = 0 < 0.01$ ). The best percent IAG attain in  $T_3 = 106.12 \pm 1.98\%$  and BAG also in  $T_3 = 90.77 \pm 3.14\%$  during 100 days culture (Table 5). [10] used 45 days culture of two species, with initial and final mean of body weights of  $5.62 \pm 0.30$  g and  $11.59 \pm 0.03$  g for *A. bicolor pasifica* and  $4.97 \pm 0.36$  g and  $7.18 \pm 0.80$  g for *A. marmorata* at 28°C, hence the BAG are approx. 106,2% and approx. 44,5 % respectively. Moreover, Angelidis *et al.* (2005) using *A. anguilla* have mean of daily absolute growth 5.5 % observed between 76<sup>th</sup> and 105<sup>th</sup> day of culture, and decreased to 2.9 % observed during 52 days latter. For these facts, the individual absolute growth of *A. bicolor bicolor* is quite similar to *A. bicolor pasifica*, much higher than *A. Marmorata* [10] and much lower than *A. anguilla* [19].

### 3.2.2. Weight gain

The weight gain of *A. bicolor bicolor* increased by increasing water exchange ( $\alpha = 0 < 0.01$ ) either individually or in biomass after cultured during 100 days. Mean of individual weight gain in  $T_1$  indicate only has  $0.039 \pm 0.003$ ,  $T_2 = 0.050 \pm 0.001$ , and  $T_3 = 0.076 \pm 0.001$  kg. Mean while the biomass gain shows  $7.087 \pm 0.443$ ,  $11.051 \pm 0.894$ , and  $19.722 \pm 0.831$  kg for  $T_1$ ,  $T_2$ , and  $T_3$  respectively (Table 4). It means that IWG of of this study equal to 0.39, 0.50, and 0.76 g d<sup>-1</sup> in  $T_1$ ,  $T_2$ , and  $T_3$  respectively (calculated from Table 4).

**Table 4.** Individual and biomass weight gain (IWG & BWG in kg), individual and biomass absolute growth (IAG & BAG in %) of *A. bicolor bicolor* race during 100 days in recirculating water system (where:  $I_{0..100}$  : Individual weight of fish at day 0 to 100,  $B_{0..100}$ : biomass weight of fish in tank at day 0 to 100)

Treat-ments	$I_0$ (kg)	$I_{100}$ (kg)	IWG (kg)	IAG (%)	$B_0$ (kg)	$B_{100}$ (kg)	BWG (kg)	BAG (%)
$T_{1.1}$	0.070	0.112	0.041	58.77	20.877	28.475	7.598	36.40
$T_{1.2}$	0.070	0.107	0.036	51.46	19.858	26.667	6.809	34.29
$T_{1.3}$	0.069	0.107	0.038	56.04	19.789	26.643	6.854	34.64
Mean±SD			$0.039 \pm 0.003$	$55.42 \pm 3.69$			$7.087 \pm 0.443$	$35.11 \pm 1.13$
$T_{2.1}$	0.069	0.119	0.050	73.09	20.479	32.130	11.651	56.89
$T_{2.2}$	0.071	0.119	0.048	68.40	21.869	31.892	10.023	45.83
$T_{2.3}$	0.071	0.121	0.050	71.59	20.465	31.944	11.479	56.09
Mean±SD			$0.050 \pm 0.001$	$71.02 \pm 2.40$			$11.051 \pm 0.894$	$52.94 \pm 6.17$
$T_{3.1}$	0.072	0.148	0.076	104.67	21.672	41.452	19.779	91.27
$T_{3.2}$	0.073	0.152	0.079	108.37	21.917	42.439	20.522	93.63
$T_{3.3}$	0.071	0.145	0.075	105.32	21.580	40.444	18.864	87.41
Mean±SD			$0.076 \pm 0.002$	$106.12 \pm 1.98$			$19.722 \pm 0.831$	$90.77 \pm 3.14$

*A. bicolor pasifica* can attain DWG of  $2.63 \pm 0.12$  g d<sup>-1</sup>, while  $1.19 \pm 0.38$  g d<sup>-1</sup> for *A. marmorata* which both achieved at 28°C of captive water [10]. Nevertheless, [20] reported that European eel *Anguilla anguilla* fed with different types of food i.e. trash fish (TF), slaughterhouse waste (SW), pelleted trout feed (PF), and combination of them (TF+PF and SW+PF) only have daily growth of  $0.21 \pm 0.01$ ,  $0.32 \pm 0.03$ ,  $0.29 \pm 0.01$ ,  $0.30 \pm 0.17$ , and  $0.0.36 \pm 0.23$  g respectively. [21] use different sources of diet, diet from fish (CB) source seems to be effective to give better weight gain instead of combination between fish and beef tallow (BT) or fish with soybean (SB). The results show that the CB give 42% absolute growth, while BT and SB only 37 and 30 % respectively. [22] conducted an eel farm in recirculating water system, the 20,000 elvers of *A. anguilla* with mean initial weight of 5.89g

(total weight 117.8 kg) produced a total weight of 478.8 kg after 335 days of farming with mortality 27.1%. It means that the biomass weight gain only attain 361 kg for almost one year with 14,580 elver survive. Hence individual weight gain only 24.76 g or additional growth 0.074 g d<sup>-1</sup>. In this study daily weight gain seems to have lower result than [10], but much better results than [20] and much better than [22].

Nevertheless, further comparison of the results conducted by [10], they used *A. marmorata* with individual initial weight range 4.97±0.36 g, *A. bicolor pasifica* 5.62±0.30 g and stock with 20 eels in each tank of 105 l water volume. This means that [10] used initial biomass density 0.95 g l<sup>-1</sup> for *A. marmorata*, and 1.07 g l<sup>-1</sup> for *A. bicolor pasifica*, whilst for this study used *A. bicolor bicolor* with initial biomass density approx. 21.72 g l<sup>-1</sup>. For this fact, the initial density of this study is 22 times more than that applied to *A. marmorata* and almost 21 times more than that applied to *A. bicolor pasifica*. When using equal comparison of culture duration, during 100 days culture, *A. bicolor pasifica* will attain total biomass of approx. 377.73 g, while *A. marmorata* 197.62 g in 105 l water volume. Hence in term of water column efficiency, during 100 days culture of eels species, this study can prove that T3 can maintain the biomass density of *A. bicolor bicolor* as much as 41.45±0.99 g l<sup>-1</sup>, whilst for *A. bicolor pasifica* and *A. marmorata* only can hold approx. 3.60 and 1.88 g l<sup>-1</sup> (calculated from [10]).

#### 4. Conclusion

Feeding rate of *A. bicolor bicolor* apparently follows the volume of water exchange in maximum of 200% water exchange. Higher water exchange will lead higher feeding rate and growth performance. These water exchange will correspond the water quality as well as growth performance either absolute growth and weight gain. Mass culture using at least 1000 l of water column can perform stable aquaculture in term of water column efficiency. Efficiency value of this study represents by biomass density per litre of water volume, which having a value of 41.45±0.99 g l<sup>-1</sup> during 100 days culture.

#### Acknowledgements

Authors thank to every single person who help and involve for this study. This work was financially supported by Diponegoro University, Funding source of PNBP DIPA UNDIP fiscal year of 2016. Special thanks to Mr. Tri Ananto who help in setting up the recirculating system.

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