



## The Osmotic Response and Hydromineral Status of Transported *Anguilla bicolor bicolor* Glass Eels with Various Ratios of Biomass and Water Volume

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### ABSTRACT

This research was conducted to determine the closed transportation system with biomass and water volume ratio that can support the osmoregulation ability of glass eel significantly. Glass eel was obtained from catching activities in the Cimandiri River, Pelabuhan Ratu (mean length  $52.40 \pm 0.84$  mm and weight  $0.10 \pm 0.01$  g) and acclimatized for 24 hours at salinity 6 mg L<sup>-1</sup> and temperature 20°C gradually. The research design was a completely randomized design. The applied treatment was glass eel closed transportation system with various ratio of glass eel biomass (kg): water volume (L), i.e. 1:11; 1:12; 1:13; and 1:14 during 24 hours of land transportation and continued with a 3-day recovery period. The water was used refer to the above acclimatization method, with ratio the water part of pure oxygen in a plastic bag was 1:3. The measured parameters include survival rate, osmotic gradient, body hydromineral, and water quality in transport packaging and recovery medium. Survival rates were generated in the high range until recovery period ( $P < 0.05$ ), but the transportation method caused differences significantly ( $P < 0.05$ ) in osmotic response and hydromineral status (sodium, chloride, potassium, and water content), so that it will affect production performance in the next stage of culture. Ratio of glass eel biomass: water volume of 1:13 has been able to support the ability of osmoregulation glass eel to maintain homeostatic condition during the transportation until 3 days of recovery period. There was no drastic decline in physical and chemical value of water in the transport bag so it could reduce the mortality rate for 24 hours.

**Keywords:** biomass, glass eel, osmoregulation, transport, volume

### 1. Introduction

The transport method of glass eel, *A. bicolor bicolor*, in Indonesia generally still refers to closed system for various types of fish larvae and shrimp seed. Incorrect application of transportation method can cause the poor condition of post-transport glass eel, so that high mortality occurs on arrival (death on arrival) and during the recovery process at the destination (death after arrival) (Schmidt and Kunzmann, 2005). The common procedures used for live fish transportation was 24 to 168 hours fasting condition, which depend on species of fish, temperature, density, transportation system, unloading packaging, and post transportation handling (Boerrigter et al., 2013). The convenience of transporting aquatic organisms is an important issue of live

fish transportation activities, especially on export destination countries of eel originating from Indonesia, one of which relates to the technical documentation of mortality during the transportation of aquaculture organisms and all its products between and or out of the cultivation location. This is necessary to be serious attention from all stakeholders, so that the quality products of eel from Indonesia can be widely accepted in the global market in the future.

One of the determining factors of success and sustainability of eel culture is the continuity of seed supply so that its production can run all the time. Until now the main focus on the glass eel stadium of *A. bicolor bicolor* is to increase the survival and growth that have not been maximized during the maintenance in the early stages of culture (Haryono and Wahyu

dewantoro, 2016), without comprehensively analyzing the condition of post transported seeds. Distribution of eel seeds from capture areas to different culture locations requires varying time depending on distance. An alternative solution that can be done to minimize mortality of post transported glass eel is to develop appropriate transport methods through optimization of environmental factors that can support the transportation efficiency with the right ratio of glass eel biomass, water volume and oxygen. The transport method of eel seeds is declared appropriate and successful when the biomass depreciation occurrence is no more than 5%, because in some cases mortality from transportation may reach 100% (Bogdan and Waluga, 1980).

Glass eels stadium is susceptible to stress that can generate high mortality right after transporting, particularly on arrival and recovery period. This is related to the condition of glass eel in poor performance because of the lack of transport method that can not support physiological functions during the transportation. The effects of stress due to long transport for 24 hours are reported to cause 50% mortality of *A. rostrata* glass eels (Appelbaum et al., 1998) and 53% of *A. Anguilla* elvers at 18 days recovery post transported (Bogdan and Waluga, 1980). Physiological response studies related to the recovery of cultured European eel (*A. anguilla*) sizing 150 g that was transported by road has been done by Boerrigter et al. (2013), but scientific information the transport method of wild Indonesian shorted-finned glass eel, *A. bicolor bicolor*, has not been explored widely. The condition of transportation medium for *A. anguilla* glass eel less support was reported can cause higher stress levels so that mortality increases, the vulnerable exposed to the disease and difficulties in weaning (Rodriguez et al., 2005). Physical and chemical stress responses in fish are the result of activation of the neuroendocrine system which can cause osmoregulation disturbance, thus affecting the hydromineral balance (sodium, chloride, and water) (Barton, 2002).

Indicators of physiological response associated with osmotic response on live fish transportation was the changes of plasma ions during period of starvation, transportation and post transportation (Boerrigter et al., 2013). Various studies have indicated the urgency of salt addition or the use of the salinity medium in an attempt to reduce fish stress levels during transportation, but its role in supporting osmoregulation capacity and hydromineral status is still limited only for a few fish species (Carneiro and Urbinati, 2002; Oyoo-okoth et al.,

2011; Urbinati and Carneiro, 2006). The results of Martemyanov's research (2015) showed that the initial period of acute stress of roach fish, *Rutilus rutilus*, occurs on post capture, transportation and acclimatization in a controlled environment can lead to an increase in the fraction of body water on this fish.

Recently, studies and publications on the transportation of post captured *A. bicolor bicolor* glass eel in the salinity medium for a quite a long time duration without substitution of water and the addition of pure oxygen are still very limited, especially the influence on the osmotic response and the hydromineral dynamics of the body that can support maximum survival. Therefore, this research needs to be done with the aim of determining the transport method of *A. bicolor bicolor* glass eel with an appropriate ratio of biomass and water volume, so that it can support osmoregulation activity during transportation and recovery period.

## 2. Materials and Methods

### *Glass eels procurement*

The wild glass eels were captured from catching activities in the Cimandiri River, Pelabuhan Ratu, West Java (long: 52.40±0.84 mm, weight: 0.10±0.01 g). The glass eels were adapted for one day in an aquarium at the Laboratory of Production Technology and Aquaculture Management of Aquaculture Department, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, before transporting during 24 hours by road. The experiment was conducted during February to March 2018.

### *Research design*

The design of this experiment used a completely randomized design, i.e. closed transportation system of glass eel with ratios of glass eel biomass (kg): water volume (L) were 1: 11; 1:12; 1:13; and 1:14, respectively, and repeated as many as three times. The ratio of the combination water and glass eel to the oxygen portion applied in the transport bag was 1: 3. Post transported glass eels were maintained for 3 days of the recovery period in aquarium to evaluate the osmotic response and hydromineral status.

### *Research procedure*

The closed transportation system used a container of polyethylene (PE) plastic bag measuring 25 x 45 cm. Prior to the use of

plastic bags, it must be double coated and the top of plastic bags were fastened with rubber bands to prevent leakage. Furthermore, the plastic bags filled with water at salinity 6 g L<sup>-1</sup> and a temperature of 20°C according to the treatments applied, based on the best acclimation method in previous as many as 935; 1020; 1105; and 1190 mL, respectively. Each of as many as 85 g glass eels (mean length 52.40±0.84 mm and weight 0.10±0.01 g) that has been acclimatized for 24 hours at salinity and temperature medium of 6 g L<sup>-1</sup> and temperature 20°C (Taqwa et al., 2018), placed in a plastic bags according to the ratio biomass and water volume to be tested.

Pure oxygen was added as much as 3 parts of the water volume that used in the plastic bag. Further, the plastic bag randomly inserted in a 75 x 42 x 32 cm polystyrene box, which was added with 650 g of ice cubes in a commercial mineral bottle and coated with paper to maintain temperature during transportation. Then the polystyrene box was sealed and simultaneously transported over 24 hours by vehicle.

After 24 hours transport test, each plastic bag that containing glass eel was acclimatized in a recovery aquarium sizing 80 x 45 x 40 cm which contains water as much as 42 L at salinity 6 mg L<sup>-1</sup> and temperature 29±1°C. Recovery of glass eels for 3 days was conditioned without feeding on a closed recirculation system that are equipped with a shelter from ropes and siphoned every day.

#### *Data collection*

Observations of glass eel mortality on arrival and continued for 3 days recovery period was conducted immediately after the end transportation process, which was performed under starvation conditions to calculate survival rate. Physiological responses of glass eel were observed before or prior to transportation, immediately after transport and post recovery of glass eels that includes osmotic gradient, water content and body minerals (sodium, chloride, and potassium). The sample of experimental animal was in whole body condition, which previously had been stunned in water at 6°C for about 1 minute. Subsequently the glass eel sample was homogenized and centrifuged at a rate of 15,000 g L<sup>-1</sup> for 5 minutes at 5°C. Supernatant was taken and stored in a refrigerator at -20°C before further analysis.

The measurement of osmolarity was conducted by osmometer (Osmometer Automatic Roebling Type 13). The water content of glass eels was measured by drying the tested sample using the oven at 102-105°C

to constant weight of the sample for 6 hours. The measurement of sodium, chloride and potassium content of the glass eel body was conducted based on wet ashing preparation followed by AAS analysis (Shimadzu type AA-7000).

During recovery after transporting, glass eels were fastened to evaluate survival rates, osmotic responses, water content and body minerals, and the physical and chemical value of water in recovery medium. The measurements of physical and chemical parameters in plastic bags and recovery medium includes temperature using thermometer, salinity using salinometer, pH using pH-meter, CO<sub>2</sub> with titration and spectrophotometer, dissolved oxygen using DO-meter, ammonia with phenate and spectrophotometer methods, and minerals content (sodium, chloride and potassium) using atomic absorption spectrophotometer, that were performed before and immediately after transport, as well as at the beginning and the end of 3 days recovery period.

#### *Test parameters*

The parameters and its formulations or reference methods include survival rate (Luo et al., 2013), osmotic gradient (Lukas et al., 2017), hydromineral parameters for water content based on AOAC (2010), while sodium, chloride, potassium of glass eel body referring to Reitz (1960). The measurement of physical and chemical value of water during transport and recovery period based on APHA method (2012).

#### *Statistical analysis*

The collected data were presented in the form of mean value and its standard deviation. Analysis of mean value of parameters were tested using analysis of varian by SPSS Ver.22 at P<0.05. If there were significant differences between mean value of parameters, then tested by the least significant difference (LSD) test (Steel and Torrie 1991).

### **3. Results and Discussion**

#### *Physical and chemical of water during transportation*

The mean value of the physical and chemical of water in the plastic bags of post transported glass eel are presented in Table 1. Most of physical and chemical values in the plastic bags showed significant differences

( $P < 0.05$ ), unless the value of salinity, pH, and sodium levels that are relatively homogeneous in all mediums. The mean value of the physical and chemical of water used for the transportation activity includes temperature was  $20.13 \pm 0.06^\circ\text{C}$ , salinity was  $6.03 \pm 0.01 \text{ g L}^{-1}$ , pH  $6.90 \pm 0.10$ , dissolved oxygen was  $7.10 \pm 0.10 \text{ mg L}^{-1}$ , carbon dioxide was  $6.93 \pm 1.00 \text{ mg L}^{-1}$ , and ammonia was  $0.004 \pm 0.001 \text{ mg L}^{-1}$ . The result of the measurement of mineral content in the initial transport medium, i.e. sodium was  $502.05 \pm 6.64 \text{ mg L}^{-1}$ , chloride was  $3,536.91 \pm 20.56 \text{ mg L}^{-1}$ , and potassium was  $55.21 \pm 4.50 \text{ mg L}^{-1}$ .

$\text{L}^{-1}$ , carbon dioxide was  $6.93 \pm 1.00 \text{ mg L}^{-1}$ , and ammonia was  $0.004 \pm 0.001 \text{ mg L}^{-1}$ . The result of the measurement of mineral content in the initial transport medium, i.e. sodium was  $502.05 \pm 6.64 \text{ mg L}^{-1}$ , chloride was  $3,536.91 \pm 20.56 \text{ mg L}^{-1}$ , and potassium was  $55.21 \pm 4.50 \text{ mg L}^{-1}$ .

**Table 1.** The mean value of physical and chemical of water in plastic bag immediately after transportation 24 hours. Number with different letter on the same row showed a significant difference ( $P < 0.05$ )

Parameters	Treatment (ratio of glass eels biomass: water volume = kg: L)			
	A (1:11)	B (1:12)	C (1:13)	D (1:14)
Temperature ( $^\circ\text{C}$ )	$24.53 \pm 0.06^a$	$24.33 \pm 0.06^b$	$24.17 \pm 0.06^c$	$24.07 \pm 0.06^c$
Salinity ( $\text{g L}^{-1}$ )	$6.03 \pm 0.01^a$	$6.03 \pm 0.01^a$	$6.03 \pm 0.01^a$	$6.03 \pm 0.01^a$
pH (unit)	$6.73 \pm 0.12^a$	$6.87 \pm 0.06^a$	$6.87 \pm 0.12^a$	$6.90 \pm 0.10^a$
Dissolved oxygen ( $\text{mg L}^{-1}$ )	$5.03 \pm 0.26^a$	$7.03 \pm 0.58^b$	$9.38 \pm 0.15^c$	$11.50 \pm 0.36^d$
Carbon dioxide ( $\text{mg L}^{-1}$ )	$16.72 \pm 1.10^a$	$14.98 \pm 1.73^{ab}$	$12.92 \pm 1.00^{bc}$	$11.43 \pm 0.35^c$
Ammonia ( $\text{mg L}^{-1}$ )	$0.047 \pm 0.005^a$	$0.031 \pm 0.004^b$	$0.014 \pm 0.011^c$	$0.005 \pm 0.003^c$
Sodium ( $\text{mg L}^{-1}$ )	$491.50 \pm 4.75^a$	$493.91 \pm 6.27^a$	$495.33 \pm 2.83^a$	$497.25 \pm 4.07^a$
Chloride ( $\text{mg L}^{-1}$ )	$2,872.26 \pm 20.56^a$	$2,929.23 \pm 8.96^b$	$2,971.16 \pm 18.13^c$	$3,038.42 \pm 20.56^d$
Potassium ( $\text{mg L}^{-1}$ )	$43.23 \pm 0.77^a$	$44.42 \pm 0.36^b$	$45.18 \pm 0.39^b$	$46.25 \pm 0.51^c$

Efficiency of live fish transportation can be done by increasing carrying capacity by the addition of a number of fish or water reduction in the transportation packaging. However, the increasing density of fish in transport packaging can lead to hyperactivity and stress on fish, as well as declining water quality due to accumulation of metabolic products, so it exhibited a reduce ability to survive and recover (Singh et al., 2004). The mean value of salinity, pH and sodium medium in the plastic bags during this test were relatively homogenous and still eligible to support survival of *A. bicolor bicolor* glass eel during transport. There were significant differences in temperature, dissolved  $\text{O}_2$ ,  $\text{CO}_2$ , ammonia, chloride and potassium medium during transport. The higher of water volume was used in the plastic bags causes increasing level of dissolved oxygen, chloride, and potassium media. The increasing of water volume have been used was inversely proportional to the level of carbon dioxide and ammonia, which were declining immediately after transport. Fish metabolism products during transport such as mucus accumulation, feces, residual feed that spewed out can cause a decrease in the value of physical and chemical water, such as depletion of dissolved oxygen content and increasing carbon dioxide due to respiration, decreasing pH due to carbon dioxide production, and increasing ammonia level (Shrivastava et al., 2017). It further stated

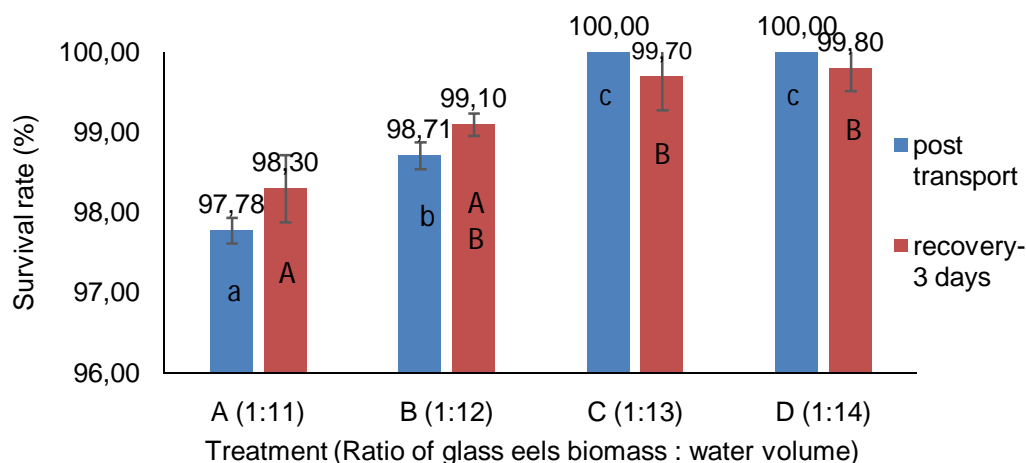
that the activities of live fish transportation for aquaculture or restocking, firstly fish are generally fastened to reduce metabolic rate and decrease water quality. However, prolonged fasting may inhibit the physiological function of the fish during the transportation period and recovery capabilities in the new environments.

### Survival rate

The mean value of survival rate of post transported and the end of the recovery period of glass eel showed a significant difference ( $P < 0.05$ ), where the survival rate of transported glass eel with the ratio of biomass and water volume of 1:13 and 1:14 showed the highest value and significantly different compared to other treatments (Figure 1). The closed transportation system with ratios of biomass and water volume over a 24 hours period in this experiment resulted in a high overall survival rate ( $>97.78\%$ ). The high survival rate of various all treatments were mainly due to the dissolved oxygen content and the temperature of the medium in the plastic bags within the supporting range to maintain homeostatic activity due to transport stress (Table 1). On the higher level of dissolved oxygen in plastic bag, there was a tendency to increase the survival rate of post transported glass eel and at the end of the 3 days recovery period. In general, the usage

of pure oxygen in the transport of live fish aimed to ensure maximum survival of fish (Da Silva et al., 2017). The water temperatures during transport in the plastic bags were in

the fairly low range (20.00-24.6°C). It could reduce the metabolic rate (Berka, 1986), so fish can be transported for a longer periods without water exchange.



**Figure 1.** Survival rate of glass eels after transport and recovery period. The different letters in the same color of bars showed a significant difference ( $P < 0.05$ )

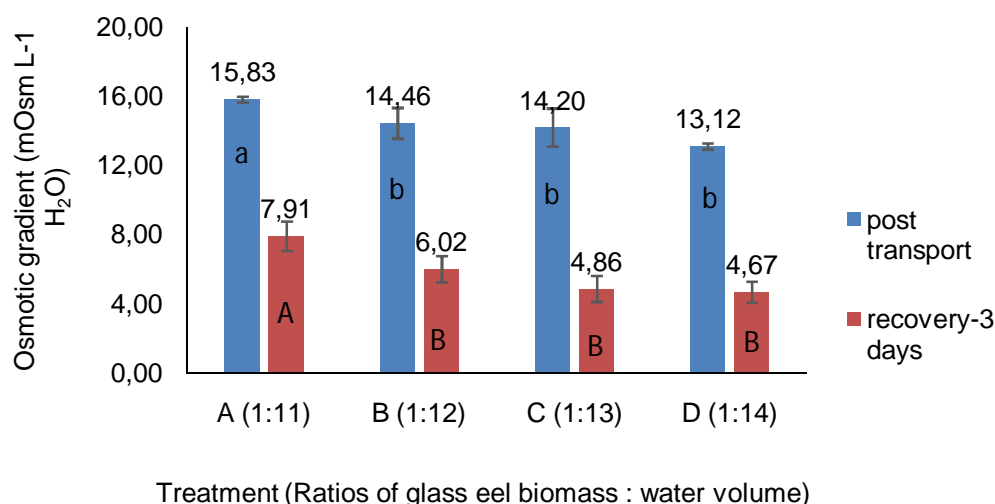
#### *The osmotic response and hydromineral status*

In addition can be used as the description of the energy portion for the metabolism activity, the rate of oxygen utilization is also related to the energy used in the osmoregulation activity (Boeuf and Payan, 2001; Lukas et al., 2017; Morgan and Iwama, 1998). Under increasing stress conditions, the required energy for metabolism will be higher in order to achieve a homeostatic condition during transportation and recovery, so that the energy portion for maintenance and growth will decrease.

The result showed that the transported glass eel with the highest ratio value of biomass and water volume (1:11) caused the highest osmotic response of *A. bicolor bicolor* glass eel and significantly different compared to other treatments ( $P < 0.05$ ), either immediately after transport ( $15.83 \pm 0.16$  mOsm  $L^{-1}$   $H_2O$ ), as well as at the end of 3 days recovery period ( $7.91 \pm 0.85$  mOsm  $L^{-1}$

$H_2O$ ) (Figure 2). It showed that the higher ratio value of glass eel biomass and the water volume in plastic bags in this experiment could lead to decreasing capability of glass eel on the osmoregulation activity. The difference in the ability of glass eel's osmoregulation was closely related to the dissolved oxygen availability on different transport media so that the available of energy portion will affect the ability to regulate osmotic pressure of body fluids.

Differences osmotic gradient of glass eels on this research could lead to regulating ability of the body hydromineral significantly different until the recovery period ( $P < 0.05$ ), except water content relatively homogeneous on glass eel at the end of the recovery period ( $P > 0.05$ ) (Table 2). The result from measurement of the mean value of hydromineral content of the whole body glass eel before transport were water content  $79.13 \pm 0.01\%$ , sodium  $1,057.86 \pm 6.54$  mg  $L^{-1}$ , chloride  $117.14 \pm 2.16$  mg  $L^{-1}$ , and potassium  $1,467.09 \pm 82.19$  mg  $L^{-1}$ .



**Figure 2.** Osmotic gradient on glass eel after transport and recovery period. The different letters in the same color of bars showed a significant difference ( $P < 0.05$ ).

**Table 2.** Hydromineral status of whole body glass eel after 24 hours transport and the end of 3 days recovery period. Number with different letter on the same row showed a significant difference ( $P < 0.05$ ).

Parameters	Treatment (ratio of glass eels biomass : water volume = kg: L)			
	A (1:11)	B (1:12)	C (1:13)	D (1:14)
After transport :				
Water (%)	82.08±0.24 <sup>a</sup>	82.01±0.84 <sup>ab</sup>	81.05±0.31 <sup>bc</sup>	80.98±0.45 <sup>c</sup>
Sodium (mg L <sup>-1</sup> )	1216.58±3.99 <sup>a</sup>	1181.55±5.90 <sup>b</sup>	1161.59±4.07 <sup>c</sup>	1128.88±8.84 <sup>d</sup>
Chloride (mg L <sup>-1</sup> )	262.96±8.60 <sup>a</sup>	261.46±4.64 <sup>a</sup>	192.46±4.81 <sup>b</sup>	140.94±11.16 <sup>c</sup>
Potassium (mg L <sup>-1</sup> )	1861.11±4.70 <sup>a</sup>	1649.86±13.02 <sup>b</sup>	1568.50±31.81 <sup>c</sup>	1516.01±22.78 <sup>d</sup>
Recovery :				
Water (%)	81.15±0.29 <sup>a</sup>	80.91±0.05 <sup>a</sup>	80.83±0.06 <sup>a</sup>	80.82±0.22 <sup>a</sup>
Sodium (mg L <sup>-1</sup> )	1174.17±30.20 <sup>a</sup>	1137.89±3.25 <sup>b</sup>	1105.48±1.21 <sup>c</sup>	1062.12±11.89 <sup>d</sup>
Chloride (mg L <sup>-1</sup> )	223.27±3.88 <sup>a</sup>	218.20±10.54 <sup>a</sup>	190.03±2.14 <sup>b</sup>	120.72±7.89 <sup>c</sup>
Potassium (mg L <sup>-1</sup> )	1719.46±32.89 <sup>a</sup>	1640.93±37.15 <sup>b</sup>	1514.55±64.97 <sup>c</sup>	1422.16±15.46 <sup>d</sup>

Even though the osmotic response is rarely used as an indicator of stress in fish transport, but the dynamics that occur can describe homeostatic mechanism and correlated with hormone that plays a major role in regulating osmoregulation in fish, i.e. cortisol (Sampaio and Freire, 2016). Status of hydromineral on body fluids, especially sodium and chloride ions are often used as an indicator of stress due to transport, because these are the major component of osmolarity in teleostei fish (Carneiro and Urbinati, 2002). Apart from sodium and chloride ions, potassium ion is also important components that contribute to the ion exchange of the body fluids and environment through the chloride cells in the gills and the activity of Na<sup>+</sup>K<sup>+</sup>ATPase enzyme during the process of regulating the osmotic pressure

and ion balance of aquatic organisms (Wilson et al., 2004).

Stress on fish due to transport can lead a change in physiological responses (Gomes et al., 2003), which will affect the osmoregulation mechanism by activating the Na<sup>+</sup>K<sup>+</sup>ATPase enzyme in the gills associated with the regulation of the body's hydromineral balance (Arjona et al., 2009; Martemyanov, 2015; Shrivastava et al., 2017; Stewart et al., 2016). The present research showed there were significant decreasing in water content, sodium, chloride and potassium of glass eel ( $P < 0.05$ ) on post transport and post recovery 3 days with a lower ratio value of glass eel biomass and water volume, except for water content of glass eel body after the recovery period ( $P > 0.05$ ) (Table 2). The higher osmotic gradient on glass eel at a higher ratio value of biomass and water



volume on this experiment showed that glass eel under high pressure of physiological stress will cause an increasing absorption of water, sodium, chloride and potassium (Ando and Takei, 2014; Iversen et al., 1998; Martemyanov, 2015). Other than that, the stress response of fish associated with the hormone corticosteroid will affect the mechanism of osmoregulation, metabolism, and hydromineral balance so that it can lead the difference in ability of fish to absorb salt from environment (McCormick and Bradshaw, 2006).

#### *The recovery period*

The performance of glass eels at the end of the recovery period, in general showed a significantly different response between treatments ( $P < 0.05$ ), except for content of body water ( $P > 0.05$ ) (Table 2). The survival rate of glass eel was significantly different at the end of the recovery period, but still within the high

range ( $98.33 \pm 0.31$ – $99.87 \pm 0.23\%$ ). This indicated that the closed recirculation system can keep water physical and chemical parameters at optimum level for glass eel's recovery after transporting. The water was used for the recovery medium of glass eel maintained with recirculation system and strived in optimal conditions for culture. The mean value of physical and chemical of water at the beginning of the recovery period includes temperature was  $28.62 \pm 0.12^\circ\text{C}$ , salinity was  $6.20 \pm 0.08 \text{ g L}^{-1}$ , pH  $6.70 \pm 0.06$ , dissolved oxygen was  $7.10 \pm 0.07 \text{ mg L}^{-1}$ , carbon dioxide was  $9.32 \pm 1.15 \text{ mg L}^{-1}$ , ammonia was  $0.001 \pm 0.001 \text{ mg L}^{-1}$ , sodium was  $504.85 \pm 1.25 \text{ mg L}^{-1}$ , chloride was  $4,201.57 \pm 17.80 \text{ mg L}^{-1}$ , and potassium was  $55.21 \pm 4.50 \text{ mg L}^{-1}$ . The mean value of water physical and chemical parameters in recovery medium for 3 days was not significantly different between treatment, except for potassium level ( $P < 0.05$ ) (Table 3).

**Table 3.** The mean value of physical and chemical of water during 3 days of there covery period. Number with different letter on the same row showed a significant difference ( $P < 0.05$ ).

Parameters	Treatment (ratio of glass eels biomass : water volume = kg: L)			
	A (1:11)	B (1:12)	C (1:13)	D (1:14)
Temperature ( $^\circ\text{C}$ )	$28.40 \pm 0.10^a$	$28.47 \pm 0.06^a$	$28.50 \pm 0.10^a$	$28.33 \pm 0.15^a$
Salinity ( $\text{g L}^{-1}$ )	$6.11 \pm 0.01^a$	$6.31 \pm 0.34^a$	$6.19 \pm 0.04^a$	$6.17 \pm 0.06^a$
pH (unit)	$6.70 \pm 0.10^a$	$6.63 \pm 0.15^a$	$6.77 \pm 0.06^a$	$6.67 \pm 0.12^a$
Dissolved oxygen ( $\text{mg L}^{-1}$ )	$6.90 \pm 0.10^a$	$7.03 \pm 0.15^a$	$6.90 \pm 0.10^a$	$7.00 \pm 0.10^a$
Carbon dioxide ( $\text{mg L}^{-1}$ )	$11.99 \pm 2.00^a$	$12.99 \pm 1.00^a$	$12.29 \pm 2.06^a$	$13.98 \pm 2.00^a$
Ammonia ( $\text{mg L}^{-1}$ )	$0.004 \pm 0.001^a$	$0.004 \pm 0.002^a$	$0.005 \pm 0.002^a$	$0.004 \pm 0.001^a$
Sodium ( $\text{mg L}^{-1}$ )	$492.99 \pm 2.70^a$	$495.47 \pm 6.24^a$	$499.14 \pm 3.28^a$	$500.44 \pm 7.04^a$
Chloride ( $\text{mg L}^{-1}$ )	$3,489.44 \pm 17.80^a$	$3,501.31 \pm 10.28^a$	$3,507.24 \pm 17.80^a$	$3,513.17 \pm 0.28^a$
Potassium ( $\text{mg L}^{-1}$ )	$42.24 \pm 0.44^a$	$42.94 \pm 0.08^b$	$43.51 \pm 0.41^b$	$44.28 \pm 0.31^c$

The physical and chemical values of water that supported the survival rate of glass eels in culture medium based on references for temperatures was  $29.0 \pm 1^\circ\text{C}$  (Luo et al., 2013), salinity was  $6.5 \pm 0.5 \text{ g L}^{-1}$  (Affandi and Riani, 1995), pH was  $7.0 \pm 1$  (Ritonga et al., 2014), dissolved oxygen was  $5.5 \pm 0.5 \text{ mg L}^{-1}$  (Affandi and Suhenda 2005), carbon dioxide was  $< 30 \text{ mg L}^{-1}$  (Wedemeyer, 1996), and ammonia was  $< 0.01 \text{ mg L}^{-1}$  (Wahyudi et al., 2015). The osmotic gradient and potassium level of whole body glass eel at the end of recovery period which significantly different between treatments indicated that application of these transports method could disturbing the activity of osmotic and ion regulation up to 3 days of recovery. Eel is capable of maintaining osmotic and ionic homeostatic through osmoregulation activity, but that process requires energy (Tseng and

Hwang, 2008). Therefore, optimization of the closed transportation system with an appropriate ratio of glass eel biomass and water volume is one of the right solutions that can be applied to support the mechanism of glass eel osmoregulation so the energy usage for a homeostatic state more efficiently.

#### **4. Conclusions**

The closed transportation system to *A. bicolor bicolor* glass eel for 24 hours with the ratio of biomass (kg) and water volume (L) by 1:13 has been able to support performance osmoregulation optimally until the recovery period. The value of physical and chemical of the water during the transportation and the recovery period is still on conditions that can be

tolerated glass eel, so it can suppress the level of mortality.

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