Optimization of physiological status of glass eel (*Anguilla bicolor bicolor*) for transport by salinity and temperature acclimatization

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Abstract. The purpose of this research was to optimize the physiological status of post captured glass eels (*Anguilla bicolor bicolor*) through salinity and temperature acclimatization before transporting. Glass eels were obtained from Cimandiri river, Pelabuhan Ratu, West Java, Indonesia, with body length and weight of 52.65±0.35 mm and 0.11±0.02 g, respectively. The experimental design was completely randomized design with two stages of research, i.e. 1) acclimatized of glass eel in salinity medium 0, 3, 6, 9, 12, and 15 g L^{-1}, and 2) acclimatized of glass eel in temperature medium 26, 24, 22, 20, 18, and 16°C. Acclimatization of salinity and temperature in the medium was conducted gradually and continuously for 24 hours. The result of acclimatization method, gradually over 24 hours in medium at salinity 6 g L^{-1} and temperature from 16°C to 20°C showed the best physiological status of glass eels, while still maintaining maximum survival rate ($p > 0.05$), minimizing osmotic gradient, glucose level, and oxygen consumption level significantly ($p < 0.05$). This physiological status caused the best performance of glass eel before transporting. Increasing salinity of the acclimatization medium, increased sodium level, chloride and water content in glass eels at the end of the acclimatization period ($p < 0.05$). The physical and chemical values of acclimatization medium such as temperature, pH, dissolved oxygen, alkalinity, ammonia, sodium and chloride were still within the range that supporting survival of glass eel during the 24 hours acclimatization period.
Key Words: glucose, hydromineral, osmotic gradient, oxygen consumption, short-finned eel.

Introduction. Eel (*Anguilla* sp.) is one of Indonesian fisheries product which has a high economic value due to high global market demand (Nijman 2015), but its availability still depends on annual migration cycle (Kuroki et al 2006; Aoyama 2009; Aarestrup et al 2010; Aoyama et al 2015) and the reproduction technology was still developed with various experimental tests (Tanaka et al 2003; Kagawa et al 2005; Butts et al 2014; Mordenti et al 2014). Therefore, sustainability and success of eel farming production depend on the quantity and quality of seeds from fishing activities. The existence of various regulations which limiting eel seeds distribution in some countries (Nijman 2015) made this commodity had higher economic value in several countries in East Asia and potentially made an illegal trade (Shiraishi & Crook 2015; Stein et al 2016). Indonesian government has banned the exports of eels (*Anguilla* spp.) with size of $\leq$150 g eel^{-1}. This led to the occurrence of the shifting activity of captured glass eel to farmed.

One of Indonesia area with potential glass eel resources was on the south coast of Java island, i.e. the estuary of Cimandiri river, Pelabuhan Ratu, West Java. The exploitation of glass eel is a long-standing activity that has been done long enough by the fishermen to supply the high demand for farming. The catching of glass eel was conducted at night before seawater tide using traditional fishing equipment such as lift net or scoop net (Sriati 1998; Muthmainnah et al 2016). However, the acclimatization method to the captured glass eels by fishermen did not consider physiological condition of these seeds. The fishermen generally moved them directly into the freshwater medium without appropriate acclimatization method. This could lead to high mortality, susceptible to disease and difficulties in the weaning glass eel during acclimatization, so they were being a factor in the low quality of seed for farming (Rodriguez et al 2005).

Handling of wild glass eel is the first step that will determine the quality of seeds that would be cultivated. Therefore, it is necessary to acclimatize glass eel with the appropriate method before being distributed to various regions or areas of farming, so that the performance of cultivation production can be maximized. An appropriate environmental condition is important to support the physiological function for optimal cultivation, so that it will produce a maximum production performance. The important physical parameters of water related to the glass eel stadium during migration from seawater to freshwater were salinity and temperature (Arai & Chino 2012), therefore in acclimatization process to captured glass eel, optimization of both environmental parameters was major priority. Previous studies showed that the ideal value range of salinity that can support the survival and growth of fish varies greatly, depending on the fish species (Affandi & Riani 1995; Tzeng et al 2003; Kearney et al 2008; Lukas et al 2017), particularly its role in minimizing osmotic gradient (Wilson et al 2004; Pittman et al 2013). Water temperature had an important role to the basal metabolism in over a longer period without feeding (Degani et al 1989), patterns of swimming behavior and climbing activity of *Anguilla* spp. glass eel (Linton et al 2007), migration time and distance between sites (Martin 1995; White & Knights 1997), whereas temperature experiments in the laboratory were generally conducted on a preference test (Tosi et al 1990). The physiological stress of glass eel will be lower to maintain the homeostatic condition when the salinity and temperature acclimatization medium are optimum. This research aimed to optimize physiological status of post captured glass eels when acclimatized in various salinity and temperature mediums before being distributed to various farming areas.

Material and Method. The research was carried out in the collection unit of wild glass eel, *Anguilla bicolor bicolor*, at Pelabuhan Ratu, West Java and Laboratory of Production Technology and Aquaculture Management of Aquaculture Department, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Indonesia in February 2018. The research consisted of 2 stages, i.e. acclimatization of glass eel in various salinities (0, 3, 6, 9, 12 and 15 g L^{-1}) and temperatures medium (16, 18, 20, 22, 24 and 26°C).

Experiment 1. Research design was completely randomized design with six treatments of glass eel acclimatization for 24 hours on various salinities, i.e. 0; 3; 6; 9; 12; and 15 g L^{-1} with three replications. The aquarium for the salinity acclimatization were 18 units, each sizing 60 x 28 x 30 cm which was equipped with salinity and temperature regulator, aerator and shelter. At the top of the aquarium was installed container of freshwater or seawater to drain it into each aquarium of treatment during salinity acclimatization. Determination of salinity medium was based on modification of dilution method from Taqwa et al (2008). The water was used according to the salinity value of the fishing ground (5 g L^{-1}), filled 10 L into each aquarium and aerated for 1 day, so that the oxygen solubility was > 5 mg L^{-1}. The freshwater was used for diluting salinity taken from ground water that has been precipitated and aerated for 3 days. The experimental animal was wild glass eel, *A. bicolor bicolor* from capturing activity in the Cimandiri river, Pelabuhan Ratu, West Java with the mean value of length and weight 52.90±1.52 mm and 0.12±0.01 g, respectively. Glass eels were placed in an acclimatization aquarium with a density of 2 g L^{-1} per treatment unit (Aziz 2014). The process of achieving salinity in the medium during the acclimatization period was conducted gradually and continuously within 24 hours. The water discharge was adjusted by setting on the valve, so that salinity will be achieved according to the each treatment at the end of the acclimatization period. During the acclimatization period, glass eels were fastened. The maintenance of acclimatization medium was carried out by regulating the temperature level at 28±1°C using automatic water heater and aeration using a central blower.

Experiment 2. The second stage of the research was acclimatization of glass eels for 24 hours at various temperatures in medium using completely randomized design that consisting of six temperature treatments for transport purpose, i.e. 16; 18; 20; 22; 24; and 26°C, respectively, as many as 3 replications. Preparation of aquarium, water and the tested animals refered to the research procedure of the salinity acclimatization method on the previous stage. The wild glass eels were captured from Cimandiri river, Pelabuhan Ratu, West Java with the mean value of length 52.40±1.57 mm and weight 0.09±0.02 g. The experiment of the temperature acclimatization was conducted by placing 20 g glass eel in an aquarium sizing 60 x 28 x 30 cm, that had been filled with 10 L water in the salinity and temperature, i.e. 5 g L^{-1} and 28°C, respectively. Temperature was decreased by the addition of ice cubes that packaged in plastic bags; each aquarium was equipped with a thermometer. The temperature of the acclimatization medium was arranged gradually and continuously, so that the temperature fluctuation did not exceed 0.5°C h^{-1} and adjusted simultaneously during the salinity acclimatization up to 6 g L-^{1} by setting the water valve.

Data collection. Survival rate was measured by observing glass eel mortality during 24 hours of acclimatization period. Whole body fluid was obtained by grinding glass eels in a mortar, then centrifuged at 15 x 10^3 g for 5 minutes and stored at -20°C before further analysis. The osmotic gradient of glass eel was measured based on the osmolarity value of whole body fluid and medium using osmometer. The whole body glucose level was performed using a glucose test kit and a spectrophotometer. The glass eel metabolism rate during the acclimatization period was determined by measuring the level of oxygen consumption in the basal state at the end of the acclimatization period for 3 hours. Measurements of the level of sodium, chloride and water in the whole body glass eel were conducted with wet ashing method, before and after the acclimatization test of each treatment, whereas the sample of sodium and chloride content on the acclimatization medium were analyzed by atomic absorption spectrophotometer (AAS). The chemical and physical water parameters of the acclimatization medium consisted of temperature, salinity, pH, dissolved oxygen, alkalinity, ammonia, sodium and chloride. Temperature, salinity, pH and dissolved oxygen were measured *in situ* every hour, meanwhile the level of alkalinity, ammonia, sodium and chloride were measured at the beginning and at the end of the acclimatization period. References to the formulation and procedural techniques were used, i.e. survival rate (Luo et al 2013), osmotic gradient and oxygen consumption level (Lukas et al 2017), whole body glucose (O'Connor et al 2011), sodium, chloride and water content (Reitz et al 1960) and physico-chemical of water during acclimatization (APHA 2012).

Data analysis. Data on survival rate, osmotic gradient, oxygen consumption level, glucose, sodium, chloride and water content of glass eel during the acclimatization period were presented as mean value and its standard deviation. These data were subjected to one-way analysis of variance at significant test 5% using statistic software SPSS Ver.22. When differences were detected ($p < 0.05$), data were analyzed by Duncan's test (Steel & Torrie 1980). The physical and chemical water parameters during acclimatization were presented in range values and analyzed descriptively.

Results and Discussion

Experiment 1. Data on survival rate, osmotic gradient, oxygen consumption level, glucose, sodium, chloride and water level of glass eels over the 24 hours acclimatization period in various salinities are presented in Table 1. Biological data of post captured glass eel consist of osmotic gradient was 6.35±0.52 mOsm L H_2O^{-1}, glucose was 175.90±6.37 mg dL^{-1}, sodium was 966.05±10.95 mg L^{-1}, chloride was 195.37±26.73 mg L^{-1} and water content was 81.08±0.52%.

Table 1

The physiological status of glass eel after acclimatization on different salinity medium for 24 hours

Parameters*	Salinity of acclimatization medium ($g\ L^{-1}$)					
	0	*3*	*6*	*9*	*12*	*15*
Survival rate (%)	100± 0.00^{a}	100± 0.00^{a}	100± 0.00^{a}	100± 0.00^{a}	100± 0.00^{a}	100± 0.00^{a}
Osmotic gradient (mOsm L H_2O^{-1})	8.67± 0.27^{a}	8.29± 0.57^{a}	5.50± 0.23^{b}	21.81± 0.47^{c}	25.48± 0.78^{d}	30.49± 0.77^{e}
Oxygen consumption (mg O_2 g^{-1} h^{-1})	0.653± 0.002^{a}	0.596± 0.003^{c}	0.538± 0.016^{d}	0.566± 0.014^{e}	0.624± 0.013^{b}	0.644± 0.011^{ab}
Glucose (mg dL^{-1})	126.22± 6.37^{a}	109.44± 7.99^{b}	86.00± 4.73^{c}	102.07± 5.27^{b}	146.58± 7.58^{d}	159.71± 2.37^{e}
Sodium (mg L^{-1})	780.51± 2.13^{a}	862.33± 0.50^{b}	1036.72± 31.88^{c}	1042.43± 0.91^{cd}	1077.99± 7.00^{d}	1266.72± 39.03^{e}
Chloride (mg L^{-1})	126.76± 7.56^{a}	174.72± 3.76^{b}	241.71± 7.07^{c}	276.44± 7.89^{d}	286.27± 8.91^{e}	352.26± 36.88^{f}
Water content (%)	79.34± 0.12^{a}	82.09± 0.30^{b}	81.32± 0.39^{b}	82.14± 0.85^{b}	81.08± 0.70^{b}	81.36± 0.92^{b}

* The mean value with different superscript on the same row showed a significant difference ($p < 0.05$).

The survival rate of glass eels were not significantly different ($p > 0.05$) after the salinity acclimatization period showed that on wild glass eels had a wide tolerance to the salinity value from 0 to 15 g L^{-1}. Various studies have indicated that different species of glass eels had varied in salinity preferences (Ingram et al 2001; Wilson et al 2004; Kearney et al 2008). The results of a preference test of post captured *A. rostrata* from several Canadian rivers showed that it prefered in freshwater environment and were more active in swimming than in seawater (33 g L^{-1}) (Boivin et al 2015), but those from different catching area indicated a preference in brackish water 22 g L^{-1} (Cote et al 2009). The optimal survival rate of glass eels and elvers of *A. bicolor bicolor* during the maintenance period were in the medium with salinity from 5 g L^{-1} to 8 g L^{-1} (Affandi & Riani 1995; Sutrisno 2008; Lukas et al 2017), but information regarding the optimization of salinity medium during the acclimatization period of post captured glass eel for transport purpose was still limited.

A common paradigm stated that farming of *Anguilla* sp. should be conducted in freshwater because life cycle of eel as a catadromous organism was not absolutely right (Fontaine et al 1995). Not all catadromous organisms could enter freshwater environment due to geographical condition, as did some tropical Indonesian short-finned eel, *A. bicolor bicolo*r, based on microchemical assay in otolith (Chino & Arai 2010). This phenomenon indicated that condition of catadromous organism were either settled or facultative, some settled on the seawater, estuary, and freshwater, whereas the other migrated (Tsukamoto & Arai 2001; Arai et al 2004).

Salinity affected on the growth of eel related to the amount of energy required during osmoregulation mechanism (Boeuf & Payan 2001), so on the isoosmotic conditions the growth and feed conversion ratio would be optimal (Imsland et al 2001). In isotonic environmental against osmolarity of whole body fluid caused a lower osmotic gradient, so the use of energy for osmoregulation was more efficiently, because almost more than 50% of the fish would allocate energy to osmoregulation under non-isotonic conditions (Boeuf & Payan 2001). In this experiment, salinity 6 g L^{-1} produced the lowest osmotic gradient and significantly different from other treatments, so the energy portion for osmoregulation would be lower. Tzeng et al (2003) showed that the growth of the Japanese eel, *A. japonica*, was maximum when in estuary habitat than in freshwater, because there was similarity between osmolarity of the aquatic environment with internal body of eel seeds (200-400 mOsm).

The salinity acclimatization in a range of 0-15 g L^{-1} caused a significant difference in oxygen consumption level of glass eel at the end of the acclimatization period. There was a tendency in a freshwater medium up to 3 g L^{-1} or more than 6 g of L^{-1} caused the higher energy requirement for glass eel for basal metabolism because oxygen consumption level in order to support the maintenance process was greater than glass eel in the medium of 6 g L^{-1}. Measurement of oxygen consumption level could also be used as a representation of the energy usage for osmoregulation (Morgan & Iwama 1998; Boeuf & Payan 2001). The oxygen consumption level of fish varied widely because it was influenced by climate, especially temperature, acclimatization period and duration of the test (Andersen et al 1985; Laursen et al 1985; Cruz-Neto & Steffensen 1997), salinity, diet, activity and fish weight (Mantel & Farmer 1983; Brett 1979). European eel, *A. anguill*a had the ability to regulate and compensate the metabolism rate for a low level of dissolved oxygen in the medium, but not too high level of CO_2 (Cruz-Neto & Steffensen 1997).

The whole body glucose level in the acclimatization medium 6 g L^{-1} was the lowest, i.e. 86.00±0.73 mg dL^{-1} and significantly different with the other treatments. Salinity medium below or above 6 g L^{-1} caused a significant increase in glucose level of the whole glass eel body, i.e. ranged from 102.07±5.27 to 159.71±2.37 mg dL^{-1}. The change of salinity caused fish stress with various physiological responses such as released of cortisol hormone which led to the elevation of blood glucose level, so this indicator usually used to describe the stress level of fish in various studies (Pottinger 2008; Barton 2011; Pankhurst 2011). The stress level of *A. anguilla* could be caused by capturing and transport handling method, so that the glucose level reached 183.75 to 228.79 mg dL^{-1} (Leitao et al 2008). A captured fish in the east coast of Pangandaran, West Java also showed a high range of glucose level from 86.5 to 206.0 mg dL^{-1} (Malini et al 2016). Indicators of blood glucose levels in non-stress fish generally ranged from 40 to 90 mg dL^{-1} (Reichenbach-Klinke & Landol 1973), whereas secondary stress indicator in the crustacean group could be known if the hemolymph glucose level exceeded 150 mg d L^{-1} (Cuzon et al 2004).

A previous experiment showed that changes in salinity medium at the end of the acclimatization period resulted in a significant difference in the whole body sodium and chloride level of *A. japonica* glass eel ($p < 0.05$), its level increased along with the rising of salinity value (Wong & Takei 2012). The content of sodium and chloride in the medium at a certain level played a role in maintaining the mineral balance in intracellular and intercellular fluid, so the energy required in the regulation of mineral concentration in the body to maintain the homeostatic conditions would be lower. When the fish stressed for the first time, the catecholamine level on erythrocyte increased and oxygen demand was higher, but sodium on erythrocyte decreased (Martemyanov 2013). Fish responses during the salinity acclimatization included improving gill capacity in performing physiological functions, which would increase the number and size of chloride cells that was responsible for sodium and chloride secretion (McCormick & Bradshaw 2006). The whole body ion level in fish could be used as an indicator of osmoregulation ability and performance prediction of growth and survival in the salinity medium, but acute physiological changes might lead to passive displacement of water or ion regulation more active, that was caused by the handling method (acclimatization) (Stewart et al 2016). The acclimatization method of salinity medium 3-15 g L^{-1} caused the water content of glass eel at the end of the acclimatization period between 81.08±0.70% to 82.14±0.85%, while in freshwater medium (0 g L^{-1}) was 79.34±0.12% ($p < 0.05$). The higher salinity value could lead increasing of glass eel's absorption of sodium, chloride and potassium, so it will affect the water absorption in the eel intestine that acclimatized in the seawater medium (Ando & Takei 2014). This indicated that stress could cause fluctuations in the water fraction in the whole body composition of glass eel, as well as stress response of *Rutilus rutilus* L. after capture, transport and during acclimatization in the laboratory (Martemyanov 2015).

In the preliminary data, physical and chemical water parameters were indicated the value range of temperature 27.50-27.60°C, salinity 5.00-5.10 g L^{-1}, pH 6.8-6.9, dissolved oxygen 6.8-7.0 mg L^{-1}, alkalinity 52.46 mg L^{-1}, ammonia 0.004 mg L^{-1}, sodium 921.37-1040.54 mg L^{-1}, and chloride 2519.16-2723.90 mg L^{-1}. The value range of physical and chemical water water parameters during 24 hours acclimatization of glass eel in various salinities are presented in Table 2. The physical and chemical values of water parameters in acclimatization medium generally supported survival of glass eel. The feasibility range of physical and chemical of water parameters, based on maintenance for various eels were temperature ranging 28-30°C (Luo et al 2013), salinity 6-7 g L^{-1} (Affandi & Riani 1995), pH 6.0-8.0 (Ritonga 2014), dissolved oxygen 5-6 mg L^{-1} (Affandi & Suhenda 2005), alkalinity 57-68 mg L^{-1} (Lukas et al 2017), and ammonia < 0.01 mg L^{-1} (Wahyudi 2015). The results of the mineral measurement in medium showed a linear pattern along with the increasing salinity during the acclimatization period toward sodium and chloride level.

Table 2

The value range of physical and chemical of water on salinity acclimatization medium for 24 hours

Parameters	*Salinity of acclimatization medium (g L^{-1})*					
	0	*3*	*6*	*9*	*12*	*15*
Temperature (°C)	26.60-27.70	27.00-27.50	27.10-27.60	27.20-27.60	27.10-27.50	27.20-27.50
Salinity (g L^{-1})	0.00-5.00	3.04-5.00	5.00-6.17	5.00-9.30	5.00-12.30	5.00-15.10
pH (unit)	6.8-7.5	6.9-7.2	6.7-7.1	6.6-7.0	6.6-6.8	6.6-6.8
Dissolved oxygen (mg L^{-1})	6.4-7.3	6.3-7.2	6.4-7.0	6.2-7.0	6.1-6.9	6.2-7.0
Alkalinity (mg L^{-1})	38.80-54.32	42.68-54.32	54.32-62.08	54.32-77.60	54.32-77.60	54.32-78.80
Ammonia (mg L^{-1})	0.006-0.012	0.005-0.064	0.002-0.045	0.004-0.005	0.001-0.005	0.001-0.004
Sodium (mg L^{-1})	17.21-921.37	851.69-1040.54	921.37-1040.54	921.37-1456.57	921.37-1621.74	921.37-1798.97
Chloride (mg L^{-1})	89.02-2723.90	1450.96-2723.90	2519.16-2928.63	2519.16-4085.85	2519.16-4762.37	2519.16-6032.25

Experiment 2. Data on survival rate and oxygen consumption level of glass eels for 3 hours after 24 hours temperature acclimatization in the salinity 6 g L^{-1} are presented in Table 3.

Table 3

Survival rate and oxygen consumption level of glass eel after temperature acclimatization

*Parameters**	*Temperature of acclimatization medium (°C)*					
	26	*24*	*22*	*20*	*18*	*16*
Survival rate (%)	100 ± 0.00^a	100 ± 0.00^a	100 ± 0.00^a	100 ± 0.00^a	100 ± 0.00^a	100 ± 0.00^a
Oxygen consumption (mg O_2 g^{-1} h^{-1}):						
hour-1	0.592 ± 0.002^a	0.571 ± 0.004^b	0.363 ± 0.015^c	0.281 ± 0.012^d	0.216 ± 0.001^e	0.157 ± 0.019^f
hour-2	0.263 ± 0.012^a	0.197 ± 0.001^b	0.191 ± 0.010^b	0.150 ± 0.011^c	0.137 ± 0.001^{cd}	0.131 ± 0.011^d
hour-3	0.184 ± 0.011^a	0.164 ± 0.010^b	0.145 ± 0.011^b	0.124 ± 0.011^c	0.124 ± 0.011^c	0.125 ± 0.011^c

* The mean value with different superscript on the same row showed a significant difference ($p < 0.05$).

The value range of physical and chemical parameters of water on 24 hours temperature acclimatization in salinity 6 g L^{-1} are presented in Table 4.

Table 4

The value range of physical and chemical of temperature acclimatization medium for 24 hours

Parameters	*Temperature of acclimatization medium (°C)*					
	26	*24*	*22*	*20*	*18*	*16*
Temperature (°C)	26.10-26.90	24.00-26.80	22.10-26.80	20.30-26.80	18.00-26.80	16.10-26.80
Salinity (g L^{-1})	6.01-6.10	6.03-6.07	6.04-6.10	6.05-6.08	6.00-6.08	6.01-6.06
pH (unit)	6.5-6.7	6.3-6.7	6.5-6.7	6.5-6.7	6.3-6.7	6.4-6.7
Dissolved oxygen (mg L^{-1})	6.3-6.9	6.1-6.8	6.6-6.8	6.4-6.8	6.7-7.0	6.0-6.9
Alkalinity (mg L^{-1})	58.2-65.96	58.2-73.72	62.08-65.96	65.96-69.84	65.96-77.60	62.08-65.96
Ammonia (mg L^{-1})	0.001-0.007	0.001-0.003	0.001-0.003	0.002-0.004	0.001-0.003	0.001-0.003

Water temperature and fish weight in the packaging transport affected metabolism activity of poikilothermic organism during transportation, especially to suppress oxygen consumption rate as low as possible (Berka 1986). Although eels in nature have a wide tolerances range to aquatic temperature, however water temperature in a culture medium that can support maximum survival and growth was in narrower tolerance (Tosi et al 1990; Heinsbroek 1991; Imsland et al 2001). The optimum survival rate at the end of the temperature acclimatization ranged from 16 to 26°C, indicated that glass eel had a wide temperature tolerance with 24 hours gradually salinity decreasment. The appropriate method of fish handling prior to transport would make fish more adaptable to stress response, as each fish had a different adaptation capability (Schmidt & Kunzmann 2005; Lekang 2007).

The appropriate range of medium temperature for different species of eel quite varied, i.e. ranged between 25-28°C for *A. japonica*, and ranged 23-25°C for *A. anguilla* (Seymour 1989; Heinsbroek 1991), and at a temperature 26.5°C for *A. australis* and *A. diffenbachii* (Kearney et al 2008). However, the temperature range on the plastic bag that could support the maximum survival of live fish during transport was in a lower value range (Golombieski et al 2003). Although the use of lower temperature on the hypothermia condition might lead to a lower fish metabolism rate during transport, but there was weakness on the limitation of temperature range and duration (Ross & Ross 1984).

The medium temperature was a factor that affected the growth of fish indirectly through the energy efficiency for metabolism. The energy portion used for basal metabolism could be determined by measuring the oxygen consumption level of fastened fish (Cruz-Neto & Steffensen 1997), whereas the higher oxygen consumption level indicated the higher metabolism energy demand (Lukas et al 2017). The lowest rate of glass eel's oxygen consumption for 3 hours after temperature acclimatization were found in the treatment of 16, 18, and 20°C, and glass eel looked calmer at the bottom of the aquarium. This result showed that specific range of temperature, salinity and duration could suppress a lower metabolism rate (Golombieski et al 2003; Kim et al 2006) and reduced swimming or climbing activity (Linton et al 2007), so that physiological status of glass eel was more optimal for transportation. The lower range of water temperature of various fish transport tests showed not only able to minimize the metabolites and stress level of fish, but also able to prevent a drastically decline of water quality and support survival rate of various species during transport (Syamdidi et al 2006; Tahe 2008; Harmon 2009; Rosten & Kristensen 2011; Kamalam et al 2017). The other value of physical and chemical of water during temperature acclimatization were still in a reasonable range to support the physiological function of glass eel for 24 hours.

Conclusions. The acclimatization method of post captured wild glass eel, *A. bicolor bicolor,* in salinity 6 g L^{-1} and temperature ranged from 16°C to 20°C, gradually and continuously over 24 hour, resulted the best physiological status for transporting glass eel. An appropriate acclimatization method can support better homeostatic mechanism for osmoregulation, whole body glucose level, oxygen consumption level, and hydromineral status of glass eel, so the vitality of fish is more excellent for shipment.

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