Survival rate of transported ricefield eels, *Monopterus albus* (Synbranchidae), in open and closed system at water salinity level of 0 and 9 g L^{-1}

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Abstract. This research aimed to study the effects of water salinity on the survival of transported ricefield eels (*Monopterus albus*). The eels were car transported at a density of 1000 kg m^{-3} using both open and closed systems each with water salinity level of 0 and 9 g L^{-1}. After 6 hours of transport duration, the eels were unloaded and placed in an acclimatization unit at the same salinity level they had been transported at. The results showed that the survival rates of all the eels were high on arrival. After the 5-week acclimatization period, the survival rate of the previously transported eels in the open system with freshwater was decreased significantly ($p<0.05$), i.e. 58.26±16.79%, compared to the other treatments. Transportation of the eels using the closed system with freshwater generated an equal statistically of high survival with the two systems of saline transportations. Evidently, the addition of sodium chloride to the water could increase the survival of the eels by 55% in the open systems and by 14% in the closed systems. Acclimatization of the post-transported *M. albus* at a salinity level of 9 g L^{-1} increased the plasma sodium content. Delayed mortality syndrome was concentrated high in the first two weeks after the transportation. It can be concluded that the application of 9 g L^{-1} NaCl to water is an effective method for increasing the survival rate of *M. albus* during transportation.

Key Words: acclimatization, condition factor, cortisol, delayed mortality syndrome, NaCl.

Introduction. Recently in Asia, live transportation of ricefield eels (*Monopterus albus*) has been increased, triggered by expansion of the eels marketing (Hasan et al 2012; Nico et al 2011) and for aquaculture purposes (Alit 2009; Idris et al 2015; Khanh & Ngan 2010). In Indonesia, there are two types of *M. albus* transportation systems. The first one is the open system, which uses a jerrycan with holes in the lid for air circulation, and the second one is the closed system, which is oxygenated and tied up in a transparent plastic bag. Both systems can have a high loading density of 1,000 kg m^{-3}. Previously, mortality due to transportation was not considered because the dead eels are still marketable in the form of processed products. However, in aquaculture the fish mortalities should be avoided because they affect productivity, which leads to economic loss. Unfortunately, the mortality of the *M. albus* is not easy to observe due the muddy culture system, and the facts showed that there were no reports on the successful cultivation of the eels, especially regarding the survival rate of the individuals (Alit 2009; Idris et al 2015). Hence, the causes of mortality should be explored, including an exploration of the negative effects of transportation. It is known that the transport of fish involves of many activities that are stressful to the subjects, such as pre-transportation handling, packaging, loading and unloading, which are carried out in a short period of time. This has led to the demand for the development of practical techniques to reduce this stress and improve survival rate.

The stressful transportation of *M. albus* begins when they are harvested due the catching methods and equipment, as reported by Barman et al (2013), and the stressful conditions continue along the marketing chain (Hasan et al 2012), as the fish are exposed to sudden changes in environmental conditions during packaging, delivering, and storing after transport. Fish exposed to stress undergo physiological changes (Gomes et al 2003; Wedemeyer 1996) such as induced sodium leakage from the blood plasma (Martemyanov 2013) and delayed mortality syndrome (Urbinati & Cameiro 2006) Wedemeyer 1996). Fish losses that occur after transportation can be categorized as death on arrival (DoA), which refers to fish that die up to the point when the bags are opened, and death after arrival (DaA), which refers to fish that die during acclimatization period (Schmidt & Kunsmann 2005).

In order to overcome low levels of blood salt due to sodium leakage, fish absorbs salt directly from the environment or from feed (Wurts 1995). However, food is not generally provided during transportation, so the addition of salt to the water is an alternative way to provide a supply of environmental salt for absorption to replace the missing blood salt. The use of sodium chloride (NaCl) has continued to gain acceptance as a fish transport additive in order to reduce the osmotic gradient, stimulate mucous production and minimize stress (Carneiro et al 2009; Gomes et al 2003; Tsuzuki et al 2001). Salt has been used for fish transportation at concentrations of 1-3 g L^{-1} for pirarucu *Arapaima gigas* (Gomes et al 2003) and 1-5 g L^{-1} for rainbow trout *Oncorhynchus mykiss* (Tacchi et al 2015). The addition of NaCl at a concentration of 6 g L^{-1} to the water used for transportation reduced the cortisol and glucose levels of silver catfish, *Rhamdia quelen* (Carneiro et al 2009). *M. albus* are known to live in an environment with a salinity range of 0-16 g L^{-1} (Schofield & Nico 2009) and they experience optimum growth at a salinity range of 0-9 g L^{-1} (Pedersen et al 2012). The osmotic gradient of *M. albus* is 0.350 Osm in freshwater, but it is reduced to 0.150 Osm with salinity levels of 6 and 9 L^{-1} (Syarif 2015). At salinity levels of 0 and 5 g L^{-1}, the osmolality of *M. albus* is 0.275 Osm, with plasma sodium levels of 135 mmol; however, both parameters increase when the fish are exposed to higher levels of environmental salinity (Pedersen et al 2012).

This research aims to study the effects on the survival rate of *M. albus* when they are transported in water with salinity levels of 0 and 9 g L^{-1}.

Material and Methods. The research was conducted from May to July 2015. *M. albus* were collected from ricefield areas in Cianjur and transported to Bogor, West Java, a distance of 92 km, which took 6 hours. The fish were transported in water with a salinity of 0 and 9 g L^{-1} in the open and closed systems. *M. albus* with a length of 24.44±0.57 cm and a weight of 12.54±5.01 g were caught using electrofishing equipment at night before the experiment was conducted. The open system of transportation used a rectangular plastic container of 50x30x30 cm in size, which had air holes in the lid. The closed system used polyethylene bags of 100×40 cm in size; these bags were oxygenated, with a ratio of 1:3 water to oxygen, by volume. Freshwater and water with added NaCl was used.

Transportation mode. Five kg *M. albus* were placed in 5 L of water (at a density of 1000 kg m^{-3}), which was placed in the transport system and transported by a vehicle for 6 hours. On arrival, the fish were held for 5 weeks at 2.5 kg in 25 L of water (at a density of 500 kg m^{-3}). The water level in the holding container was keep at 10 cm. The water was changed every day and the salinity was kept at the salinity used during transportation. The eels were fed daily with chopped fillet of walking catfish (*Clarias* sp.), at feeding rate of 5% of their biomass.

Sample collections and measurements. The *M. albus* were anesthetized before blood collection with Ocean Free Special Arowana Stabilizer (Qian Hu Corporation Ltd, Singapore) at a dose of 1-2 mL L^{-1}. Blood plasma was obtained by centrifuging the blood at 3000 rpm and it was stored in the freezer before the subsequent procedures were carried out. Cortisol measurements were made by radioimmunoassay using IZOTOP Cortisol [125I] RIA KIT (Ref: RK-240CT) (Institute of Isotopes Ltd, Budapest). Measurements of plasma glucose and liver glycogen were conducted using a spectrophotometer at a wavelength of 635 nm on standard D-(+)-glucose (C6H12O6) (K34425937.606, Merck, Germany). Measurements of plasma sodium were prepared using a wet washing method and they were analyzed using an Atomic Absorption Spectrophotometer (AAS). The water quality parameters measured were salinity (using a salinometer), temperature (using a temperature meter), pH (using a pH meter), oxygen (using a Dissolved Oxygen meter) and ammonia (method of APHA 2012).

Data analysis. The average values ± standard deviations are displayed, and the data were tested using ANOVA and post hoc multiple comparison tests with Duncan's multiple range tests at $p<0.05$ in Excel 2007 and SPSS 16.0. Significant differences between treatments are marked with different superscript letters, as explained beneath each tabulation.

Results and Discussion. As shown in Table 1, the transportation system did not affect the survival rates of the *M. albus* (see p-values). On arrival, the survival rates were high for all transportation modes and salinity levels. However, after 5 weeks of acclimatization, the eels exhibited post-transportation delayed mortality syndrome. The survival rates of the eels decreased, although these decreases were only statistically significant for the eels that had been transported in open systems with freshwater ($p<0.05$). Rearing the eels that had previously been transported in closed systems with freshwater still maintain high survival rates, as well as those fish reared in saline water. Increasing the salinity of the transport water from 0 to 9 g L^{-1} increased the survival rates of *M. albus* by 55% in the open systems and by 14% in the closed systems.

Table 1

Survival rate of *Monopterus albus* transported in open and closed systems with a water salinity of 0 and 9 g L^{-1}

Transportation system	*Survival rate (%) of post-transportation M. albus*	
	On arrival	*5 weeks post-transportation*
Open system at S of 0 g L^{-1}	98.45 ± 2.19^a	58.26 ± 16.79^b
Open system at S of 9 g L^{-1}	100.00 ± 0.00^a	90.17 ± 9.10^a
Closed system at S of 0 g L^{-1}	100.00 ± 0.00^a	86.37 ± 2.74^a
Closed system at S of 9 g L^{-1}	100.00 ± 0.00^a	98.03 ± 0.19^a
ANOVA: two-factor with replication	p-value	p-value
Systems	0.374	0.058
Salinities	0.374	0.033
Interaction	0.374	0.212

S = salinity. Means with different superscript letters are significantly different at $p<0.05$, n = 2 replications.

Water salinity affected the physiological status of the *M. albus*, as shown in Figures 1-3. At an average body length of 24.44±0.57 cm, the condition factor (CF) of the *M. albus* was higher at a water salinity level of 9 g L^{-1} compared to freshwater (Figure 1). The CF is an index used to measure an individual fish health and it reflects interactions between biotic and abiotic factors that indicate the physiological condition of the fish. Low osmotic gradients of *M. albus* in saline water decrease the osmoregulation required by the fish, so there are lower osmoregulation costs, which allow body weight to be maintained (Syarif 2015).

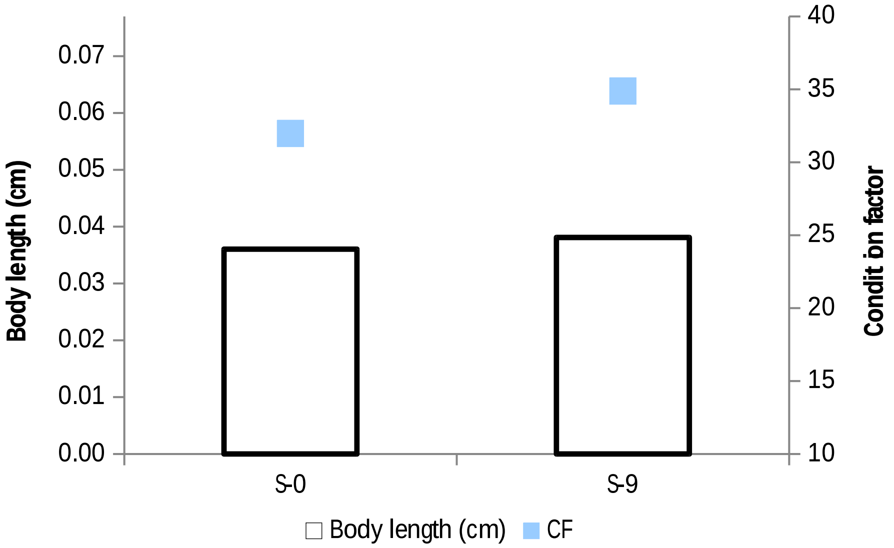

Figure 1. Condition factor (CF) compared to body length of *Monopterus albus* acclimatized at water salinity of 0 and 9 g L^{-1}.

Saline water at a salinity level of 9 g L^{-1} was beneficial to *M. albus* in that they had lower glucose levels and they retained high liver glycogen concentrations (Figure 2). This indicates that the eels had a better physiological status so that the fish could cope with environmental changes during the transportation and acclimatization activities and therefore their survival rate was higher.

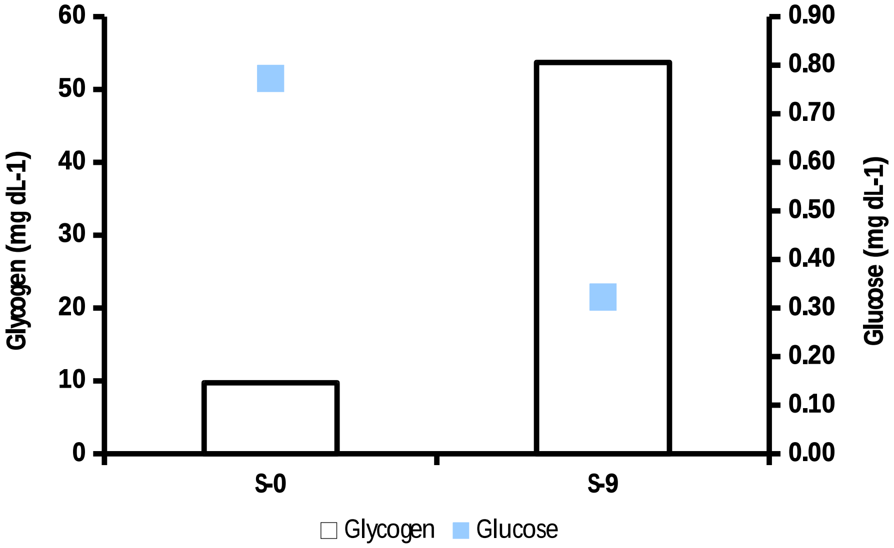

Figure 2. Blood plasma glucose and liver glycogen concentration of *Monopterus albus* acclimatized to water salinity of 0 and 9 g L^{-1}.

M. albus are freshwater fish with a characteristic behavior, they bury themselves in mud and inhabit in earthen dike holes. However, immersing the eels to the freshwater resulted high cortisol levels of 22.76±8.5 ng mL^{-1}, whereas at salinity of 9 g L^{-1} the cortisol level was 14.74±9.10 ng mL^{-1}. This indicates that the fish is able to reduce its stress response in saline water. In contrast, the sodium level of the eel blood plasma is lower in freshwater compared to the eel in saline water, as plasma sodium is retained due to the low osmotic gradient.

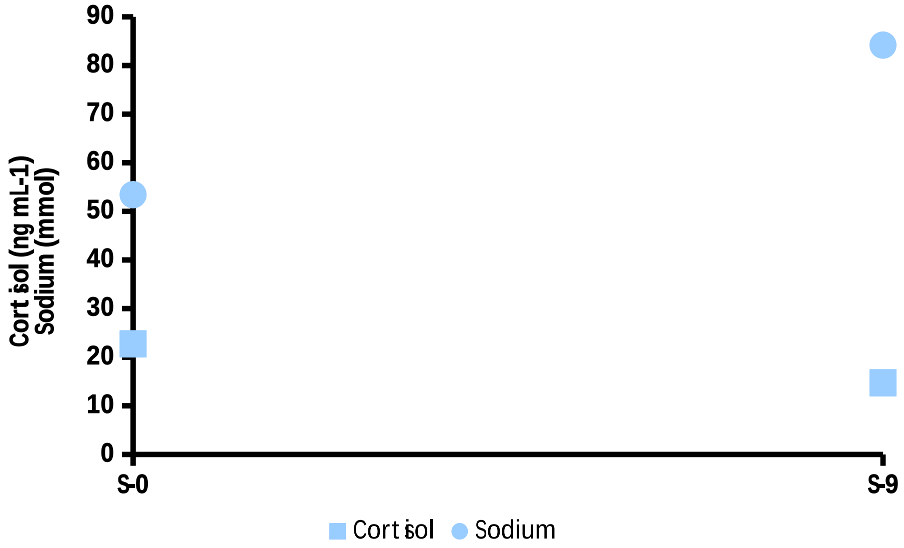

Figure 3. Cortisol and sodium concentration of *Monopterus albus* blood plasma acclimatized to water salinity of 0 and 9 g L^{-1}.

Further explanation of the effect of salinity levels on plasma sodium can be seen in Table 2.

Table 2

Plasma sodium of the transported *Monopterus albus*

Transport system	*Plasma sodium (mmol L^{-1}) of M. albus*			
	Pre-transport	*Post-transportation*		
		On arrival	*Week 1*	*Week 2*
Open system at S of 0 g L^{-1}	118.66 ± 10.53^{bcd}	103.04 ± 4.09^{d}	125.51 ± 3.26^{abcd}	$118.07 \pm 18{,}03^{bcd}$
Open system at S of 9 g L^{-1}		125.40 ± 7.95^{abcd}	118.25 ± 13.91^{bcd}	136.91 ± 8.21^{ab}
Closed system at S of 0 g L^{-1}		133.88 ± 19.61^{abc}	123.46 ± 20.45^{abcd}	111.47 ± 21.44^{cd}
Closed system at S of 9 g L^{-1}		144.73 ± 6.59^{a}	126.81 ± 6.86^{abcd}	130.12 ± 1.50^{abc}

S = salinity. Means with different superscript letters are significantly different at $p<0.05$, n = 4.

On arrival of the transported *M. albus*, the fish transported in closed systems with saline water had the highest levels of plasma sodium, and the lowest levels were experienced by the fish transported in open systems with a water salinity level of 0 g L^{-1}, while acclimatizing the eels experienced increased plasma sodium more rapidly in saline environments. According to Pedersen et al (2012) the plasma sodium level of *M. albus* was 135 mmol at water salinity levels of up to 5 g L^{-1}, hence the lower value of sodium plasma in this transportation experiment could be assumed that the eels undergo sodium leakage due to stress and show effort to do recovery. The fish absorbed sodium when they were in hyperosmotic environments but they suffered a sodium leakage when they were surrounded by dilute water, such freshwater. The ability to absorb salt from the environment is also affected by corticosteroid hormones that play a role in osmoregulation, metabolism, balance and the overall hydromineral stress response (McCormick & Bradshaw 2006). The response of the eels to salinity levels could explain the reason behind the fact that the eels tend to hide in mud holes, as this could be an attempt to avoid sodium leakage.

Delayed mortality syndrome in the post-transportation *M. albus* is shown in Table 3. DoA was low and not significant in all the treatments, whilst DaA fluctuated over 4 weeks. The mortality of the fish was high at week 2 and 3 in all the treatments, but statistically significant ($p<0.05$) only for the eels previously transported in the open system with freshwater, whereas transporting the eels in the closed system with saline water led to the lowest mortality rates each week. This indicated that the environment was suited to the acclimatization of the eel and it promoted better recovery from the transportation stress.

Table 3

Delayed mortality syndrome of post-transportation *Monopterus albus* during 5 weeks of acclimatization

Transportation system	*Delayed mortality syndrome (%)*				
	Death on arrival (DoA)	*Death after arrival (DaA)*			
		Week 1	*Week 2*	*Week 3*	*Week 4*
Open system at S of 0 g L^{-1}	1.5 ± 2.19^{a}	26.23 ± 13.21^{c}	11.39 ± 2.26^{b}	0.77 ± 1.09^{a}	1.03 ± 1.46^{a}
Open system at S of 9 g L^{-1}	0.00 ± 0.00^{a}	3.31 ± 4.69^{ab}	6.21 ± 3.99^{ab}	0.30 ± 0.43^{a}	0.00 ± 0.00^{a}
Closed system at S of 0 g L^{-1}	0.00 ± 0.00^{a}	4.97 ± 1.24^{ab}	6.55 ± 3.48^{ab}	2.11 ± 0.50^{a}	0.00 ± 0.00^{a}
Closed system at S of 9 g L^{-1}	0.00 ± 0.00^{a}	0.31 ± 0.43^{a}	1.10 ± 0.68^{a}	0.26 ± 0.37^{a}	0.00 ± 0.00^{a}

S = salinity. Means with different superscript letters are significantly different at $p<0.05$, n = 2.

Six hours of transportation changed the temperatures, levels of dissolved oxygen and the salinity levels of the water being transported (Table 4). The temperature increased from 24°C to 28°C, which was caused by the temperature in the transport vehicles reaching 29.0±1.0°C. The levels of dissolved oxygen in the closed system showed an increase due to the addition of pure oxygen, whereas in the open system the levels of dissolved oxygen tended to diminish. The transported freshwater exhibited a slight increase in salinity while the transported saline water exhibited a slight decrease in salinity. The increased salinity was assumed to be due to the sodium leakage from the eels and the decreased salinity was assumed to be due to absorption by the fish.

Table 4

Water quality in the *Monopterus albus* transport systems

Transportation system	*T (°C)*	*DO (mg L^{-1})*	*S (g L^{-1})*
Pre-transportation			
Open system at S of 0 g L^{-1}	24.4±0.2	7.6±0.1	0.3±0.0
Open system at S of 9 g L^{-1}	24.4±0.1	7.9±0.1	9.0±0.1
Closed system at S of 0 g L^{-1}	24.3±0.1	7.7±0.2	0.3±0.0
Closed system at S of 9 g L^{-1}	24.4±0.1	7.6±0.3	9.0±0.1
Post-transportation			
Open system at S of 0 g L^{-1}	28.0±0.8	3.2±0.4	0.5±0.0
Open system at S of 9 g L^{-1}	28.1±0.8	7.1±0.4	8.4±0.2
Closed system at S of 0 g L^{-1}	28.1±0.6	13.7±2.0	0.4±0.0
Closed system at S of 9 g L^{-1}	27.9±0.8	13.5±3.5	8.5±0.3

T = temperature, DO = dissolved oxygen, S = salinity.

However, the dissolved oxygen was also used in order for the eels to cope with the environmental changes. In the transported freshwater, presumably more oxygen was consumed by the eels. According to Awal et al (2012), the oxygen consumption of tilapia in a freshwater environment is up to 300 times higher than in a saline water environment with a salinity level of 10 g L^{-1}. The oxygen consumption rate then decreased with increasing salinity, and the lower oxygen consumption at high salinity levels may be a result of reduced activity, which in itself was modulated by salinity levels. The oxygen consumption rates were related to the energetic cost of ionic and osmotic regulation in case of salinity differences in the transported water. In stressful conditions, such as during transportation, eels require more oxygen in order to cope with stress and maintain homeostasis. Apparently the addition of oxygen in a closed system is an effective stress mitigation technique when transporting eels. According to Berka (1986), transportation of fish in a closed system with the addition of oxygen in high pressure ensures that the oxygen is not a limiting factor and the amount of oxygen consumed depends on the amount of oxygen available, which is at a stable level when oxygen availability is high and low when the availability of oxygen is low despite the fact that the need is high

(Berka 1986). In the open transport system with the highest mortality of eels, low levels of oxygen (hypoxia) may have exacerbated the problems. This is supported by Berka (1986), who showed that when the transportation system has insufficient oxygen levels, this makes the fish shift their metabolism to anaerobic metabolism and use up the stored oxygen in their bodies, causing oxygen debt. Three hours of hypoxia experienced by cichlid Amazonian fish *Astronotus ocellatus* resulted in depression of their metabolic rate by 50% followed by a decrease of up to 50%-60% in protein synthesis in their livers, hearts and gills (Lewis et al 2007). Hypoxia results in changes to the physiological status of fish, such as hematocrit and blood hemoglobin, as well as glucose and sodium concentrations in the plasma (Silkin & Silkina 2005).

Conclusions. The survival rate of the *M. albus* was not affected by the transportation system but it was affected by the water salinity during the post-transportation acclimatization of the fish. Freshwater should be avoided during *M. albus* transportation as it causes an alteration of the physiological status of the eels. The addition of NaCl at a dose of 9 g L^{-1} to the water during transportation was effective at mitigating stress and it increased the survival of the *M. albus*.

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