Effects of different loading densities during transport on survival rates of Asian seabass (*Lates calcarifer* Bloch, 1790) juvenile

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ABSTRACT

This study was carried out to evaluate effects of loading density during transport on water quality and survival rate of Asian seabass (Lates calcarifer) juvenile. The experiment included four treatments of different loading densities: 50 kg/m^3 (T1), 70 kg/m^3 (T2), 90 kg/m^3 (T3) and 110 kg/m^3 (T4) with three replicates for each treatment. The fish with an average weight of 20.50 ± 0.25 g was transported in an aerated and oxygenated heat-insulated tanks. The water temperature in transport was set at 22°C and the concentration of isoeugenol-50% was 6 ppm. Water quality, blood glucose and survival rate of the fish were recorded at the beginning, after transport 6 h and 12 h, and 3 and 7 days after the end of transporting. The results showed that the water quality was declined expressed by the decrease of DO and pH, and the increase of CO_2, TAN and NO_2 during transport but still in suitable ranges for seabass. The blood glucose content of fish increased during transportation due to stress. The survival rates of the fish of all treatments were reduced following transport duration. The fish was well recovered after the transport. At the end of the study, the survival rate of the fish of T1 was highest (96.00%), followed by T2 (95.33%), T3 (90.00%), and T4 (87.63%). Based on the accumulated mortality, loading densities of 70 to 90 kg/m^3 and 50 to 70 kg/m^3 were recommended for transport of seabass juvenile in cooling water (22°C) and isoeugenol-50% (6 ppm) during 6 and 12 h, respectively.

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1. Introduction

Asian seabass or barramundi (*Lates calcarifer* Bloch, 1790) is an euryhaline fish widely distributed in the Indo-West Pacific, from the Arabian Gulf to China, Taiwan, and Northern Australia (FAO, 2020). According to GOAL, seabass production for selected survey countries was about 108,000 MT in 2019, up by 20% compared to 2018, and the forecast for 2019 was at an increase of 6% to around 115,000 MT (Tveteras et al., 2019). In Vietnam, *L. calcarifer* species is distributed in the Eastern of Northern Gulf and the Central Coast. It has been successfully farmed in many coastal provinces in the countries such as Quang Ninh, Hai Phong, Thua Thien - Hue, Quang Nam, Da Nang, Binh Dinh, Khanh Hoa, Binh Thuan, Ba Ria - Vung Tau, Ben Tre, and Soc Trang (Nguyen, 2009; Ly et al., 2016; Nguyen & Nguyen, 2018).

Seabass farming systems in Vietnam are include brackish water ponds and cages suspended in coastal water bodies. However, the major system in the Mekong river delta is seabass farming in ponds (Ly et al., 2016). Currently, seabass hatcheries are mainly located in South Central provinces (Khanh Hoa, Ninh Thuan, Binh Thuan) and Eastern (Ba Ria - Vung Tau) (Tran et al., 2019). Live fish transportation usually produces negative effects on fish due to the degradation of water quality (Rimmer et al., 1997a). One of them is the stressful state of transported

fish. Stress can lead to high incidence of disease, decrease in feeding and growth, changes of behavior, and mortality in critical cases (Rucinque et al., 2017). Methods to make the fish sedative, movement, and stress reduction are important in live fish transportation. Cooling water and using anesthetics are the most common methods applied in live fish transport (Yoshikawa et al., 1989; Coyle et al., 2004; Lili et al., 2020). There are many anesthetics used in the laboratory as well as in aquaculture such as tricaine methanesulfonate (MS-222), benzocaine, clove oil, eugenol, and isoeugenol (Rucinque et al., 2017; Priborsky & Velisek, 2018; Schroeder et al., 2021).

Recently, AQUI-S was proposed as an immediate or reduced withdrawal time sedative and used in fish propagation and transportation (Javahery & Moradlu, 2012; Cupp et al., 2017). The common method of anesthetic used in live fish transportaion was immersion (Neiffer & Stamper, 2009). Safely transporting higher loading densities of fish would benefit seed producers by increasing efficiency and reducing costs, but research evaluating transport for individual species is generally lacking (Cupp et al., 2017). This study aimed to evaluate effects of loading densities on water quality and survival rate of seabass juvenile during and post live fish transport.

2. Materials and Methods

2.1. Experimental animals

Asian seabass (*Lates calcarifer*) juvenile with an average weight of 20.50 ± 0.25 g and length of 11.52 ± 0.22 cm was used in this experiment. The fish used in the live transporting experiment were relatively uniform size, good appearance, no signs of disease, and scratch. The fish was fasted for 24 h prior to transporting.

2.2. Water and anesthetic

Water used in fish transport was taken from a treated reservoir with quality parameters as following: salinity = 18‰, pH = 8.2, dissolved oxygen (DO) > 4.5 ppm, total ammonia nitrogen (TAN) < 0.2 ppm, nitrite (NO_2) < 0.01 ppm and transparency > 150 cm.

Insulated tanks (30 cm × 40 cm × 38 cm) for transporting fish, containing 40 L of water, were equipped with tubes connected to an air compressor and a liquid oxygen cylinder for aeration and oxygenation (Berka, 1986). All the tubes were installed valves to control air and oxygen volume injection to ensure the even distribution and saturation of DO in each tank. A small hole was created on tank lids to let excess air and oxygen escape without water leaking. The air compressor, oxygen cylinder, and fish tanks were loaded in an insulated truck which air temperature was adjusted around 20 to 22°C during the transport.

Before the experiment, two trials imitated a live fish transportation was carried out to evaluate effects of cooling temperatures and anesthetic of isoeugenol-50% (AQUI-S®) concentrations on seabass juvenile. Based on survival rates of the fish at the end of the trials and on the seventh-day post transporting, appropriate values of 22°C for cooling temperatures and of 6 ppm for isoeugenol-50% concentrations were identified.

2.3. Experiment design

This experiment comprised of four treatments of different loading biomass of fish for transporting: 50 kg/m^3 (T1), 70 kg/m^3 (T2), 90 kg/m^3 (T3) and 110 kg/m^3 (T4), with three replicates for each treatment. The water temperature in all transport tanks was set at 22°C and the concentration of isoeugenol-50% was 6 ppm. The fish was acclimatized to the experiment temperature in a holding container by gradually cooling water with ice from room temperature to 22°C for 30 min and immediately transferred to the experiment tanks and closed with lids. Then all the tanks were loaded into the truck for transporting. In fact, the time to transport fingerlings usually took from 6 h to 12 h.

At the end of the transport (12 h), the water temperature of all tanks was gradually raised to pond water temperature by adding pond water for 30 min to protect the fish from heat stress caused by quick temperature change. After transporting, the fish in each treatment was stocked in 2 m^2 hapas suspended in pond for routine management and fed ad libitum with Uni President's floating seabass pelleted feed of 43% crude protein at 6:00 AM and 17:00 PM.

2.4. Recorded data

Environmental parameters including temperature, DO and pH were measured with an AZ8602 (AZ Instruments); of which, the degree of accu-

racy of temperature, DO and pH measurement was of 0.1°C, 0.1 ppm, and 0.1, respectively. CO_2 was measured with an EA80 (EXTECH-USA) meter, TAN with a HI97700 (HANNA) meter and NO_2 with a NO_2-30 meter (China); of which the degree of accuracy of CO_2, TAN and NO_2 was of 1 ppm, 0.01 ppm and 0.01 ppm, respectively.

Fish blood glucose was measured with the Medismart Sapphire Plus test kit (Switzerland), using an automatic test strip to take a very small amount of blood 0.6 µL from the fish's tail, measuring range from 20 - 630 mg/dL. Results are displayed for 5 sec. Blood samples were collected at initial, after 6 and 12 h of transport, 3 and 7 days after transport to assess the stress level of fish before, during, and after transport. At each time of data collection, 9 samples were collected for each treatment. After collecting the samples, the fish were returned to the treatment.

The water parameters in the tanks were recorded at 0, 6, and 12 h of the transport, and in the pond on the days of 1, 3, and 7 post transporting. The survival rate (%) of the fish was also recorded at 6 and 12 h of the transport, and on the days of 3 and 7 post transporting.

2.5. Data analysis

Statistical analysis was performed with Microsoft Excel 2010 and SPSS 20.0 for Window software. Data were analyzed with one-way analysis of variance (ANOVA) at the significance level of $P = 0.05$, and when effects were found to be significant, LSD was used to determine differences for each paired treatment. Percentage values were converted to arsin$\sqrt{}$ prior to analyzing. The data in tables were presented as mean ± standard deviation.

3. Results

3.1. Water quality

Water parameters of the tanks recorded in the 6 and 12 h transport times were presented in Table 1.

The water quality of fish tanks was reduced following transport time expressed by the significant decrease of pH and DO, and the increase of CO_2, TAN and NO_2 ($P < 0.05$) compared to initial time, except for NO_2 after transported 6 h (Table 1). The water quality was also reduced following increased stocking densities. In the 6 h transport time, pH means between T1 and T2 treatments, T2 and T3 treatments, T3 and T4 treatments were not significantly different. No significant difference of DO means was found between T1 and T2, T3 and T4 treatments; and of CO_2 means between T2 and T3 treatments, T3 and T4 treatments. TAN means between all treatments were significantly different. The significant difference of NO_2 means was also found between all treatments, except for T1 and T2 treatments (Table 1).

At the end of the transport, no significant difference ($P > 0.05$) was found for pH means between T1 and T2 treatments, T2 and T3 treatments; for DO means between T1 and T2 treatments, and T2, T3, and T4; for CO_2 means between T1 and T2 treatments, T2 and T3 treatments, and T3 and T4; and for NO_2 means between T1 and T2, and T2 and T3 treatments. TAN means between all treatments were significantly different ($P < 0.05$), except for T1 and T2 treatments (Table 1).

In general, there was no considerable fluctuation of the water quality parameters in the pond between different times posts transporting. Temperature, pH and DO values in the afternoon were higher than those in the morning (Table 2).

The glucose content (mg/dL) in fish blood was investigated at the time before transport, after 6 and 12 h of transport, 3 and 7 days post transporting (PT) in the experiments. The data were presented in Figures 1 and 2.

3.2. Glucose indicator

At the time before transport, the blood glucose content of fish fluctuated in the range of 68.3-73.3 mg/dL. The glucose content tends to increase during transportation. After 6 h of transportation, the highest concentration of glucose in the blood of fish in the treatment 110 kg/m^3 (100.8 ± 7.9 mg/dL) was significantly higher ($P < 0.05$) compared with the other treatments. After 12 h of transportation, the glucose content continued to increase, the treatment of 110 kg/m^3 was highest (122.2 ± 10.9 mg/dL), followed by the treatment of 90 kg/m^3 (98.8 ± 8.0 mg/dL) was different from the other 2 treatments. At the time of 3 days post transporting, the glucose content decreased and by the time of 7 days post transporting, the glucose content almost returned to the

Table 1. Water quality parameters in the 6 and 12 h transport time

Time	Treatment	Parameters				
		pH	DO (ppm)	CO_2 (ppm)	TAN (ppm)	NO_2 (ppm)
6 h	Initia	8.20 ± 0.0^{d}	7.55 ± 0.13^{c}	0.00 ± 0.0^{a}	0.50 ± 0.0^{a}	0.01 ± 0.0^{a}
	T1	7.60 ± 0.20^{c}	6.37 ± 0.15^{b}	11.67 ± 1.53^{b}	1.13 ± 0.12^{b}	0.03 ± 0.01^{ab}
	T2	7.47 ± 0.06^{bc}	6.17 ± 0.15^{b}	14.17 ± 0.58^{c}	1.43 ± 0.12^{c}	0.04 ± 0.01^{b}
	T3	7.33 ± 0.12^{ab}	5.80 ± 0.20^{a}	15.80 ± 1.00^{cd}	1.73 ± 0.12^{d}	0.07 ± 0.02^{c}
	T4	7.13 ± 0.12^{a}	5.50 ± 0.30^{a}	16.67 ± 1.53^{d}	2.20 ± 0.20^{e}	0.09 ± 0.0^{d}
12 h	Initia	8.20 ± 0.0^{d}	7.55 ± 0.0^{c}	0.00 ± 0.0^{a}	0.50 ± 0.0^{a}	0.01 ± 0.0^{a}
	T1	7.27 ± 0.25^{c}	6.20 ± 0.26^{b}	15.83 ± 2.52^{b}	1.27 ± 0.25^{b}	0.05 ± 0.01^{b}
	T2	7.03 ± 0.25^{bc}	5.80 ± 0.20^{ab}	19.83 ± 2.52^{bc}	1.53 ± 0.31^{b}	0.07 ± 0.02^{bc}
	T3	6.77 ± 0.25^{b}	5.63 ± 0.15^{a}	22.47 ± 2.08^{cd}	2.20 ± 0.20^{c}	0.09 ± 0.01^{c}
	T4	6.23 ± 0.25^{a}	5.33 ± 0.42^{a}	25.33 ± 3.25^{d}	3.00 ± 0.20^{d}	0.13 ± 0.03^{d}

Means within the same column with different superscript letters are significantly different at $P < 0.05$ where a < b < c < d.

Table 2. Water quality parameters in the pond at different times post transporting

Parameters	Time	Day post transporting			Mean ± SD
		1	3	7	
Temperature (°C)	8:00	28.50	28.30	28.50	28.43 ± 0.09
	16:00	29.50	29.40	29.30	29.40 ± 0.07
pH	8:00	8.00	7.90	8.00	7.97 ± 0.04
	16:00	8.50	8.40	8.60	8.50 ± 0.07
DO (ppm)	8:00	5.20	5.40	5.50	5.37 ± 0.11
	16:00	6.70	6.50	6.70	6.63 ± 0.09
CO_2 (ppm)	8:00	9.50	10.50	10.00	10.00 ± 0.33
	16:00	1.60	1.20	0.50	1.10 ± 0.40
TAN (ppm)	8:00	1.30	1.50	1.20	1.33 ± 0.11
NO_2 (ppm)	8:00	0.01	0.02	0.05	0.03 ± 0.02

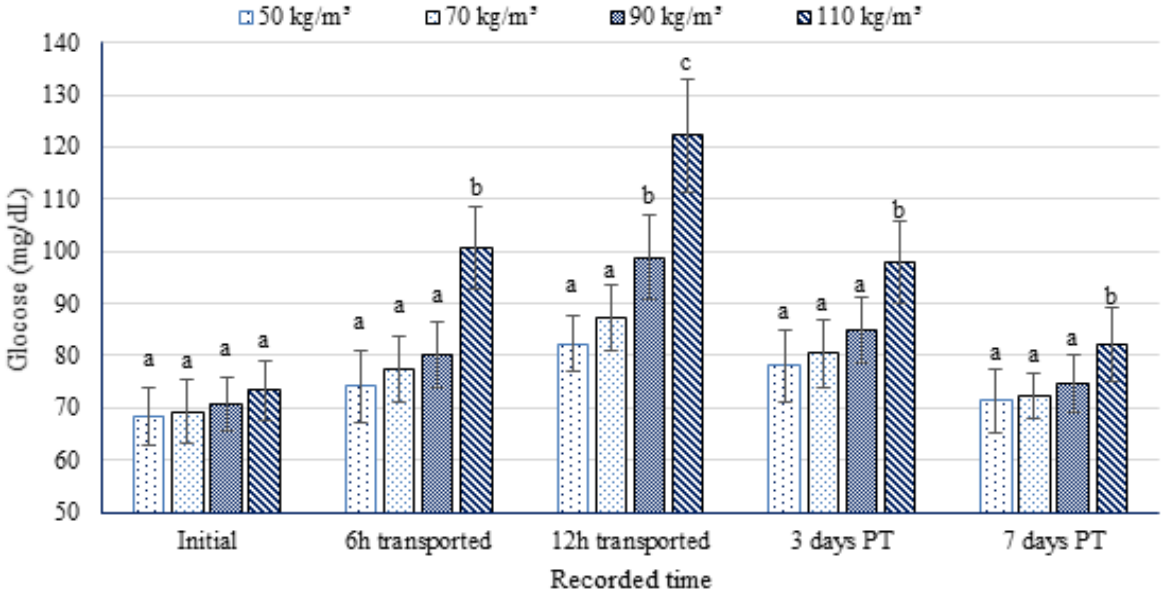

Figure 1. Variation of glucose content (mg/dL) in fish blood at the time of survey.
Columns containing the same letters present no significant difference of mean blood glucose at $P < 0.05$ where a < b < c.

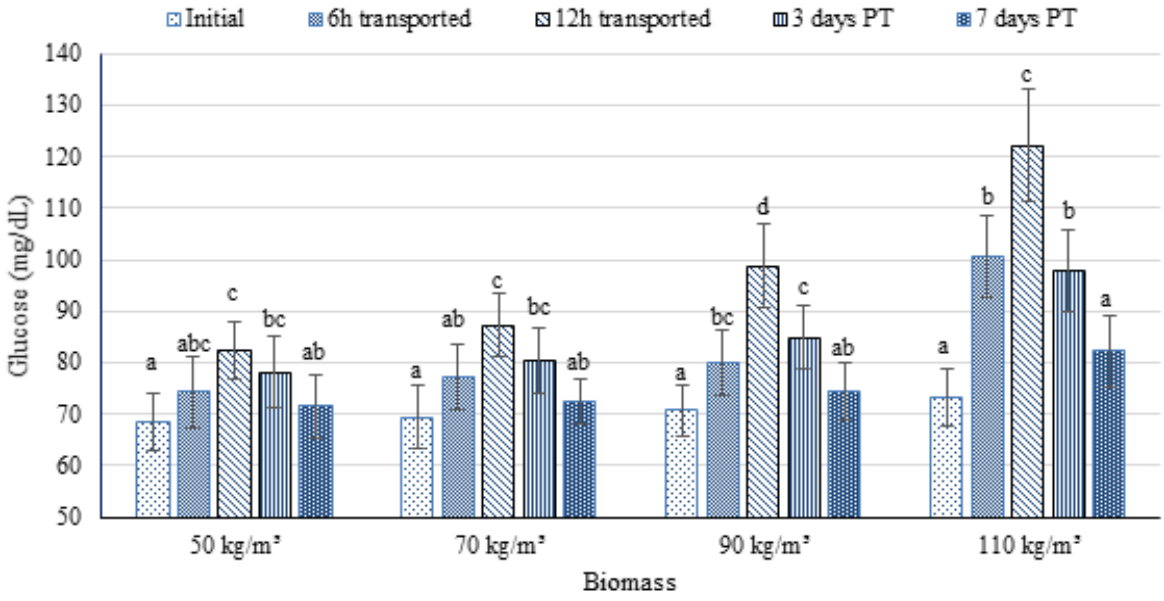

Figure 2. Variation of glucose content (mg/dL) in fish blood in different transport biomass.
Columns containing the same letters present no significant difference of mean blood glucose at $P < 0.05$ where a < b < c < d.

original value before transportation. However, in the treatment of 110 kg/m^3, the glucose content was still significantly higher ($P < 0.05$) compared with the other treatments (Figure 1).

Examining the variation of glucose content in each treatment, it showed that the glucose content tended to increase during transport and reached the highest value at the end of the transport duration (12 h). Then the glucose content tended to decreasing during post-transportation. In the treatment with higher biomass, the change in blood glucose concentration was stronger (Figure 2).

3.3. Survival rate

Survival rate of the fish in the 6 and 12 h transport, 3 and 7 days post transporting was presented in Figure 3.

In general, the survival rate (SR) of the fish reduced following transport and post transporting times. In the 6 h transport time, SR means of the fish in T4 treatment (92.67%) was lowest and significantly different ($P < 0.05$) from the others but there was no significant difference ($P > 0.05$) of the SR means between T3 (97.33%), T2 (98.67%) and T1 (98.67%) treatments. At the end of the transport (12 h), SR means of the fish T1 treatment (98,00%) was highest and followed by T2 (97,33%), T3 (92,00%), and T4 (84,00%) treatments. There was a significant difference of the SR means between the treatments, except for T1 and T2 treatments. A same trend of the SR of the fish was found for 3 days post transporting time (with T1 = 96.67%), T2 = 95.33%, T3 = 90.00% and T4 = 80.00%), as well as for 7 days post transporting (with T1 = 96.00%, T2 = 95.33%, T3 = 90.00% and T4 = 78.67%). At these times, the SR means of the fish between the treatments were significantly different, except for T1 and T2 treatments (Figure 3).

4. Discussion

Fish health state during live transportation is affected by several combined factors including dissolved oxygen (DO), pH, carbon dioxide (CO_2), ammonia (NH_3), and temperature. Respiration by the fish and bacteria causes depletion of DO and production of the toxic metabolite CO_2 and NH_3 accumulated in the transport water. The increase in CO_2 causes water pH to decrease and low pH increases the proportion of the toxic form of CO_2, but decreases the proportion of the toxic form of NH_3 (Berka, 1986; Rimmer et al., 1997a). Rimmer et al. (1997a) found that in closed seabass transport without water cooling and fish anesthesia, dissolved oxygen lev-

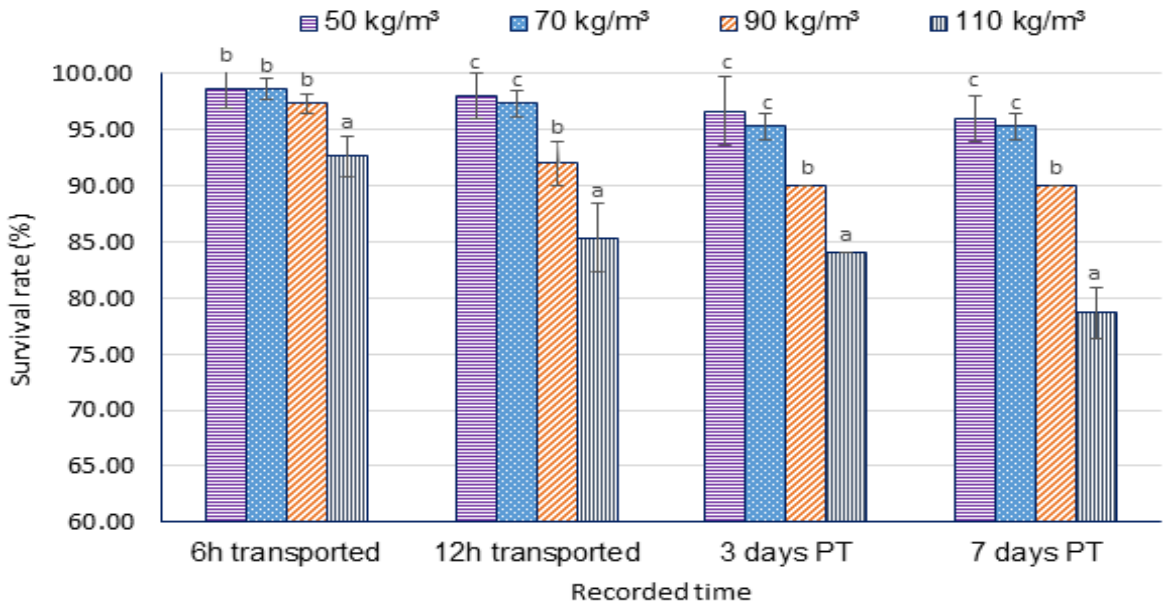

Figure 3. Survival rate of the fish at different time during transport and post transporting (PT). Columns containing the same letters present no significant difference of mean survival rates at $P < 0.05$ where a < b < c.

els at packing were 7.2 mg/L and dropped rapidly to only 3.5 mg/L; CO_2 also built up rapidly, from 13 to 38 mg/L; pH dropped rapidly from an initial value of 8.1 to 6.8 within 1.2 h after packing; in contrast to these variables, ammonia accumulated at a relatively constant rate throughout the experiment. In this study, DO levels of the treatments at packing were high (7.55 mg/L) and dropped consecutively (6.37 - 5.50 mg/L and 6.20 - 5.33 mg/L in the 6 and 12 h transporting times, respectively); in the same recorded times, CO_2 concentrations were low (0 mg/L) and increased (11.67 - 16.67 mg/L and 15.83 - 25.33 mg/L); pH values were high (8.2) and decreased (7.60 - 7.13.67 mg/L and 7.27 - 6.23 mg/L); and TAN concentrations were also low (0.5 mg/L) and increased (1.13 - 2.20 mg/L and 1.27 - 3.00 mg/L) (Table 1 and 2). However, the increase of CO_2 and TAN concentrations and decrease of DO levels and pH values were lower and slower compared to those in the study of Rimmer et al. (1997a). The lower and slower changes of these water parameters in this study could be explained by the cooling water and fish anesthesia which resulted in reduction of the metabolic rate of the fish thereby reducing oxygen consumption and the production of NH_3 and CO_2. High DO levels of the treatments at the end of the transport were due to the continuous oxygenation by the pure oxygen. The fluctuation of the water quality parameters in this study was also similar to that of studies of Simões et al. (2011) and of Gil et al. (2016) in live fish transportation of Nile tilapia (*Oreochromis niloticus*) and olive flounder (*Paralichthys olivaceus*) with the anesthetic of clove oil. The values of pH, DO, NH_3, and NO_2 of the transport water in this study were in suitable ranges for seabass recommended by Tookwinas & Charearnrid (1988). Thereby, the fish had high survival rates (92.67 - 98.67%) across all loading biomass in the 6 h transporting time (Figure 3).

In general, cooling water is only to make the fish sedated - reducing movement and maintaining equilibrium (Yoshikawa et al., 1989). An ideal anesthetic stage in live fish transportation is perfect sedation expressed by only opercular movement which can be achieved with anesthetic use (Simões et al., 2011; Gil et al., 2016). Cupp et al. (2017) found that at high loading densities of yellow perch (Perca flavescens) (240 g/L) and Nile tilapia (*O. niloticus*) (480 g/L), AQUI-S 20E (10% eugenol) concentrations (100 and 200 mg/L) decreased rapidly in transport tank water regardless anesthetic levels, and fish showed no signs of sedation by the end of the transport (6 h). According to Park et al. (2018), lowered temperature was effective in reducing stress measured by plasma cortisol in juvenile and adult

red spotted grouper (*Epinephelus akaara*) after exposure to 50 ppm clove oil for 48 h in various temperatures. However, for longer transport the mortality of the fish was increased, particularly at high loading densities of the T3 and T4 treatments. Elevated carbon dioxide concentrations are detrimental to fish and can be a limiting factor in fish transport (Berka, 1986). The survival of fish transported live is directly influenced by carbon dioxide and dissolved oxygen levels in the transport medium, either singly, or in combination (Rimmer et al., 1997b). The increase in the mortality of the fish by the end of the transport (12 h) in this study may be due to the stress of the fish caused by the increase of CO_2 (Table 1). Berka (1986) also noted that aeration of the water will reduce concentrations of dissolved CO_2, if there is adequate ventilation. Moreover, Alabaster et al. (1979) found that high levels of DO decreased the toxicity of ammonia in transport tanks. In the present study, applying aeration and oxygenation in the transport tanks could not prevent fish death but may mitigate the problem of over-accumulation of carbon dioxide and reduce the toxicity of NH_3, particularly at high loading biomass.

The change in blood glucose content of animals is considered as a hematological indicator to assess the stress level of animals. When animals are stressed, the adrenal glands are activated to release glucose to provide more energy to fight stressors, which often leads to increased blood glucose levels (Nguyen, 2005). Nguyen & Do (2014) showed that glucose content of pangasius fingerlings fluctuated and increased during transportation. When transporting for a long time, the glucose content increases due to stress. Dang (2019) when stressing pangasius fingerlings by changing temperature and salinity also showed an increase in blood glucose of fingerlings.

In this experiment, the blood glucose concentration of fish increased during the transport and reached the highest value at the end of the transport. When transporting fingerlings with higher biomass (90 to 110 kg/m^3), the fish was more stressed and the recovery was slower than the treatments with lower biomass (50 to 70 kg/m^3).

In general, the fish well recovered expressed by low mortality, particularly in the T1, T2, and T3 treatments, after the transport. Based on survival rate, loading biomass of 70 to 90 kg/m^3 were proper for live transport for 6 h and 50 to 70 kg/m^3 were proper for live transport for 12 h of seabass juvenile in cooling water of 22°C and sedated with AQUI-S® (containing 50% isoeugenol) at the concentration of 6 ppm.

5. Conclusions

The quality of tank water was declined following transport duration presented by the decrease of DO and pH, and the increase of CO_2, TAN and NO_2. Fish blood glucose levels increase and change with respect to transit time and fish biomass contained in the transport equipment.The survival rates of the fish decreased following times in transport duration and post transport, and following increased loading densities. The suitable loading biomass for live transport of seabass juvenile were 70 to 90 kg/m^3 and 50 to 70 kg/m^3 with a water temperature of 22°C, concentration isoeugenol-50% of 6 ppm and for 6 h and 12 h respectively. This study suggests for carrying out trials on live fish transport of marketable size of seabass.

Conflict of interest

The authors declare that they have no any conflict of interest in this paper.

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