The application of nanobubbles and charcoal in the closed transportation of *Litopenaeus vannamei* seeds

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Abstract. The application of closed transportation to vannamei seeds in the hatchery is far from being effective as shown by a number of problems that arise in the field. For instance, it is shown that the survival rate of the seeds after transportation has yet to reach a high level. To increase the survival rate of prawn seeds, this research applied nanobubbles and charcoal. This research aimed at determining the survival rate of prawn seeds and how the nanobubble-charcoal interaction affects it. This research used a factorial experiment using a basic completely randomized design with 2 factors. Factor A referred to the charcoal (0 g, 5 g, and 10 g), while Factor B referred to whether or not nanobubbles were used. The vannamei (*Litopenaeus vannamei*) seeds used were PL_9 in size and density was 1,600 seeds * 1.8 L-1 of water. Results show that, the charcoal treatment showed a significant effect, nanobubble treatment showed a highly significant effect, and the interaction of both had a highly significant effect on the survival rate of prawn seeds after 24 hours transportation and after seven days of rearing.
Key words: Nanobubbles, Charcoal, Prawn Seeds, Closed Transportation.

Introduction. Vannamei prawns are a strategic issue that the Ministry of Marine Affairs and Fisheries (MoMAF) Indonesia is currently developing in the 2020–2024 fishery development program concerning prawn farming cluster pond modelling. This fact, therefore, opens up a considerable opportunity for middle-class prawn farmers in vannamei prawns (*Litopenaeus vannamei*) rearing. Vannamei prawns are one of the fishery commodities recently much farmed (Arsad et al. 2017). In the last five years, the national prawn production has seen a highly significant growth trend with a yearly average of 15.7%, growing by an average of 6.43% as recorded by the Statistics Indonesia (2013–2017). As of the end of 2018, the prawn export volume has touched 180 thousand tons, up from 147 thousand tons in 2017 (Syah et al 2017). Vannamei prawns are superior in many ways: they grow rapidly; have a high level of resistance disease resistance; and demonstrate adaptability to environmental changes (Widodo et al 2011). This proves that it is fairly potential to develop vannamei prawns as they have an excellent prospect and a vast market share.

Seeds’ continuous, year-round availability and distribution from one location to another are among the significant issues in the farming activity (Afriansyah & Sri Mumpuni 2016). Thus, the presence of a hatchery is expected to be of use to pond prawn farmers in meeting their prawn seed demand. The primary problem encountered by farmers all this time is low survival rate during seeds transportation that is caused by the carbon dioxide (CO_2) produced by te respiratory process, reduced dissolved oxygen (O_2) level, and ammonia (NH_3) production from fecal decomposition that is released during the travel (Berka 1986). The steps that may be taken to minimize mortality during the transportation process are as follows: determine the number of seeds transported, apply oxygen in the transport vessels, optimize the use of low temperatures to suppress the metabolism activity, and, in this case, transport the seeds in a living state (Ikasari & Singgih Wibowo 2006). To deal with reduced oxygen level during the transportation process, nanobubble technology may be used, and to suppress the ammonia accumulation and reduce the carbon dioxide level, charcoal may be added.

Nanobubbles are a newly-found technology in the micro gas domain in solid and liquid forms from which finer bubbles are produced than traditional bubbles (Prabowo et al 2019). They are a technology currently developed to improve the aquaculture quality that enables the oxygen contained in the water to endure longer, hence increasing the oxygen availability and maintaining the stability of dissolved oxygen in the water (Fuadi et al 2020). The advantage of this technology is that oxygen distribution may be increased. In the case, oxygen is produced in the form of fine nanobubbles capturing suspended pollutants in liquids and floating on the surface. Nanobubbles sized smaller than 200 nm, are capable of penetrating small cavities in contaminants and wrapping up solids, and causing them to be lifted (Meegoda et al 2019).

Reduced water quality is marked by the increase of carbon dioxide (CO_2) and ammonia (NH_3) levels as well as lowered dissolved oxygen level, causing prawn seeds some stress that can lead to death. The carbon dioxide (CO_2) and ammonia (NH_3) contained in the seeds bag are toxic, thus necessitating some treatment to reduce the toxicity level that may be harmful to the survival rate of the prawn seeds. Iriani and Heryadi (2014) mentioned that active carbon can be used to absorb carbon dioxide, and (Anandasari et al 2015) used active carbon to maintain the water quality parameters in the transport media to minimize the stress levels of the giant freshwater prawn (*Macrobrachium rosenbergii*) seeds that were transported for 24 hours. This research aimed at figuring out the survival rate of prawn seeds after transportation and the nanobubble-charcoal interaction on water quality conditions during transportation.

Materials and Methods

Experimental location. This research was conducted for 30 days from 25 November to 25 December 2020 at Agape Hatchery of PT. Ki Semar Mas in Kalianget Village, Banyuglugur District, Situbondo Regency, East Java Province. The location selected for the research is a company engaged in fisheries, particularly in vannamei prawn (*Litopenaeus vannamei*) seeding.

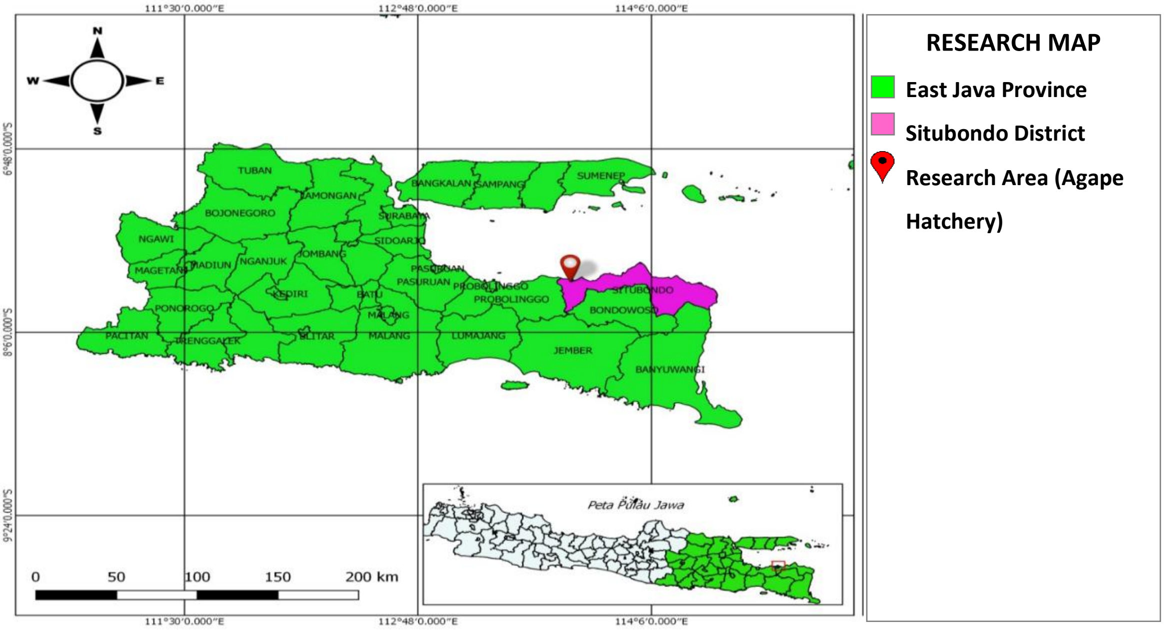

Figure 1. The location of research area at Situbondo district, East Java, Indonesia. Source: Authors' personal archive

Experimental design. The current study was conducted by a factorial experiment using a basic completely randomized design with 2 factors (factors A and B). Factor A is a source of oxygen (nannobubbles and non-nannobubbles). While factor B is the dose of charcoal (0 g, 5g, and 10g) with three replications for each treatment. Every combination of factors was repeated 3 times, making up a total of 36 experiment units. The transportation lasted 24 hours.

Figure 2. Nannobubbles tools and charcoal used for research.
Source: Authors' personal archive

Experimental shrimp. The shrimp used in this study were *Litopenaeus vannamei*. The vannamei (*Litopenaeus vannamei*) seeds used were PL_9 in size and density was 1,600 seeds * 1.8 L^{-1} of water.

Experimental containers. The study used 36 styrofoam containers with the size 70x49x40 cm. The clean containers were filled with clean brackish sea water at 33–34 ppt salinity, sterilized. Then, aeration installation was set up as a source of oxygen. This aeration installation was run continuously. This stage was followed by sorting healthy, relatively homogenous prawn seeds. The charcoal and transportation tools were cleaned.

Experimental transportation. In the execution stage, measurement of packing water media was conducted prior to transportation. The elements subjected to the measurement were pH, salinity, nitrite, nitrate, total amonia nitrogen (TAN), ammonia, carbon dioxide, dissolved oxygen (DO), and temperature levels. The seeds to be used first were subjected to 24-hour fasting. This was done because fasting 24 hours helps to empty the stomach of the fish so that during transportation the fish do not vomit or excrete feces (Ismi et al 2016). Double-layer plastic bags were tied at their two ends, into which charcoal was added at dosages following the treatments. For factor A with previously prepared brackish water nanobubbles with a salinity of 33-34 ppt, the nanobubbles were given for 1 hour before being put into plastic packing. Furthermore, factor A without nannobubbles is also packed into plastic packing. The volume of water inserted into the plastic packing is 1,6 L, after that input the shrimp seeds that have been prepared as much as 1 sedds L^{-1}. And then the bags were tied at their ends with rubber bands. The ready plastic packing is then put into a styrofoam container that has been filled with ice cubes beforehand. Ice cubes are useful for maintaining the temperature in the optimum range of 25 °C. The styrofoam containers were then sealed with duct tape.

In the next stage, the styrofoam containers are arranged in pick-up cars for 24-hour transportation. After the transportation process for 24 hours, glucose content was observed by sending a sample of fry to the laboratory, testing water quality parameters by sending water samples to a water quality laboratory, and observing the survival rate.

Experimental post-transport. 36 styrofoam containers were prepared 24 hours before the transportation took place. The styrofoam containers were filled with 35 L of brackish water with 33 ppt salinity. The seeds were maintained in the 36 containers for seven days after transportation. Feeding was carried out five times a day at 7 am, 1 pm, 4 pm, and 12 am.

Experimental Parameters. The parameters observed in this research were water quality, survival rate of the prawn seeds, and stress level of the prawn seeds, measured by glucose testing.

Water quality parameters. Water quality parameters observation was conducted at the start and end of transportation. The water quality parameters observed in this research are presented in Table 1.

Table 1.

Water quality parameters during 24-hour observation of transportation and seven days rearing

Parameters	*Data collection method*	*Method specification*
Salinity (ppt)	Insitu	Refaktometer
Temperature (°C)	Insitu	Termometer
pH	Laboratory analysis	IKM/7.2.15.K/BPBAPS*
DO (mg L^{-1})	Insitu	DO meter
Ammonia (mg L^{-1})	Laboratory analysis	IKM/7.2.14.K/BPBAPS*
Nitrite (mg L^{-1})	Laboratory analysis	IKM/7.2.13.K/BPBAPS*
Nitrate (mg L^{-1})	Laboratory analysis	Colorimetric
Carbon dioxide (ppm)	Laboratory analysis*	Titrimetric
TAN (mg L^{-1})	Laboratory analysis	IKM/7.2.14.K/BPBAPS*

BPBAPS: Balai Perikanan Budidaya Air Payau Situbondo; DO : Disolved Oxygen; ppt: part per thousand; ppm: part per milion; TAN: Total Amonia Nitrogen.

Survival rate of the prawn seeds. The survival rate was calculated by comparing the number of living individuals at the end of the experiment, which is after being kept for seven days, against the number of individuals at the start of experiment. The calculation was conducted following Effendie (1997) in Ernawati & Rochmady (2017) as given below:

$$SR\ (\%): \frac{Nt}{N0} \times 100\%$$

Where:

SR = Survival rate (%),

N_0 = Number of prawn seeds at the start of experiment (seeds), and

Nt = Number of living prawn seeds at the end of experiment (seeds).

Stress level of prawn seeds. Following Widodo et al. (2011), sampling of the hemolymph of vannamei seeds was conducted at 0.05 mL^{-1}, and using a 1 mL^{-1} disposable syringe 3.8% trisodium citrate ($Na_3C_6H_5O_7$) was added as an anticoagulant to a 1.5 mL^{-1} tube. Blood plasma and red blood cells were separated by centrifuging the hemolymph at 3,500 rpm for 10 minutes at 4 °C. The plasma obtained was used to measure the glucose concentration in the blood. The blood glucose measurement used Glucose liquicolor (GOD-PAP Method Enzymatic Colorimetric Test for Glucose Method without Deproteinization). It was conducted using the following formula:

$$C\ (mg\ dL):\ 100 \times \frac{\Delta A\ sample}{\Delta A\ STD}$$

Where:

C = Blood glucose concentration (mg dL^{-1})

ΔA_{sample} = Sample absorbance

ΔA_{STD} = Standard solution absorbance

Effects of treatments on stress. Transport of shrimp seeds lasts for 24 hours. After transportation, the rearing of shrimp fry was carried out for seven days in a rearing container to determine the effect of treatment on stress levels and survival rates of shrimp fry after being reared for seven days.

Data analysis. The effect of the variance of the combination of nannobuble and charcoal on the survival of shrimp fry was analyzed using two-way ANOVA with F test at 95% confidence interval. To find out the difference between treatments significantly, further tests were carried out by the Duncan and Tukey tests. Water quality parameters and glucose measurements are presented Table 4 and 5.

Results

Survival Rate after 24 hours transportation. Fish were transported for 24 hours and after that they were observed for one month to register the survival rate. The ANOVA results for the survival rate of the prawn seeds is presented in Table 2.

Table 2.
The results of two-way ANOVA (mean and standard deviation) regarding differences between treatments for the survival rate of the prawn seeds after 24 hours transportation

Treatments (oxygen)	*Charcoal (g)*		
	0 (A1)	*5 (A2)*	*10 (A3)*
Nannobuble (N1)	85.02 ± 1.02^{c}	84.89 ± 0.69^{a}	83.44 ± 0.68^{b}
Without nannobuble (N2)	84.50 ± 0.49^{b}	82.43 ± 0.45^{ab}	83.77 ± 1.08^{ab}

Note: Different superscripts in the same column show that there are significant differences (p<0.05). The difference between charcoal and nannobubble can be seen with the different superscript values. Charcoal is denoted by A (1,2,3) and nannobubble is denoted by N (1,2).

The ANOVA results show that the charcoal treatment had a significant effect, the nanobubble treatment had a highly significant effect, and the interaction of both had a highly significant effect on the survival rate of prawn seeds after transportation.

Survival rate of the prawn seeds after seven days of rearing. After unpacking, the prawn seeds were reared in rearing containers for seven days to observe the effects of the treatments on the survival rate of the prawn seeds. The containers used were styrofoam measuring 75x42x32 cm and were filled with 35L of seawater. The results of the analysis of variance of the survival rate of the prawn seeds after rearing are presented in Table 3.

Table 3.
The results of ANOVA test regarding differences between treatments for the survival rate of the prawn seeds after seven days of rearing

Treatments (oxygen)	*Charcoal (g)*		
	0 (A1)	*5 (A2)*	*10 (A3)*
Nannobuble (N1)	90.98 ± 0.36^{b}	86.49 ± 0.55^{b}	84.59 ± 0.36^{ab}
Without nannobuble (N2)	85.75 ± 0.72^{b}	84.43 ± 0.13^{a}	83.51 ± 0.29^{a}

Note: Different superscripts in the same column shows that there are significant differences (p<0.05)

Based on the table above, the charcoal treatment had a highly significant effect, the nanobbuble treatment had a significant effect, and the interaction of both had a highly significant effect on the survival rate of the prawn seeds after rearing. Table 3 shows that different sources of oxygen and different doses of charcoal had a significant effect (p<0.05) on the survival of seeds reared for seven days. The survival of seeds given nannobuble (N1) was higher than those without nannobuble (N2). At at a small dose of charcoal (A1) it was higher than that of a high dose of charcoal (A3). This was due to the high glucose content during maintenance, which resulted in stress during transport.

Water quality parameters. The water quality parameters at the end of seven days of rearing are presented in Table 4.

Table 4.

The mean water quality parameters at the end of 24 hours transportation

Water quality variables		*Charcoal (g)*		
		0 (A1)	*5 (A2)*	*10 (A3)*
DO (mg L^{-1})	N1	14,28±0,76	15,66±0,71	16,14±0,72
	N2	25,75±1,30	25,97±1,35	25,47±0,60
Temperature (°C)	N1	28,20±0,00	28,23±0,06	28,27±1,79
	N2	28,43±0,21	28,26±0,21	27,50±1,13
pH	N1	6,92±0,44	7,19±0,03	7,27±0,23
	N2	6,99±0,43	7,19±0,05	7,31±0,04
Salinity (ppt)	N1	32,67±2,31	32,7±2,31	32,7±2,31
	N2	32,0±0,00	32,0±0,02	32,0±0,05
NO_2 (mg L^{-1})	N1	0,00±0,00	0,00±0,00	0,00±0,00
	N2	0,06±0,01	0,23±0,17	0,58±0,00
NO_3 (mg L^{-1})	N1	16,47±1,45	13,67±3,00	23,80±2,79
	N2	15,30±2,86	17,17±5,23	16,57±4,06
TAN (mg L^{-1})	N1	5,13±0,15	4,95±0,29	5,29±0,15
	N2	5,29±0,15	5,19±0,09	4,95±0,29
CO_2 (mg L^{-1})	N1	11,71±0,61	7,85±1,22	5,31±0,11
	N2	10,38±0,69	6,79±1,99	5,03±0,07

Note: A1 (Charcoal 0g); A2 (Charcoal 5g); A3 (Charcoal 10g); N1 (Nannobuble); N2 (None Nannobuble)

Glucose level of the prawn seeds. The measurement results of the glucose level of the prawn seeds after 24-hour transportation are presented in Table 5.

Table 5.

The measurement results of the glucose level of the prawn seeds after 24-hour transportation

Treatments (oxygen)	*Charcoal (g)*		
	0 (A1)	*5 (A2)*	*10 (A3)*
Nannobuble (N1)	37±0,02[a]	38±0,01[a]	40±0,03[a]
Without nannobuble (N2)	38±0,01[a]	39±0,03[a]	37±0,01[a]

Note: Different superscripts in the same column show that there are significant different ($p<0.05$). The difference between charcoal and nannobubble can be seen with the different superscript values. Charcoal is denoted by A (1,2,3) and nannobubble is denoted by N (1,2).

Discussion

Survival rate of the prawn seeds after transportation. Based on the results of the Tukey's test on the effects of the two treatments, nanobubble and charcoal treatments, on the survival rate of the prawn seeds after 24-hour transportation, the highest effect was resulted from the A1N1 treatment (0 g charcoal-nanobubbles) at 85.02%, followed by the A2N1 treatment (5 g charcoal-nanobubbles) at 84.90%, the A1N2 treatment (0 g charcoal-no nanobubbles) at 84.50%, the A2N2 treatment (5 g charcoal-no nanobubbles) at 83.77%, the A3N1 treatment (10 g charcoal-nanobubbles) at 83.44% and the lowest was resulted in the A3N2 treatment (10 g charcoal-no nanobubbles) at 82.44%. This is in line with Indonesian National Standard (BSN, 2010), which determines that the survival rate of prawn seeds after transportation should be ≥ 80%. Research by (Yustiati et al 2017) found that the survival rate generated after fish transportation at a low temperature was 88.33%. This is consistent with the research by (Munandar et al 2017), which found that the survival rate of giant freshwater prawns during closed transportation was 88%. Nanobubbles were discovered to be the best aerator in increasing the survival rate to 76.5% during fish seeds release (Aghnia et al 2016).

The prawn seeds survived after transportation demonstrating active swimming and agile movements. Visually, the bodies of the prawn seeds appeared bright in color. However, some prawn seeds were found weak and dead, showing that the deaths of the prawn seeds were not caused by non-feeding, but by a change in the water quality parameters in the packing bags. As expressed by (Nirmala et al 2012), deaths of experiment seeds during closed transportation are caused by changes in the chemical factors of water quality. One of the factors that trigger changes in water quality during the transportation is the ongoing metabolism process, causing the feces produced to decompose fast. The decomposition process is rendered by the increase in temperature, leading to the melting of the ice cubes in the styrofoam containers and eventually lowered pH and increased ammonia level. As mentioned by Ismi et al (2016), the accumulation of ammonia is caused by the raise of the metabolism rate.

Regarding the effect of the interaction between the two treatments, the nanobubble and charcoal treatments, on the survival rate of the prawn seeds during transportation, it is assumed that the oxygen supply was adequate and so it stayed for a long time during transportation. As conveyed by Susanti et al (2020), the dissolved oxygen level in the aquaculture media can be increased by supplying DO into the water using nanobubble technology, so the gas resulted is in the form of fine bubbles in liquids with diameters on nano/microscale. The gas bubbles produced from nanobubbles are a technology that is developed to increase the DO concentration in the water (Saputra et al 2018). Meegoda et al (2019) said that nano- or ultra-fine bubbles in the water are gas cavities in solutions smaller than 200 nm in size that are electrically charged and capable of producing electrical field and influencing the distribution of ions in the mass solution; hence, there is a high concentration of charged ions in contrast to the distribution of scattered ions. According to Nghia et al (2021), nanobubbles are a technology that can improve the potential of significantly reducing oxygen consumption through ozone bubbles.

Charcoal proved able to reduce the carbon dioxide level, hence capable of suppressing the stress level of the prawn seeds. As stated by Murtiadji et al (2020), the active charcoal used in the research was capable of absorbing carbon dioxide, hence reducing the rate of water quality degradation. Munandar et al (2017) observed that the addition of active charcoal in the 24-hour transportation process maintained the water quality in the packing media in good condition and that the survival rate of giant freshwater prawns after transportation was 88%. The elements contained in active charcoal are 82.55% C (carbon), 8.36% O (oxygen), 6.95% Si (silica), and less than 1% N, Zr, Cu, Al, and K, and based on the proximate testing, the ash contents in granular and powdered active charcoal are 3.77% and 4.69%, respectively, the water contents in granular and powdered active charcoal are 6.37% and 5.69%, and the C contents in granular and powdered active charcoal are 54.83% and 55.13%, respectively (Murtiadji et al 2020). The predominant contents are carbon, oxygen, and silica; therefore, active charcoal is more effective in absorbing carbon dioxide. Supriyono et al (2011) observed that the use of activated charcoal increased to 83.11% the survival rate of shrimp fry after transportation. Similarly, Nirmala et al (2012) found that charcoal addition increased to 100% the survival rate in seeds transportation.

The metabolism of the prawn seeds that produced feces during transportation decreased the water quality. Furthermore, the oxygen level in the packing bags was reduced as it was used for the prawn seeds' respiration during transportation. DO is one of the chemical parameters important in the aquaculture water environment as it can influence the other water quality parameters (Anandasari et al 2015). The results of the DO measurement during the research were still in the acceptable DO range in transportation. Nirmala et al (2012) showed that the increase of oxygen level in the packing bags resulted from the oxygen diffusion from the air through oxygen injection. In Midihatama et al (2018) study, the dissolved oxygen level in fish transportation was 3.33–3.78 mg L^{-1}. Galang et al (2019) observed that the use of nanobubble after 48 hours decreased the DO level from 10.8 ± 0.07 mg L^{-1} to 8.25 ± 0.09 mg L^{-1}. Other research (Ebina et al 2013) discovered that nanobubbles were able to increase the DO level significantly fourfold from 7.7 mg L^{-1} in control to 31.7 mg L^{-1}. In another study (Wu et al 2019), similar results were discovered, pointing to the fact that that nanobubbles use could increase the DO level ninefold from 0.60 mg L^{-1} to 5.00 mg L^{-1}. In the research by Faudzi et al (2021), the dissolved oxigen level in fish transportation was 13,0-16,7 mg L^{-1}.

The rise in temperature led to lowered oxygen level in the water, hence driving up the metabolism waste and the oxygen consumption in the transportation media. This is in line with the statement of Sarah et al (2018), that the biochemical activity increase in a prawn's body occurs as a result of the rise of temperature, and the metabolism process in the prawn would go down when the temperature goes down as well. The temperature in the packing bags at the start and end of 24-hour transportation was still in an acceptable range. As stated by Munandar et al (2017), the elevation of packing temperature is caused by the prolonged time taken for transportation. According to SNI (2010), the optimum temperature for transportation that takes less than six hours is in the range 26–28 °C, while in transportation that takes 18–24 hours, in the range 20–22 °C. Hence, the temperature in the packing media during transportation has trespassed the optimum limit, driving up the metabolism rate of the prawn seeds as can be seen in the feces that was found in a great amount during unpacking. Ismi et al (2016) discovered that the seeds transportation temperature in the range 27.8–28.1 °C generated the highest survival rate, namely 99%.

As for pH observation, the pH decreased with the increase in the transportation time and the prawn seed density in the packing bags. Yet, the survival rate of the prawn seeds was still considered high as it was in the optimum range. As stated by Syamsunarno et al (2019), the pH during transportation should be in the range 6.5–8.5. SNI (2009) determines that the range of pH for prawn nauplii and seeds production is 7.5–8.5.

These results comply with SNI (2009), which determines that the salinity for extension seeds should be in the range 29–34 ppt. According to (Supriatna et al 2020), the salinity in vannemei prawn farming ranges from 27 ppt to 30 ppt. Vannamei prawn seeds are highly tolerant to salinity. The higher the salinity rate the bigger the osmotic pressure. Similarly, (Rakhfid et al 2017) stated that salinity is closely related to osmotic pressure.

The nitrite measurement results during the transportation are in line with SNI (2009), which states that the nitrite level for prawn nauplii and seeds production should be 0.1 mg L^{-1} at the highest. It is stated in the research by (Ratnawati et al 2020) that the nitrite level in the water is 0.001–0.009 in small- and big-scale prawn seeding at the hatchery. Excessive nitrite levels may result in toxicity since they may cause methemoglobin to form and therefore cause hemoglobin to be unable to bind oxygen. Prawns have hemocyanin to transport oxygen and nutrients, in which case its function imitates the function of hemoglobin. Thus, the body may be deprived of oxygen and nutrients if nitrite binds to hemocyanin.

The final results showed increases, but they were still within the tolerated range of nitrate levels in transportation, hence not harming the prawn seeds in the packing bags. As stated by Makmur *et al* (2018), the optimum level of nitrate for vannamei prawn farming is < 20 mg L^{-1}. Boyd and Clay (2002) added that nitrate levels below 50 L^{-1} are not harmful to prawns.

This shows that there were increases as described by Boyd and Clay (2002) in Susanti et al (2020), according to whom TAN increases would range between 5 and 15 mg L^{-1}. In transportation, TAN has resulted from metabolism during the travel in the form of prawn seeds' excretion. The increases in TAN are caused by the competition for a room for movement due to the high density of prawn seeds in the packing bags, putting the prawn seeds under stress. This is in line with Ismi et al (2016) who stated that increased metabolic activity could give fish in closed transportation some stress.

According to Ebeling et al (2006), ammonia is a fish's excretion from food protein catabolism. It is excreted through the gills as unionized ammonia. The values in this study were still in the tolerated ammonia range in prawn seeds transportation. As said by

Midihatama et al (2018), the ammonia level during fish transportation is in the range 0.0002–0.0137 mg L^{-1}. Wahyuningsih et al (2020) stated that ammonia is an requirement in a chemical process, but in water environments, it can be toxic if it is present at certain values; ammonia levels above 1.5 mg L^{-1} can be toxic to fish. The low levels of ammonia in the packing bags are presumed to be due to the fasting process the prawn seeds were subjected to before transportation; therefore, the feces produced were still tolerable. It is stated by Pahlawi et al (2019) that seeds should go through fasting before transportation to empty the digestive tract since such metabolism waste as feed residue that is released through feces or thrown up will be accumulated in the media.

The carbon dioxide level increased during the transportation process since the prawn seeds in the packing media took oxygen for their movements. According to Sunardi et al (2016) CO_2 level of 12 mg L^{-1} causes the fish to suffer from stress, and if it reaches 30 mg L^{-1}, some fish of some sorts will die. However, this is different from the findings of Supriyono et al (2011), who stated that the carbon dioxide level of 50.42 mg L^{-1} in the packing media in 72-hour transportation resulted in a survival rate of 83.11%.

Glucose level of the prawn seeds. According to Tang et al (2018), the increase of glucose level in the plasma is an indicator of stress fish is undergoing. The increase of blood glucose level starts with a change in the blood glucose level after the receptor receives information on the stress factor, which is communicated to the brain in the hypothalamus through the nerves, leading to the command for chromaffin cell to secrete catecholamine to activate the enzymes involved in the catabolism in the sympathetic nerve fibers. Blood glucose is one of the blood measurement parameters that reflect a response to stress. In the Rachmawati et al (2010) study, the glucose level measured tended to increase. Stress in fish is a result of a high level of energy need. In the present study, there was no statistically significant difference between the glucose yields in the charcoal treatment with nannobubbles or non-nannobubbles (Table 5). This is because the condition of the shrimp during the observation had almost the same level of stress symptoms.

Survival rate of the prawn seeds after rearing. Mauladani et al (2020) demonstrated that nanobubble use increased vannamei prawn production in a pond with a survival rate reaching 92%. In the present study, rearing was conducted to figure out the effects of the treatments on the survival rate of the prawn seeds. It was conducted for seven days because these first seven days after transportation are critical for prawn seeds in adapting to a new environment. Deaths of prawn seeds in the packing bags were caused by high levels of carbon dioxide and glucose in the prawn seeds (Supriyono et al 2011). Similarly, one of the factors that cause death in fish during the transportation is a high level of CO_2. Adiyana et al (2014) pointed out that high level of blood glucose is an indicator of an increased stress level. The deaths in each treatment were assumed to be caused by the prawn seeds limpness after prolonged transportation and adaptation to a new environment. Midihatama et al (2018) observed that after undergoing transportation, fish placed in a new environment were faint.

Water quality during rearing. With regard to water quality parameters during rearing in each treatment, the temperature was within the recommended optimum range, the salinity was still in the recommended acceptable range (as per (Brito et al 2014)), the Total Ammonia Nitrogen (TAN) was still in the recommended range, and the ammonia level (NH_3) was in the optimum range. This is the reason why the prawn seeds were sound and healthy and were active in their movements (Umiliana et al 2016). This also explains why the mortality rate of the prawn seeds during rearing was not excessively high. As stated by (Prabowo et al 2019), the rearing survival rate when releasing vannamei seeds could reach 93.63% when an aeration system is applied.

Conclusions. The results of this study show that the charcoal treatment had a significant effect, the nanobubble treatment had a highly significant effect, and the interaction of both had a highly significant effect on the survival rate of prawn seeds after 24 hours transportation. The results also show that the effect on the survival rate of prawn seeds after seven days of rearing was as follows: the charcoal treatment had a highly significant effect, the nanobubble treatment had a significant effect, and the interaction of both had a highly significant.

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