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Research Article

The effect of stocking density on the transport of pink shrimp *Farfantepenaeus brasiliensis* (Crustacea: Decapoda), as live bait for sport fishing in Brazil

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ABSTRACT. The capture of juvenile shrimp as live bait for sport fishing has intensified, as has the trade of shrimp in different locations. The transport of shrimp to regions, other than those in which they are captured, is often poorly performed due to the lack of information regarding effective transport, resulting in high mortality of the transported animals. The aim of this study was to determine the optimum stocking density for the transport of juvenile *Farfantepenaeus brasiliensis* (weight: 5.53 ± 1.20 g) and to evaluate the effect of the addition of hydrated lime in the transport water. Four stocking densities were tested for transport (1, 2, 3 and 4 ind L^{-1}). Following the analysis of the results obtained in the density experiment, the addition of hydrated lime (0.15 g L^{-1}) in the transport water was also tested. Water quality and the final survival were negatively correlated with increasing stocking density. The results of this study demonstrated that the highest density that can be used to avoid mortality is 3 ind L^{-1} for a maximum period of 10 h. The use of hydrated lime in the transport water attenuated the observed effects on water quality parameters.

Keywords: live bait, sport fishing transport, stocking density, penaeid, *Farfantepenaeus brasiliensis.*

El efecto de la densidad de población sobre el transporte de camarón rosa, *Farfantepenaeus brasiliensis* (Crustacea: Decapoda), como carnada viva para la pesca deportiva en Brasil

RESUMEN. La captura de juveniles de camarón como carnada viva para la pesca deportiva se ha intensificado, al igual que el comercio de camarón en diferentes ciudades. El transporte de camarones a regiones distintas de aquellas en las que se capturan, a menudo se realiza en forma inapropiada, debido a la falta de información sobre el transporte eficaz, lo que resulta en una alta mortalidad de los animales transportados. El objetivo de este estudio fue determinar la densidad óptima para el transporte de camarón rosa *Farfantepenaeus brasiliensis* (peso: 5,53 ± 1,20 g), y evaluar el efecto de la adición de cal hidratada en el agua de transporte. Se hicieron pruebas con cuatro densidades de siembra para el transporte (1, 2, 3 y 4 ind L^{-1}), y luego del análisis de los resultados obtenidos en el ensayo de densidad, se realizó una prueba con la adición de cal hidratada (0,15 g L^{-1}) en el agua de transporte. La calidad del agua y la sobrevivencia final se correlacionaron negativamente con el aumento de la densidad de población. Los resultados de este estudio demuestran que la densidad más alta que se puede utilizar, para evitar la mortalidad en el transporte de camarón, es de 3 ind L^{-1} por un periodo máximo de 10 h. El uso de cal hidratada en el agua del transporte atenúa los efectos observados en los parámetros de calidad del agua.

Palabras clave: carnada viva, pesca deportiva, transporte, densidad de población, peneidos, *Farfantepenaeus brasiliensis.*

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INTRODUCTION

Recreational fishing is an important economic and social activity that creates jobs and income. In the USA, it is estimated that approximately 30 million individuals were fished recreationally in 2006, totaling a $42 billion investment in recreational fishing (Kempthorne *et al.*, 2006). In Brazil, recreational fishing has expanded since the 1990s, and it is estimated that there are 25 million recreational fishers nationally (Fabri, 2006).

Tourism in Brazil has increased in recent years, and recreational fishing and live bait capture to support this activity have been considered threats in certain regions due to a lack of appropriate monitoring, thereby contributing to the decline of natural stocks (Mendonça, 2007). The catch of juvenile shrimp as live bait has intensified in recent years, and many regions in Brazil do not currently supply this demand. Therefore, it is required that shrimp be transported from other regions to supply the local trade for live bait (Beccato, 2009). Often, this transport is poorly performed, resulting in high shrimp mortality rates, a consequence of scarce information on the subject of effective transport. One difficult aspect of transporting live bait is the paucity of available information regarding the proper stocking density.

Although stocking densities have been widely studied for different shrimp species (Allan & Maguire, 1992; Wasielesky *et al.*, 2001; Preto *et al.*, 2009; Fóes *et al.*, 2011; Krummenauer *et al.*, 2011), specific data regarding the optimum transport densities of live bait are still scarce, particularly with respect to the indigenous species living along the Brazilian coast. According to Peixoto *et al.* (2008), the shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817) is a handling resistant species during capture, an important and suitable characteristic for use as live bait. In addition, this species has a wide distribution, occurring from North Carolina (USA) to the coast of Rio Grande do Sul (Brazil) (Buckup & Bond-Buckup, 1999).

Water quality parameters tend to change rapidly during the transport of crustaceans and can often reach unsuitable values for live organisms (Smith & Wannamaker, 1983; Schmitt & Uglow, 1996; Barajas *et al.*, 2006). To minimize these variations in water quality, certain substances are added to the transport water, thereby reducing stress in transported aquatic organisms (Kubitza, 1997; Sperandio, 2004). Barbieri & Ostrensky (2002) reported that it is recommended to add microalgae (80,000 cells mL^{-1}) and activated charcoal (0.3 g L^{-1}) for the transport of post-larval penaeids in plastic bags. TRIS (Hydroxymethyl amino-methane) can also be used to avoid pH changes during transport (Smith & Wannamaker, 1983; Barbieri & Ostrensky, 2002).

According to Van Wyk & Scarpa (1999), high alkalinity reduces the pH fluctuations resulting from respiration processes and carbon dioxide (CO_2) release from organisms. Certain products, such as limestone ($CaCO_3$) and dolomite ($CaMg\ (CO_3)_2$) are commonly used in tanks and ponds to achieve the correct pH levels and alkalinity for fish and shrimp culture. Hydrated lime ($Ca(OH)_2$) is also used, most often to disinfect ponds by causing a sudden increase in pH (Boyd, 1990; Vinatea, 1997; Barbieri & Ostrensky, 2002). However, the use of ($Ca(OH)_2$) in suitable concentrations to raise the pH and alkalinity of water culture can be effective without causing shrimp mortality (Furtado *et al.*, 2011); nevertheless, the effects of this compound have not been evaluated for shrimp transport.

Therefore, the aim of this study was to determine the optimum stocking density for the transport of *F. brasiliensis* juveniles and to evaluate the effect of the addition of hydrated lime to the transport water.

MATERIALS AND METHODS

Juvenile *F. brasiliensis* with an average weight of 5.53 ± 1.20 g were obtained from outdoor ponds at the Marine Station of Aquaculture - Federal University of Rio Grande (EMA-FURG), Rio Grande-RS, Brazil. The shrimp were stocked in a tank (4,000 L) with 5 µm of filtered water at a temperature of 22°C and a salinity of 22 g L^{-1}. The animals were allowed to acclimatize for four days prior to the beginning of the experiment. Following the acclimatization period, the animals were submitted to a transport simulation of 12 h, time enough to ship shrimps to different markets along the coast. The experiment was carried out in different stocking densities (treatments of 1, 2, 3 and 4 ind L^{-1}), with three replicates each. The shrimp were fed twice daily *ad libitum* during the acclimatization period prior to the transport simulation.

The experimental units consisted of transparent plastic bags (50 L) with 10 L of water (temperature of 22°C, and salinity of 22 g L^{-1}). The bags were inflated to two-thirds full with oxygen and tied closed with rubber strings. Prior to sealing the plastic bags, a 1 m long and 0.3 cm diameter transparent hose was inserted, and water samples (60 mL) were collected from this hose every 2 h to monitor the water quality during the transport simulation. Three experimental units without shrimp were used to determine the concentration of the dissolved oxygen in the plastic bags. The rate of pure oxygen incorporation was determined based on these measurements.

After defining the optimum stocking density for transport, a second experiment using three plastic bags (15 L) containing 3 L of water and a density of 3 ind L^{-1} was conducted. The same salinity and temperature conditions were used, and 0.15 g of $Ca(OH)_2$ L^{-1} was added (Furtado *et al.*, 2011) to increase the alkalinity of the transport water from 125 to 200 mg $CaCO_3$ L^{-1}. This experiment was performed in order to reduce variations in alkalinity and pH of the transport water. The addition of the $Ca(OH)_2$ was performed 15 min prior to the storage of shrimp in the plastic bags; the shrimp were therefore not acclimatized to the new alkalinity (200 mg $CaCO_3$ L^{-1}). The shrimp feeding was suspended 12 h prior to initiation of the experiment. The water, in both experiments, was heated using submerged heaters with attached thermostats, and the plastic bags were partially immersed in a water bath to aid in temperature maintenance. The water was constantly aerated to maintain homogenization.

The water quality parameters of temperature, salinity, dissolved oxygen concentration and pH was monitored using an YSI 556 multi-parameter instrument (Yellow Springs Instruments, Yellow Springs, OH, USA). The total ammonia level was monitored according to UNESCO (1983). The alkalinity was determined using the method described by Baumgarten *et al.* (1996), and the CO_2 concentration was determined as described by Summerfelt (1996).

The shrimp survival was continuously monitored. At the end of 12 h transport simulation, survival was determined for each treatment. The shrimp were considered to be dead if they did not respond to mechanical stimuli.

Following the experiments, the shrimp were submitted to a salinity stress test for 24 h, to assess the influence of the different treatments on their health. Three different salinities: 12, 22 (control) and 32 g L^{-1} were tested and the survival was determined during the stress test following 2, 12 and 24 h. The shrimp were transferred directly from the bags into experimental units (15 rectangular boxes), without any acclimation, where they remained for 24 h. The boxes contained 30 L of seawater at a temperature of 22°C with constant aeration. To perform this test, for each treatment two shrimps per bag were collected, totaling six shrimp on each box by salinity tested (12, 22 and 32), totaling 18 shrimp per treatment.

The statistical analysis of the water quality parameters among treatments was performed by analysis of variance (one-way ANOVA), followed by Newman-Keuls *post-hoc* comparisons, with a minimum significance level of 0.05. All tests were performed after the confirmation of homogeneity of variance (Levene's test) and normality of data distribution (Kolmogorov-Smirnov test). An arcsine square root transformation was applied to the final survival data, which were then analyzed with a one-way ANOVA and a subsequent Newman-Keuls test.

RESULTS

The survival and the water quality parameters that were monitored during the experimental period are presented in Table 1 and Figure 1. The salinity of the transport water did not change over the 12 h of the experiment, and the mean temperature inside of the experimental units was 22.2 ± 0.1°C.

The mean dissolved oxygen concentration differed significantly ($P < 0.05$) between treatments (Table 1), and a decrease in the dissolved oxygen levels was observed in the first 2 h in treatments with 2, 3 and 4 ind L^{-1} (Fig. 1a). Following 5 h of transport simulation, the 4 ind L^{-1} treatment exhibited a dissolved oxygen concentration of 1.99 mg L^{-1}; after 12 h, the oxygen concentration in this group was 1.41 mg L^{-1} (Fig. 1a). The 3 ind L^{-1} treatment exhibited dissolved oxygen concentrations below 2 mg L^{-1} beginning at the tenth hour of transport, at which point two of the replicates exhibited concentrations of 1.78 and 1.97 mg L^{-1}; at 12 h, the mean oxygen concentration in this group was 1.96 mg L^{-1} (Fig. 1a). However, no values below 2 mg L^{-1} were observed during the experimental period in the other treatments. The oxygen incorporation rate in water alone (*i.e.*, without shrimp) was 12.39 ± 2.88 mg L^{-1} on the average, with a minimum rate of 7.45 mg L^{-1}, and a maximum rate of 16.16 ± 2.17 mg L^{-1}. At the end of transport simulation, mean dissolved oxygen was 8.71 mg L^{-1}, with an average of 0.72 mg L^{-1} h^{-1}.

The pH data were similar to those observed with respect to dissolved oxygen and exhibited significant differences between treatments ($P < 0.05$) (Table 1). The water in the 1, 2, 3 and 4 ind L^{-1} treatments exhibited pH variations of 1.2, 1.42, 1.58 and 1.59 during the 12 h of the experiment, with 52.5, 73.2, 79.7 and 79.9% of these changes occurring in the first two hours of the simulation, respectively (Fig. 1b). In the treatment with 3 ind L^{-1} and hydrated lime addition, the decrease in pH was not as strong, and a variation of 19.13% was observed following the first two hours of transport (Fig. 1b). Although pH changes in the treatment with 3 ind L^{-1} and hydrated lime were less physiologically stressful than the 1, 2, 3 and 4 ind

Table 1. Change to Physical and chemical water quality parameters during the transport simulation and final survival for the different treatments. Data reported as mean ± SD. Different letters indicate significant differences between different densities ($P < 0.05$).

Parameters	Treatments (ind L^{-1})				
	1	2	3	3 (hydrated lime)	4
Dissolved oxygen (mg L^{-1})	7.02 ± 0.8^{a}	4.55 ± 1.5^{b}	3.48 ± 1.9^{bc}	4.71 ± 1.4^{b}	2.65 ± 2.0^{c}
pH	7.40 ± 0.4^{a}	7.15 ± 0.5^{ab}	6.99 ± 0.5^{ab}	8.62 ± 0.6^{c}	6.97 ± 0.5^{b}
Alkalinity (mg L^{-1})	105.35 ± 9.7^{a}	103.33 ± 11.4^{ab}	95.47 ± 14.4^{b}	172.32 ± 12.9^{c}	80.35 ± 19.1^{d}
Dissolved carbon dioxide (mg L^{-1})	8.97 ± 4.6^{a}	19.01 ± 9.0^{b}	26.08 ± 12.5^{c}	1.65 ± 2.0^{d}	23.34 ± 10.8^{bc}
Total ammonia (mg L^{-1})	2.75 ± 1.4^{a}	4.53 ± 2.3^{b}	5.26 ± 2.8^{b}	0.95 ± 1.2^{c}	4.67 ± 2.3^{b}
Final survival (%)	100 ± 0.0^{a}	100 ± 0.0^{a}	95.5 ± 1.9^{a}	100 ± 0.0^{a}	85.0 ± 7.5^{b}

L^{-1} treatments over the period of transport, the hydrated lime-treated group exhibited a similar degree of variation (1.62) in pH by the end of the experiment (Fig. 1b).

The alkalinity was reduced over the first two hours in the treatments in the 1, 2, 3 and 4 ind L^{-1} treatments, for which there was a reduction of 12.0, 13.6, 20.0 and 34.7% of the initial alkalinity (125 mg $CaCO_3$ L^{-1}) and a final reduction of 22.0, 26.6, 34.6 and 42.6%, respectively (Fig. 1c). In the treatment with 3 ind L^{-1} and hydrated lime, the reduction in alkalinity in the first two hours was 14.2%. At the end of the experiment, this group exhibited a 19.2% decrease in alkalinity (Fig. 1c) relative to the initial value (200 mg $CaCO_3$ L^{-1}). The mean alkalinities at the simulation were significantly different ($P < 0.05$) between treatments (Table 1).

Treatments with higher shrimp densities (2, 3 and 4 ind L^{-1}) exhibited a more pronounced increase in carbon dioxide concentrations in the first two hours compared to the 1 ind L^{-1} treatment (Fig. 1d). In the treatment with addition of hydrated lime, the final CO_2 concentration was lower (5.20 ± 1.68 mg L^{-1}) compared to the treatments of the same density in which no hydrated lime was added (35.57 ± 9.65 mg L^{-1}) (Fig. 1d). The mean CO_2 concentrations at the simulation were significantly different ($P < 0.05$) between treatments (Table 1).

There were no significant differences ($P > 0.05$) with respect to the total ammonia concentrations in the 2, 3 and 4 ind L^{-1} treatments (Table 1), all of which demonstrated a significant increase in ammonia concentrations in the first two hours (Fig. 1e). Although the ammonia concentration in the 1 ind L^{-1} treatment exhibited a peak in the first two hours, the concentration continued to increase during the trial. With respect to the treatment with 3 ind L^{-1} and hydrated lime, the water exhibited lower ammonia levels compared to treatments with the same density but in which no lime was added; however, the shrimp in the hydrated lime treatment were subjected to a period of fasting prior to the experiment.

The survival rate does not differ statistically among treatments until the eleventh hour of the trial. After 5 h, the 4 ind L^{-1} treatment exhibited 2.5% mortality; at the end of the experiment, a mean survival of 85.0 ± 7.5% was observed ($P < 0.05$) for this treatment. The 1 and 2 ind L^{-1} and the 3 ind L^{-1}, with hydrated lime treatments, all exhibited a survival rate of 100%. In the 3 ind L^{-1} treatment the mortality rate after 11 h was 4.5%, resulting in a survival rate of 95.5 ± 1.9% at the end of the experiment.

In the saline stress test, no mortality was observed in any of the treatments in the first two hours. One death was observed in the salinity 12 g L^{-1} treatment after 12 h (3 ind L^{-1}). In the 4 ind L^{-1} group that was exposed to the same salinity, mortality was observed after 12 h, and 50% survival was observed at 24 h.

DISCUSSION

Transport is a traumatic process consisting in a succession of adverse stimuli, including capture, the transport itself and storage (Robertson *et al.*, 1988). According to Vadhyar *et al.* (1992), the combination of decreased levels of dissolved oxygen and increased CO_2, ammonia and bacteria within the transport packaging may be responsible for shrimp mortality. The combination of these factors is likely worse than any of them individually.

The physical and chemical parameters of water quality can directly affect the performance and survival of aquatic organisms. Therefore, the adequate maintenance of water quality parameters is extremely important (Vinatea, 1997; Van Wyk & Scarpa, 1999; Barbieri & Ostrensky, 2002). However, little information is available regarding the biological and

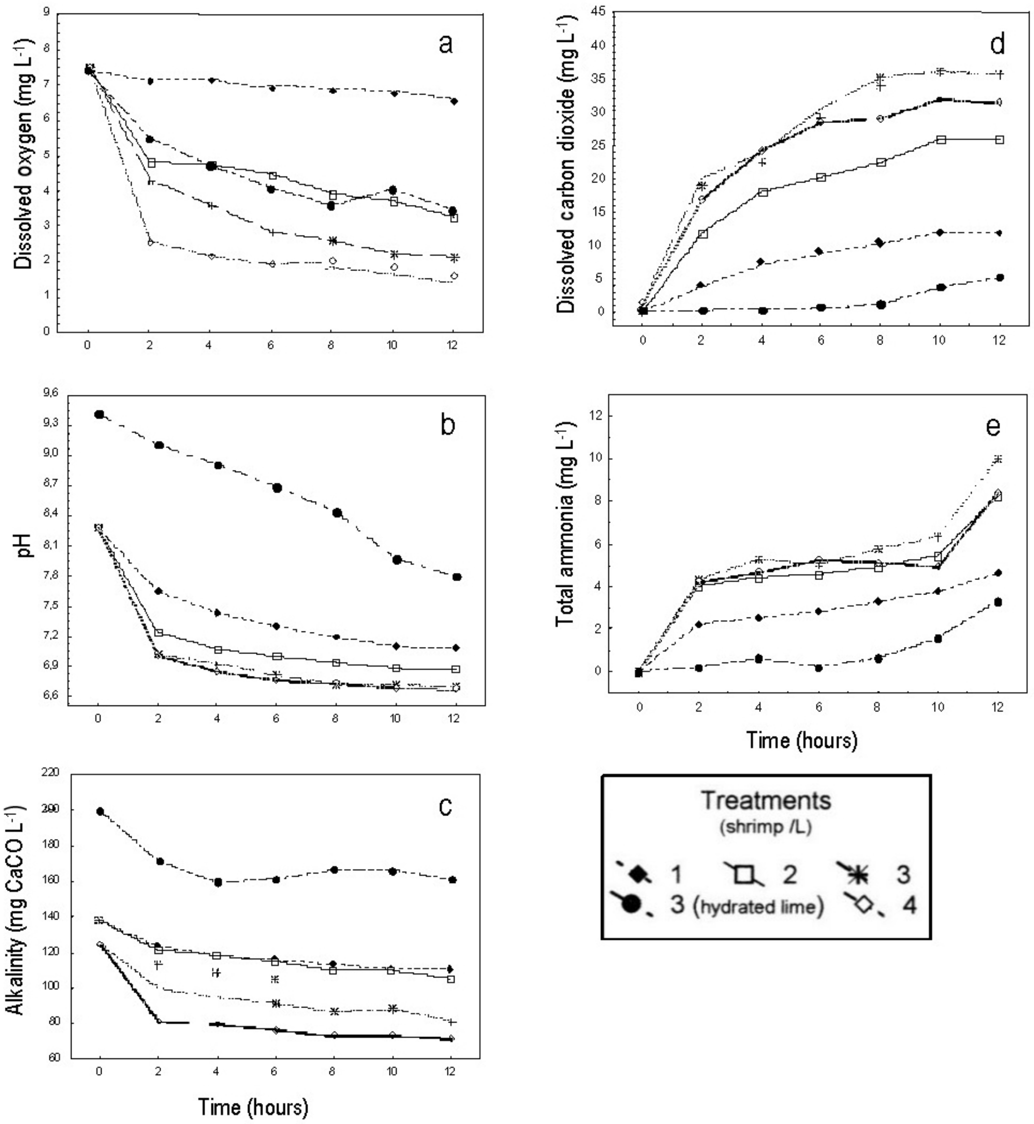

Figure 1. Variations in the average a) dissolved oxygen concentration, b) pH, c) alkalinity, d) dissolved carbon dioxide, e) concentration and total ammonia in the transport water during the transport simulation.

physiological aspects of culture and transport in the pink shrimp *F. brasiliensis*.

The dissolved oxygen concentration is one of the most important environmental factors with respect to the transport-induced stress of live animals (Rosas *et al.*, 1997; Li *et al.*, 2006; Zhang *et al.*, 2006). The respiration of aquatic organisms and the accumulation of organic matter can lead to conditions of hypoxia or anoxia in the water (Zhang *et al.*, 2006). Sperantio (2004) reported on the transport of juvenile *Macrobrachium amazonicum* (0.09 ± 0.03 g) for 8 h at different densities (5.8, 11.6, 17.4 and 23.2 g L^{-1}) and verified that increased stocking density may reduce both the dissolved oxygen concentration in the transport water (7.3, 6.2, 5.1 and 2.3 mg L^{-1}, respectively) and the survival rate (98.9, 99.2, 52.7 and 9.6 %, respectively).

Dissolved oxygen concentrations below 2 mg L^{-1} are classified as lethal to shrimp, especially in the context of long-term exposure (Boyd, 1990). Thus, in the present study, the exposure of shrimp to dissolved oxygen concentrations under 2 mg L^{-1}, at higher stocking densities (3 and 4 ind L^{-1}), may have influenced the survival of these animals. It should be noted that after 5 h in the 4 ind L^{-1} treatment, the oxygen levels fell below 2 mg L^{-1}. The dissolved oxygen concentration in the transport water varied depending on the stocking density and the addition of hydrated lime, the latter of which apparently results in lower oxygen consumption. This lower consumption may be associated with a condition of transport that is less stressful for the shrimp, as higher alkalinity and pH levels were observed in the hydrated lime treatment when compared to the treatment with the same stocking density (3 ind L^{-1}) but without hydrated lime.

The pH level is an important parameter to be considered in the context of the transport of any live aquatic organism, as it affects their metabolism and other physiological processes (Vinatea, 1997; Barajas *et al.*, 2006). In addition, the pH level also strongly influences the toxicity of certain chemical parameters, such as unionized ammonia, which becomes more abundant at an alkaline pH (Van Wyk & Scarpa, 1999) and hydrogen sulfide (H_2S), which increases proportionally to increasing acidity (Vinatea, 1997). Certain studies have demonstrated an increased sensitivity of animals when exposed to low pH and dissolved oxygen levels (Allan & Maguire, 1991; Zhang *et al.*, 2006), confirming the importance of pH control within adequate levels for shrimp transport. Van Wyk & Scarpa (1999) reported that in cropping systems, the optimum development of marine shrimp occurs in the pH range between 7.0 and 9.0, with levels below 6.5 and above 10.0 being harmful to the shrimp's gills.

Girotto (2010) analyzed the toxicity of ammonia excretion on the transport of *Litopenaeus vannamei* and *L. schmitti* juveniles and observed that increased stocking density caused a decrease in pH levels in both species following 150 min of transport. Sperantio (2004), examined the transport of juvenile *M. amazonicum* (0.09 ± 0.03 g) for 8 h at different stocking densities (5.8, 11.6, 17.4 and 23.2 g L^{-1}). This previous study reported a decrease in the pH of the transport water from 7.8 to 6.5 in lower densities and to 6.3 at higher densities, with a decrease in the shrimp survival rate due to increased stocking density (92.3, 81.7, 34.0 and 7.5%, respectively).

In the present study, increased stocking density also reduced pH levels. None of the pH values that were obtained in the different treatments were within the range considered harmful to penaeids. However, in the 3 and 4 ind L^{-1} treatments, the mean pH levels at the end of the experiment were 6.67 and 6.66, respectively, values that are close to the harmful limit. The survival rate in the treatments with the addition of hydrated lime was 100% at the end of the experiment. This result suggests that an acclimation of shrimp in an intermediate pH and alkalinity reduces the initial stress of the transport.

According to Van Wyk & Scarpa (1999), *L. vannamei* requires alkalinity values above 100 mg $CaCO_3$ L^{-1} for proper development. According to Ebeling *et al.* (2006), in systems with limited water exchange, the alkalinity should be between 100-150 mg $CaCO_3$ L^{-1} to avoid decreased pH levels and reductions in the organism's performance. In the present work, the alkalinity levels decreased in all of the treatments due to increased stocking density. Moreover, in the beginning from the two hour of the experiment, in the 3 and 4 ind L^{-1} treatments, values below 100 mg $CaCO_3$ L^{-1} were detected. After the eighth hour, the observed levels were below those recommended by Van Wyk & Scarpa (1999), except for the 3 ind L^{-1} with hydrated lime treatment. The use of hydrated lime to increase the alkalinity was determined to be efficient, given that this group exhibited high alkalinity and no mortality at the end of the 12 h simulated transport.

Dissolved carbon dioxide levels below 5 mg L^{-1} are considered ideal, and water levels above 20 mg L^{-1} of CO_2 are considered acceptable for penaeid shrimp. The CO_2 concentrations between 20 and 60 mg L^{-1} are not lethal but can interfere in the exchange of CO_2 in the gills. Concentrations above 60 mg L^{-1} CO_2 can be fatal (Van Wyk & Scarpa, 1999). CO_2 becomes toxic to aquatic organisms due to the reduction in the hemolymph's ability to transport oxygen. The hemolymph therefore acidifies, generating metabolic stress. The concentration of dissolved carbon dioxide can be affected by changes in the total amount of carbonate carbon in the solution, the pH of the water, and the temperature and salinity of the solution. However, CO_2 levels tend to increase due to organism respiration as a consequence of increasing biomass (Timmons & Ebeling, 2010).

One strategy to minimize the accumulation of dissolved carbon dioxide is the renewal of water, which does not naturally occur in the transport of living organisms. Another strategy to mitigate this problem is the application of alkaline compounds in the water, thereby shifting the balance of CO_2 to the formation of bicarbonates and carbonates. In the present study, an increase in the levels of dissolved CO_2 was observed with increasing stocking density, as were reduced alkalinity and pH levels. However, the highest carbon dioxide levels were within a range that is considered to be harmful, but not lethal, to penaeids (Van Wyk & Scarpa, 1999). Lower levels of CO_2 were recorded in the treatment in which hydrated lime was added. This effect was a consequence of the higher pH values and higher alkalinity in this treatment. Thus, the proportion of free CO_2 in the water was lower, resulting in concentrations within the range considered optimal for penaeids (Van Wyk & Scarpa, 1999).

Besides the inclusion of hydrated lime to soften the changes in the parameters of water quality, another important action that can be done is to control the temperature of the water transport of animals. Kir *et al.* (2004) observed an increase in the tolerance of juvenile *Penaeus semisulcatus* to total ammonia levels by reducing the water temperature from 26°C to 14°C. According to Barajas *et al.* (2006), this result can be explained by the increased excretion of ammonia in crustaceans at higher temperatures (Regnault, 1987); moreover, a temperature increase causes an increase in the concentration of unionized ammonia, which is more toxic (Bower & Bidwell, 1978).

Ammonia toxicity is related to several metabolic processes. Ammonia decreases the absorption of sodium by the shrimp, inhibiting the function of the Na^+-K^+ pump, an important mechanism in regulating the osmotic balance of these organisms. In addition, ammonia alters the pH of the cells, affecting all enzymatic metabolic processes in the animal (Barbieri & Ostrensky, 2002). High concentrations of ammonia in the water can irritate the shrimp's gills and may cause gill hyperplasia, reducing the ability of the shrimp to capture oxygen from the water. In addition, high ammonia levels in the water can lead to an increased concentration of ammonia in the hemolymph, reducing the affinity of hemocyanin, an oxygen-carrying pigment. Together, these latter two effects of ammonia can reduce the tolerance of shrimp to low-oxygen conditions (Van Wyk & Scarpa, 1999).

It is known that ammonia tolerance is species-specific (Vinatea, 1997; Baldisserotto, 2009) and varies with the age of the animal; certain studies have reported an increased of ammonia tolerance with increasing age (Van Wyk & Scarpa, 1999; Barajas *et al.*, 2006). According to Ostrensky & Wasielesky (1995), the lethal concentrations of total ammonia ($LC_{50\text{-}24\,h}$) for post-larvae (PL_1) juvenile (5.45 ± 0.4 g) and adult (31.43 ± 1.3 g) *F. paulensis* are 24.19, 51.87 and 61.63 mg L^{-1}, respectively. Girotto (2010) analyzed ammonia-related toxicity and ammonia excretion of *L. vannamei* and *L. schmitti* juveniles following 150 min of transport, and observed a variation between species and an increased concentration of total ammonia in relation to stocking density (2, 3 and 5 ind L^{-1}), with mean total ammonia concentrations of 0.77, 1.23 and 1.60 mg L^{-1} for *L. schmitti*, and 0.47, 0.68 and 0.85 mg L^{-1} for *L. vannamei*, respectively.

According to Campos *et al.* (2012), the lethal concentration of total ammonia ($LC_{50\text{-}24\,h}$) for juvenile (0.3 g) *F. brasiliensis* is 24.77 mg L^{-1}. In this same study, the authors recommended that the safe level of ammonia for *F. brasiliensis* be set at 10% of the LC_{50} to avoid mortality. In the present study, the values of total ammonia were considered high when compared to the results obtained by Girotto (2010), reaching values above 10.82 mg L^{-1} in the 3 ind L^{-1} treatment. However, due to the low pH (6.67) in this treatment, the percentage of toxic ammonia was also low (0.05 mg L^{-1}). We believe that the high levels of total ammonia observed in the present study were a consequence of handling prior to the transport simulation, which allowed the shrimp access to food. In the treatment with addition of hydrated lime, the concentration of total ammonia was similar to that observed by Girotto (2010). In this previous study, feeding was suspended prior to the transport. Our results suggest that the suspension of shrimp feeding prior to transport is an important factor in decreasing excretion and, therefore, the ammonia concentration in the water.

Several authors reported negative correlations between stocking density and survival on transport (Smith & Wannamaker, 1983; Kubitza, 1997). These authors observed lower survival rates with increasing stocking density. Cobo (2003) verified that during the transport of *L. vannamei*, post-larvae exhibit a decrease in survival with increasing stocking density and the duration of transport. Sperandio (2004) examined the transport of post-larval and juvenile *M. rosenbergii* and *M. amazonicum* at different densities, observing the same negative correlation. In the present study, we also observed a negative correlation between the stocking density and survival; however, this correlation was only observed after the eleventh hour of the experiment. Lower survival was observed at higher densities (4 ind L^{-1}) than for the other treatments.

During the salinity stress test, the shrimp from the 3 and 4 ind L^{-1} treatments exhibited lower resistance than those from the lower density treatments, and higher mortality was observed when transferred from a salinity of 22 g L^{-1} to one with a salinity of 12 g L^{-1}. Brito *et al.* (2000) examined the effect of abrupt salinity changes on the survival of post-larval *F. brasiliensis*, and observed no effect when the post-larvae were transferred from a salinity level of 37 g L^{-1} to salinities between 35 and 15 g L^{-1}. Mortalities were observed only for transfers to salinities of below 15 g L^{-1}. These authors estimated that the lethal salinity (SL_{50}) of post-larval *F. brasiliensis* for 96 h is 10 g L^{-1}. This limited tolerance to low salinities can be explained by the euryhaline habits of this species, which has a natural distribution in more saline regions (Brito *et al.*, 2000).

CONCLUSIONS

The results of this study demonstrated that the highest density that can be used for shrimp transport to avoid mortality is 3 ind L^{-1} for a maximum period of 10 hours. The density of 4 ind L^{-1} is not recommended for transport in order to avoid the exposure of shrimp to dissolved oxygen concentrations below 2 mg L^{-1}. This density is also not recommended due to the low survival (50%) in the saline stress test. The use of hydrated lime in the transport water efficiently attenuated the variation of water quality parameters (oxygen, pH, alkalinity and carbon dioxide). Its use is recommended to increase the alkalinity and pH levels of the transport water, thereby reducing shrimp stress and mortality during transport.

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