









Transport simulation of Brazilian sardine *Sardinella brasiliensis* (Steindachner, 1879)

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ABSTRACT

The fish transport is a routine operation in aquaculture, and the practice has a significant cost, as well as a considerable risk during execution. Numerous factors are related to fish transport, and density is one of the factors that must be considered first. The aim of this study was to investigate the ideal density to use in transport operations for Brazilian sardines *Sardinella brasiliensis* (Steindachner, 1879). For this purpose, 4,020 juveniles *S. brasiliensis* (0.51 ± 0.10 g and 3.89 ± 0.32 cm) were randomly distributed in 60L-polyethylene bags filled with 20 L of water (salinity 35 g/kg) at densities of 15, 10, 5, 2.5 and 1 g·L⁻¹, all in triplicate. The transport bags were inflated with pure oxygen (about 2/3) and sealed with elastic bands. The duration of the simulated transport was 24 h. Then, survival rate, as well as analyses of water quality, was performed. The results showed that density had a considerable influence on survival and water quality. At the density of 15 g·L⁻¹ dissolved oxygen reached 0.0 mg·L⁻¹, causing 100% mortality at the end of the experiment, while the density of 10 g·L⁻¹ showed a higher amount of N-NH₃ in the water. Survival was the same at densities below 10 g·L⁻¹. Densities ≤ 10 g·L⁻¹ are recommended for the transport of *S. brasiliensis* lasting up to 24 h.

Keywords: Transporting live fish; Clupeidae; Ammonia; Low-trophic level species.


Simulação do transporte da sardinha brasileira *Sardinella brasiliensis* (Steindachner, 1879)

RESUMO

O transporte de peixes vivos é uma operação rotineira na aquicultura, e a prática tem custo significativo, bem como um risco considerável durante a execução. Inúmeros fatores são relacionados ao transporte de peixes, e a densidade é o fator que deve ser considerado em primeiro lugar. O objetivo deste estudo foi investigar a densidade ideal para uso em operações de transporte de sardinha brasileira *Sardinella brasiliensis* (Steindachner, 1879). Para tanto, 4.020 juvenis de *S. brasiliensis* ($0,51 \pm 0,10$ g e $3,89 \pm 0,32$ cm) foram distribuídos aleatoriamente em sacos de polietileno de 60 L contendo 20 L de água (salinidade 35 g/kg) em densidades de 15, 10, 5, 2,5 e 1 g·L⁻¹, todos em triplicata. As bolsas de transporte foram infladas com oxigênio puro (cerca de 2/3) e seladas com elásticos. A duração do transporte simulado foi de 24 h. Em seguida, foram realizadas análises da taxa de sobrevivência e da qualidade da água. Os resultados mostraram que a densidade teve influência considerável na sobrevivência e na qualidade da água. Na densidade de 15 g·L⁻¹ o oxigênio dissolvido atingiu 0 mg·L⁻¹, causando 100% de mortalidade ao final do experimento, enquanto a densidade de 10 g·L⁻¹ apresentou maiores quantidades de N-NH₃ na água. A sobrevivência foi a mesma em densidades ≤ 10 g·L⁻¹. Recomendam-se densidades ≤ 10 g·L⁻¹ para o transporte de *S. brasiliensis* com duração de até 24 h.

Palavras-chave: Transportando peixes vivos; Clupeidae; Amônia; Espécies de baixo nível trófico.

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INTRODUCTION

Low-trophic marine species, such as *Sardinella brasiliensis*, represent approximately 30% of all global fisheries production, directly contributing to world food security (Smith et al., 2011; FAO, 2022). The genus *Sardinella* is widely distributed throughout the world in the Atlantic and Indo-Pacific oceans (FAO, 2022). However, the species *S. brasiliensis* is geographically isolated in the Atlantic Ocean from other groups of the same genus (Cergole and Dias-Neto, 2011). In Brazil, *S. brasiliensis* occurs along the coast between the states of Rio de Janeiro (Cape São Tomé, 22° S) and Santa Catarina (south of Cabo de Santa Marta, 29° S) (Schroeder et al., 2022).

Research reporting the larval condition and growth of *S. brasiliensis* in laboratory (Rossi-Wongtschowski et al., 2003), spawning in captivity (Passini et al., 2013; Magnotti et al., 2020), breeder management and reproduction control (Cerqueira et al., 2017, 2020), nutrition (Baloi et al., 2016; Sterzelecki et al., 2017, Baloi et al., 2017b; Sterzelecki et al., 2018, 2021a, 2021b), effects of abiotic factors such as acute toxicity of ammonia in juvenile (Baloi et al., 2017a), influence of temperature (Angelo et al., 2021) and salinity (Owatari et al., 2023) on growth and survival of juveniles, as well as the control of parasites (Owatari et al., 2020), gained prominence in research related to the cultivation of *S. brasiliensis* in Brazilian aquaculture.

The transport of live fish is necessary in many aquaculture operations, in which fish are commonly transferred between facilities and/or between properties, mainly using roads for this purpose (Broom, 2008; King, 2009). The basic principles for transporting live fish can be affected by species and size of specimens, duration of transport, temperature, oxygen levels, storage density, and by products of fish metabolism such as ammonia (Berka, 1986). In short-term transport, operational problems are related to the reduction of water pH, while, in long-term transport, dissolved oxygen levels and increased ammonia in the water are the main concerns (Sampaio and Freire, 2016). In this way, the establishment of specific transport protocols for each species is needed in aquaculture (Amend et al., 1982; Sampaio and Freire, 2016; Vanderzwalmen et al., 2019; Faust et al., 2023), allowing the animals to arrive at their destination in physiological conditions close to the normal state.

In view of the aforementioned facts, this study aimed to evaluate the resistance of the Brazilian sardine *S. brasiliensis* during simulated transport in polyethylene bags, monitoring survival, dissolved oxygen, and the levels of nitrogenous compounds accumulated in the water during the transport period.

MATERIALS AND METHODS

All fish used in this research were handled in accordance with the guidelines approved by the Ethics Committee on Animal Experimentation of the Universidade Federal de Santa Catarina (UFSC), under protocol PP0861.

Experimental design

The experiment was carried out at the Marine Pisciculture Laboratory of the Aquaculture Department of the UFSC, Barra da Lagoa, Florianópolis, SC, Brazil. A total of 4,020 specimens of Brazilian sardine *S. brasiliensis* were used, with an average weight of 0.51 ± 0.10 g and length of 3.89 ± 0.32 cm from natural spawning obtained at the laboratory. The fish were conditioned to a commercial diet (45 % crude protein) and stored in a fiber tank with capacity of 5,000 L, with continuous flow of ocean water, collected at Mozambique beach, Florianópolis, Brazil ($27^{\circ}34'02''\text{S}$, $48^{\circ}25'44''\text{W}$), and constant aeration, maintaining dissolved oxygen levels at 6.54 ± 0.63 mg·L⁻¹.

After fasting for 24 h, the fish were randomly separated and packed in 60-L polyethylene bags filled with 20 L of sea water (salinity 35 g/kg) at densities of 15, 10, 5, 2.5 and 1 g·L⁻¹, all in triplicate. The bags were inflated with pure oxygen (about $\frac{2}{3}$) and sealed with elastic bands and placed in boxes under a vibrating platform, in which the simulated transport was performed for 24 h. At the end of the period, physicochemical analyses of the water were performed, as well as the number of dead fish in each treatment. Ammonia analyses were performed with protocols and reagents from Alfacit, dissolved oxygen and temperature with multiparameter probe (YSI Pro Plus). The data were submitted to the Levene's test to verify homoscedasticity, followed by the Shapiro-Wilk's test, to verify the normality, and submitted to analysis of variance (ANOVA), with average compared using the Tukey's test ($p < 0.05$). All statistical analyses were performed using the GraphPad Prism 6 software.

RESULTS AND DISCUSSION

Stocking density and duration of transport must be carefully planned, using suitable vehicles, conducted with extra care so that disease susceptibility is not increased (Broom, 2008). The results of the present study showed that the density significantly influenced the survival of *S. brasiliensis* during simulated transport, significantly altering the water quality in the transport bags.

The dissolved oxygen concentration and saturation reduced proportionally to the stocking density, with the highest levels ($p < 0.05$) in the lower density treatment (1 g·L⁻¹) (Fig. 1). Most fish

species depend on oxygen dissolved in water to breathe, and the oxygen consumption varies according to species, size, activity level, and water temperature (Boyd, 2020). Thus, during the transport of live fish, it is essential to provide adequate levels of dissolved oxygen to support the animals' basic respiration (King, 2009).

A fish at resting requires minimal amounts of oxygen, however, in a transport operation, in which homeostasis is disrupted, fish will require more than the minimum amount, possibly increasing oxygen consumption close to the maximum (Berka, 1986). This situation was observed in the present study, in which in the treatment with the highest density (15 g·L⁻¹) the oxygen concentration reached 0, causing total fish mortality before the 24-h period, explaining the lower concentrations of ammonia in this group. On the other hand, after 24 h of simulated transport, the 10-g·L⁻¹ density treatment showed high survival rates even with low concentrations of dissolved oxygen in the water, indicating a high consumption of oxygen in a situation of physiological stress close to the limit.

After 24 h, ammonia levels were significantly lower ($p < 0.05$) in treatments with densities 1, 2.5 and 5 g·L⁻¹, when compared to density 10 g·L⁻¹ (Fig. 1). This can be explained by lower metabolic activity related to lower fish biomass. According to Grøttum et al. (1997), the transportation time of turbot *Scophthalmus maximus*, maintained at high densities (0.5 kg·L⁻¹), can be increased by reducing the pH, either by raising carbon dioxide levels or by adding acid to the water, probably owing to reduced toxicity of total ammonia (NH₃ and NH₄⁺), and *S. maximus* mortality was recorded at NH₃ concentrations above 0.4 mg·L⁻¹. In the present study, the highest density groups, i.e., 15 g·L⁻¹, in which the N-NH₃ concentration did not exceed 0.2 mg·L⁻¹, showed 100% mortality at the end of the experimental period. However, the 10 g·L⁻¹ density groups had a higher amount of N-NH₃, close to 0.6 mg·L⁻¹, but high mortality rates were not observed in this group. This probably indicates that the fish in the 15 g·L⁻¹ density groups died even before the ammonia levels increased.

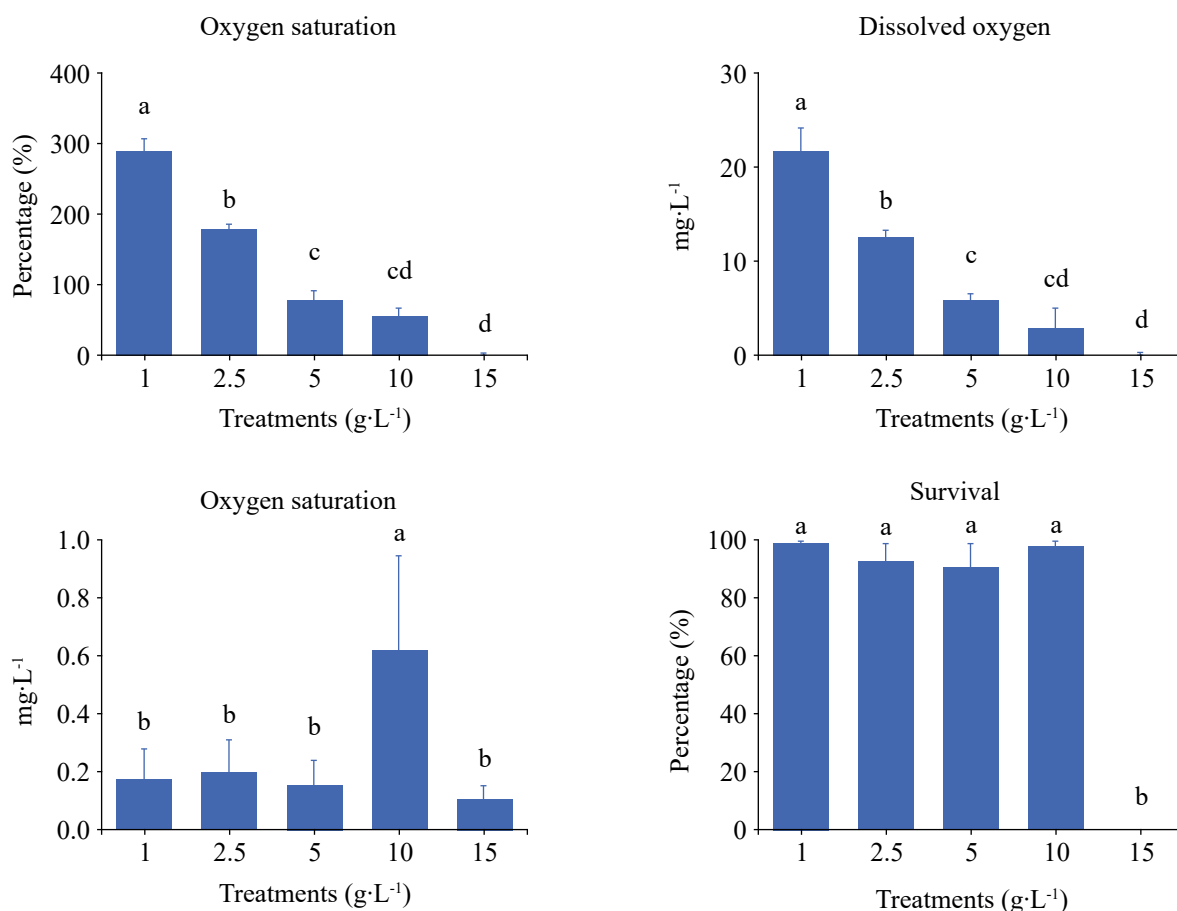


Figure 1. Water quality variables of dissolved oxygen, oxygen saturation, ammonia, and survival rate of Brazilian sardine *Sardinella brasiliensis* during simulated transport. Data are presented as average \pm standard deviation. Different letters indicate significant differences between treatments

According to Jarboe (1995), dissolved oxygen consumption and total ammonia nitrogen (TAN) production are influenced after feeding juvenile channel catfish (*Ictalurus punctatus*) at different times; oscillating TAN production from 10.1 to 64.4 mg N·kg⁻¹ for fish fed at 8 a.m.; while rates for fish fed at 4 p.m. ranged from 8.9 to 105.4 mg N·kg⁻¹. Average oxygen consumption per hour ranged from 148.7 to 326 mg·kg⁻¹ for fish fed at 8 a.m., and from 295.8 to 462.0 mg·kg⁻¹ for fish fed at 4 p.m. (Jarboe, 1995). Such information highlights the importance of a fasting period for the fish, as performed in the present study, before transport.

Methods based on the addition of Clinoptilolite buffer at 14 g·L⁻¹ to control the development of toxic by-products in fish transport water, such as ammonia accumulation, can reduce mortality rates even when fish are transported at higher densities for longer periods of time (Amend et al., 1982). These procedures can be useful for the transportation of *S. brasiliensis* at 10 g·L⁻¹ to maintain lower ammonia levels through transportation.

CONCLUSION

Considering the transport characteristics proposed in the present study and the results obtained related to survival rates, oxygen consumption, and nitrogenous compounds in water, we recommend densities ≤ 10 g·L⁻¹ for the transport of *S. brasiliensis* lasting up to 24 h.

CONFLICT OF INTEREST

Nothing to declare.


DATA AVAILABILITY STATEMENT

Data will be made available upon reasonable request.


AUTHORS' CONTRIBUTION

Conceptualization: Owatari MS, Sterzelecki FC, Cerqueira VR, Magnotti C; **Methodology:** Sterzelecki FC, Manhães JV, Palma US, Cerqueira VR, Magnotti C; **Investigation:** Owatari MS, Sterzelecki FC, Manhães JV, Palma US; **Data curation:** Owatari MS, Sterzelecki FC; **Formal Analysis:** Owatari MS, Sterzelecki FC, Palma US, Magnotti C; **Funding acquisition:** Cerqueira VR, Magnotti C; **Project administration:** Cerqueira VR, Magnotti C; **Resources:** Magnotti C; **Supervision:** Magnotti C; **Writing – original draft:** Owatari MS, Magnotti C; **Writing– review & editing:** Owatari MS, Magnotti C; **Final approval:** Owatari MS.


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