



Influence of salinity on growth and survival of juvenile *Sardinella brasiliensis*

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ABSTRACT

The aim of the study was to evaluate the influence of changes in seawater salinity on juvenile Brazilian sardine (*Sardinella brasiliensis*). Through two assays, the LC_{50} (96 h) and the zootechnical performance (42 days) were determined, respectively. In the first assay, six treatments of salinity 0, 7, 14, 21, 28 and 35 parts per thousand (ppt) with three replicates were established. For this, 100-L cylinder-conical tanks were used, with 30 individuals with 45-day after eclosion (DAE) per unit, without water renewal and feeding. In the groups of salinity 0 and 7 ppt, all fish died on the first day. The LC_{50} was estimated at salinity 11.13 ppt. The second trial was designed with five treatments (salinity 7, 14, 21, 28 and 35 ppt), with three replicates. Two thousand-L cylindrical-conical tanks were used, with 30 individuals (58 DAE) per unit, with water renewal and feeding until apparent satiation. In the treatment salinity 7 ppt, all fish died by the second day. At salinity 35 ppt, the highest growth rates (2.78 g) and survival (100%) were observed. Salinity 14 ppt had the lowest survival (83%) and growth (1.48 g). We concluded that the juvenile sardines can be adapted to environments with salinity from 14 ppt, with significant losses. However, salinity 35 ppt showed the highest survival and growth rates.

Keywords: Clupeidae; Tolerance; LC_{50} ; Water quality; Zootechnical performance.

Influência da salinidade no crescimento e sobrevivência de juvenis de *Sardinella brasiliensis*

RESUMO

O objetivo do estudo foi avaliar a influência da variação da salinidade na água do mar em juvenis de sardinha brasileira (*Sardinella brasiliensis*). Por meio de dois ensaios, determinaram-se a CL_{50} (96 h) e o desempenho zootécnico (42 dias), respectivamente. No primeiro ensaio foram estabelecidos seis tratamentos de salinidade 0, 7, 14, 21, 28 e 35 *parts per thousand* (ppt) com três repetições. Para isso, foram utilizados tanques cilindro-cônicos de 100 L com 30 peixes (45 dias após eclosão — DAE) por unidade, sem renovação de água e sem alimentação. Nos grupos de salinidade 0 e 7 ppt todos os peixes morreram no primeiro dia. O LC_{50} foi estimado na salinidade 11,13 ppt. O segundo ensaio foi delineado com cinco tratamentos (salinidade 7, 14, 21, 28 e 35 ppt), com três repetições. Foram empregados tanques cilíndrico-cônicos de 200 L, com 30 indivíduos (58 DAE) por unidade, com renovação de água e alimentação até a saciedade aparente. No tratamento salinidade 7 ppt, todos os peixes morreram no segundo dia. Na salinidade 35 ppt foram observadas as maiores taxas de crescimento (2,78 g) e sobrevivência (100%). A salinidade 14 ppt teve a menor sobrevivência (83%) e crescimento (1,48 g). Concluímos que os juvenis de sardinha podem se adaptar a ambientes com salinidade de 14 ppt em diante, com perdas significativas, no entanto a salinidade 35 ppt apresentou as maiores taxas de sobrevivência e crescimento.

Palavras-chave: Clupeidae; Tolerância; CL_{50} ; Qualidade da água; Desempenho zootécnico.

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INTRODUCTION

Small-sized pelagic fish of the Clupeidae family play a fundamental role in marine ecosystems due to their important value in the food chain (Peck et al., 2021). In addition, intended for human consumption, they are essential in the stability of world food security, as well as in the use for the production of fish meal and oil, used as ingredients in agricultural or aquatic feed (FAO, 2022; Peck et al., 2021). Species of the Clupeidae family are found throughout the world, commonly in warmer marine waters. Moreover, some species are anadromous or perennial inhabitants of freshwater environments (Coad, 2019).

Brazilian sardine *Sardinella brasiliensis* is the main species caught in pelagic fisheries on the southeastern coast of the country (Isaac-Nahum et al., 1988). The species Brazilian sardine is a small omnivorous fish, with a laterally elongated and silvery body that forms schools and inhabits coastal waters, entering bays and estuaries (Cergole and Dias-Neto, 2011). It is found along Brazil's southeast coast, from Cabo de São Tomé, RJ, to Cabo de Santa Marta Grande, SC (Cergole and Dias-Neto, 2011; Jablonski, 2007).

However, intrinsic and extrinsic factors, including abiotic factors such as temperature, salinity, pH, dissolved oxygen and density, can influence the population dynamics of small Clupeidae pelagic fish (Peck et al., 2021). In addition, the high variation in the biomass of populations throughout the year, susceptibility to climate change and recruitment are obstacles to the control of sustainable fishing of these species (Pikitch et al., 2012).

The conditions of estuarine ecosystems directly influence the initial stage of life and distribution of ichthyoplankton belonging to the Clupeidae family (Breux et al., 2019; De Lima et al., 2022). The spatio-temporal variations are related to environmental factors such as pH, temperature and salinity, with salinity being influenced by rainfall (Davis et al., 2022; De Lima et al., 2022).

Sudden changes in salinity greater than 10 parts per thousand (ppt) can cause an irreversible collapse and make it impossible for animals to adapt to change, even if exposed for only a few minutes or hours. It is ideal that change be gradual, so that fish have time for the structural and metabolic reorganization needed to satisfy the increased energy demand associated to exposure to the new environment, allowing greater adaptation to these changes (Boyd, 1990; Marshall et al., 2019).

Studies on the effects of salinity variations in aquaculture environments are essential, as they generate information that allows for the improvement of zootechnical performance indexes (survival, growth, etc.), in prevention of diseases, and allow the

fish farming in artificially manipulated environments, differently from their natural habitat (Baldisserotto, 2002; Noga, 2021). In recent years, the Brazilian sardine has aroused interest in Brazilian aquaculture (Angelo et al., 2021; Baloi et al., 2016; Cerqueira et al., 2020; Sterzelecki et al., 2017) due to its economic importance for the canning industry and tuna fisheries, as well as to address the decline in wild populations (Baloi et al., 2017).

Thus, considering the economic importance and ecological relevance, as well as the importance of the species in the Brazilian aquaculture scenario, the objective of this study was to evaluate the influence of salinity variation in seawater on the survival, growth and morphology of juvenile Brazilian sardine (*S. brasiliensis*).

MATERIALS AND METHODS

This study was approved by the Committee for Ethical Use of Animals of Universidade Federal de Santa Catarina (UFSC) (protocol number: PP0861).

Biological material

Juvenile *S. brasiliensis* were obtained from induced spawning of breeding stock kept at the Marine Fish Farming Laboratory (LAPMAR), at UFSC, in 8,000-L tanks with a maximum biomass of 1 kg·m⁻³, and constant water renewal (19-20 L·min⁻¹) supplied with sea water (35 ppt) from Barra da Lagoa beach, Florianópolis, SC, Brazil. The photoperiod and water temperature vary naturally, minimum 10 and maximum 14 h light, and minimum 17 and maximum 30°C, for winter and summer (latitude 27°S), respectively.

For hormonal induction, the fish were anesthetized with 50 ppm of benzocaine. After being anesthetized, the reproducers were induced with an intramuscular application of an analog of the hormone that liberates luteinizing hormone (LHRHa Sigma-AldrichCo.) at the dose of 50 µg per kg of fish. After induction, the females and males were transferred to a 2,000-L tank with a continuous water flow system. After 36 h, at 26 ± 1°C, the fish spawned naturally in the tank. The egg collection was conducted at a water outlet on the upper portion of the tank that led to a conical shaped collector. The eggs were then transferred to the larviculture tanks with water temperature between 26 and 29°C, salinity 35 ppt, dissolved oxygen (6.02 ± 0.51 mg L⁻¹), and total ammonia nitrogen and nitrite below 0.05 mg L⁻¹ according to Cerqueira et al. (2020). At the second day after eclosion (DAE), the first food provided was the rotifer (*Brachionus rotundiformis*), which was later combined with nauplius/metanauplius of

Artemia sp. and commercial feed (NRD 3/5, INVE Aquaculture, Salt Lake City, UT, United States of America) 300-500 μm , with 52% crude protein, 9.5% ether extract, and 1.8% fiber, following the protocol described by Cerqueira et al. (2017).

Acute salinity change test: determination of LC_{50} at 96 hours

To determine LC_{50} at 96 h, a test was conducted to verify the tolerance of fish in a large range of salinity. First, a sample of 20 fish at 45 DAE was taken to determine initial weight and length (2.48 g \pm 0.57 and 5.5 \pm 0.5 cm). Seven salinities were tested (0, 7, 14, 21, 28 and 35 ppt) each in triplicate. The tests were conducted using conical-cylindrical tanks of 100 L of useful volume, with 30 fish per experimental unit (0.3 fish·L⁻¹). The experimental units had constant aeration, temperature control, and a natural photoperiod (14 h light : 10 h dark). During the experiment, the fish were not fed, and water was not renovated. In addition, in the first 12 h of the experiment, mortality was monitored hourly, and then at 24, 48, 72 and 96 h. The dead individuals were removed and their number registered. Temperature, dissolved oxygen, and total ammonia were measured daily.

The salinities were obtained through stoichiometric calculation of the dilution of freshwater with seawater. In the freshwater, chlorine was eliminated through constant aeration for 24 hours. The salinity measurements were conducted using a portable refractometer (Instrutherm RTS-101ATC-03137). Using the Trimmed Spearman Karber program, developed by Hamilton et al. (1977), the median lethal salinity concentration and the respective confidence intervals (95%CI) were estimated using the mortality data observed at each salinity.

Chronic salinity change test

The chronic experiment was conducted over six weeks, at five salinities (7, 14, 21, 28 and 35 ppt) in triplicate. Conical-cylindrical tanks with 200 L of useful volume and 30 fish per experimental unit (0.15 fish·L⁻¹) were used.

Juveniles with 51 DAE were allocated in tanks with water at 35 ppt salinity, in which they were acclimatized for seven days. During the acclimatization period, the water in the experimental units was renewed 100% every 48 hours. Feed was provided three times daily (9 h, 13 h and 17 h) until apparent satiety. The tanks had constant aeration and natural photoperiod (14 h light : 10 h dark). Temperature, total ammonia, dissolved oxygen, and the number of dead were measured daily.

Fifty fish with 58 DAE and average weight of 2.68 g \pm 0.62 were sampled, without replacement, from the larviculture

tank to then start the test. On the last day of the experiment, a biometry was conducted using all of the surviving fish. The biometry used benzocaine (50 ppm) as anesthesia. Every 48 h, 100% of the water was renovated. A commercial feed (45% crude protein, 4,500 cal/kg, 13% umidity, 9% lipid and 3.6% fiber) was provided three times per day until apparent satiety (at 9 h, 13 h and 17 h). The tanks had constant aeration and a natural photoperiod (14 h light : 10 h dark). Temperature, dissolved oxygen (HANNA HI 9146), total ammonia (kits Indotest ALFAKIT, Florianópolis, SC, Brazil), and the number of mortalities were measured daily.

For growth performance analysis, the juveniles were weighed on a precision scale and measured with an ichthyometer (cm). The parameter evaluated was survival (S), and the juveniles were counted at the end of the experiment (Nf). The calculations were conducted with the following formulas: Weight Gain (GP) difference of final and initial weight (g), and Fulton's condition factor (Eq. 1), according to Froese (2006).

$$K = 100 \times (W/L^3) \quad (1)$$

The coefficient of variation in weight (%) and coefficient of variation in length (%) were calculated with the following formulas, respectively (Eqs. 2, 3, 4 and 5):

$$CV \text{ weight} = \text{standard deviation in weight} / \text{mean weight} \quad (2)$$

$$CV \text{ length} = \text{standard deviation in length} / \text{mean length} \quad (3)$$

$$\text{Specific growth rate} = [(\ln \text{ weight total final}) - (\ln \text{ with total initial}) \text{ time of experiment (days)}] \times 100 \quad (4)$$

$$\text{Apparent feed intake} = \text{total feed consumed per treatment} / \text{time of experiment (days)} \quad (5)$$

For statistical analyses, the data were submitted to a analysis of variance (ANOVA), and the means were compared by Tukey's test. All tests were performed at a significance level of 5% using Statistica 10.0 software.

Histological analyses

For the histological analyses, at the end of the chronic experiment the fish were euthanatized by immersion in anesthesia (benzocaine 300 ppm). Twenty fish were used for the initial sample. At the end of the chronic salinity changes experiment, samples of 10 fish were removed per experimental

unit. The secondary lamella were removed and fixed in 10% buffered formalin and preserved in ethanol 70%. The samples were then dehydrated in an increasing gradation of ethanol, diaphanized in xylol, impregnated and included in regular paraplast, following the routine methods for histological preparation. Histological cuts of 3 μm were made using a manual microtome that were stained with hematoxylin-eosin, following the methodology developed by Michalany (1980). For each slides, three random images were captured, for later analysis with the software Image J 1.45e (National Institutes of Health, United States of America), 1,000 x magnification.

RESULTS

Acute salinity change test: LC_{50} determination in 96 hours

The water quality variables monitored throughout the experiment did not show significant differences between treatments ($p > 0.05$). The temperature remained at 25.1 ± 0.8 °C, dissolved oxygen at $6.02 \text{ mg} \pm 0.7 \cdot \text{L}^{-1}$, and total ammonia below $0.03 \text{ mg} \cdot \text{L}^{-1}$.

The survival of the juvenile Brazilian sardine was affected by the sudden change at salinities 0 and 7 ppt, with 100% mortality in the first hour for the treatment at salinity 0 ppt. For the treatment at salinity 7 ppt, the first mortalities were registered after six hours of monitoring, and at 10 hours mortality reached nearly 50%. After 24 hours, mortality reached 100%.

In the treatment at salinity 14 ppt, the first mortalities started at 10 h, and at 24 h 9% of the juveniles were dead. At 48 and 72 h, mortality increased by 6 and 2%, respectively. In the treatment at salinity 21 ppt, the first mortalities started at 12 h. In 24 hours the mortality rate reached 3% and at 48 h grew by 2%. Meanwhile, in salinity groups 28 and 35 ppt, no mortalities were recorded in 96 hours.

At 96 h, the treatments at salinities 28 and 35 ppt had significantly higher survival ($p < 0.05$) than the other treatments, but without differences between them. Survival at salinity 21 ppt was significantly higher than at salinity 14 ppt ($p < 0.05$). The treatments at salinities 0 and 7 ppt were not included, since mortalities reached 100% within 24 hours (Table 1).

After 96 h of exposure to different salinities, higher survival was found beginning at salinity 14 ppt, at which it was 83%. At salinity 21 ppt survival was 95%, and in the treatments of 28 and 35 ppt survival was 100%. At salinities of 0 and 7 ppt, mortality was 100% in the first 24 hours. Based on this data, the low mean lethal salinity was estimated at 11.13 ppt (95% CI 10.61–11.67).

Table 1. Survival (%) of juvenile Brazilian sardine (*Sardinella brasiliensis*) reared at different salinities in a acute salinity change test for 96 hours*.

Time (h)	Salinity					
	0	7	14	21	28	35
24	0	0	91.1 ± 1.7^c	97.6 ± 1.2^b	100.0 ± 0^a	100.0 ± 0^a
48	-	-	85.3 ± 1.2^c	95.8 ± 0.6^b	100.0 ± 0^a	100.0 ± 0^a
72	-	-	83.2 ± 2.4^c	95.2 ± 0.6^b	100.0 ± 0^a	100.0 ± 0^a
96	-	-	83.2 ± 2.4^c	95.2 ± 1.2^b	100.0 ± 0^a	100.0 ± 0^a

*Values are expressed as mean \pm standard deviation. Means followed by different letters on the same line indicate significant difference by Tukey test ($p < 0.05$).

Chronic salinity change test: cultivation at low salinity

Preliminarily, in the treatment at salinity 7 ppt, anomalous behavior of fish was seen after stocking in the experimental units, with the juveniles swimming erratically. In the first measurement, at 24 h into the experiment, 95% of the individuals were dead. After 48 h of exposure, mortality was 100%.

In the treatment at salinity 14 ppt, in the first measurement after 24 hours of experiment, 10% of the individuals were dead, and at 48 h mortality increased 5%. The remaining mortality occurred in the first week of the trial. Meanwhile, in treatment with salinity 21 ppt, in the first 24 hours of the trial, there was mortality of 4%. At 48 h, mortality increased 2%. In the treatments with salinities 28 and 35 ppt, no mortality was registered during the experiment. In terms of growth statistics, the treatment at salinity 7 ppt was not included, since mortalities reached 100% in 48 hours.

For final weight, weight gain, specific growth rate and feed intake, the treatment at salinity 35 ppt had the best results, with significant differences ($p < 0.05$) from the other treatments. The treatments at salinities 28 and 35 ppt did not have significant differences ($p > 0.05$) between them, although there were significant differences ($p < 0.05$) in relation to the treatment at salinity 14 ppt (Table 2).

There were no significant differences ($p > 0.05$) between the treatments in the water quality parameters monitored during the experiment and in the acclimatization period. Temperature remained at 25.4 ± 0.7 °C, dissolved oxygen at $6.22 \text{ mg} \pm 0.6 \cdot \text{L}^{-1}$, and total ammonia less than $0.03 \text{ mg} \cdot \text{L}^{-1}$.

Histological analyses

In the initial sample, the size of the chloride cells in the interlamellar region was small and did not stand out from the other cells (Fig. 1a). In the treatment at salinity 7 ppt (Fig. 1b), it was possible to observe lesions such as detachment of the gill lamellae, rupture of the respiratory epithelium, extravasation

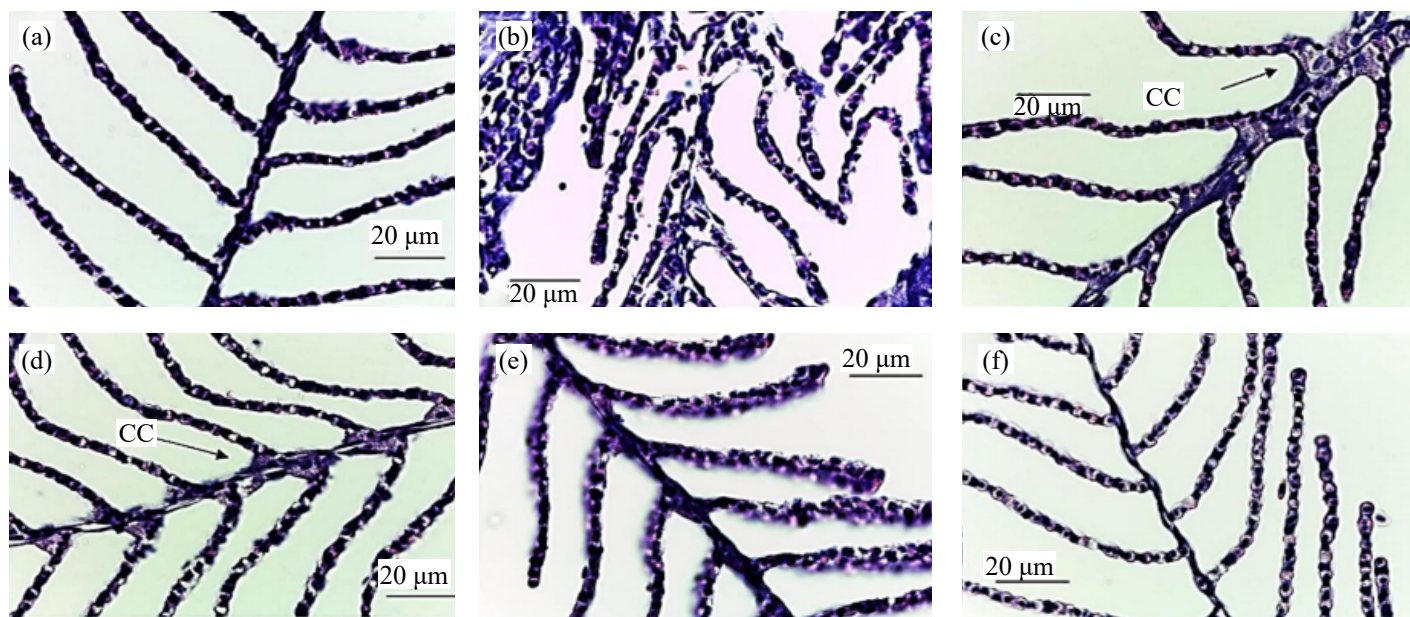
of blood to the external environment and a destructuring of the secondary lamellae. In the treatments at salinities 14 and 21 ppt, there was an increase in the number and development of chloride cells in the intralamellar region (Figs. 1c and 1d).

In the treatments at salinity 28 and 35 ppt, a similar situation was found as in the initial sample, in which the chloride cells under the light microscope were small and similar to the other cells of the lamellae (Figs. 1e and 1f).

Table 2. Growth performance of juvenile Brazilian sardine at 100 days post-eclosion, after six weeks of experiment, at different salinities*.

	Water Salinity (ppt)			
	14	21	28	35
Survival (%)	83.20 ± 0.90 ^c	94.40 ± 1.20 ^b	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a
Final weight (g)	4.16 ± 0.60 ^c	4.68 ± 0.65 ^b	4.65 ± 0.79 ^b	5.46 ± 0.67 ^a
Specific growth rate (%)	1.04 ± 0.38 ^c	1.32 ± 0.41 ^b	1.31 ± 0.35 ^b	1.69 ± 0.37 ^a
Weight gain (g)	1.48 ± 0.29 ^c	2.00 ± 0.25 ^b	1.97 ± 0.10 ^b	2.78 ± 0.09 ^a
Length (cm)	7.60 ± 0.50 ^b	7.90 ± 0.50 ^a	8.00 ± 0.50 ^a	7.90 ± 0.40 ^a
Feed intake (g/day)	1.28 ± 0.30 ^c	1.62 ± 0.40 ^b	1.68 ± 0.42 ^b	2.08 ± 0.20 ^a
Condition factor	0.97 ± 0.07 ^b	0.94 ± 0.08 ^b	0.90 ± 0.06 ^b	1.11 ± 0.06 ^a
CV weight (%)	15.08 ± 1.10 ^a	14.01 ± 0.90 ^a	13.98 ± 0.70 ^a	12.42 ± 0.30 ^a
CV length (%)	6.64 ± 1.20 ^a	6.50 ± 1.30 ^a	6.70 ± 1.00 ^a	5.80 ± 0.90 ^a

*Mean values for survival, final weight, specific growth rate, weight gain, final length, feed intake, condition factor, coefficient of variation (cv) of weight and coefficient of variation in length. Values are expressed as mean ± standard deviation. Means followed by different letters on the same line indicate significant difference by Tukey test ($p < 0.05$).



CC: chloride cell.

Figure 1. Micrographs of secondary lamellas of *Sardinella brasiliensis* after 42 days in a chronic salinity changes experiment. (a) Initial sample: the chloride cells in the interlamellar region was small and did not stand out from the other cells. (b) Salinity 7 ppt: intense detachment of the lamellas can be noticed, rupture of the respiratory epithelium, extravasation of blood to the outer environment and a destructuring of the secondary lamellas, which led to necrosis and generalized cell death, and thus loss of cell structure, without development of the chloride cells, due to the aggressivity of the treatment. (c and d) Salinities 14 and 21 ppt: an increase in the number and development of chloride cells was found in the intralamellar region due to the high osmoregulatory activity. (e and f) Salinities 28 and 35 ppt: the gill lamellae present a structure similar to the initial sample model, structurally small and ovoid, similar to other cellular structures of the lamellae. Magnification 1,000 ×. Hematoxylin-eosin staining.

DISCUSSION

Salinity variations can cause functional imbalances in an ecosystem, generating disturbances to estuarine communities (Breux et al., 2019). The present study verified the effects of low salinity (< 35 ppt) on the physiology of juvenile Brazilian sardine. The acute and chronic salinity changes tests of juvenile Brazilian sardine in the present study revealed that, although the species is oceanic, it can withstand a wide range of salinity (14 ~ 35 ppt). However, at lower salinities we observed losses in growth performance. Thus, such negative indexes can make the commercial cultivation of the species unfeasible. After prolonged exposure of 42 days at salinity 14 ppt, there was 83% survival and the mean lethal salinity (LC50) was estimated to be 11.13 ppt. This result shows a probable ability of this species to transit in estuarine or lagoon environments, with salinities above 14 ppt.

According to Saccardo et al. (1988) in a study to determine the age and growth of Brazilian sardine, during capture, it was found that the young specimens were found in greater frequency in the estuarine-lagoon regions. Matsuura (1971), in a study of the initial phase of the lifecycle of the sardine, found that spawning did not occur at more than 100 meters of depth and concluded that it occurs most frequently between depths of 15 and 100 meters (Saccardo and Rossi-Wongtschowski, 1991). However, environmental predictors such as salinity, pH, dissolved oxygen and temperature can influence the distribution of ichthyoplankton (De Lima et al., 2022).

The specific growth rate in the present study ranged from 1.04 to 1.69%, which is an interesting result compared with other marine fish species such as sea bream *Sparus aurata*, in which it was 0.65% (Izquierdo et al., 2005), Silverside *Odontesthes argentinensis*, at 1.60% (Tesser and Sampaio, 2006), the fat snook *Centropomus parallelus*, 1.75% (Sanches et al., 2011). Likewise, Baloi et al. (2016) found similar specific growth rates in a laboratory-scale study of Brazilian sardine feeding frequency (1.35 to 1.69%). It is worth noting that the maximum specific growth rate was obtained at salinity 35 ppt, and decreased at lower salinity, which indicates that salinities below 35 ppt reflect an increase in metabolic costs for ionic and osmotic regulation. Thus, this energy is reallocated to physiological processes other than growth (Altinokand and Grizzle, 2001; Morgan and Iwana, 1991). Notwithstanding, higher salinity can influence the condition factors of some estuarine fish species, and have adverse effects on growth (Biswas et al., 2018).

The literature indicates the importance of the gills, which are an essential organ that help fish to adapt to changes in salinity. Gills contain chloride cells, which are sensitive to modifications in salinity, which can lead to an increase in cell size, density, and morphological changes (Carmona et al., 2004; Hirose et al., 2003; Mylonas et al., 2009).

The analyses of the secondary lamellae of the fish allowed us to examine the branchial responses to exposure to different salinities. Morphological changes and proliferations of chloride cells were observed in the secondary lamellae of juveniles. This is due to the fact that the species is inserted in a salinity different from its natural habitat. That is, there is greater effort by chloride cells in the osmoregulatory process, increasing the development and proliferation of these cells. This response may be regulated by factors internal to the gills or be part of a systemic stress response (Mallat, 1985). Thus, the changes that occurred in the gills explain the responses of the juvenile Brazilian sardine to the various salinities tested in this study.

CONCLUSION

The environmental conditions tolerated by the Brazilian sardine found in the present research, despite the losses in growth performance, are indicators that the species is versatile and manageable in aquatic environments with non-oceanic waters, that is, with salinities lower than 28 and higher than 14 ppt. This is a strong indication that the species can be maintained and cultivated together with other estuarine species or in places where obtaining marine water is difficult. Further investigations should be carried out to verify the production cycle (from larviculture to harvesting) of *S. brasiliensis* at the lowest salinities investigated in this research (14 ~ 21), and in this way make aquaculture an environmentally friendly tool in the supply of this species for human consumption.

CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

Data will be available upon request.

AUTHORS' CONTRIBUTION

Conceptualization: Owatari M S, Sterzelecki F C, Cerqueira V R; **Data curation:** Owatari M S, Magnotti C; **Formal Analysis:** Vargas J H; Sterzelecki F C; **Investigation:** Sterzelecki F C; **Methodology:** Magnotti C, Vargas J H, Carvalho C V A, Sterzelecki F C, Cerqueira V R; **Funding acquisition:** Cerqueira V R; **Writing – original draft:** Owatari M S, Magnotti C, Vargas J H, Carvalho C V A; **Writing – review & editing:** Magnotti C, Cerqueira V R.

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