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METABOLIC AND HISTOLOGICAL ALTERATIONS AFTER EXPOSING *Deuterodon iguape* TO DIFFERENT SALINITIES*

Edison BarbieriI[1]
Karina Fernandes Oliveira Rezende[2]
Julia Schulz Carneiro[3]
Marcelo Barbosa Henriques[3]

[1]Instituto de Pesca-Governo do Estado de São Paulo, Av. Professor Wladimir Besnard, s/n, CEP 11990-000, Cananéia, SP, Brasil. E-mail: edisonbarbieri@pesca.sp.gov.br (corresponding author)

[2]Laureate International Universities, R. Quatá, 67, CEP 04546-040, São Paulo, SP, Brasil.

[3]Instituto de Pesca-Governo do Estado de São Paulo, Av. Bartolomeu de Gusmão, 192, Ponta da Praia, CEP 11030-906, Santos, SP, Brasil.

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ABSTRACT

The decrease of the live bait stock for the sport fishing, in estuarine and marine environments, has stimulated studies looking for species tolerant to the different salinities. Lambari *Deuterodon iguape* Eigenmann, 1907 has been widely used as live bait in Brazilian estuaries has a significant market and being a native animal to Brazil, it becomes a promising substitute. In order to analyze tolerance, the objective was to evaluate routine metabolism (oxygen consumption and ammonia excretion), hematological parameters (glucose, hemoglobin and total proteins), and histological parameters (gills and kidneys) of *D. iguape* after exposure to different salinities. The data were evaluated according to the means and standard deviations obtained by ANOVA (one-way) analysis followed by the Tukey post-test, after verification of the normal distributions (Kolmogorov-Smirnov test) and homoscedasticity (Levene test), $p<0.05$. In the higher salinities (12.5) tested, increased oxygen consumption, serum glucose, hemoglobin levels, decreased ammonia and total protein excretion were observed. It was concluded that 1 hour of exposure to different salinities, changes the metabolism of *D. iguape*, characterized by increased oxygen consumption and decreased ammonia excretion. Changes in hematological parameters (serum glucose, hemoglobin, and total protein) are also observed in groups exposed in the higher salinities (7.5, 10 and 12.5). Gill and kidney histological alterations were classified as mild to moderate, showing that *D. iguape* adapted well to the saline environment, which can make its use as live bait possible in estuarine sport fishing, preserving the natural stocks of *Litopenaeus schimitti* shrimp.

Key words: live bait; oxygen consumption; excretion of ammonia; hematology; gills; kidney.

ALTERAÇÕES METABÓLICAS E HISTOLÓGICAS EM *Deuterodon iguape* APÓS EXPOSIÇÃO A DIFERENTES SALINIDADES

RESUMO

A diminuição do estoque de iscas vivas para a pesca esportiva, em ambientes estuarinos e marinhos, estimulou estudos em busca de espécies tolerantes às diferentes salinidades. O lambari *Deuterodon iguape* Eigenmann, 1907 tem sido amplamente utilizado como isca viva em estuários brasileiros, possui um mercado significativo e sendo um animal nativo do Brasil, torna-se um substituto promissor. Para analisar a tolerância, o objetivo foi avaliar o metabolismo de rotina (consumo de oxigênio e excreção de amônia), parâmetros hematológicos (glicose, hemoglobina e proteínas totais) e parâmetros histológicos (brânquias e rins) de *D. iguape* após exposição a diferentes salinidades. Os dados foram avaliados segundo as médias e desvios padrão obtidos pela análise ANOVA (one way) seguida do pós-teste de Tukey, após verificação das distribuições normais (teste de Kolmogorov-Smirnov) e homocedasticidade (teste de Levene), $p <0,05$. Nas maiores salinidades (12,5) testadas, foram observados aumento do consumo de oxigênio, glicemia sérica, níveis de hemoglobina, diminuição da amônia e excreção total de proteínas. Concluiu-se que 1 hora de exposição a diferentes salinidades, altera o metabolismo de *D. iguape*, caracterizado pelo aumento do consumo de oxigênio e diminuição da excreção de amônia. Alterações nos parâmetros hematológicos (glicose sérica, hemoglobina e proteína total) também são observadas nos grupos expostos nas maiores salinidades (7,5, 10 e 12,5). As alterações histológicas das brânquias e dos rins foram classificadas como leves a moderadas, mostrando que *D. iguape* se adaptou bem ao ambiente salino, o que pode viabilizar iscas vivas na pesca esportiva estuarina, preservando os estoques naturais de camarão *Litopenaeus schimitti*.

Palavras-chave: isca viva; consumo de oxigênio; excreção de amônia; hematologia; brânquias; rim.

INTRODUCTION

Sport fishing is defined as a sport or leisure activity, with no commercial purpose, with a secondary goal of catching fish for personal use (FAO, 2012). Approximately 730 million people practice worldwide (Arlinghaus and Cooke, 2009), which represents a catch of 2 to 10.9 million tons per year (Cooke and Cowx, 2004). However, these estimates are not concrete data because some countries do not record their catches (Freire, 2010).

In Brazil, sport fishing is one of the most practiced sport and leisure activities (Soares, 2001), generating resources for the economy of several regions (Freire et al., 2012). Along the southeastern coast, sport fishing is present from the rivers, that form the estuaries, to the marine regions. For sport fishing, live bait (small shrimp and other types of organisms) are used in addition to artificial bait. Among the main species traded as live bait in the estuarine and marine environment is white shrimp (*Litopenaeus schimitti*) (Barros et al., 2014).

Litopenaeus schimitti are caught while still young to be marketed as live bait (Chaves and Robert, 2003). The capture of juveniles may compromise recruitment, as pointed out by Santos et al. (2008) after analyzing the population structure of this species in the Santista lowlands, a southeastern region of Brazil. The decline in shrimp stocks is reported by artisanal fishermen on the southeastern coast of Brazil, for various reasons: devastation of nursery areas, large amounts of litter in the estuary, overexploitation of adults by industrial fishing, and significant numbers of young shrimp fishermen during the harvest period. Some artisanal fishermen interviewed stated that at the time of greatest abundance of white shrimp juveniles (December to February), the number of collectors working in the estuary reaches 200 boats per day (Motta et al., 2016). The decrease in shrimp stocks led to research on freshwater species tolerant to different salinities to be used as live bait in the marine and estuarine regions. Of these species, the exotic species *Oreochromis niloticus* stands out because they present great tolerance to salinity (Kamal and Mair, 2005).

According to Carlton (2001), the discarding of live bait is one of the vectors for introducing exotic species into the marine environment, entailing an environmental and economic risk, since they are introduced in favorable environmental conditions and are free of predators, parasites, and competitors.

In view of the facts, it is important to search for new native species to be used as live bait in estuarine and marine environments. The lambari is much sought after as live bait for sport fishing in fresh water, being the most significant market for its cultivation (Silva et al., 2011). However, there are no studies aimed at analyzing the tolerance of the rainforest lambaris *(Deuterodon iguape*) to different salinities.

Henriques et al. (2018) compared the efficiency of using lambari *D. iguape* as live bait with the shrimp *L. schmitti*, while fishing for common snook (*Centropomus* spp.) in estuarine areas. The results showed that there was no significant difference of catch between the live baits used (*L. schmitti* vs *D. iguape*). *D. iguape* appears as effective as *L. schmitti* and can be successfully used as live bait; and from an ecological point of view, it reduces the impact of fishing on juvenile shrimp.

Among the important analyzes to evaluate this tolerance are the analyzes in relation to the bioenergetic state of the fish, and analyzes that provide information to interpret the functioning of various organs and systems of the animal (González and Silva, 2006), mainly related to the animal's capacity in keeping the osmolarity of body fluid at a relatively constant level (Brennan et al., 2016).

Osmoregulation in fish occurs mainly in the gills and kidney, creating ionic and osmotic gradients between body fluid and external environments (McCormick, 1995), with energy demand (Barbieri et al., 2014; Lisboa et al., 2015). Therefore, histological changes in gills and kidney, in addition to changes in oxygen consumption and ammonia excretion, are results of the acclimatization of fish in relation to different salinities (Barbieri et al., 1998; Baldisserotto, 2009). However, it was hypothesized that, in the face of different salinities, *Deuterodon iguape*, a common lambari species in southeastern Brazil (Valladão et al., 2016), tends to adapt through changes in routine metabolism, in hematological parameters, and in the gills and kidney histology, and can also be used as live bait in recreational fisheries in estuarine regions. An increase in the oxygen consumption related to the increase in the energy demand and, also, an increase in the concentrations of hemoglobin, glucose and total proteins were predicted.

In the gills, hypertrophy and hyperplasia of chloride cells were expected, since these cells are responsible for osmoregulation through Na^+/K^+-ATPase activity, associated with increased epithelial thickness. In addition, changes in salinity could stimulate the production of mucus as a form of protection, characterized by mucosal cell hypertrophy with the presence of these cells along the secondary lamellae (Uchida et al., 2000). In the kidney, knowing that the glomeruli have the function of filtration to eliminate excess water and the renal tubules have the function of reabsorbing salts, changes in salinity would result in a glomerular and tubular disorganization, followed by a decrease in ammonia excretion.

According to Ostrensky et al. (2008), Brazilian marine fish farming will only be established if public policies, together with the private initiative, manage to overcome the low production of juveniles of different promising species, reaching on a commercial scale. Due to the scarcity of species of marine fish that could be used as live bait, and because the lambari is used empirically by some estuarine fishers, for this reason the objective of the present study is to determine oxygen consumption and ammonia excretion, hematological parameters, and to quantify and qualify possible histological changes of the *Deuterodon iguape* gills and kidney after exposure to different salinities.

MATERIAL AND METHODS

Test organism

This study is in accordance with the ethical principles in animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and has authorization No. 06/2016 of the Committee of Ethics in Animal Experimentation of the Fisheries Institute.

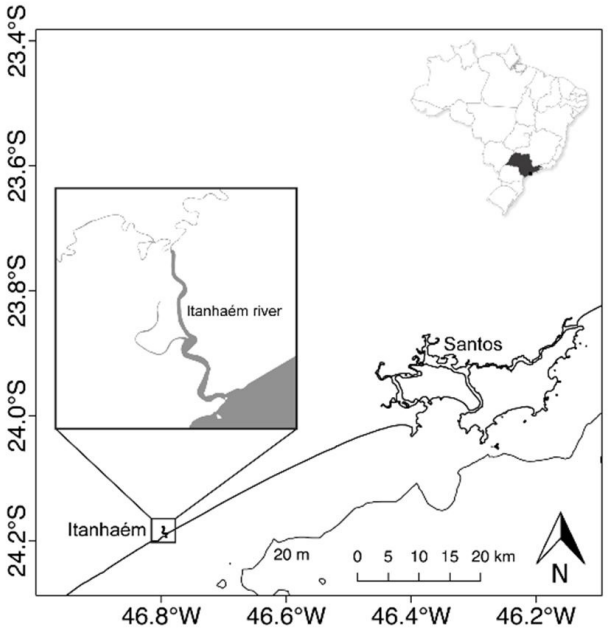

Figure 1. Location and geographical coordinates of the source of *Deuterodon iguape* used in this study.

Deuterodon iguape with an average mass of 8g ± 0.5 g (mean ± standard deviation) were obtained from a fishery in the region, located on the coast of Sao Paulo (Figure 1), and maintained at the Cananéia Base - Fishery Institute, on the southeast coast of Brazil, in 500L tanks for one week for their acclimatization. The animals were fed commercial feed once a day during this period. The feeding was suspended 24 hours before the test. No animal was used more than once.

Oxygen consumption and ammonia excretion

A total of 36 lambaris (*Deuterodon iguape*), were randomly divided into 6, 6-liter glass aquariums per fish with different salinities (2.5, 5, 7.5, 10 and 12.5 g L^{-1}), besides a control group with a salinity of 0.5 g L^{-1}. The salinities chosen for the experiment are those found in the fishing areas where lambari is used as live bait. The salinity was determined by conductivity.

After exposure to the aquariums for one hour, the fish were first acclimatized in cylindrical glass respirometers for a period of one hour with a continuous circulation of water to attenuate the stress caused by handling. Subsequently, the water flow was interrupted and the respirometers were closed, remaining in this condition for a period of one hour. The feed was suspended 24 hours before starting the experiments to avoid any kind of inference in metabolism.

Thereafter, a sample of water was drawn from each respirometer. The difference between the concentrations of oxygen and ammonia determined at the beginning and end of confinement was used to calculate the specific oxygen consumption ($mLO_2/L/g/h$) and specific ammonia excretion (mg/L/g/h) during the period, considering the volume of the respirometer, the wet weight of the animal, and the time of confinement. Were used 6 respirometer with one fish per respirometer.

To minimize the effects of low oxygen concentration and accumulation of metabolites in metabolism, the duration of the experiments was regulated so that the oxygen concentration at the end was greater than 70% of its initial concentration. The dissolved oxygen was determined with the digiMed brand oximeter and the ammonia excretion by the Nesler method (Standard Methods for the Examination of Water and Waste Water).

Hematological parameters

Thirty juvenile lambaris were used for measurements of hematocrit parameters (HTC). After 1 hour of exposure to various salinities (0.5 (control), 2.5, 5, 7.5, 10 and 12.5 g L^{-1}) the animals were anesthetized with benzocaine (4%) and blood samples were obtained by a caudal puncture with a needle and heparinized syringe.

Haematological parameters were estimated according to routine clinical methods (Wintrobe, 1978). The hematocrit was determined using a microhematocrit centrifuge and the hemoglobin concentration was determined by spectrophotometry according to the Drabkin method (Drabkin, 1949). The samples were centrifuged (12,000 rpm / 15 min) to separate the plasma, which, with appropriate dilution, would be used for enzyme assays and determining metabolic intermediates. Blood glucose was analyzed with a Medisense precision TM QID blood glucose sensor. Hemoglobin concentrations were also determined from the technique established by Tonks (1983), based on the homogenization of 4 µL of whole blood in 1 mL of Working Color Reagent of the Hemoglobin Bioclin kit, kept for 5 minutes at room temperature and processed in the Thermo Scientif Multiskan spectrophotometer.

Histology

A total of 20 lambaris (*Deuterodon iguape*), with a mean weight of 13.11 g (± 3.07) and mean total length of 9.68 cm (± 0.84), were randomly divided into 4 glass aquariums with a 6-liter capacity, with different salinities (5, 10 and 12.5 g L^{-1}), in addition to a control group with a salinity of 0.5 g L^{-1}.

After 1 hour of exposure, the animals were anesthetized with 2% benzocaine and sacrificed via a medullary section, the gills and kidneys removed and fixed in Metacarn (methanol: chloroform: acetic acid, 6:3:1) for 12 hours. Subsequently, the gills and kidneys were submitted to alcoholic dehydration and were placed in Historesina (Leica Microsystems Nussloch, Heidelberg, Germany).

Using the Hyrax M25 microtome model (Zeiss®), 4 µm thick slices (random and non-sequential) were made for the preparation of slides. About 5 cuts were placed on each slide and stained with hematoxylin / eosin (H./E.).

The slides were scanned using an ICS-Standard 25 (Zeiss®) microscope with an AxioCam HRC Camera (Zeiss®). There were 5 random pictures of 5 different cuts, resulting in 25 photos for each animal.

The gills and kidneys were evaluated (qualitative and quantitative analysis) and classified by degree of severity according to the Histological Alteration Index (HAI) (Poleksic and Mitrovic-Tutundzic, 1994) and by the Average Value of Alteration (AVA) (Schwaiger et al., 1997).

The HAI consists of two criteria, where the first criterion assesses the location and the type of change and the second criterion assesses the stage of severity. HAI was calculated using the formula: $HAI = 1 \times \sum I + 10 \times \sum II + 100 \times \sum III$, with I, II and III corresponding to alterations of stage I, II and III. The average HAI was divided into five categories: 0–10 = normal functioning of the tissue; 11–20 = mild to moderate alteration; 21–50 = moderate to severe alteration; 51–100 = severe alteration; ≥ 100 irreparable alteration.

The AVA is based on the degree of lesion severity and occurrence scale in which score 1 organs do not present pathological alterations, score 2 organs have slight or mild punctual alterations, and score 3 organs have severe and extensive pathological alterations. For the quantitative analysis, the images were submitted to the Image J® program, in which the width of the capillaries and the areas of the epithelial, mucus and chloride cells were measured in the gills; and in the kidneys, the Bowman's capsule width and tubular cell and tubular lumen areas were measured (Rezende et al., 2014; Campos-Garcia et al., 2016).

Statistical analysis

The data were evaluated according to the means and standard deviations obtained by ANOVA (one-way) analysis, after verification of the normal distributions (Kolmogorov-Smirnov test) and homoscedasticity (Levene test). The differences were considered significant when $p < 0.05$.

RESULTS

The specific oxygen consumption in individuals exposed to salinity increased in a dose-dependent manner up to the concentration of 12.5 g L^{-1}. This increase was twice as high when compared to the control (Figure 2). There was a clear trend of increasing oxygen consumption as the salinity concentration increased. However, there was statistical difference in relation to the control only at salinity concentrations of 10 and 12.5 g L^{-1}.

The results obtained for the excretion of ammonia in relation to the increase of the salinity concentrations were proven by a tendency of elevated excretion rates in relation to the control until 5 g L^{-1} of salinity. However, from 7.5 g L^{-1}, the excretion decreased, and when using the statistical test there was not a difference in relation to the control at the three concentrations (7.5, 10 and 12.5 g L^{-1}) (Figure 3).

The blood glucose concentrations of individuals exposed to different salinities increased in the 12.5 g L^{-1} concentration. This increase was 2.6 times greater when compared to the control (Figure 4). There was a clear trend of increased glucose concentration as the salinity concentration increased. However, only in the 7.5, 10 and 12.5 g L^{-1} concentrations of was there a statistical difference in relation to the control.

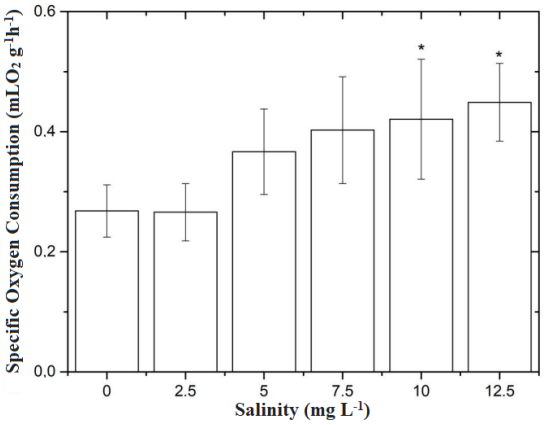

Figure 2. Specific oxygen consumption ($mLO_2\ g^{-1}h^{-1}$) in relation to different salinity concentrations. The bars are the respective standard deviations. *Indicates statistical difference in relation to the control group.

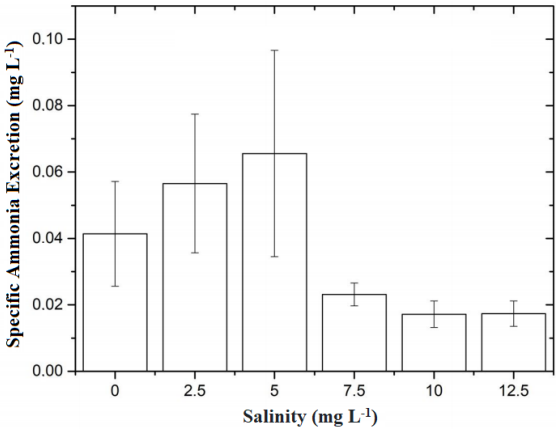

Figure 3. Specific ammonia excretion (mg L^{-1}) relative to different salinity concentrations. The bars are the respective standard deviations.

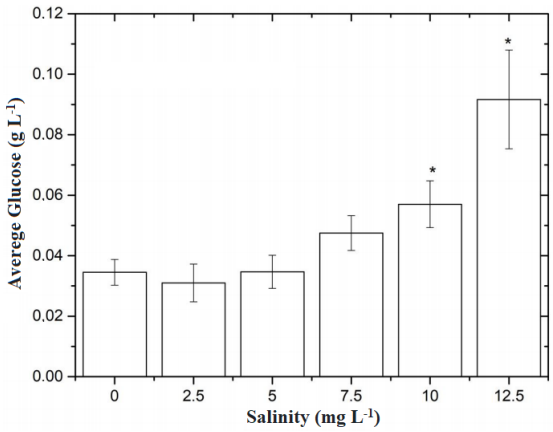

Figure 4. Blood glucose increase of *Deuterodon iguape* in relation to elevation of NaCl concentration (g L^{-1}). The vertical bars are the respective standard deviations. *Indicates statistical difference in relation to the control group.

The hemoglobin averages of individuals exposed to different salinities increased in a dose-dependent manner up to the 12.5 g L^{-1} concentration. This increase was twice as high when compared to the control (Figure 5). There was a clear trend of increasing oxygen consumption as the salinity concentration increased. However, there was a statistical difference in relation to the control only in 7.5, 10 and 12.5 g L^{-1} the concentrations.

Quantitatively analyzing the gill histology, it was observed that, for the analyzes performed, no statistical differences were seen between the groups (Table 1).

No significant statistical differences were also observed in the quantitative renal histological analysis. However, it is worth noting a trend of decreasing tubular lumen area of the groups exposed to salinities 10 g L^{-1} (29.85 ± 10.98 µm²) and 12.5 g L^{-1} (30.40 ± 10.16 µm²) when compared to the control group (49.28 ± 20.37 µm²) (Table 2).The control group presented, in the qualitative gill analysis, secondary lamellae formed by blood capillaries supported by pillar cells and covered by lamellar epithelial cells. (Figure 6A).

Exposure over a period of 1 hour to different salinities resulted in histological changes of the gills in *Deuterodon iguape*, as shown in Table 3 and Figure 6B-F.

It was observed that in the control group epithelial cell hyperplasia was not observed at the base of the secondary lamella, however, the exposure to different salinities presented an occurrence of 10, 33 and 40% for salinities 5, 10 and 12.5 g L^{-1}, respectively (Table 3). It is also worth noting that 13% of lamellar aneurysms occurred in the groups exposed to salinities 10 and 12.5 g L^{-1} (Table 3).

Applying HAI and AVA, the gills of the animals belonging to the control group and to salinity 5 g L^{-1} were classified as functionally normal organs with a grade 1 occurrence (without pathological alterations). The animals exposed to salinities 10 and 12.5 g L^{-1} were classified as organs with mild to moderate changes with a grade 1 occurrence (without pathological changes) (Table 3).

In relation to the qualitative renal histological analyzes, the animals of the control group and the 5 g L^{-1} salinity group, obtained a pattern of normality, represented in Figure 7A. Proximal and distal tubules surrounded by hematopoietic cells, glomeruli, and Bowman capsule were observed.

However, animals exposed to salinities of 10 and 12.5 g L^{-1} presented renal histological changes (Table 4 and Figure 7B-D).

It is worth noting the occurrence of 10 and 20% of tubular disorganization in the groups exposed to salinities of 10 and 12.5 g L^{-1}, respectively (Table 4).

The kidney HAI and AVA revealed that the animals belonging to the control group and the 5 g L^{-1} salinity group were classified as functionally normal organs with a grade 1 occurrence (without pathological changes). The animals exposed to salinities of 10 and 12.5 g L^{-1} were classified as organs with mild to moderate changes with a grade 1 incidence (without pathological alterations) (Table 4).

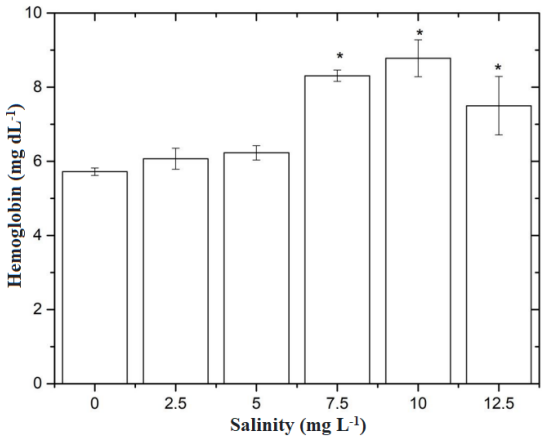

Figure 5. Elevation of *Deuterodon iguape* hemoglobin (mg dL^{-1}) in relation to increased NaCl concentration (g L^{-1}). The vertical bars are the respective standard deviations. *Indicates statistical difference in relation to the control group.

Table 1. Mean (± SD) of the quantitative histological analysis of the gills of *Deuterodon iguape* exposed to different salinities.

Quantitative Gill Analysis	Salinity (g L^{-1})			
	Control	5	10	12.5
Capillary width (µm)	6.33 (±1.17)	5.74 (±1.32)	6.69 (±1.75)	6.18 (±1.39)
Area of epithelial cells (µm²)	5.30 (±1.24)	6.32 (±1.40)	6.89 (±1.64)	7.30 (±1.52)
Mucus cell area (µm²)	30.90 (±7.16)	34.19 (±6.58)	37.13 (±9.78)	34.48 (±7.83)
Area of chloride cells (µm²)	40.88 (±7.28)	39.11 (±8.44)	37.80 (±6.68)	40.81 (±6.53)

Table 2. Mean (± SD) of the quantitative histological analysis of the *Deuterodon iguape* kidney exposed to different salinities.

Quantitative Kidney Analysis	Salinity (g L^{-1})			
	Control	5	10	12.5
Area of tubular cells (µm²)	8.81 (±1.37)	8.23 (±1.36)	7.89 (±1.48)	7.66 (±1.45)
Tubular lumen area (µm²)	49.28 (±20.37)	39.40 (±19.35)	29.85 (±10.98)	30.40 (±10.16)
Bowman Capsule width (µm)	2.18 (±0.76)	2.17 (±0.48)	2.07 (±0.51)	2.57 (±0.78)

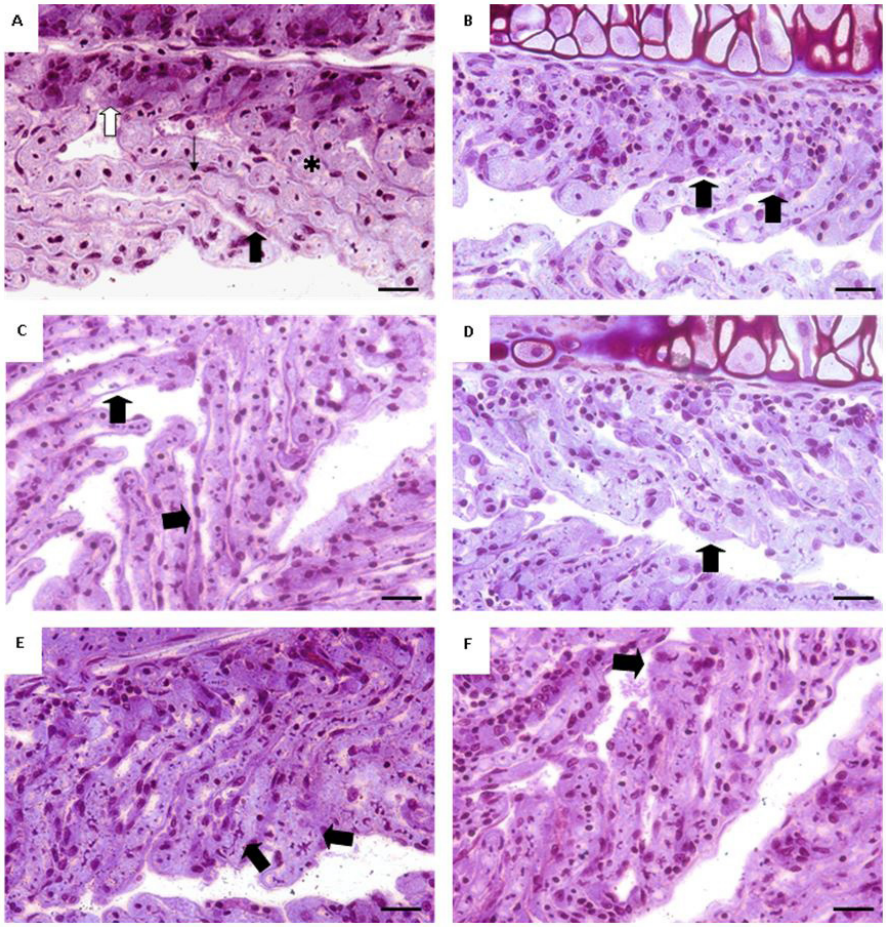

Figure 6. Photomicrograph of histological cut in resin of *Deuterodon iguape* gills. A. Control Group. The thin black arrow indicates a pillar cell, the thick black arrow indicates lamellar epithelial cells, the white arrow indicates epithelial cells at the base of the secondary lamella, and the asterisk indicates the blood capillary. B. Group exposed to a salinity of 5 g L^{-1}. Arrows indicate epithelial cell hyperplasia. C. Group exposed to a salinity of 5 g L^{-1}. The arrows indicate the displacement of lamellar epithelial cells. D. Group exposed to a salinity of 10 g L^{-1}. The arrow indicates the presence of chloride cells along the secondary lamella. E. Group exposed to a salinity of 10 g L^{-1}. The arrows indicate disorganization of the capillaries. F. Group exposed to a salinity of 12.5 g L^{-1}. The arrow indicates vascular congestion. Blue Toluidine/ Fuchsin Coloration. Scale 10 µm.

Table 3. Mean of the intensity of branchial changes, according to the Histological Alteration Index (HAI) and Average Value of Alteration (AVA) for *Deuterodon iguape* exposed to different salinities.

	Stage	Salinity (g L^{-1})			
		Control	5	10	12.5
Gill Histological Alterations					
Displacement of epithelial cells	I	0.13	0.07	0.10	0.33
Hyperplasia of epithelial cells at the base of the secondary lamella	I	0.00	0.10	0.33	0.40
Hyperplasia of epithelial cells along the secondary lamella	I	0.00	0.00	0.20	0.27
Presence of mucus cells in the secondary lamella	I	0.07	0.10	0.20	0.23
Presence of chloride cells in the secondary lamella	I	0.03	0.13	0.17	0.23
Breakdown of two capillaries	I	0.07	0.03	0.23	0.27
Vascular Congestion	I	0.00	0.00	0.00	0.03
Lamellar aneurysm	II	0.00	0.00	0.13	0.13
Gill HAI		**4**	**5**	**16**	**17**
Gill AVA		**0.04**	**0.05**	**0.17**	**0.24**

Table 3. Continued...

Legend			
HAI		**AVA**	
Values	**Classification**	**Values**	**Classification**
0-10	Organs with normal functionality	**0.00-0.33**	Grade 1 - Organs without pathological changes
11-20	Organs with mild to moderate changes	**0.34-0.66**	Grade 2 - Organs with occasional, mild and moderate pathological changes
21-50	Organs with moderate to severe changes		
51-100	Organs with severe changes	**0.67-1.00**	Grade 3 - organs with severe and extensive pathological alterations
> 100	Organs with irreversible damage		

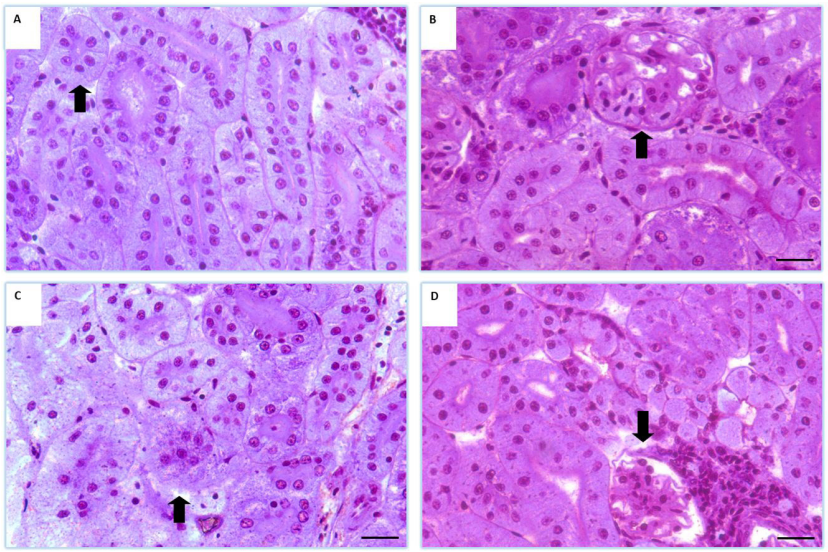

Figure 7. Photomicrograph of histological cut in resin of *Deuterodon iguape* kidney. A. Control Group. The arrows indicate renal tubules. B. Control Group. The arrow indicates glomerulus. C. Group exposed to salinity of 10 g L^{-1}. The arrow indicates tubular disorganization. D. Group exposed to salinity of 12.5 g L^{-1}. The arrow indicates glomerular disorganization. Blue Toluidine / Fuchsin Coloration. Scale 10 µm.

Table 4. Mean of the intensity of kidney changes, according to the Histological Alteration Index (HAI) and Average Value of Alteration (AVA) for *Deuterodon iguape* exposed to different salinities.

	Stage	**Salinity (g L^{-1})**			
		Control	**5**	**10**	**12.5**
Kidney Histological Alterations					
Tubular disorganization	I	0.00	0.00	0.10	0.20
Glomerular disorganization	I	0.00	0.00	0.07	0.03
Cytosplasmic degeneration of tubular cells	II	0.00	0.00	0.07	0.07
Kidney HAI		**0**	**0**	**12**	**12**
Kidney AVA		**0.00**	**0.00**	**0.08**	**0.10**

Legend			
HAI		**AVA**	
Values	**Classification**	**Values**	**Classification**
0-10	Organs with normal functionality	**0.00-0.33**	Grade 1 - Organs without pathological changes
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> 100	Organs with irreversible damage		

DISCUSSION

The specific oxygen consumption results showed that salinities 10 and 12.5 g L^{-1} alter the energy metabolism of *Deuterodon iguape*, increasing the demand for oxygen. This increase may be related to a compensatory response, not only caused by stress, but as a result of the decrease of oxygen solubility in salt water (Christensen et al., 1998, Barbieri et al., 2013). Studies show that after the decrease in the oxygen level caused by increased salinity, an increase in blood flow occurs, making metabolic respiration more effective, with a consequent increase in hemoglobin concentration (Pereira et al., 2016); which are pigments present in erythrocytes, responsible for the transportation of oxygen and carbon dioxide (Ranzani-Paiva et al., 2013).

The concentration of glucose and hemoglobin is one of the most used indicators to evaluate the stress of an animal (Bosisio et al., 2017). According to Barbieri et al. (2016), the stress in fish causes hematological variation followed by increased glucose and hemoglobin, a consequence of cortisol release leading to increased hepatic glycolysis. In the analyzes performed with lambari in this work, both glucose and hemoglobin presented significant elevations in relation to the control, when salinity increased.

The increase in salinity affected the total plasma protein concentration in lambaris only at the salinities of 5 and 12.5 g L^{-1} in relation to the control. According to Centeno et al. (2007), the alteration of the total plasma protein is a compensatory mechanism to direct tissue fluids to the blood vessels, thus reducing plasma viscosity. Studies by Tsuzuki et al. (2007), Centeno et al. (2007) and Bosisio et al. (2017) also reported changes in total plasma protein concentration in other fish species.

As a consequence of increased blood flow, changes in the capillaries may occur, as observed in the *Deuterodon iguape* gills of the groups exposed to the highest salinities. The occurrence of 23 and 27% of capillary breakdown in the groups exposed to salinities 10 and 12.5 g L^{-1}, respectively, and the occurrence of 13% of aneurysms in the 10 and 12.5 g L^{-1} groups were recorded.

Nero et al. (2006) correlate capillary breakdown with increased gas exchange due to vasoconstriction occurring towards the end of the secondary lamella to the base. Vasoconstriction would be controlled by the pillar cells by regulating the diameter of the intracellular gaps (Wilson and Laurent, 2002), thus increasing blood flow and consequently making gas exchange more efficient, resulting in increased oxygen consumption as observed in the present study. However, increased blood flow, according to Xiong et al. (2011), can cause pillar cell rupture leading to an accumulation of blood cells and the appearance of an aneurysm (occurrence of 13% in the groups exposed to salinities of 10 and 12.5 g L^{-1}). The regions with aneurysms can affect the gas exchange, however, there was no decrease in oxygen consumption in the present study and this may be related to the low occurrence of aneurysms.

In addition to increased blood flow, a higher rate of ventilation may occur simultaneously to increase water-gill contact, improving gas exchange (Boyd and Tucker, 1998) and increasing oxygen consumption. However, the more direct contact of saline water with the gills of *Deuterodon iguape* stimulated histological changes in order to maintain their osmoregulatory function.

According to Baldisserotto (2009), during the adaptation of euryhaline fish to seawater, it is possible to observe an increase in the number and size of chloride cells. In the present study, chloride cells were observed along the secondary lamella (hyperplasia), but no area increase (hypertrophy) was observed.

Studies have observed chloride cell hyperplasia after exposing the animals to different salinities (Carmona et al., 2004). Pereira et al. (2016) observed that in *Oreochromis niloticus* there is a direct relationship occurred between the number of cells (hyperplasia) and the increase in salinity levels (20 and 25 g L^{-1}). Chloride cells have the osmoregulatory function, in which, in freshwater animals, these cells have the function of absorbing salts, and in marine animals have the function of secreting excess salts (McCormick, 1995).

Uchida et al. (2000), evaluating the tolerance of *Oreochromis mossambicus* to different salinities, concluded that chloride cells are activated as the salinity increases. This conclusion was based on increasing the size and density of the chloride cell related to the higher Na^+/K^+-ATPase activity. Therefore, chloride cell hyperplasia observed in the present study may be related to increased Na^+/K^+-ATPase activity. Urbina et al. (2013), studied Na+, K+-ATPase activity in *Galaxias maculatus* showed a decreasing trend over the first 72 h following seawater exposure, but activity increased after 240 h. Their results indicate that *G. maculatus* is an excellent osmoregulator, an ability that is conferred by the rapid activation of physiological and molecular responses to salinity change. Unlike the *D. iguape* that proves not to be a good osmoregulador However, increased salinity stimulated mucosal cell hyperplasia (mucus-promoting cells), which, according to Perry and Laurent (1993), corresponds to a protection against lamellar damages, reducing the permeability of the gills to saline waters, as observed in the present study. Mucus is an important barrier in fish and possesses antibacterial peptides in addition to proteases like trypsin or cathepsin (Aranishi and Mano, 2000). Studies have shown that mucus production is significantly increased when fish are subjected to stress (Demers and Bayne, 1997; Rezende et al., 2018).

Moreover, all groups exposed to different salinities showed epithelial cell hyperplasia. Takashima and Hibya (1995) state that hyperplasia is caused by increased physiological activities. In addition, epithelial displacement was observed (33% occurrence in the group exposed to a salinity of 12.5 g L^{-1}), which is an alteration characterized by elevation of an epithelial lamina (Thophon et al., 2003). Hyperplasia and epithelial displacement increase the diffusion barrier to pollutants (Erkmen and Kolankaya, 2000), impairing the gas exchange process. This, according to Fernandes and Mazon (2003), increases the respiration rate of the fish, increasing the oxygen consumption (as observed in the present study), thus compensating for the low oxygen input.

The gills are also responsible for 85% of ammonia excretion (Wood and Part, 1997). In aqueous solution, it is normally balanced between the ionized (NH_4) and non-ionized (NH_3) forms (Boyce, 1999). The kidneys also have this function, but the elimination of nitrogen compounds by the urine is very much reduced (compared to the gills). In marine animals, this elimination is further reduced

because of low urine production to maintain water control (Wood and Part, 1997).

Ammonia is the main product of fish excretion (Westers, 2001; Damato and Barbieri, 2012), however, studies have shown that gill histological changes may lead to increased blood diffusion into water (Bombardeli et al., 2003), and may decrease the specific ammonia excretion as observed in the present study. In addition, Bombardeli et al. (2003) correlate the decrease in specific ammonia excretion with a decrease in protein metabolism, thus maintaining the fish's energy balance. The ammonia excretion of *D. iguape* decreases, Urbina and Glover (2015) studied another fish (*G. maculatus*) they conclued that decreases in ammonia excretion at salinities close to the isosmotic point, and variation in oxygen to nitrogen ratios did, however, suggest changes in fuel use.

With increasing salinity, the environment becomes hyperosmotic in relation to the fish, causing, according to Singer (2001) and Patterson et al. (2012), the loss of water from the body to water. The loss of water by osmosis decreases the filtration function of the kidney glomeruli. In the present study, it was observed that in the lambari groups exposed to salinities of 10 and 12.5 g L^{-1}, the possible decrease in glomerular functions resulted in glomerular disorganization.

Hyperosmotic adaptation also resulted, in the present study (10 and 20% occurrence in the groups exposed to salinities of 10 and 12.5 g L^{-1}, respectively), in disorganization of the renal collecting tubules and cytoplasmic degeneration of tubular cells (7% occurrence in the groups exposed to salinities of 10 and 12.5 g L^{-1}).

In freshwater fish, tubular reabsorption of salt occurs to compensate for loss of salts caused by the hypo osmotic environment (Freire et al., 2008), however, with increasing salinity, fish water intake occurs and, consequently, the accumulation of salts. In this way, fish do not need to reabsorb salts, decreasing tubular function.

Authors claim that for freshwater species to tolerate increased salinity two physiological adaptations are fundamental: (1) secrete salts by means of chloride cells; which may have occurred in *Deuterodon iguape* after an increase in the number of chloride cells, and (2) ensure the maintenance of body tissue hydration, reducing kidney filtration; also observed in the present study after glomerular disorganization (Wood and Part, 1997; Sakamoto et al., 2001; Freire et al., 2008).

Moreover, histological changes of gills and kidneys classified as mild to moderate, reinforce the hypothesis that *Deuterodon iguape* generated mechanisms of adaptation to the saline environment.

CONCLUSION

It is concluded that 1 hour of exposure to different salinities, changes the metabolism of *Deuterodon iguape*, characterized by increased oxygen consumption and decreased ammonia excretion.

Changes in hematological parameters (serum glucose, hemoglobin, and total protein) are also observed in groups exposed to different salinities.

Gill and kidney histological alterations were classified as mild to moderate, showing that Deuterodon iguape no adapted well to the saline environment, but for one hour can resist, which can make its use as live bait possible in estuarine sport fishing, preserving the natural stocks of Litopenaeus schimitti shrimp.

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