Salinity effects in metabolic rate and behavior in lambari *Astyanax bimaculatus*

Efeitos da salinidade no metabolismo de rotina e comportamento do lambari *Astyanax bimaculatus*

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**ABSTRACT**

Lambari (Characidae) is a small fish, common in Brazil and it has potential for aquaculture. It is used as live-bait in sport fishing in inland waters. The most commonly used live-bait in marine and estuarine sport fishing is the white shrimp (*Litopenaeus schmitti*), but the current demand caused by overfishing has depleted natural stocking. This study aimed to evaluate the lambari fish *Astyanax bimaculatus* resistance in different salinities so it might be considered an alternative live-bait in oligohaline waters. It was determined Lethal Concentration (LC50), routine metabolism and...
swimming behavior. To determine LC 50 (96h), 120 fish were divided in eight treatments: 0 (control), 5, 10, 15, 20, 25, 30 and 35 g L\(^{-1}\) of salinity. Afterwards, individuals were exposed in salinities of 0, 5 and 10 g L\(^{-1}\) (n=10) and analyzed specific oxygen consumption and ammonia excretion in 1 and 24 hours of exposure. Swimming behavior was tested in 90 fish, divided into three groups in the same salinities (0, 5 and 10 g L\(^{-1}\)), after 2 and 45 minutes of exposure. LC 50 result was 12.73 g L\(^{-1}\). Ammonia excretion \((p=0.8)\) and oxygen consumption \((p=0.09)\) did not show difference in 24 hours to all tested salinities. Ammonia excretion presented a difference in 1 hour treatment in salinity of 10 g L\(^{-1}\) comparing to control \((p=0.0003)\). It occurred with oxygen consumption also showing a difference in 1 hour of exposure. When comparing control with the other salinities, there was a statistical difference \((p = 0.02\) to 5 g L\(^{-1}\) and 0.006 to 10g L\(^{-1}\)). Lambari fish swimming acceleration decreased, showing a statistical difference in 45 minutes of exposure \((p <0.05)\) comparing to control. \textit{A. bimaculatus} showed resistance to salty waters (10 g L\(^{-1}\)) and it maybe considered as alternative live-bait for estuarine sport fishing.

**Keywords:** oxygen specific consumption, specific ammonia excretion, sport fishing, Characidae, live bait.

**RESUMO**
Lambaris (Characidae) são peixes de pequeno porte com potencial para a aquicultura que também podem ser utilizados como isca viva na pesca esportiva. A isca viva mais usada na pesca esportiva estuarina é o camarão branco (Litopenaeus schmitti), mas a atual alta demanda provocada pela sobre pesca vem acarretando a diminuição dos estoques naturais dessa espécie. O presente estudo teve como objetivo avaliar a resistência do lambari Astyanax bimaculatus para que no futuro possa ser considerado como alternativa de isca viva em águas oligohalinas. Foram determinados: Concentração Salinidade Letal 50 (CL50), metabolismo de rotina e comportamento natatório. Para a CL50, 120 peixes foram divididos em oito tratamentos: 0 (controle), 5, 10, 15, 20, 25, 30 e 35 g L\(^{-1}\). Posteriormente, os indivíduos foram expostos em salinidades de 0, 5 e 10 g L\(^{-1}\) (n=10) e foram medidos o consumo de oxigênio e excreção de amônia em 1 e 24 horas de exposição. O comportamento natatório foi testado com 90 lambaris nas salinidades (0, 5 e 10 g L\(^{-1}\)), após 2 e 45 minutos de exposição. O resultado de LC50 foi 12.73 g L\(^{-1}\). A excreção de amônia \((p=0.8)\) e o consumo de oxigênio \((p=0.09)\) não mostraram diferença em 24 horas em todas as salinidades testadas. Excreção de amônia mostrou diferença em 1 hora de exposição em salinidade 10 g L\(^{-1}\) comparado com controle \((p=0.0003)\). O mesmo ocorreu com o consumo de oxigênio em exposição de 1 hora. Comparando controle com outras salinidades, houve diferença significativa \((p = 0.02\) para 5 g L\(^{-1}\) e 0.006 para 10g L\(^{-1}\)). A aceleração dos lambaris decresceu mostrando uma diferença significativa em 45 minutos de exposição \((p < 0.05)\) comparando com controle. \textit{O. bimaculatus} mostrou resistência à água salinas de até (10 g L\(^{-1}\)) e pode ser considerado como isca viva alternativa para pesca esportiva estuarina.

**Palavras-chave:** consumo de oxigênio, excreção de amônia, pesca esportiva, Characidae, isca viva

**1 INTRODUCTION**
Lambaris are small-sized fish and belong to the Characiformes order, in the Characidae family. Genera Astyanax species, the most abundant in Brazil, presents high geographic distribution (Lucena & Soares, 2016; Valladão, Gallani, & Pilarski, 2016). Lambaris are studied in different approaches: reproduction, dispersion, phylogenetic relation, genetic patterns, feeding behavior,
bioindicators (Prado, Souza, Bazzoli & Rizzo, 2011; Casane & Rétaux, 2016; Barbieri, Rezende, Henriques & Carneiro, 2019; Tincani et al. 2019; Araújo, Nascimento, Gomes, Sales & Oliveira, 2019).

Lambari potential for fresh water fish farming was evaluated and considered promising; however, its cultivation as human food is not common due to high production and commercialization costs (Silva et al. 2011). Its consumption as a food product can be related to entertainment and cultural activities, where they are prepared as appetizers (Fonseca, Costa-Pierce & Valenti, 2017). The commercialization of live individuals for the fish market provides more profit when compared to the market for human consumption (Sabbag, Takahashi, Silveira & Aranha, 2011; Castilho-Barros, Barreto & Henriques, 2014a).

There are plenty other organism live baits in sport fishing (Henke & Chaves, 2017). The majority of these baits come from extraction caused by artisanal anglers as complementary income (Castilho-Barros, Alves, Silva & Henriques, 2014b). Sport anglers use Brazilian white shrimp *Litopenaeus schimitti* the most for marine and estuarine fishing (Henke & Chaves, 2017).

Castilho-Barros et al. (2014b) reported an increase in the demand for white shrimp from sport fishers in regions with great tourist activity like the Brazilian Southeast shore. In many cases, fishers keep these organisms in adapted structures and transport them in inappropriate ways, which causes stress, leads to high mortality rates and, consequently, leads to loss of money and time for artisanal fishers (Henriques et al. 2018).

In cases where there is a great availability and low demand of white shrimp for commercialization, a significant amount is lost, making this activity economically impracticable. Such fact contributes to juvenile natural stocking constant depletion in their raising habitats (Zeineddine, Barella, Rotundo, Clauzet & Ramires, 2015).

Sport fishing supply chain provides financial increment in the coastline region. Castilho-Barros et al. (2014b) states that the activity practiced in Santos bay, Brazilian Southeast shore, increases economy, when considering travel expenses, bait acquisition, fuel, housing and food, among others. Fish such as the snook fish (*Centropomus* sp.) and the blacktail basher fish (*Cynoscion por Macrodon* sp.) are pursued by fishers, mainly in estuarine areas (Castilho-Barros et al. 2014b; Barrella, Cachola, Ramires & Rotundo, 2016; Henke & Chaves 2017).

Fresh water species like tilapia (*Oreochromis niloticus*) had been tested as a possible live bait for marine and estuarine sport fishing for presenting great resilience to high salinities (Júnior, Vahrlich, Hoinkes & Tebaldi, 2010; Bosisio, Rezende & Barbieri, 2017). However, its
commercialization as bait is not suggested because it is a non-native species and could compromise the natural ecology of the habitat (Fonseca et al. 2017).

The hypothesis of this present study was that the yellow tail lambari *A. bimaculatus* presents resistance to survive in low salinities, being considered an alternative for live-bait sport fishing. Thus, the objective of this study was to evaluate the metabolic rate and swimming behavior of *A. bimaculatus* submitted in different salinities concentrations.

2 METODOLOGY

Lambari fish *A. bimaculatus* weigh 5.55 ± 1.9 g and have a size of 6.91 ± 0.75 cm. Fish were kept in 500L tanks in Mariculture Laboratory of Fishery Institute, in Santos, Brazilian Southeast shore (23°96’S, 46°33’W). The animals were fed with commercial fish food (31% protein) twice a day at 3% of live weight during the experiment.

**Determination of LC50 for *A. bimaculatus* in different salinities**

LC50 was calculated by using the statistic method Trimmed Spearman-Karber (Abbott correction), proposed by Hamilton *et al.* (1977) for 24, 48, 72 and 96 hours.

It was used 120 fish, divided into 24 units of 10L glass tanks, with three replicas. Eight different salinities were used during the experiment: 0 (control), 5, 10, 15, 20, 25, 30 and 35 g L⁻¹, distributed randomly with 5 lambaris each. Different salinities from seawater (35 g L⁻¹) were obtained and diluted with fresh water at the same place the individuals were kept, by using refractometer (RTS 28 – 03159 Instrutherm Instrumentos de Medicação Ltda).

Mortality was registered every 30 minutes in the first 12 hours. After this period, it was checked every 12 hours until 96 hours of experiment.

**Salinity effect in metabolic rate in lambari fish *A. bimaculatus***

From the results showed by LC50, metabolic rate was analysed by using salinities concentration that allowed the individuals survival over 24 hours of exposition: 0 (control), 5 and 10 g L⁻¹. Before the experiment, fish were acclimated during 24 hours in recirculation water recipients and constant airing, in a maximum density of 2.5 animals L⁻¹ (*American Public Health Association* – A.P.H.A., 1989). In the experiment it were used 27 individuals in 10 L glass tanks (duplicate), totalizing nine fishes per salinity.
Fish were maintained in recirculation water respirometers for 60 minutes so it could decrease the possible stress caused by handling. Afterwards, the water supply was suspended and all respirometers sealed so the individuals would consume the present oxygen in the water for an hour.

The oxygen and ammonia concentrations difference, determined by values calculated at the beginning and at the end of confinement, represented specific oxygen consumption (mLO$_2$ g$^{-1}$ h$^{-1}$) and specific ammonia excretion (mg L$^{-1}$ g$^{-1}$ h$^{-1}$).

To minimize the lack of oxygen effects over the metabolism, the experiment's duration was regulated in a way so that the experiment's final oxygen concentration was higher than 70% comparing to initial concentration (Barbieri et al 2019b). Dissolved oxygen was measured by using YSI proplus and Nessler ammonia determination method was applied for standard curve.

**A. bimaculatus swimming behavior**

A total of 90 lambaris with of 6.18 ± 0.59 cm length and 5.32 ± 1.51 g weight. Individuals were distributed in three different groups (n=10) exposed in salinities of 0 (control), 5 and 10 g L$^{-1}$ in 20 L glass fish tanks. This experiment was conducted according to Henriques et al. (2018). Fish movements were filmed frontally and laterally for 2 minutes at 2 and 45 minutes of exposition.

All filming was analysed by using Tracker software (Open Source). This program allows the analysis of an object movement frame to frame in a determined space and time in coordinates axes. The position on the Y-axis shows the depth that the animals were at in the aquarium. In addition, the displacement of the animals was calculated by means of the X and Y axes using the Pythagorean theorem: $d = (y_2 - y_1)^2 + (x_2 - x_1)^2$, where: ($d$) stands for displacement; ($y_2$) stands for the position of the fish on the y axis in the second frame; ($y_1$) stands for the position of the fish on the y-axis in the first frame; ($x_2$) stands for the position of the fish on the x-axis in the second frame; and ($x_1$) stands for the position of the fish on the x-axis in the first frame (Henriques et al 2018).

**Statistical analysis for lambari A. bimaculatus experiments**

For routine metabolism analysis (specific ammonia excretion and oxygen consumption) and swimming behavior, normality test Shapiro – Wilkand Leven’s test for homogenity of Variance were applied. Later, variance was analyzed by using One-way ANOVA and posttest Tukey HSD. We considered difference when p<005. PAST software was used to conduct analyses.
3 RESULTS

LC50 (96h) result for *A. bimaculatus* was 12.73 g L⁻¹. It was observed 10% mortality in salinity of 10g L⁻¹. LC90 (96h), which means when 90% of lambari could not survive, was 16.82 g L⁻¹ (Figure 1).

Figure 1. Lethal Concentration (LC) levels in different salinities for lambari *Astyanax bimaculatus* in 96 hours of exposure.

The exposed lambaris in salinities of 20, 25, 30 and 35 g L⁻¹ did not survive the first two hours of experiment. Individuals submitted to salinity of 15 g L⁻¹ showed mortality at the first hour, with records of 2 hours exposure (Figure 2).

Figure 2. Mortality percentage of lambari *Astyanax bimaculatus* in the first 2 hours of experiment in salinities of 15, 20, 25, 30 and 35g L⁻¹.
Specific oxygen consumption and specific ammonia excretion

There was a tendency in specific oxygen consumption decrease in relation to exposure time, with statistical difference in salinities of 5 and 10 g L^{-1} in 1 hour of exposure ($p = 0.02$ e $0.006$, respectively).

There was an oxygen consumption tendency to increase in these two salinities by comparing to control after 24 hours testing. There was no difference in any of 24-hour experiments ($p = 0.09$) when compared to control (Figure 3).

Figure 3. *Astyanax bimaculatus* specific oxygen consumption (mLO$_2$ g$^{-1}$ h$^{-1}$) at 1 and 24 hours in salinities of control (0), 5 and 10 g L$^{-1}$. ($p<0.05$).

Specific ammonia excretion decreased in control treatment between both exposure times. At 1 hour exposure, 10 g L$^{-1}$ treatment showed difference compared to control (0 g L$^{-1}$) ($p = 0.0003$). There was no difference between control and 5 g L$^{-1}$ in 1 hour of exposure ($p = 0.8$) and neither treatments at 24 hours exposure ($p = 0.13$) (Figure 4).
Figure 4. *Astyanax bimaculatus* specific ammonia excretion (mg g⁻¹ h⁻¹) during 1 and 24 hours in salinities of control (0), 5 and 10 g L⁻¹. (*p*<0.05).

Swimming behavior

Lambari acceleration was found at 8.28 (± 1.4) and 7.35 (± 1.71) cm s⁻² for control, 7.36 (± 1.3) and 5.61 (± 0.39) cm s⁻² for 5 g L⁻¹ and 7.49 (± 1.9) cm s⁻² and 5.04 (± 0.8) cm s⁻² for 10 g L⁻¹ in 2 and 45 minutes of exposure, respectively. Lambari fish exposed to control treatment did not show statistical difference in either exposure times (*p* = 0.07). However, they showed statistical difference when tested in salinities of 5 and 10 g L⁻¹ at 45 minutes of exposure (*p* < 0.05) (Picture 5).

Figure 5. *Astyanax bimaculatus* acceleration when exposed to control (0), 5 and 10 g L⁻¹ salinities at 2 and 45 minutes of exposure (*p*<0.05).

4 DISCUSSION

Fresh water fish resistance to acute exposure in salinity concentrations is related to specific species factor (Moreira, Ferreira, Zuanon & Salaro, 2011; Barbieri & Doi, 2012; Barbieri et al, 2019a; Bosisio et al, 2017; Mattioli et al, 2017). Yellow tail lambari *A. bimaculatus* showed tolerance in
salinity levels lower than 10 g L$^{-1}$. Comparing to other fish species, Moreira et al. (2011) found lethal salinity of 11.11 g L$^{-1}$ for the species *Pterophyllum scalare*, determining safe concentration at 7.5 g L$^{-1}$ for this species. Mattioli et al. (2017) studied the species *Lophiosilurus alexandri* and obtained LC50 of 11.6 g L$^{-1}$ for 24 hours of exposure and total mortality between 12.5 and 15 g L$^{-1}$. According to Imanpoor, Najafi e Kabir (2012), goldfish *Carassius auratus* shows good adaptation up until 12 g L$^{-1}$ of salinity, keeping specific growth rate, final biomass and feeding conversion rate at similar levels to fresh water, its natural habitat, similar behavior showed by *A. bimaculatus*.

Changes in the environment can alter metabolism in aquatic organisms (Uliano et al. 2010). Water salinity increase for fresh water fishes proposes to decrease osmoregulation energetic spend once it decreases the gradient between the individual and external environment (Souza-Bastos et al. 2016).

Salinity addition may influence body behavior, transport stress, fish metabolism, but there is not enough information aiming live baits (Plaut, 2000; Guo, Yan, Ma, Xiao & Zhang, 2013; Souza-Bastos, Bastos, Carneiro & Freire, 2016). Henriques et al. (2018) claim some fishers already use lambari species in estuarine sport fishing, however, there are no studies detailing resistance to low salinity concentrations for these species yet.

Environmental factors may also influence ammonia excretion, which can be toxic when accumulated in the fish circulatory system (Damato & Barbieri, 2012). Ammonia is a product derived from protein digestion and its decrease is related to protein catabolism reduction, which causes high-energy costs aiming energetic balance in the fish (Diniz & Honorato, 2012; Wright & Wood, 2012). This fact explains ammonia excretion decreases between both exposures tested times.

Barbieri et al. (2019c) exposed rain forest lambari *Deuterodon iguape* to salinities of 0; 2.5; 5; 7.5; 10 and 12.5 g L$^{-1}$. This species showed an increase in ammonia excretion until 5 g L$^{-1}$ and decrease from 7.5 g L$^{-1}$. In this present study, ammonia excretion for *A. bimaculatus* decreased in all treatments, but in salinity of 10 g L$^{-1}$ it was four times higher in 1 hour comparing to results with *D. iguape* (Barbieri et al., 2019b). This reduction may occur when the fish is found in isosmotic point in contrast with the environment. Lambari *D. iguape* increased its excretion faster in a lower salinity. Lambari *A. bimaculatus*, on the contrary, increased its metabolic rate at the highest tested salinity (10 g L$^{-1}$) and decreased its excretion in 24 hours, a result similar to lambari *D. iguape* at the same concentration.

Oxygen consumption in lambari *A. bimaculatus* also decreased along exposure time. Salinity increase tends to reduce dissolved oxygen availability in the water and raise demand for oxygen (Barbieri et al., 2019a). This consumption tends to increase when fish are in conditions of stress as a
way of maintaining homeostasis (Gutierre, Vitule, Freire & Prodocimo, 2014). The same was not noticed in our results. The only treatment in which the oxygen consumption increased along exposure time was at 10 g L$^{-1}$ of salinity with a difference of 0.031 mLO$_2$ g h$^{-1}$, still not showing difference comparing to remaining treatments. Altinok e Grizzle (2003), observed the same oxygen consumption reduction in juvenile rainbow trouts (*Onchorynchus mykiss*) (6.3 ± 1g) noting fish tended to keep low metabolic rate when found in salty waters avoiding energetic expenditure and this rate tends to raise when there is an increase in salinity (Zhao et al 2015; Sampaio & Freire 2016).

Behavioral tests are well accepted to corroborate toxicity for routine metabolism tests (Armstrong et al., 2019). Swimming activity in fish reflects a series of predictable physiological and biochemical responses of organisms related to environment. Some response changes may occur when there are alterations in water quality, which changes locomotion, reaction to predators, breathing and feeding (Faimali et al., 2016). This behavior may rise or lower energetic costs and, consequently, its metabolism (Uliano et al., 2010).

Henriques et al. (2018) tested *D. iguape* behavior, evaluating its dislocation (cm) and speed (cm s$^{-1}$) at speeds of 0, 5, 10 and 15 g L$^{-1}$, observing speed increase, but they found difference only in salinity of 10 g L$^{-1}$ comparing to control. Lambari *A. bimaculatus* tested in this study had their movement speed significantly reduced at 45 minutes for salinities of 5 and 10 g L$^{-1}$ comparing to control. These results corroborated with the result obtained by Hassan, Mi, Wahab, Muhammad, Idris e Jasmani (2013) for tilapia species (*Oreochromis* sp.) which showed osmorregulatory capacity in salinities of 0 and 5 g L$^{-1}$ keeping its swimming activity stable.

The authors affirm this phenomenon occurs when the individual is capable of keeping its body fluids without losing water to the high saline environment and, when this kind of abrupt change occurs, there are possible cases of convulsion and hyperactivity.

Henriques et al. (2018) show the importance of using endemic species aiming the local fauna conservation and avoiding ecological imbalance. In the same study, the authors compare fishing efficiency by using *D. iguape* and Brazilian native white shrimp *Litopenaeus schmitti*, both used as live bait in sea bass fishing (*Centropomus* sp.) in estuarine areas in Brazilian Southeast shore. In their results, it was reported that there was interest from anglers about the use of lambari as an economic alternative comparing to shrimp and demonstrated there was no difference when both were used as live baits in sea bass fishing. They found lambari resistance at 10 g L$^{-1}$ of salinity of sea bass fishing by using lambari as live bait comparing to shrimp.

The result found in this study showed *A. bimaculatus* maintains reduced metabolism showing resistance to salty waters. This fact may catch the interest of small lambari producers and artisanal
fishers in shore regions who may see this production as income supplement. The cultivation of endemic species in Brazilian water bodies, which is the case of *A. bimaculatus*, fulfills a market niche not yet well explored in sport fishing which the live bait is provide (Fonseca et al., 2017).

The results obtained from LC50, routine metabolism and swimming behavior confirmed this work's hypothesis, indicating *A. bimaculatus* shows resistance to salty waters lower than 10 g L$^{-1}$ of salinity, and consequently, it has potential to be used as live bait in oligohalyne environments.

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This study is in agreement with ethical principles in animal experimentation taken by Animal Experimentation Brazilian College (COBEA) and it has authorization nº 02/2018 from the Ethic Committee in Fishery Institute Animal Experimentation, agency of Agriculture and Supply Agency of São Paulo State.
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