# Physiological responses of jundiá *Rhamdia quelen* juveniles anesthetized with different concentrations of lidocaine hydrochloride (Lidostesim® 3%)

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**Abstract**

The aim of this trial was to determine the effect of lidocaine hydrochloride-based immersion anesthesia in concentrations of 1,000, 1,500, 2,000 and 2,500 mg $L^{-1}$ on physiological responses of jundiá *Rhamdia quelen*, with the control group anesthetized with clove oil (50 mg $L^{-1}$) bath anesthesia. Ten jundiá juveniles were used in each experimental and control groups in order to analyze the hematocrit, blood glucose levels and induction and recovery times. Jundiás anesthetized in 2,000 and 2,500 mg $L^{-1}$ solutions showed percentual hematocrit increase (42.7 ± 0.9% and 43.7 ± 0.7 %, $P < 0.05$). It was observed inverse association between blood glucose levels and lidocaine concentration, with values ranging from 57.3 ± 14.7 mg $dL^{-1}$ to jundiá anesthetized in 1,000 mg $L^{-1}$ and 39.5 ± 12.5 mg $dL^{-1}$ in 2,500 mg $L^{-1}$ anesthetic baths, with higher values detected in the control group (78.1 ± 12.5 mg $dL^{-1}$, $P < 0.001$). Likewise, it was observed inverse relationship between lidocaine hydrochloride concentration and induction times, but direct association with recovery times. Higher concentrations were the most effective as blood glucose reduction and maintenance of hematocrit levels, with promising induction and recovery times for future anesthetic trials.

**Keywords:** local anesthetic, glucose level, recovery time, Pimelodidae, stress.

# Respostas fisiológicas de juvenis de jundiás *Rhamdia quelen* anestesiados com diferentes concentrações de hidrocloreto de lidocaína (Lidostesim® 3%)

**Resumo**

O objetivo deste estudo foi determinar o efeito da anestesia por imersão à base de cloridrato de lidocaína em soluções de 1.000, 1.500, 2.000 e 2.500 mg $L^{-1}$ nas respostas fisiológicas de jundiá *Rhamdia quelen*, sendo o grupo controle anestesiado com óleo de cravo (50 mg $L^{-1}$). Foram utilizados dez juvenis de jundiá em cada grupo experimental e controle para análise do hematócrito, glicemia e tempos de indução e recuperação. Jundiás anestesiados com 2.000 e 2.500 mg $L^{-1}$ apresentaram aumento percentual do hematócrito (42,7 ± 0,9% e 43,7 ± 0,7%, $P < 0,05$). Foi observada associação inversa entre os níveis de glicemia e a concentração de lidocaína, com valores variando de 57,3 ± 14,7 mg $dL^{-1}$ para jundiá anestesiado a 1000 mg $L^{-1}$ e 39,5 ± 12,5 mg $dL^{-1}$ a 2500 mg $L^{-1}$, sendo detectados maiores valores no grupo controle (78,1 ± 12,5 mg $dL^{-1}$, $P < 0,001$). Da mesma forma, foi observada relação inversa entre concentração de lidocaína e tempos de indução, mas associação direta com tempos de recuperação. As concentrações mais altas foram as mais eficazes uma vez que reduziu a glicemia e manteve níveis do hematócrito, com tempos relativos de indução e recuperação promissores para futuros testes anestésicos.

**Palavras-chave:** anestésico local, glicemia, tempo de recuperação, Pimelodidae, estresse.

## 1. Introduction

The jundiá *Rhamdia quelen* (Teleostei, Pimelodidae) is a siluriform fish (catfish) found from central Argentina to southern Mexico (Gomes et al., 2000). This species inhabits lentic waters, mainly benthic areas on the banks of rivers. Therefore, they can be found in colors ranging from reddish brown to slate gray, facilitating camouflage and night feeding (Gomes et al., 2000).

Jundiá breeding has economic advantages once *R. quelen* is a stenohaline and eurithermic species, enduring up to 9 g $L^{-1}$ of common salt (NaCl) and temperatures ranging from 15 to 34 ºC, respectively (Gomes et al., 2000). Jundiás are adapted to subtropical and temperate climates (Montanha et al., 2011), being most exploited in southern Brazil, Uruguay and Argentina. It is reported a direct relationship between water temperature and fish growing (Gomes et al., 2000). Indeed, feeding frequency (Carneiro; Miklos, 2005) and rearing systems do not increase weight gain in jundiás juveniles (Iunes et al., 2023).

Fish health may be evaluated using blood laboratorial analysis, showing blood cells counting and their tinctorial characteristics, hematocrit levels and hematimetric factors evaluation (Tavares-Dias et al., 2002). To reduce stress and its consequences during blood collection, anesthetic solutions based on eugenol, menthol, benzocaine, tricaine, quinaldine and phenoxyethanol are emploied (Sneddon, 2012). Among local anesthetics often used in mammals, lidocaine hydrochloride is an inexpensive drug used in fish immersion anesthesia and analgesia protocols (Martins et al., 2019), however its using is poorly studied (Ross; Ross, 1999).

Thus, the aim of this trial was to determine the effect of different concentrations of lidocaine hydrochloride-based anesthetic solutions on the blood glucose and hematocrit levels, in addition to the induction and recovery times of jundiá *Rhamdia quelen* juveniles.

## 2. Materials and Methods

### *2.1 Acquisition of fish, acclimatization and feeding*

Fifty jundiá *Rhamdia quelen* juveniles were purchased from Fish and Aquaculture Laboratory of the Federal University of Pampa (UNIPAMPA), Dom Pedrito, Brazil with an average weight of 90.76 ± 4.53 g and kept at the Aquatic Animals facility of the Institute of Biomedical Sciences, University of São Paulo (ICB/USP), São Paulo, Brazil. One-week acclimation took place in 50-liter boxes connected to a closed thermoregulated recirculation system. Fish were fed twice a day at a feeding rate of 3% of live weight with a diet containing 32% crude protein and 3,100 Kcal gross energy.

### *2.2 Analysis*

To evaluate water quality, the following physical-chemical parameters were analyzed: temperature (aquarium thermometer), dissolved oxygen (digital oximeter), pH (bench digital pH meter), electrical conductivity (bench digital conductivity meter), salinity (refractometer) and ammonia, nitrite and alkalinity analyzed twice during the week, using commercial colorimetric kits (Alfakit®, Brazil).

Anesthetic solutions with lidocaine hydrochloride in association with norepinephrine hemitartrate (Lidostesim® 3%, Dentsply Pharmaceutical, Brazil) in concentrations of 1,000, 1,500, 2,000 and 2,500 mg $L^{-1}$ were prepared. Ten jundiá juveniles were exposed to each of the anesthetic concentrations individually in plastic boxes until the stage of deep anesthesia, with total loss of muscle tonus and equilibrium with slow but regular opercular beat (Ross; Ross, 1999). The jundiás in the control group were subjected to an anesthetic bath based on clove oil at 50 mg $L^{-1}$ (Diemer et al., 2012). Anesthetic induction and recovery times were recorded (in seconds) and, after reaching the anesthetic stage, the jundiás were transferred to recovery boxes containing only water. After recovery, the fish were subjected to blood collection by puncture of the dorsal caudal vessels using a heparinized hypodermic needle (1.2 x 0.5 mm) attached to 3 ml syringe to determine blood glucose (mg $dL^{-1}$) using a portable glucose monitor (Accu-Chek®, Roche, Germany) (Crosby et al., 2010) and hematocrit analysis (in percentage) using the microhematocrit technique (Borges et al., 2004).

### *2.3 Statistical analysis*

The experimental design was completely randomized with four treatments and one control, with ten replications each. Data were subjected to analysis of variance and *F-test* with 95% confidence interval. The means were compared by ANOVA and *Tukey's post-hoc test* and the results were subjected to *Pearson's correlation analysis*

(SAS, 2001); subsequently linear regressions were performed using the statistical package GraphPad Prism 6.01 (GraphPad Software, 2012).

## 3. Results

### *3.1 Biological parameters*

Limnological parameters during this trial were: dissolved oxygen: 5.65 ± 0.12 mg $L^{-1}$; temperature: 23.76 ± 0.19 °C; pH: 7.58 ± 0.06; conductivity: 5.32 ± 0.79 mS $Cma^{-1}$ at 25 °C; salinity: 6.58 ± 0.71‰; alkalinity: 56.66 ± 5.16 mg $L^{-1}$ $CaCO_3$; total ammonia: 0.05 ± 0.05 mg $L^{-1}$; nitrite: 0.02 ± 0.008 mg $L^{-1}$. Those parameters are within the ranges considered ideal for jundiás (Conrado et al., 2019).

### *3.2 Blood parameters*

The hematocrit of jundiá *Rhamdia quelen* juveniles anesthetized with lidocaine hydrochloride at concentrations of 1,000 and 1,500 mg/L showed no difference when compared to the control group (41.3 ± 0.5% and 41.5 ± 0.5% vs. 40.5 ± 0.8%, respectively; $P > 0.05$), but with increasing values in fishes anesthetized in solutions of 2,000 and 2,500 mg $L^{-1}$ (42.7 ± 0.9% and 43.7 ± 0.7% vs. 40.5 ± 0.8%; $P < 0.001$) (Figure 1a). An inversely proportional relationship was observed between blood glucose levels and lidocaine hydrochloride concentrations, with values varying between 57.3 ± 14.7 mg $dL^{-1}$ for jundiás anesthetized with 1,000 mg $L^{-1}$ and 39.5 ± 12.5 mg $dL^{-1}$ at 2,500 mg $L^{-1}$ ($P < 0.001$), resulting in negative correlation (Figure 1b). The control group recorded the highest values (78.1 ± 12.5 mg $dL^{-1}$) when compared to the experimental groups.

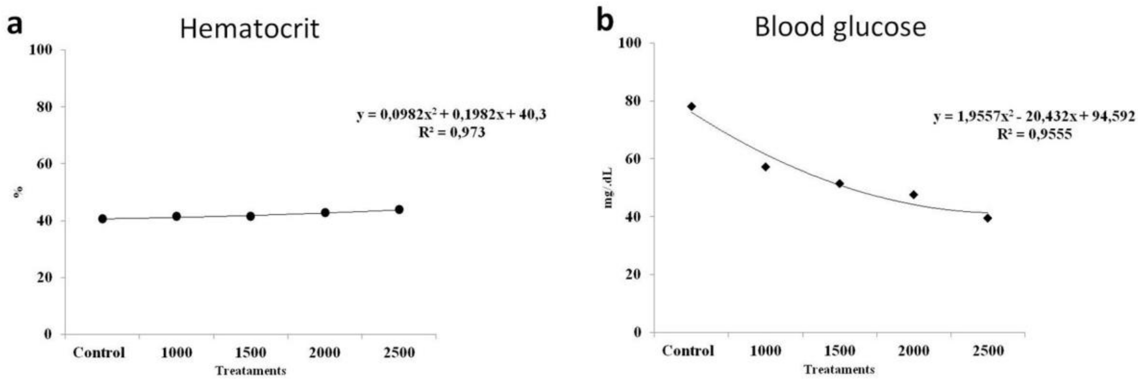


Figure 1. Polynomial regression for hematocrit (a), with positive correlation, and polynomial regression for blood glucose (b), with negative correlation, for jundiá *Rhamdia quelen* juveniles anesthetized with different concentrations of lidocaine hydrochloride associated with norepinephrine hemitartrate (Lidostesim® 3%). Source: Authors, 2023.

### *3.3 Induction and recovery times of jundiás anesthetized*

When determining the induction and recovery times of jundiás anesthetized with lidocaine, an inversely proportional behavior was observed between the concentration and induction time and directly proportional in relation to the time required for recovery (Table 1).

Table 1. Induction and recovery times of jundiá *Rhamdia quelen* juveniles anesthetized with different concentrations of lidocaine hydrochloride in association with norepinephrine hemitartrate (Lidostesim® 3%).

| Lidocaine (mg/L) | Induction (sec)* | Recovery (sec)* |
|---|---|---|
| 1,000 | 462.8 ± 40.9[a] | 49.2 ± 9.2[a] |
| 1,500 | 360.7 ± 46.3[b] | 59.0 ± 10.7[a] |
| 2,000 | 343.5 ± 46.1[b] | 60.7 ± 10.0[a] |
| 2,500 | 276.9 ± 46.9[c] | 79.0 ± 5.4[b] |

Note: In columns, different letters indicate statistical difference ($P < 0.05$) between the times recorded for induction and recovery. ANOVA with *Tukey's* post-hoc test. *Data shown as mean ± standard deviation. Source: Authors, 2023.

**4. Discussion**

Here we reported the low variation in hematocrit values and inverse correlation between blood glucose levels and lidocaine hydrochloride concentrations, in addition to induction and recovery times for jundiá *Rhamdia quelen* juveniles. Hematological analyses in juvenile and adult jundiás showed hematocrit interval ranging between 37% and 51% (Borges et al., 2014; do Nascimento et al., 2016), and blood glucose levels between 43 and 78 mg $dL^{-1}$ (Borges et al., 2014). As high blood glucose levels and hematocrit are factors that indirectly demonstrate the cortisol releasing consequences (Wendelaar-Bonga, 1997), there are conflicting literature data after using lidocaine hydrochloride for fish anesthesia.

No changes were reported in hematocrit or plasma glucose values in kingyos *Carassius auratus auratus* (Chen, 2012) and *Hexagrammos otakii* greenlings anesthesia (Park et al., 2015), with elevated glucose levels up to 24 hours post-anesthesia in *Takifugu niphobles* grass puffer (Gil et al., 2017). Thus, the reduction in blood glucose levels combined with the small variation in hematocrit can be considered positive when compared to the use of clove oil as seen in this trial. Nevertheless, jundiá juveniles anesthetized with other essential oils-based solutions showed similar hematocrit and blood glucose increasing levels as seen here (Gressler et al., 2014; Silva et al., 2013, 2015; Souza et al., 2017; Toni et al., 2014). Clove oil is made up of between 70-90% by weight of eugenol (Ross; Ross, 1999), used in bath anesthesia in concentrations between 20 and 50 mg $L^{-1}$ for *R. quelen* (Cunha et al., 2010). This substance should be avoided in fish anesthesia as it does not promote depression of the central nervous system (Barbas et al., 2021).

In relation to induction and recovery times of jundiás anesthetized with lidocaine hydrochloride, similar results were reported by Carrasco et al. (1984) in the anesthesia of tilapia *Oreochromis mossambicus*, carp *Cyprinus carpio* and channel catfish *Ictalurus punctatus*, by Kim et al. (1988) for several species (Cyprinidae, Siluridae, Cichlidae and Salmonidae families) and by Park et al. (2015) in the anesthesia of greenlings *Hexagrammos otakii*. Regarding water temperature, it is known that fish metabolism is directly influenced by environmental temperature. The relationship between anesthetic bath concentration and water temperature reveals that the increasing in water temperature reduces the induction time but, on the other hand, there is the increasing of recovery time in fishes undergoing lidocaine-based anesthesia (Kim et al., 1988; Park et al., 2015). In the present trial, water temperature was maintained at around 23-24 ºC, this being a fixed factor that directly influenced the results of the catfish induction and recovery times. Future trials are needed to establish the ideal concentration for jundiá juvenile anesthesia under a wide range of water temperature.

Some intrinsic factors may have influenced the results, the main ones being the concentration of lidocaine hydrochloride and its association with other substances. Carrasco et al. (1984), Kim et al. (1988) and Park et al. (2015) indicated the use of low concentrations of lidocaine hydrochloride (up to 1,200 mg $L^{-1}$) associated with sodium bicarbonate ($NaHCO_3$). However, Abbas et al. (2006) and Collymore et al. (2014) indicated low concentrations of lidocaine hydrochloride, with concentration of up to 4,000 mg $L^{-1}$ in anesthesia of kingyos *Carassius auratus auratus* (Chen, 2012). In our case, lidocaine hydrochloride was used associated with norepinephrine, which may have influenced the results.

Lidocaine hydrochloride with or without association with norepinephrine is used as a local anesthetic in surgical interventions in livestock (Anderson; Edmondson, 2013) and is easily purchased in veterinary pharmacies and farming stores. Furthermore, lidocaine hydrochloride in association with norepinephrine has advantages due to its cardioprotective action (Hermanns et al., 2019), peripheral hypotension and hypoxia prevention and increased anesthetic effectiveness (Smith; Maani, 2019). Another positive factor is the short half-life of the lidocaine hydrochloride on the environment (Kostrubiak et al., 2020), which must be taken into account when disposing of anesthetic solutions in wastewater systems.

## 5. Conclusions

Higher lidocaine hydrochloride-based solutions concentrations were the most effective as blood glucose reduction and maintenance of hematocrit levels, with promising induction and recovery times for future anesthetic trials in jundiá *Rhamdia quelen* juveniles.

## 6. Authors' Contributions

*Iuri Moraes Neyrão*: conceptualization, methodology, data collection, writing – original draft, investigation, statistical analysis. *André Luiz Veiga Conrado*: conceptualization, methodology, data collection, translation, investigation, funding acquisition. *Paulo Rodinei Soares Lopes*: conceptualization, formal analysis, validation, writing – review and editing, supervision.

## 7. Conflicts of Interest

The authors declare no conflicts of interest.

## 8. Ethics Approval

The care and use of experimental animals complied with brazilian animal welfare laws, guidelines and policies as approved by Ethics Committee on the Use of Animals of the Instituto de Ciências Biomédicas da Universidade de São Paulo (ICB/USP), São Paulo, Brazil # 5420020819.

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Not applicable.

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