ECOLOGICAL, TOXICOLOGICAL AND LIMNOLOGICAL STUDIES USING TAMBAQUI EGGS (*Colossoma macropomum*)

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ABSTRACT

This study aimed to evaluate the incubation of tambaqui eggs as an ecological indicator in natural nurseries, in exposure to pesticides and as an indicator of water quality. Induction of tambaqui females and males was performed. After fertilization, the eggs were separated to carry out three trials: (i) incubation of eggs in waters of urban and natural environments, (ii) incubation in different concentrations of glyphosate, malathion, and casugamycin, considered pesticides, and (iii) with exposure of eggs incubated in different concentrations of total ammonia. In all trials, the design was completely randomized, with five replications. (i) The analysis of variance indicated that the hatching rate was higher in the water of the Solimões River, and the lowest were in the Paraíba and Buriti streams ($p<0.05$), considered polluted. (ii) Treatment with incubation water with 0.0 mg L-1 of glyphosate, malathion and kasumamycin showed the best fertilization, hatching and survival rates ($p<0.05$). (iii) The fertilization rate and hatching rate of tambaqui eggs were higher ($p<0.05$) in water containing between 0.0 and 3.5 mg L-1 of total ammonia. Larval survival was higher ($p<0.05$) in waters containing concentrations between 0.0 and 2.0 mg L-1 of total ammonia. The results indicated that the best natural water for incubating the eggs is from the Solimões River. It was verified that all pesticides showed toxicity in the incubation phase of

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tambaqui eggs. Finally, concentrations of up to 2.0 mg L-1 of total ammonia did not have implications in tambaqui eggs.

Keywords: Reproduction. Embryos. Hatching. Survival.

INTRODUCTION

The Amazon has the largest hydrographic basin in the world, with a vast and differentiated environment, with many biotopes available to aquatic communities (Goulding, 1996; Junk and Furch, 1985), providing a splendid ichthyofauna of more than 3,500 species (Lowe-McConnell, 1999; Reis et al., 2003). However, urban and agricultural expansion in the Amazon without any planning is strongly linked to the life of water resources (Waisbich et al., 2022). Therefore, the conservation of biodiversity in aquatic ecosystems will be one of the most important and difficult challenges to be faced in the coming years (Oliveira et al., 2019).

In a cultural, social and infrastructure maintenance process, the municipalities in the triple border region (Brazil, Colombia and Peru) have gradually lost the pristine characteristics of their water resources, either due to the misuse of the soil, disorderly urban expansion, the poor packaging of solid waste and the lack of treatment of agricultural effluents and domestic sewage, transforming into contaminated spaces (Costa et al., 2023; Oliveira et al., 2023).

Also, the production of vegetables has increased in the floodplain region, making it one of the main economic activities developed by the riverside dwellers during the ebb and drought. However, as these crops are not adapted to tropical conditions, susceptibility to attack by pests (insects, fungi and others) and competition with native vegetation has forced farmers to make intensive use of biocides (Waichman, 2007). The use of pesticides in crops located near water bodies and knowledge about the toxic effects produced by these compounds on non-target organisms are still scarce (Aguiar et al., 2019; Durante et al., 2024).

In addition, the region has several aquaculture facilities in a semi-intensive system in which there is a lack of technical guidance and many with inadequate management. (Mota et al., 2021). However, to ensure the success in the production of native fish species, it is of fundamental importance to know the toxicity and sensitivity of such species to water quality parameters, such as nitrogen residues, since these are limiting factors for the survival and growth of fish in confinement.

In this case, the tambaqui (*Colossoma macropomum*) was considered our biological test model due to its ability to reproduce it in captivity, being the main native species of Brazilian fish farming (PeixeBR, 2024) and one of the most important for subsistence fishing of riverside dwellers in the Amazon (Santos et al., 2009). Studies on the conservation of this species are fundamental both for fish farming and commercial and survival fishing.

Considering that floodplain areas are considered breeding areas and nursery for most Amazonian fish, studies evaluating natural nurseries, toxicity of the main pesticides used in the region and water quality as effluents on the reproduction of native fish species become extremely important. In this sense, this study aimed to evaluate the incubation of tambaqui eggs as an ecological indicator in natural nurseries, in exposure to pesticides and as a monitoring of water quality.

MATERIAL AND METHODS

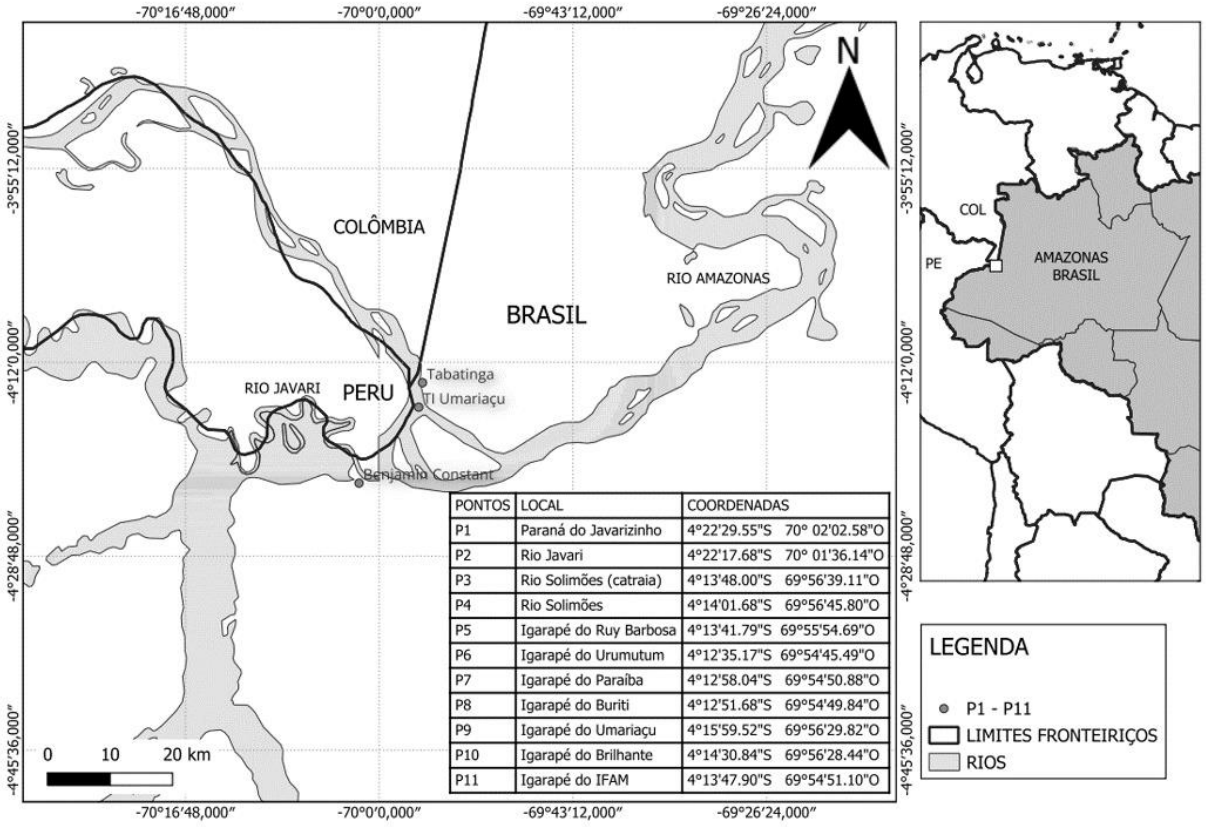
The laboratory processes, induction, gamete collection and fertilization were carried out in the Sector of Educational Production Units of the Federal Institute of Amazonas - IFAM *campus* Tabatinga (4°13'49.14"S and 69°54'50.44"W). Females (n=6, 7.16 ± 0.90 kg) and males (n=6, 6.30 ± 1.15 kg) of tambaqui (*Colossoma macropomum*) were selected when they presented reproductive characteristics of gonadal maturation (Lima et al., 2013; Matavelli et al., 2021). These fish were weighed, tagged, and separated by sex in individual tanks. Females and males were induced with carp pituitary extract (HEC) (Streit Jr et al., 2012). After 240 accumulated thermal units (AHU), gametes were collected (Oliveira et al., 2023). At this time, massage was performed in the ventral region of the animals in the cephalocaudal direction, thus collecting the oocytes in a clean and dry plastic container, where the semen was poured directly over the oocytes.

The mixture of oocytes with semen (fertilization) was hydrated with water from the egg incubation system. The fertilized eggs were kept in a gentle motion for one minute, with replacement of the water in the container to remove excess semen and incubated for one hour in fiberglass incubators with a capacity of 200 liters (Garcez et al., 2024).

TEST 1 – *ECOLOGICAL INDICATOR OF NATURAL NURSERIES OF NATIVE FISH*

Samples of 2 liters of water were collected at each established collection point (P1 - P12) in the municipalities of Tabatinga and Benjamin Constant, Amazonas. The method used to collect the water followed the standards of the American Public Health Association (APHA, 2005). During the collection, the hydrological cycle in the region was in the flood phase (Solimões River level = 9.47 m), being considered the rainy season and the reproduction period of rheophilic fishes.

Figure 1. Water collection points, geographic coordinates and location of the municipalities of Benjamin Constant and Tabatinga, Amazonas, Brazil. (Source: The authors, 2024)



PONTOS	LOCAL	COORDENADAS
P1	Paraná do Javarizinho	4°22'29.55"S 70° 02'02.58"O
P2	Rio Javari	4°22'17.68"S 70° 01'36.14"O
P3	Rio Solimões (catraia)	4°13'48.00"S 69°56'39.11"O
P4	Rio Solimões	4°14'01.68"S 69°56'45.80"O
P5	Igarapé do Ruy Barbosa	4°13'41.79"S 69°55'54.69"O
P6	Igarapé do Urumutum	4°12'35.17"S 69°54'45.49"O
P7	Igarapé do Paraíba	4°12'58.04"S 69°54'50.88"O
P8	Igarapé do Buriti	4°12'51.68"S 69°54'49.84"O
P9	Igarapé do Umariaçu	4°15'59.52"S 69°56'29.82"O
P10	Igarapé do Brilhante	4°14'30.84"S 69°56'28.44"O
P11	Igarapé do IFAM	4°13'47.90"S 69°54'51.10"O

The embryos were transferred to circular disposable plastic containers containing 50 ml of water. Each container with 10 embryos was considered as an experimental unit. The containers were kept without shaking, at room temperature (26.72 ± 0.87 °C) and controlled light (101 ± 58 luxes) throughout the experiment. In this trial, a completely randomized experimental design was used, consisting of twelve treatments and five replications. The treatments consisted of the use of water from different urban streams and the Solimões and Javari rivers.

After 12 hours after artificial fertilization, the fertilization rate (TF) was measured using the equation: TF = number of viable eggs x 100/total number of eggs. The hatching rate was measured 24 hours after artificial fertilization. To calculate the hatching rate (TE), the following equation was applied: TE = number of hatched larvae x 100/total number of eggs. Larval survival (SL) was measured 24 hours after larval hatching, using the following equation: SL = number of live larvae x 100/total number of larvae hatched.

TEST 2 – *PESTICIDE TOXICITY INDICATOR*

Three experiments were carried out: (i) concentrations of 0.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 14.0 mg $^{L-1}$ of glyphosate, (ii) concentrations of 0.0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 µg $^{L-1}$ of malathion, and (iii) concentrations of 0.0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg $^{L-1}$ of

casugamycin. The embryos were transferred to circular disposable plastic containers containing 50 ml of water from the experimental concentration. Each container containing 20 embryos was considered as an experimental unit. For the three trials, a completely randomized experimental design was used, where each agrochemical was composed of seven treatments that were the different concentrations tested, with five replications each. The containers during incubation were kept without shaking, at room temperature (28.72 ± 0.86 °C) and controlled light (151.5 ± 58 luxes) throughout the experiment.

After 12 hours after artificial fertilization, the fertilization rate (TF), hatching rate (TE) and larval survival (TSL) were measured 6 (six) hours after larval hatching.

TEST 3 – *WATER QUALITY INDICATOR*

Total ammonia was used as a parameter for water quality. The experimental concentrations of ammonia used in the experiment were 0.0; 0,25; 0,50; 1,0; 2.0, 3.5, 6.5 and 10 mg $^{L-1}$, based on the LabconTest® total ammonia reading scale. The embryos were transferred to circular disposable plastic containers containing 50 ml of water from the experimental concentration. Each container containing 20 embryos was considered as an experimental unit. In this trial, a completely randomized experimental design was used, consisting of eight treatments with the different concentrations tested, in five replications each. The containers during incubation were kept without agitation, at room temperature (28.52 ± 0.76 °C) and controlled light (161.5 ± 68 luxes, Luvimeter® Software) throughout the experiment.

After 12 hours after artificial fertilization, the fertilization rate (TF) and hatching rate (TE) were estimated. Larval survival (SLT) was measured at 12, 24 and 48 hours after larval hatching. The number of larvae that presented curvature in the spine and shortened trunks were counted after hatching to measure the larval normality rate (LN), using the following equation: NL = number of normal larvae x 100/total number of larvae hatched.

DATA ANALYSIS

In all trials, the results were expressed as mean ± standard error. The data in percentage of fertilization, hatching, normality and larval survival rates were transformed into *arcsine* $\sqrt{(x/100)}$. The assumptions of normality and homogeneity were verified by the Shapiro-Wilk and Levene tests. Subsequently, the data were submitted to Analysis of Variance (ANOVA) and Tukey's test of means for multiple comparisons. All statistical analyses were performed considering a significance of 5%. Data processing and analysis were performed using the Statistica 7.1® software (Statsoft Inc., Tulsa, OK, USA, 2007).

RESULTS AND DISCUSSION

ESSAY 1

The waters used during incubation showed different physicochemical variables. The pH ranged from 6.2 to 7.7, dissolved oxygen from 4 to 11 mg $^{L-1}$, total ammonia from 0.0 to 3.5 mg $^{L-1}$, only the Brilhante stream showed nitrite from 0.25 and the total hardness ranged from 50 to 400 ppm of CaCo3 (Table 1).

Table 1. Physicochemical variables of incubation water of tambaqui eggs (*C. macropomum*).

Collection points	ph	Dissolved oxygen (mg $^{L-1}$)	Ammonia (mg L^{-1})	Nitrite (mg L^{-1})	Total hardness (ppm CaCo3)
P1	6,6	4,0	1,00	0,00	50
P2	6,4	11,0	0,25	0,00	50
P3	6,6	8,0	0,25	0,00	50
P4	7,5	11,0	0,25	0,00	300-400
P5	7,2	6,0	3,50	0,00	150-300
P6	6,2	11,0	0,25	0,00	50
P7	6,6	6,0	0,50	0,00	50
P8	6,2	8,0	0,25	0,00	50
P9	6,2	8,0	0,00	0,00	50
P10	6,6	8,0	0,25	0,25	50-150
P11	6,6	11,0	0,00	0,00	150-300
P12	7,0	11,0	0,25	0,00	50

The hatching rate of tambaqui was influenced by the incubation water from the urban streams in relation to the Solimões River, as shown in table 2. The hatching rate was higher in the Solimões River ($p<0.05$). On the other hand, they were lower at the collection points of the IFAM Campus Tabatinga, Urumutum, Paraíba and Buriti streams ($p<0.05$). The water of the urban streams and the Solimões and Javari rivers did not influence the fertilization rate and the survival rate of the larvae ($p>0.05$).

Table 2. Reproductive rates of incubation of tambaqui (*C. macropomum*) eggs in waters of different urban streams and rivers in the municipalities of Benjamin Constant and Tabatinga, Amazonas.

Collection points	Identification	TF 12h (%)	AT 24h (%)	Under. 24h (%)
P1	Paraná do Javarizinho	74,0 ± 6,78	66,0 ± 2,45 ^{abc}	14,29 ± 6,39
P2	Rio Javari	74,0 ± 8,12	62,0 ± 4,90 ^{abc}	11,43 ± 5,35
P3	Rio Solimões (catraia)	86,0 ± 6,00	66,0 ± 5,10 ^{abc}	11,07 ± 5,31
P4	Rio Solimões	84,0 ± 7,48	72,0 ± 3,74 ^{a}	27,26 ± 5,63
P5	Igarapé do Ruy Barbosa	76,0 ± 5,10	68,0 ± 4,90 ^{ab}	15,00 ± 1,02
P6	Igarapé do Urumutum	68,0 ± 5,83	52,0 ± 5,83 ^{bc}	11,19 ± 4,90
P7	Igarapé do Paraíba	78,0 ± 7,35	50,0 ± 4,47 ^{bc}	16,67 ± 4,56
P8	Igarapé do Buriti	80,0 ± 8,37	44,0 ± 5,10 ^{c}	25,67 ± 4,77
P9	Igarapé do Umariaçu	74,0 ± 7,48	68,0 ± 6,63 ^{ab}	16,35 ± 3,37
P10	Igarapé do Brilhante	86,0 ± 5,83	68,0 ± 4,00 ^{abc}	14,19 ± 7,33
P11	IFAM Creek	78,0 ± 3,74	56,0 ± 4,90 ^{bc}	18,00 ± 5,33
P12	IFAM Incubators	80,0 ±3,16	64,0 ± 4,00 ^{abc}	30,67 ± 3,75

	p-value	0,8201	0,0070	0,1788
Values are expressed as mean ± standard error. TF: Fertilization rate; TE: Hatching rate; Under: Survival of larvae after 24 hatching. Different letters indicate differences in the means due to the treatments, according to Tukey's multiple comparison test ($p < 0.05$).				

As for the streams that are part of the sub-basin of the Urumutum stream, which is the case of the Urumutum, Paraíba and Buriti streams, they showed low rates in the hatching rate. This can be explained by the proximity of 1km to the landfill that leaches its water into the streams. According to Bezarra and Souza (2021), the Urumutum basin is useful for transporting goods, for fishing for fish, for bathing and recreation, and the use of water for domestic supply. However, the waters of the Paraíba and Buriti streams in recent years have been undergoing a growing process of environmental degradation. Including possible disappearances of ornamental fish species that previously inhabited there.

ESSAY 2

Increasing concentrations of glyphosate reduced the rates of fertilization, hatching, and survival of tambaqui larvae (Table 3). Concentrations higher than 0 mg $^{L-1}$ negatively affected the fertilization rate and hatching rate ($p<0.05$), however survival did not show significant difference between treatments with concentrations up to 4 mg $^{L-1}$.

Table 3. Effect of different concentrations of glyphosate on the reproductive rates of tambaqui (*Colossoma macropomum*).

Glyphosate (mg L^{-1})	TF (%)	TE (%)	UNDER (%)
0,0	56,00 ± 1,87a	20,00 ± 1,58[a]	48,67 ± 4,29a
2,0	48,00 ± 7,00ab	14,00 ± 3,32ab	42,00 ± 11,9a
4,0	49,00 ± 7,31ab	13,00 ± 2,00ab	43,33 ± 11,30a
6,0	48,00 ± 4,64ab	12,00 ± 4,36ab	25,33 ± 11,43ab
8,0	42,00 ± 2,55ab	16,00 ± 7,31ab	23,00 ± 10,20ab
10,0	32,00 ± 5,61ab	17,00 ± 2,55ab	36,33 ± 4,16ab
12,0	27,00 ± 7,81b	1,00 ± 1,00b	0,00 ± 0,00b
p-value	0,0149	0,0380	0,0089
Values are expressed as mean ± standard error. TF: Fertilization rate; TE: Hatching rate; SOB.: Larval survival. Different letters in the same column indicate differences in the means due to the treatments, according to Tukey's multiple comparison test ($p < 0.05$).			

Malathion also showed toxicity to tambaqui eggs (Table 4). Eggs incubated without the presence of malathion had the highest fertilization rate ($p<0.05$). On the other hand, the concentrations tested did not influence hatching and survival rates ($p>0.05$).

Table 4. Effect of different concentrations of malathion on the reproductive rates of tambaqui (*Colossoma macropomum*).

Malationa (µg L^{-1})	TF (%)	TE (%)	UNDER (%)
0,00	63,00 ± 4,64a	23,00 ± 2,55	59,67 ± 8,47
0,25	59,00 ± 2,92ab	18,00 ± 2,39	76,48 ± 8,51

0,50	60,00 ± 2,24ab	22,00 ± 6,63	71,79 ± 11,29
0,75	53,00 ± 1,22ab	21,00 ± 1,87	58,33 ± 10,85
1,00	46,00 ± 6,00abc	17,00 ± 3,74	45,00 ± 7,26
1,25	44,00 ± 2,45bc	21,00 ± 5,10	51,43 ± 15,88
1,50	35,00 ± 5,70c	12,00 ± 2,00	33,33 ± 18,26
p-value	0,0002	0,5797	0,2074
Values are expressed as mean ± standard error. TF: Fertilization rate; TE: Hatching rate; SOB.: Larval survival. Different letters in the same column indicate differences in the means due to the treatments, according to Tukey's multiple comparison test ($p < 0.05$).			

As for casugamycin, it also showed toxicity to tambaqui eggs and larvae (Table 5). The concentration of 0.25 mg $^{L-1}$ increased the fertilization rate, and the concentration of 1.5 mg $^{L-1}$ decreased the fertilization rate ($p<0.05$). Water without the presence of casugamycin increased the survival of larvae ($p<0.05$), however, the survival rate at concentrations up to 1.25 mg $^{L-1}$ showed statistically similar results to treatments at lower concentrations. The hatching rate was not influenced by the concentrations of casugamycin ($p>0.05$).

Table 5. Effect of different concentrations of casugamycin on the reproductive rates of tambaqui (*Colossoma macropomum*).

Casugamycin (mg L^{-1})	**TF (%)**	**TE (%)**	**UNDER (%)**
0,00	60,00 ± 1,87ab	24,00 ± 1,87	73,33 ± 11,30a
0,25	67,00 ± 4,85a	24,00 ± 5,79	45,71 ± 12,08ab
0,50	59,00 ± 3,67ab	23,00 ± 2,00	52,00 ± 2,00ab
0,75	61,00 ± 1,87ab	18,00 ± 3,39	53,33 ± 12,25ab
1,00	64,00 ± 4,18ab	26,00 ± 5,10	62,64 ± 11,27ab
1,25	59,00 ± 2,92ab	25,00 ± 2,74	64,67 ± 8,92ab
1,50	51,00 ± 3,00b	17,00 ± 4,06	21,67 ± 9,72b
p-value	0,0448	0,5505	0,0346
Values are expressed as mean ± standard error. TF: fertilization rate; TE: hatching rate; SOB.: survival of the larvae. Different letters in the same column indicate differences in the means due to the treatments, according to Tukey's multiple comparison test ($p < 0.05$).			

This decrease in reproductive rates in general may have caused the interruption of egg development before the closure of the blastopore, which causes embryo mortality at this stage, indicating the greater sensitivity of the early embryonic stages (before gastrulation) (Lal, 2007). In addition, it may be related to behavioral deficits of the forming embryo, which result in weakening or delay of spontaneous muscle movement (Haendel et al., 2004) and the inhibition of chorionase (Waiwood and Haya, 1983). According to Felsenfeld et al. (1990), the decrease in embryo mobility can be related to their hatching, as the loss of mobility due to exposure to toxic compounds can anticipate, delay or avoid hatching, also contributing to the increase in embryonic mortality.

ESSAY 3

High concentrations of ammonia showed toxicity to tambaqui embryos and larvae (Table 6). The fertilization rate and hatching rate of tambaqui eggs were higher ($p<0.05$) in water containing between 0.0 and 3.5 mg of NH4OH $^{L-1}$. At the concentrations at which there was hatching (up to 3.5 mg $^{L-1}$), the larval normality rate was not affected by ammonia ($p<0.05$). Larval survival up to 48 hours after hatching was higher ($p<0.05$) in waters containing concentrations between 0.0 and 2.0 mg of NH4OH $^{L-1}$.

Table 6. Effect of different concentrations of total ammonia on incubation, hatching, normality and survival of tambaqui (*C. macropomum*) larvae.

Total ammonia (mg L^{-1})	TF (%)	TE (%)	TNL (%)	SL (%) 12h	SL (%) 24h	SL (%) 48h
0,00	92,00 ± 2,00a	91,00 ± 1,87ª	96,73 ± 1,34ª	100,0 ± 0,00a	98,95 ± 1,05ª	96,78 ± 1,31ª
0,25	90,00 ± 3,67ª	88,00 ± 3,74ª	96,16 ± 2,63ª	100,0 ± 1,18ª	100.0 ±1.18th	97,78 ± 1,18ª
0,50	89,00 ± 3,32ª	88,00 ± 2,55ª	97,70 ± 1,42ª	98,95 ± 1,05ª	97,89 ± 2,11ª	95,67 ± 2,65ª
1,00	93,75 ± 2,14ª	95,00 ± 1,83ª	96,05 ± 1,18ª	97,30 ± 1,40ª	93,34 ± 2,34ª	90,77 ± 1,20ª
2,00	90,00 ± 3,16ª	89,00 ± 3,67ª	95,50 ± 2,78ª	99,00 ± 1,00a	99,00 ± 1,00a	94,70 ± 2,75ª
3,50	88,75 ± 1,12ª	88,75 ± 1,12ª	94,20 ± 3,72ª	11,11 ± 8,36b	0,00 ± 0,00b	0,00 ± 0,00b
6,50	31,57 ± 60,09b	0,00 ± 0,00b	0,00 ± 0,00b	0,00 ± 0,00b	0,00 ± 0,00b	0,00 ± 0,00b
10,00	0,00 ± 0,00c	0,00 ± 0,00b	0,00 ± 0,00b	0,00 ± 0,00b	0,00 ± 0,00b	0,00 ± 0,00b
p-value	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000

Values are expressed as mean ± standard error. TF: Fertilization rate; TE: Hatching rate; TNL: Larval normality rate; SL.: Survival of the larvae. Different letters in the same column indicate differences in the means due to the treatments, according to Tukey's multiple comparison test ($p < 0.05$).

Higher levels of ammonia contamination of the water interfered with the fertilization of the eggs. This may have occurred at the beginning of embryonic development at the beginning of blastopore closure. According to Vidal *et al*. (2013), the increase in ammonia can delay this ontogenetic moment and directly affect fertilization. The decrease in the fertilization rate reflects directly on the hatching rate. In addition, a high concentration of ammonia can accelerate the metabolism of the embryo by interacting in biochemical pathways, and thus the reserves present in the calf are consumed more quickly, contributing to accelerated development (Luckenbach *et al*., 2003).

CONCLUSION

Among the environments analyzed, the best water for incubation and hatching of tambaqui eggs is from the Solimões River, which is the natural environment for reproduction of the species. On the other hand, the streams in an area of urban expansion and close to the municipality's landfill are not in adequate conditions for hatching the eggs.

The results demonstrated in trial two that the exposure of tambaqui eggs to glyphosate, malathion and casucamicin can have negative implications on the rates of fertilization, hatching and survival of the larvae.

The results showed that the exposure of tambaqui eggs and larvae at concentrations of up to 2.0 mg of total ammonia $^{L-1}$ of water had no implications on fertilization rates, hatching, normality and survival of the larvae.

In this sense, tambaqui eggs can serve as bioindicators in water quality studies. We recommend international public policies for the conservation of water resources and rivers that border the municipalities of the triple border of the Amazon (Brazil, Peru, Colombia) so as not to affect the reproduction of native species.

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