







Reproductive aspects of the dwarf cichlid *Apistogramma agassizii* under captive conditions

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Received: November 26, 2021

Approved: May 03, 2022

ABSTRACT

Apistogramma agassizii is a native fish from the Amazon basin, which is of great interest for fishkeeping and is supplied to the retail trade from extractivism. Knowledge about the reproductive characteristics of this fish is fundamental to successful breeding. Objective: This work aimed to study the behavior and reproductive performance of this species in white and black aquariums. A total of 16 pairs were used, 8 in each treatment. The system had water recirculation, with fresh and inert food supply. Result: The fish demonstrated parental care, more accentuated by the female and partial spawning. The same coloration pattern was observed in both treatments, with coloration intensifying only during spawning. The eggs were adhesive and U-shaped/oval. The females followed the larvae, even after they left the nests, and attacked the males when they approached the offspring. Males exhibited territorial behavior. No significant differences were observed for the following parameters studied: largest and smallest egg size, spawning weight, absolute fecundity, number of hatched larvae, and hatchability rate. Conclusion: This study found that the fish are prolific and show good reproductive rates in conditions of captivity; thus, they are suitable to rear for the ornamental fish trade.

Keywords: aquarium hobby; behavior; native fish; reproduction.

Aspectos reprodutivos do ciclídeo anão *Apistogramma agassizii* em condições de cativeiro

RESUMO

Apistogramma agassizii é um peixe nativo da bacia amazônica muito apreciado pela aquariofilia; seu comércio é oriundo do extrativismo. Conhecer os aspectos reprodutivos desse peixe é fundamental para o sucesso da criação. Este trabalho teve por objetivo estudar o comportamento e o desempenho reprodutivo dessa espécie, para isso utilizaram-se aquários brancos e pretos, nos quais foram alojados 16 casais, oito em cada tratamento. O sistema adotado foi o de recirculação de água, com oferta de alimento inerte e fresco. Os peixes apresentaram cuidado parental acentuado pela fêmea e desova parcelada; o mesmo padrão de coloração para ambos os tratamentos pôde ser observado, intensificando-se na reprodução. Os ovos eram adesivos e em forma de U/ovais. As fêmeas acompanhavam as larvas, mesmo após a saída do ninho, chegando a agredir os machos quando estes se aproximavam da prole. Os machos apresentaram comportamento territorialista. Não foram observadas diferenças significativas para os seguintes parâmetros estudados: comprimento maior e menor do ovo, peso da desova, fecundidade absoluta, número de larvas eclodidas e taxa de eclodibilidade. Pôde-se ainda concluir que os peixes são prolíficos e apresentam bons índices reprodutivos em condições de cativeiro, portanto aptos para o cultivo visando ao comércio de peixes ornamentais.

Palavras-chave: aquariofilia; comportamento; peixe nativo; reprodução.

INTRODUCTION

Of the more than 1,300 species belonging to the Cichlidae family worldwide, about 450 live in South America. With representatives in the Amazon basin, the Guianas, northern Orinoco, southern Pará, and rivers in eastern Brazil, these fish are widely distributed throughout the Neotropical region and prefer lentic environments (Kullander, 2003; Sampaio, 2011).

In the Amazon region, the floodplain environments with their sets of flooded lakes, channels, and forests are very important, especially due to the diversity and richness of fish. Crampton (1999) mentioned that this ecosystem has an abundance of aquatic

macrophytes, forming favorable places for the development of both invertebrates and fish, which usually use the natural resources found for their diet.

According to Estivals et al. (2020), the *Apistogramma* genus includes about 94 species. These fish normally reach between 20 and 60 mm in standard length and have accentuated sexual dimorphism, where males are larger, more colorful, and more adorned than the females (Römer, 2001; Oliveira and Queiroz, 2017). *A. agassizii* is a species of dwarf cichlid endemic to the Amazon basin, with minimal qualitative information on its biology and ecology in its natural habitat, despite its importance in the ornamental fish trade (Anjos et al., 2009; Oliveira et al., 2017; Tribuzy-Neto et al., 2020; Mendes et al., 2021). It is found and collected throughout the floodplain of the Amazon basin and lives in all types of water, including white, black, and clear, and in degraded environments, being considered an opportunistic fish with an insectivorous tendency (Römer, 2006; Oliveira and Queiroz, 2017; Virgilio et al., 2020).

Lima et al. (2001) mentioned that a major threat to ornamental fish is that certain species are vulnerable to disappearance due to the lack of studies on their reproduction in captivity. Such studies could also help reduce the pressure of extractivism on commercialized fish.

According to Ribeiro et al. (2010), technologies have not been developed for the commercial production of fish of this genus, and their commercial exploitation is often illegal, which can impact their conservation and population stability (Mendes et al., 2021). Alves et al. (2009) described that for the formation of *Apistogramma* couples, fish must be collected in common aquariums, where juveniles choose their partners during their development.

Thus, this study aimed to evaluate the behavior and reproductive performance of *A. agassizii* under captivity conditions.

MATERIALS AND METHODS

All procedures used in this experiment were approved by the Ethics Committee on the Use of Animals at the Universidade Federal do Amazonas under number 004/2021.

Sixty specimens of *A. agassizii* were captured in aquatic vegetation (65482-3 – ICMBio/MMA), with the help of a net on the banks of streams in the municipality of Parintins, AM, Brazil. The fish were transported alive in isothermal boxes to the Aquaculture Laboratory – LAqua/ICSEZ. They were allowed to acclimate for 15 days in 1000-L water tanks, equipped with constant aeration. The fish were fed commercial feed containing 32% crude protein and supplemented with natural food, composed of a mixture of squid, octopus, shrimp, and shellfish, supplied in a proportion of 4% of live weight in two daily portions (8 and 16 h).

For the experiment, 16 couples were selected and distributed in a completely randomized design with 2 treatments and 8 replications, being observed for 6 months. The treatments

consisted of aquariums with two types of coloration – eight aquariums constructed with black ceramics and eight with white ceramics, in which only the front was made of transparent glass. The capacity of each aquarium was 20 L of water. The black and white aquariums were distributed in pairs on the bench. All experimental units received sand with 1.5-mm grain size, an artificial shelter (made of PVC, the color of the aquarium walls). The aquariums were interconnected by a water recirculation system, and the water was partially replaced upon evaporation and due to the siphoning of persistent residues from natural food.

The physical-chemical parameters of the water were monitored three times a week, at 8 a.m., for temperature ($28.07 \pm 0.37^\circ\text{C}$) and pH (7.40 ± 1.83) with a thermometer and a digital pH meter, respectively. Ammonia ($0.05 \pm 0.13 \text{ mg L}^{-1}$) and nitrite ($0.01 \pm 0.06 \text{ mg L}^{-1}$) were tested using commercial kits (Labcon Test). The food provided was the same as described during the acclimatization period.

All females were measured for their standard length and weighed with the aid of a caliper and digital scale (0.001 g), respectively. To analyze their reproductive behavior, the animals were observed daily. Courtship, posture, agonistic territorialism, hatching, and post-hatching behavior were followed. All observations were recorded and photographed.

The reproductive parameters measured were the total of spawnings obtained per treatment, the mean absolute fecundity ($Ft = \sum \text{total number of eggs} / \text{total number of spawns}$, obtained per treatment, according to Römer et al., 2001), the mean weight egg mass ($PMO = \text{total weight of the egg mass} / \text{total number of spawns}$, obtained per treatment), the average number of larvae that hatched ($N_L = \sum \text{total number of hatched larvae} / \text{total number of spawns}$, obtained per treatment), and the rate of hatchability ($TE = [N_L / Ft] \times 100$). Five hydrated eggs were measured from each spawn per treatment, to obtain the longest and shortest average lengths, with the aid of an optical microscope. The eggs were stored in 1.5-mL Eppendorf tubes containing 70% alcohol. Pearson's correlation ($p=0.05$) was performed between the weight and length of the females and the fecundity rate obtained, to verify the existence of interaction between these factors, for each treatment.

Data were confirmed for homoscedasticity and normality by Levene's and Shapiro-Wilk tests. Then, the data were subjected to analysis of variance (ANOVA) and means compared by Tukey's test at 5% significance.

RESULTS

Reproductive behavior

During the courtship period, males curved their bodies into an "S" shape and beat their fins, which have an erect appearance, with quick and short movements toward the female. The color patterns of the females and males of both treatments (white and black aquariums) did not differ, either in the period before or

during reproduction. In the latter period, the color became more intense, with females characterized by a yellowish color with black stripes and males a pattern of blue, yellow, green, silver, and iridescent, the latter only noticeable near the operculum (Figure 1A-F).

From the beginning of laying, the females always deposited their eggs in the artificial shelters, regardless of the aquarium color. The females built their nests by spreading the substrate inside the shelter and reducing the entrance, collecting the substrate with their mouths, and taking it to the opening (Figure 1D-F);

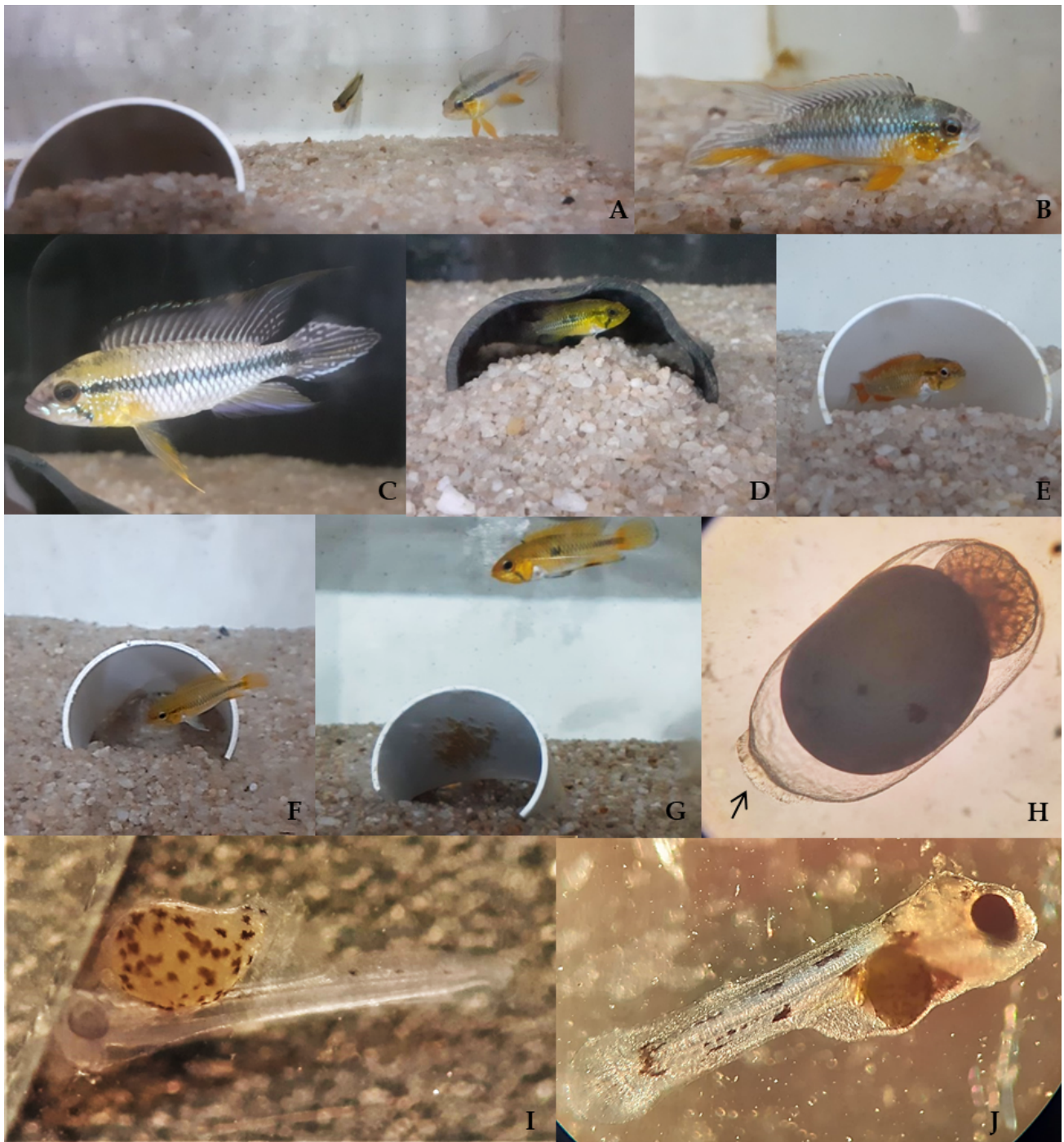


Figure 1. *Apistogramma agassizii* couple exhibiting agonistic behavior (A); male coloration during the reproductive period (B, C); females preparing the nest for spawning in black and white aquariums (D-F); spawning carried out in a white aquarium (G); U-shaped or oval adhesive egg, arrow indicating the adhesive egg membrane (40×) (H); larvae up to 3 days old observed under a stereomicroscope (50× and 20×, respectively) (I, J).

male passage was only allowed when spawning. At this time, the female laid the adhesive eggs, which had a U-shaped or oval body, on the concave region of the shelter (Figure 1G, H), without any symmetry, and then the male fertilized them.

After spawning, the females remained longer in the artificial shelters, exhibiting parental care, and the males displayed a territorial behavior around the shelter. Both individuals explored the territory in search of food, and the female made the shortest trips, returning quickly to the spawning site. The larvae hatched 48 h after laying (Figure 1I, J), and they remained inside the nest under parental care. On the third day of life, they were already free-swimming, making forays around the shelter, closely monitored by the female, who at certain times defended them from the male's approach.

Reproductive performance

Table 1 shows that aquarium color had no significant effect on the reproductive parameters analyzed. The smallest and largest females of *A. agassizii* in standard length were housed in white aquariums (24.99 and 30.42 mm, respectively) and in weight (318.0 and 885.0 mg, respectively) in black aquariums.

By the end of the experimental period, 100% of the couples were alive, 31 spawns were obtained – 18 from the white aquarium and 13 from the black aquarium (Table 2). Of the 16 couples, 7 from the white aquarium and 6 from the black aquarium had at least 1 spawn, with no significant difference between treatments ($p=4.18$). Of the *A. agassizii* females that spawned, 71.4% of the white aquarium and 83.3% of the black aquarium did so more than once, characterizing this as a split spawning species.

Table 1. Means and standard deviation of the studied reproductive parameters of *A. agassizii* couples housed in white and black aquariums.

Reproductive parameters	White aquarium	Black aquarium	$p \geq 0.05$
Final standard female length (mm)	28.51±1.69	28.98±0.25	0.56
Final female weight (mg)	684.89±110.21	704.31±163.18	0.70
Longest egg length (mm)	1.45±0.08	1.43±0.06	0.77
Shortest egg length (mm)	0.81±0.04	0.82±0.02	0.48
Egg mass weight (mg)	46.88±0.02	58.50±0.04	0.49
Absolute fecundity	123.57±40.30	137.35±28.52	0.36
Hatched larvae	77.36±49.89	88.41±39.38	0.49
Hatchability rate (%)	62.60	64.37	0.69

The most spawns of the same female (Table 2) were achieved in the white aquarium, totaling 626 eggs (125.20 ± 28.90 eggs spawn⁻¹). However, the smallest and largest number of eggs deposited in a single spawn were 20 and 249 eggs, respectively, which were obtained in the black aquarium. There was no correlation between female weight and fecundity ($p=3.42$; $r=0.79$) and between fecundity and standard length ($p=4.33$; $r=0.63$).

DISCUSSION

The physical and chemical parameters of the water agreed with those reported by Ismiño-Orbe et al. (2007) and Alves et al. (2009), when they reproduced *Apistogramma panduro* and *Apistogramma cacatuoides*, respectively, under laboratory conditions. Römer et al. (2001) stated that temperature has a significant influence on the reproduction of these fish and reported a range of thermal comfort between 25°C and 28°C.

A. agassizii has an agonistic reproductive behavior similar to other cichlids, as well as a change in color intensity during the reproductive period, which has been reported for *Mesonauta insignis* (Maan et al., 2001), *A. panduro* (Ismiño-Orbe et al., 2007), *A. cacatuoides* (Alves et al., 2009), *Cichlasoma dimerus* (Alonso et al., 2011), *Apistogramma hippolytae* (Rodrigues et al., 2012), *Heros severus* (Dias, 2016), and *Apistogramma trifasciata* (Mendes et al., 2021). According to Ready et al. (2006) and Alonso et al. (2011), changes in the fish color pattern during stress or even during the reproductive period send complex information quickly and reinforce that visual communications are very important for these organisms.

According to Estivals et al. (2020) and Souza (2021), the intense coloration in the caudal and anal regions of *A. agassizii* indicates a possible relationship with characteristics that can be

Table 2. Number of spawns obtained and their frequency by housed couples.

Fish couples	Number and frequency of spawns per treatment	
	White aquarium (%)	Black aquarium (%)
1	-	2 (15.4)
2	3 (16.7)	3 (23.1)
3	1 (5.5)	-
4	3 (16.7)	2 (15.4)
5	1 (5.5)	2 (15.4)
6	5 (27.8)	3 (23.1)
7	3 (16.7)	-
8	2 (11.1)	1 (7.6)
Total spawns	18 (100.0)	13 (100.0)

sexually selected and act as a signal, making individuals more attractive. As observed in this study and according to Maan and Sefc (2013), *Apistogramma* sp. males feature a wide variety of colors, which can vary between individuals and depend on their age and social status.

The post-spawning parental behavior observed for *A. agassizii* females is similar to that reported by Ready et al. (2006), Alves et al. (2009), Rodrigues et al. (2012), and Mendes et al. (2021) for *Apistogramma caetei*, *A. cacatuoides*, *A. hippolytae*, and *A. trifasciata*, respectively. According to Rodrigues et al. (2012), under natural conditions, the energy used by females to defend the nest can be balanced immediately after the separation of the offspring, as the cost of a lower feeding frequency can reduce the energy available for the next spawning. In this scenario, artificial incubation of eggs could be a viable strategy to increase the absolute fecundity of females, combined with a nutritionally balanced diet.

Römer and Beisenherz (2005) and Oliveira and Queiroz (2017) suggested that males with territorial characteristics, such as those observed in this study, can be important when females choose a mate. Hence, females could reduce attacks against possible predators and their energy reserves would be better preserved. More studies are needed for this observation.

Barcellos et al. (2009) and McLean (2021) mentioned that fish adapt to photic and color variations, expressing differences in visual sensitivity. The sensitivity to the color of the environment that they are reared can affect the behavior and stress response in fish, with implications for the welfare of these animals and performance at various stages of production. Thus, the effect of the environment on fish physiology and behavior has been studied as an important area for reproduction, with the effect of each color on species-specific well-being (Volpato et al., 2004; Costa et al., 2013; McLean, 2021; Vissio et al., 2021). According to Vissio et al. (2021), rearing conditions, including tank coloration, can affect a range of physiological processes, and better understanding this may improve fish productive performance, including reproduction.

Stress levels were not monitored in this work; however, both fish from white and black aquariums were apparently calm throughout the experimental period, with no records of cannibalism, both for eggs and offspring, which could be considered an adverse factor. According to Blaser and Rosemberg (2012), opaque tanks reduced anxiety in *Danio rerio*. Oliveira (2016), in studies with a preference for aquarium coloring for *Astronotus ocellatus*, found that they had a preference for blue aquariums, as this color has a maximum absorbance of 535 nm, because this species captures a frequency between 360 and 561 nm. The same work observed that individuals housed in a white environment adopted a calmer posture with other individuals.

Volpato et al. (2004) reported a higher reproductive rate for Nile tilapia in tanks with blue substrate. However, differences in egg incubation rates and hatchling survival were observed (Brian, 2015). According to McLean (2021), the apparent effect on the reproductive behavior of fish and the successful

performance of females can have consequences for breeding programs, since color can alleviate stress, reduce aggressive behavior, and improve reproductive performance.

Amazonian fish have a great diversity of reproductive strategies and tactics, which guarantee the survival of the largest possible number of offspring and are a product of the long processes of natural selection and adaptation to seasonal fluctuations in environmental variables (Silva, 2013). According to Vazzoler (1996), partial spawning is a mechanism where oocytes develop at different intervals, being released as they reach full maturation, with at least three batches of intra-ovarian oocytes. This case occurs in species that spawn periodically during their life, such as *A. agassizii*, revealing great potential for their intensive production in controlled environments. Multiple spawns were also observed by Römer et al. (2001) in *A. cacatuoides*.

The females studied in this experiment were smaller in standard length and weight than the *A. cacatuoides*, used by Alves et al. (2009). However, the mean number of eggs released per female was similar to those reported in that study. According to Oliveira and Queiroz (2017), *A. agassizii* females reach their first sexual maturity at 19.85 mm, which is in accordance with the standard size reported here. These same authors mentioned that they found an average fecundity of 161.7 oocytes per gonad for this species. Thus, the fecundity found in the present study is near that reported by these authors. Römer et al. (2001) reported a mean fecundity of 36.6 ± 15.9 eggs for *A. cacatuoides*.

The results for egg size agree with those reported by Oliveira and Queiroz (2017) for this same species (1 mm oocytes) and smaller than those reported by Römer et al. (2001) for fish of the same genus (3.29 mm). Morrongiello et al. (2012) mentioned that the size and quantity of eggs can vary between populations, between females of the same population, and even for a specific individual, decreasing the size and increasing the quantity according to environmental variability. As the environmental parameters and the food offered to the animals were the same and no significant differences were found between the treatments studied, including the correlations made between the number of eggs laid and the length and weight of the females, further studies are suggested to identify the factors that could interfere with these parameters.

Although there was no significant difference, the black aquarium trend to have a higher hatchability rate (2.83% higher than the white one). For *D. rerio*, Cruz (2020) mentioned a significantly higher hatchability rate in the black aquarium, compared to a yellow aquarium. According to Brian (2015), hatchability in tilapia was numerically higher in aquariums with a blue background, although there was no significant difference regarding other color patterns.

CONCLUSION

A. agassizii is a fish that exhibits parental care and partial spawning. No significant differences were observed between

reproductive parameters. This fish was easy to maintain in captivity conditions, adapting to both natural and artificial feeding, with satisfactory reproductive rates to be reared, which could reduce predatory pressure on natural stocks. However, further studies of the larval and juvenile phases are needed, as no data were found in the literature on the mass cultivation of this species of interest for fishkeeping.

CONFLICT OF INTERESTS

Nothing to declare.

FINANCIAL SUPPORT

The Amazonas State Research Support Foundation funded the first author's scientific initiation grant (PIB-A/0026/2020).

AUTHOR'S CONTRIBUTIONS

Silva, H.J.: Conceptualization, Data curation, Investigation, Project administration, Writing – original draft. Costa, T.V.: Conceptualization, Formal Analysis, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing. Almeida, J.P.C.: Investigation. Santos, W.G.: Investigation. Machado, N.J.B.: Conceptualization, Formal Analysis, Data curation, Validation. Vieira, R.B.: Investigation.

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