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Scientific Article

Otolith morphometry for the determination of the theoretical growth curve of fish

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ABSTRACT

During the period from November 2019 to August 2021, a total of 43 specimens of *Leporinus friderici*, 18 of *Prochilodus lineatus*, 17 of *Hoplias intermedius*, and eight of *Cichla kelberi* were caught in the Verde River basin (Mato Grosso do Sul, Brazil) using various types of fishing equipment. Data collection consisted of measuring fish lengths (LO_a, mm) and area (O_a, mm^2) of their respective sagitta otoliths. The modified Von Bertalanffy's equation (1938) was used to determine the theoretical growth curve as a function of the otolith area. The results indicated that the length of the fish increases in relation to the increase in the otolith area, resulting in a strong logarithmic regression for all species studied. The values of the theoretical growth curve of the fish (observed and estimated) as a function of the areas of the otoliths showed adjustments of $R^2 > 76$ for the measured and $R^2 > 90$ for the estimated values. Therefore, the otolith area proved to be adequate for the estimation of growth for the species, which can also facilitate the evaluation of fish length, being a useful tool for the evaluation of fish stocks.

Keywords: Von Bertalanffy; Sagitta; Otolith area; Verde River.

Morfometria de otólito para a determinação da curva de crescimento teórico de peixes

RESUMO

Durante o período de novembro de 2019 a agosto de 2021 foi capturado o total de 43 exemplares de *Leporinus friderici*, 18 de *Prochilodus lineatus*, 17 de *Hoplias intermedius* e oito de *Cichla kelberi* na bacia do Rio Verde (Mato Grosso do Sul, Brasil), por meio de diversos apetrechos de pesca. A coleta dos dados consistiu na mensuração dos comprimentos dos peixes (LO_a, mm) e da área (O_a, mm^2) de seus respectivos otólitos Sagittae. Foi utilizada a equação de von Bertalanffy (1938) modificada para determinar a curva de crescimento teórico em função da área do otólito. Os resultados indicaram que o comprimento dos peixes aumenta em relação ao incremento da área do otólito, resultando em uma regressão logarítmica forte para todas as espécies estudadas. Os valores da curva de crescimento teórico dos peixes (observados e estimados) em função das áreas dos otólitos exibiram ajustes $R^2 > 76$ para os valores medidos e $R^2 > 90$ para valores estimados. Portanto, a área do otólito mostrou ser adequada para a estimativa de crescimento para as espécies, o que pode facilitar a avaliação de comprimento dos peixes, sendo uma ferramenta útil para a avaliação dos estoques pesqueiros.

Palavras-chave: Von Bertalanffy; Sagitta; Área do otólito; Rio Verde.

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INTRODUCTION

Despite hydroelectric dams being considered a sustainable and low-cost source of energy, their presence results in impacts on fishery resources, with greater emphasis on rheophilic fish, which, due to the dams, is prevented from carrying out or having greater difficulty in continuing its reproductive migrations (Agostinho et al., 2008). In addition, changes also occur in the composition and size of the population of opportunistic and sedentary fish species, which easily adapt to the new lentic environments upstream of the dams and modify the local guilds (Salaro et al., 2006; Sousa et al., 2021).

The Verde River basin is located in the state of Mato Grosso do Sul, in the midwestern region of Brazil (Lanza et al., 2014), and has great relevance for the conservation of fishing biodiversity, especially as it is part of the ecological corridor that connects the Pantanal biome with the Paraná River basin (Silva et al., 2015). However, dams have been built along this river for hydroelectric use, and these have triggered several impacts on the aquatic environment, including a decrease in the diversity of fish species in this region (Sousa et al., 2021).

Considering that the study of population dynamics is the study of changes in the number and composition of individuals in a population and the factors that influence these changes (Gulland, 1983), it is necessary to carry out continuous studies that provide information that clarifies how the basic components work and interact, such as recruitment, age structure, sex ratio, dispersion, mortality, and growth. In this way, one can evaluate the effects of abiotic factors on the size of these populations and, consequently, predict whether a species is threatened by overfishing (King, 1995), since the anthropization of the environment can lead to abrupt changes in the environment.

In this scenario, the study of the growth of fish from a given stock helps in the correct determination of the age of its individual components (Weatherley & Gill, 1987), which can be done through the analysis of length distribution over time and by interpretation of growth marks in rigid structures, such as scales, vertebrae, opercula, and otoliths of fish (Luo et al., 2016), which are structures that are widely used by scientists (Thorrold et al., 2001).

Otoliths are concretions of aragonite, a form of calcium carbonate, located in the neurocranium of teleost fish. They have multiple functions, such as the perception of sounds and aid the balance of the fish (Andrus & Crowe, 2002). Its use in experiments gives scientists a better understanding of the life history of fish, and they provide information on the age, biology, places of growth and habitat, thus providing useful subsidies for understanding the systematics and evolution of fish and their populations (Garcez et al., 2014).

Among the otoliths, the so-called sagitta is the most used in morphometric studies and in determining the growth and age of fish (Campana, 2004), since they are easy to handle and have larger increments (Secor et al., 1992), which makes it possible to distinguish an individual or a population via the study of the shape and counting of the growth rings in these structures (Campana, 2004). It is even possible to distinguish between genera and species (Mereles et al., 2021).

Studies also point to a high precision between the measurements of length and weight of the otolith with the length of the fish's body, and with these data it is possible to estimate with high precision the age of an individual or a population (Pawson, 1990). In this sense, it is plausible to think that length is a function of the area of the otolith in the same way that we assume that length is a function of age (Hanson & Stafford, 2017).

The growth of otoliths is regulated by fish physiology, which is integrated with endogenous (reproduction, migration, spawning) and exogenous factors (temperature, food availability, environmental stress) (Rufino, 2004). On the other hand, age rings are formed by periodic events, such as climatic seasons and temperature variations (Casselman, 1983).

There is a relationship between the length of fish and the morphometric parameters of otoliths (Ozpiçak et al., 2015), but most otoliths do not have a unique morphology, and require standardized readings (Campana, 1992). There are difficulties in performing the readings of the age rings according to the structure used (Oliveira et al., 2014). In the case of the lapillus otolith, the rings are translucent and opaque, the sagitta is fragile to handling, and the asteriscus has an internal and external groove that are not very flat (Cutrim & Batista, 2005) and the occurrence of double rings with alternating intermediate marks that are not constant, which makes it difficult to interpret the annual marks (Pérez & Fabré, 2003).

Therefore, with the construction of the dams in rivers, the environment is dramatically modified. Their installation causes physical and chemical impacts on the environment, reduces the speed of the river, increases sedimentation and mainly affects the temperature of the environment (Sousa, 2000). Consequently, this leads an irregular formation of rings in the otoliths, and a variety of structures, opaque and hyaline zones and daily growth rings have been observed that can then hinder the accuracy of their readings in the otoliths (Gauldie & Nelson, 1990).

Due to the numerous difficulties encountered in performing accurate readings of growth rings in otoliths of fish from dam environments, together with the lack of studies that have analyzed the area of the otolith with the applicability of this dimension in the analysis of fish growth, the present study aimed to establish the predictive relationships between the otolith area and the length of four species of fish (*Leporinus friderici*, *Prochilodus lineatus*, *Hoplias intermedius*, and *Cichla kelberi*); and determine the growth curves using the adapted Von Bertalanffy equation, of the species that were captured in the Verde River basin, in the state of Mato Grosso do Sul, Brazil.

MATERIALS AND METHODS

Study area

The fish were collected at six sampling points in the Verde River basin. This river has an area of 23,739 km^2 and runs through an area of Cerrado biome and a small part of the Atlantic Forest (Lanza et al., 2014). The Verde River originates from the union of two tributaries, the Paraíba and Grande rivers, on the border of the states of Mato Grosso do Sul, Minas Gerais, and São Paulo, forming part of the Paraná River basin (Fig. 1).

Data collection

The fish were caught between November 2019 and August 2021 (with the *in-situ* fauna collection authorization via protocol A.A. No. 036/2019, issued by the Environmental Institute of Mato Grosso do Sul), in experimental fisheries, with fishing nets and hooks, in six collection points in the study area, followed by the identification of the fish while in the field, measurement of the total length (mm), and the extraction of the otoliths.

To perform the morphometric measurements of their area (mm^2), the otoliths were placed under a binocular stereomicroscope (Bioptika), at 10x magnification using a 0.8 objective lens under transmitted light. The otoliths were photographed with an attached camera using the TCapture software (ISCapture), and the images were measured using the ImageJ program. In this process, the calculation of the area of the otoliths in ImageJ was performed with the image of the otoliths in Tiff format, changed from 32 to 8 bits, followed by the watershed transformation, which induces the appearance of lines to the contours of the otoliths separately (Klava & Hirata, 2009), and then the calculation of the area was performed by counting pixels (Rasband, 2004).

Data analysis

The existence of a correlation between the total length of the fish (mm) and the area (mm^2) of their respective otoliths was verified via regression analysis between these variables.

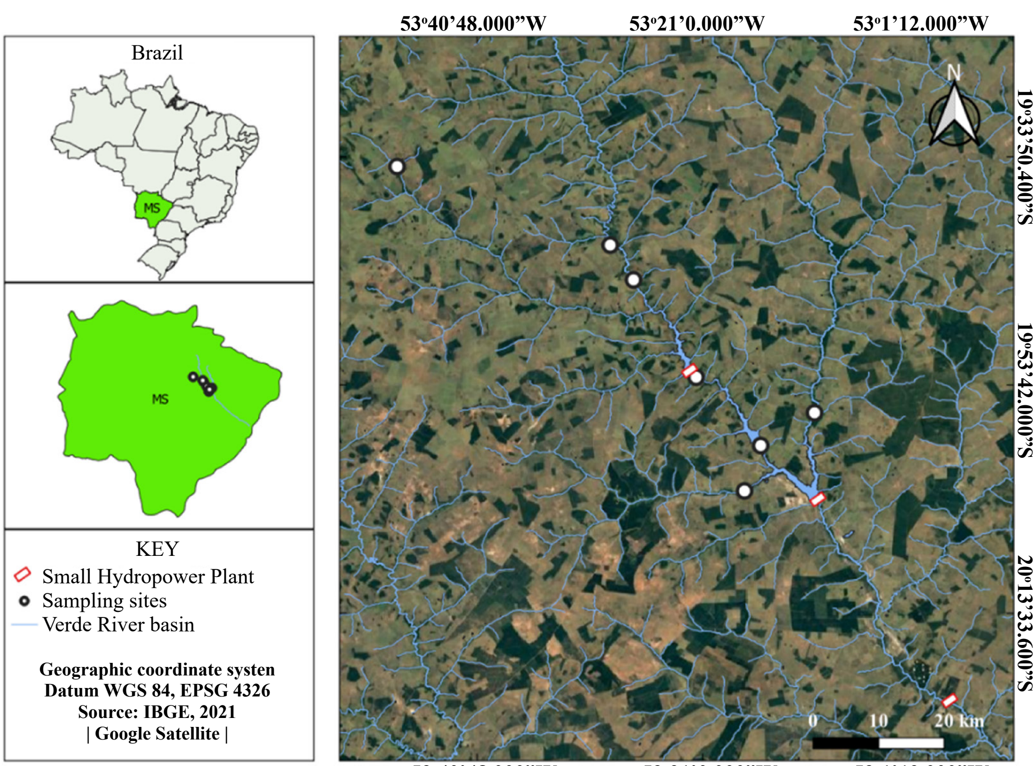

Figure 1. Location of fish collection points in the Verde River basin. Bars indicate dammed environments.

The asymptotic lengths (L_∞) of *H. intermedius* and *C. kelberi* were obtained through data published in FishBase (Froese & Pauly, 2024), and for *L. friderici* and *P. lineatus*, the L_∞ was calculated from the largest individual caught (L_{max}) according to Eq. 1, found in Pauly (1979):

$$L_\infty = \frac{Lmax}{0{,}95} \quad (1)$$

The values of *a* and *b* were obtained through the results of the linear regression equation of the total length of the fish transformed by the Von Bertalanffy's method (1938), and represented by Eq. 2:

$$-\ln(1-LO_a/l_\infty) \quad (2)$$

Where: ln = natural logarithm; LO_a = length of the fish, as a function of the area data, in accordance with the standardization of the data, in which the result of the value of *a* divided by the value of *b*, from the regression analysis is the value of Oa_0 (Oa_0 = a/b), and *b* = *k*.

For the determination of the theoretical growth curve as a function of the otolith area (Eq. 3), the modified Von Bertalanffy's equation (1938) was used:

$$L_{Oa} = L_\infty\left[1 - \exp^{-k(Oa-Oa0)}\right] \quad (3)$$

Where: LO_a = the length of the fish at the instant Oa, exp = Euler number; constant *k* = the slope of the growth curve; Oa_0 = the theoretical area of the otolith at the 0 length of the fish.

Statistical analyses were performed using RStudio 4.1.3 software, using the stats, ggplot2, dplyr, ggpmisc, and ggpubr packages. Initially, the data were submitted to the Shapiro-Wilk's test to verify normality and to the Levene's test to assess the homogeneity of variances. A significance level of $p < 0.05$ was adopted. Then, a second-order polynomial regression was performed due to the non-linearity of the models, which was accompanied by the F test, in order to test whether the variance of the samples is equal.

RESULTS

During the sampling period, a total of 43 specimens of *L. friderici*, 18 of *P. lineatus*, 17 of *H. intermedius*, and eight of *C. kelberi* were captured. The coefficient of determination (R^2) referring to the adjustment of the linear regression of the total length of the fish, transformed by the Von Bertalanffy's method, as a function of the otolith area of each species, indicated that the model explained more than 76% of the observed variation. Furthermore, the tests obtained a high statistical significance for these adjustments ($p < 0.001$). The purpose of this was to find the relationship between the otolith area and the total length of the fish, as well as to estimate the parameters *a* and *b*.

The sampling of *C. kelberi* showed the lowest coefficient of variation (CV) of the total fish length among the analyzed species, indicating lower relative variability in relation to the mean. However, this sample also had a higher residual standard error in the linear regression, suggesting greater dispersion of the data around the fitted line. Even so, the results followed a significant pattern ($p < 0.001$) and showed a satisfactory coefficient of determination ($R^2 > 76\%$). The other species showed a slightly better relationship, which was attributed to a larger sample size (n) and the growth modeled by the Von Bertalanffy's model in relation to the otolith area (Fig. 2).

The relationship between the total length of the fish as a function of the otolith area also showed a strong linear adjustment ($R^2 \geq 0.76$). In the Table 1, we present the results of the otolith area (Oa), total fish length (TLm), logarithmic regression coefficients, and the parameters for the growth curve (L_∞, k, and Oa_0) of Eq. 3 (Table 1).

With the determination of the values of L_∞, k, and Oa_0, it was possible to generate the Von Bertalanffy's theoretical growth curve, whose adjustments for all specimens had a coefficient of determination of $R^2 > 76$ for the measured and $R^2 > 90$ for the estimated values with a confidence interval of 95% and the t-test with a statistical significance of $p < 0.001$ for all samples. However, this logarithmic relationship was limited to the maximum growth (L_∞) described in FishBase (Froese & Pauly, 2024). In Fig. 3, each species shows marked growth in the first years of life, which stagnates as the fish approach their asymptotic length.

DISCUSSION

Analysis of otolith structures are more accurate when compared to other rigid structures, such as scales, because otoliths are formed before them (Werder & Soares, 1984). However, some of the methods used in studies with otoliths and vertebrae are more complex, and have high costs according to the technique and degree of sensitivity of the equipment to be used for the analyses (Mereles et al., 2020).

The morphometry technique with the use of images for the calculation of otolith area (Rasband, 2004) is a more practical and cheaper methodology, and its validation from the results obtained allows us to infer that the growth of the species

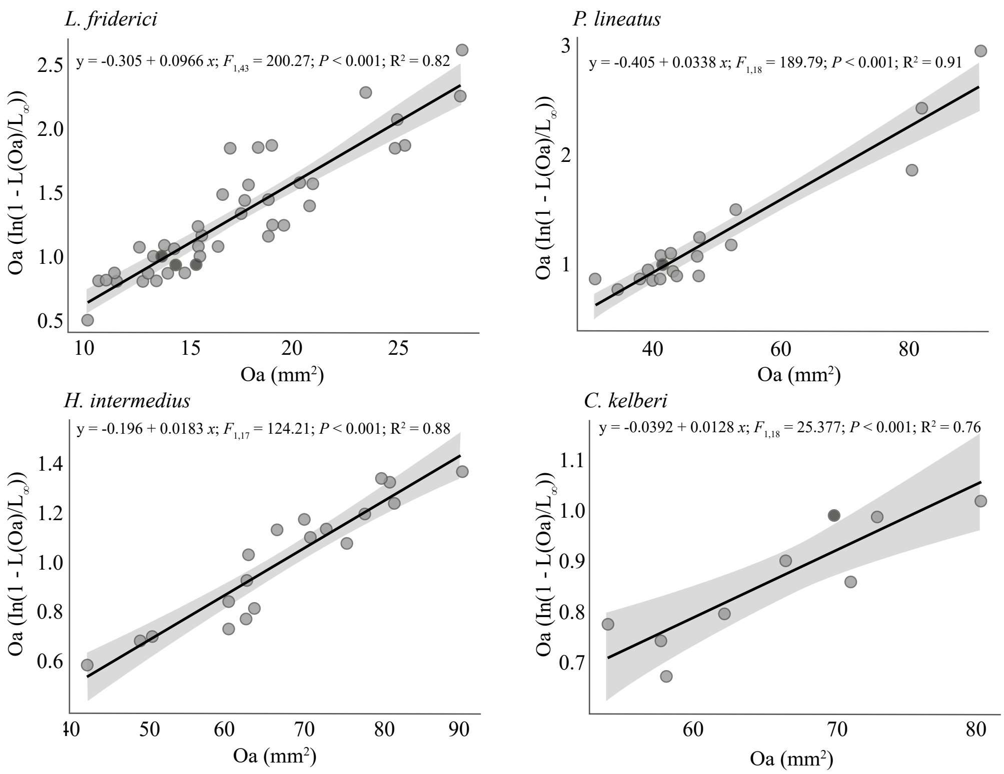

Figure 2. Linearized relationship between log-transformed species length data as a function of otolith area.

Table 1. Input data for the modified Von Bertalanffy's growth curve.

Species	N	Oa (mm^2)	TLm (mm)	R^2	R^{2*}	Growth parameters		
						L_∞ (mm)	k	Oa_0 (mm^2)
L. friderici (Bloch, 1794)	43	16.8 ± 4.4	283.1 ± 45.2	0.82	0.91	392	0.0966	3.15
P. lineatus (Valenciennes, 1837)	18	48.6 ± 16.3	405.9 ± 68.3	0.91	0.99	600	0.0338	11.97
H. intermedius (Günther, 1864)	17	67.1 ± 12.4	380.0 ± 53.9	0.88	0.96	600	0.0183	10.73
C. kelberi (Kullander & Ferreira, 2006)	8	66.2 ± 8.3	341.9 ± 29.9	0.76	0.93	585	0.0128	3.06

N: number of fish; Oa: area of the otolith and its respective standard deviation values (±); TLm: mean total length; R^2: logarithmic regression of the total length of the fish as a function of Oa; R^{2*}: logarithmic regression of the estimated total length of the fish as a function of Oa; L_∞: asymptotic length; k: growth constant; Oa0: theoretical area of the otolith when the length of the fish is close to 0.

studied are negative allometrics due to the greater increase in otolith area in relation to length. This factor can be explained via Worthmann's (1979) study, in which the author described that, for a group of individuals of the same species, older fish had larger and heavier otoliths, which indicates that fish with slower development have the largest otoliths. This means that the otoliths of younger fish tend to grow in a more adjusted way to the total length and start to tend to less adjustment when the fish approach their maximum growth (Hanson & Stafford, 2017).

The fact that the smallest adjustment was observed for *C. kelberi* may be related to factors not considered in the study, such as the low number of individuals sampled. However, the values obtained for the estimated lengths from the otolith

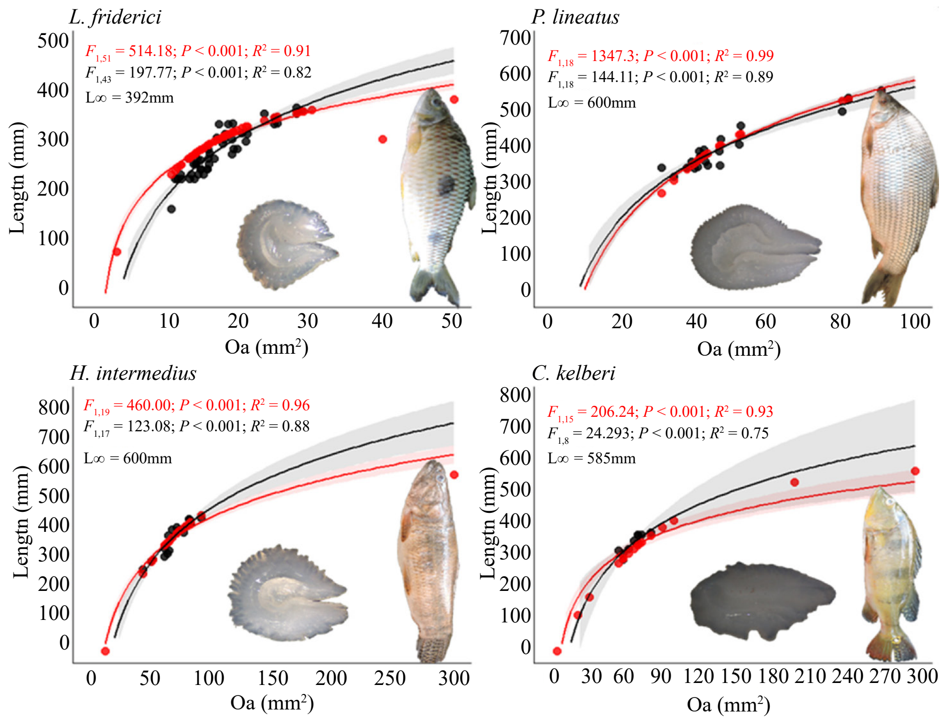

Figure 3. Von Bertalanffy's theoretical growth curve for fish caught in the Verde River basin. Logarithmic adjustment of the observed total length of the fish (black line and dots) and the estimated total length (red line and dots) as a function of the otolith areas.

areas studied have greater adjustment to the data and amplify the adjustment between the otolith area and the length of the analyzed fish. This relationship had a similar adjustment to that found for marine species (Lombarte, 1992), whose morphometric relationships also demonstrated that they can be used as an isometric standard for fishery management studies. However, these factors are little studied in Brazilian continental waters (Garcez et al., 2014; Mereles et al., 2020; Mereles et al., 2021).

Thus, it is also impossible to mitigate the possible phenotypic differences (specifically the amplitude of the total length range) resulting from interactions in an environment with different characteristics; the age of the specimens analyzed, since younger fish tend to have a higher growth rate than older ones; the difference in accessibility to environmental resources; the intrinsic factors of each organism; and the non-differentiation of the genus, since the females of some species have different growth when compared to males (Aydin et al., 2004; Froese et al., 2011).

Thus, the results obtained allowed us to infer that the equations proposed using the otolith area is another tool that can be applied to estimate the length of the species studied and, therefore, studies of population dynamics. This expands the possibilities already presented in the different methodologies employed for the use of otoliths related to the unit of measurement, mass, and microchemistry of fish (Battaglia et al., 2010; Campana, 2004; Garcez et al., 2014; Li, 2022; Park et al., 2018).

Given the difficulty in counting the age rings of fish located in dammed environments due to seasonal instability of river levels, the results presented here can serve as an alternative in estimating fish growth through the morphometry of the otolith area, given its wide correlation with fish growth, and can also subsidize important information for the study of biology, feeding, paleontology, monitoring of fishery sources, and fisheries management (Reis-Santos et al., 2022).

This study demonstrated that otolith area is an effective tool for estimating the growth of different fish species in the Verde River. The results indicated there is a positive relationship between fish length and otolith area, with a strong logarithmic regression observed in all the species analyzed. In addition, the adjustments to the theoretical growth curve, using the modified Von Bertalanffy's equation, were adequate since they presented R^2 values greater than 76 for the measured data and greater than 90 for the estimated ones. This suggests that otolith area can be a useful tool in the assessment of fish stocks and in understanding the population dynamics of the species studied.

However, although the present study does not have sufficient data to fully validate the model, it does offer support for improving new study techniques. We highlight here the importance of continuing research on population dynamics and the impacts of dams on the aquatic environment, especially in migratory fish, which are most affected by these interventions.

CONFLICT OF INTERESTS

Nothing to declare.

DATA AVAILABILITY STATEMENT

All data relevant to the study are included in the article.

AUTHORS' CONTRIBUTION

Conceptualization: Bezerra Neto EB, Faria Junior CH; **Formal Analysis:** Sousa RGC; **Investigation:** Faria Junior CH; **Resources:** Faria Junior CH; **Data curation:** Bezerra Neto EB, Zanchi FB; **Writing – original draft:** Bezerra Neto EB; **Writing – review & editing:** Bezerra Neto EB, Sousa RGC; **Final approval:** Sousa RGC.

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