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Who Is in Handicrafts: Tooth Morphology and Age Determination as Tools to Assess Vulnerable Dolphins *Sotalia* spp. Supplying the Trade of Artisanal Charms in Brazil

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In Brazil, dolphins' body parts are commonly used as traditional amulets, named as love-charms, while dolphins' teeth are commercialized in handcrafted necklaces and bracelets. Recent studies on forensic genetics confirmed the Guiana dolphin, *Sotalia guianensis*, as the principal target of the love-charms trading, with specimens incidentally captured in gillnets suggested as the primary source. As additional support for these investigations, we characterized tooth morphotypes and ages of dolphins used in the construction of traditional crafts, with the main objective of detecting the dolphin species and population groups most affected by this trade. Teeth collected from necklaces, bracelets, and earrings, sold in public markets from three major cities of Northern and Northeastern Brazil (Belém = 99, São Luís = 4, and Fortaleza =15), were analyzed using four morphological measurements: tooth total length (TL), root diameter (RD), crown length (CL), and cingulum diameter (CID). An unweighted pair group method using arithmetic means (UPGMA), with Euclidean distances as a measure of dissimilarity, multivariate analysis of variance (MANOVA), and principal component analysis (PCA) were used to detect tooth morphotypes in the samples. Ages were obtained by counting the growth layer groups (GLGs) deposited in dentine. Two different groups of teeth were detected presumably belonging to the species *Sotalia guianensis* and *S. fluviatilis*. Handicrafts showed a diverse age composition, ranging from 2 to 30 years, with a multimodal distribution (Mean =11 ± 7 SD, Median = 10, Mode = 4, 8, 19), similar to dolphin

populations affected by fishery interaction. Results suggested that juveniles individuals and adults younger than 20 years, from both *Sotalia* species, probably incidentally captured, are the principal source of handicrafts, and raise concern about the greater vulnerability of young age categories presented in this trade.

Keywords: age, sotalia, guiana dolphin, tucuxi, love-charms, teeth, northern Brazil, amazon

INTRODUCTION

The regular trade of cetacean products is long-standing in Brazil, still today. Folkloric beliefs from the Amazon region have the riverine dolphins (*Sotalia* spp. and *Inia* sp.) as central characters, fomenting a ritualistic usage of dolphin body parts with believed magical powers, renowned as "love charms" (Siciliano et al., 2018). These charms are commonly used and sold as traditional amulets, principally in Amazonian city markets, but have also been reported in coastal regions of the North, Northeast, and Southeast of the country (Siciliano, 1994; Sholl et al., 2008; Siciliano et al., 2018).

In Northern Brazil, the demand for boto (*Inia* sp.) derived amulets is high (Smith, 1996) and includes genital organs, eyes, skin, and teeth as principal products (Gravena et al., 2008; Siciliano et al., 2008). Since local traditional legends talk about boto dolphins changing to human form and seducing young women, it is believed that possessing one of these body parts brings luck in love and good sexual performance to the carrier (Cravalho, 1999; Vidal et al., 2019). However, recent research using genetic material from love charms collected in traditional markets of eight main cities from Northern to Southeastern Brazil indicated the estuarine dolphin *Sotalia guianensis* as the only cetacean species supporting this dolphin-derived charms trade (Siciliano et al., 2018).

Dolphins of the genus *Sotalia* are small-size odontocetes with geographical distribution restricted to Central and South America. The genus includes two species, *Sotalia guianensis*, a marine species limited to shallow coastal and estuarine habitats from Nicaragua to Southern Brazil, and *Sotalia fluviatilis*, a relatively smaller riverine species, exclusively found in the Amazon basin, including southern Peru, eastern Ecuador, southeastern Colombia, and northern Brazil (Flores et al., 2018) (**Figure 1A**). Both species are widely affected by incidental catch caused by the interaction with the intense fishing activity existing throughout their geographic range (Siciliano, 1994; Zappes et al., 2009; Meirelles et al., 2010; Iriarte and Marmontel, 2013a). Despite being fully protected by federal laws, intentional killing has been also documented for some regions of Brazil, related to their use as bait, in traditional medicine, and for human consumption (Alves and Rosa, 2008; Siciliano et al., 2008; Iriarte and Marmontel, 2013b; Barbosa-Filho et al., 2018, Dos Santos-Filgueira et al., 2021). In addition, *S. guianensis* and *S. fluviatilis* are intensely threatened by habitat degradation and pollution related to anthropic action, such as dam constructions and mining in the Amazon, besides city growth, boat traffic, and the destruction of mangroves and salt marshes in coastal areas (Flores et al., 2018; Secchi et al., 2018).

Genetic forensic researches, such as Siciliano et al. (2018), are significantly important to assess the species more vulnerable or affected by illegal human trade, mainly when this trading uses body parts with no diagnostic characteristics or when the commercialized species are more difficult to identify. This achieves greater importance for the conservation effort of species classified as endangered, which is the case of *Sotalia* genus in Brazil (Flores et al., 2018; Secchi et al., 2018; Da Silva et al., 2020). As additional support to these genetic investigations, morphological characterization of specimens, as well as age determination could be valuable tools, making it possible to obtain specific information about demographic groups and populations of species that have been more exposed to this human activity. As part of the market of dolphin-derived charms, dolphin teeth are another widely traded item in the Northern Brazil region, mainly as ornaments for handmade necklaces, earrings, and bracelets, and often sold in streets and traditional markets. The morphology of teeth from aquatic mammals has been used before in the identification of populations stock (*Stenella longirostris* - Akin, 1988), for identification of species (Brunner, 2004), and sexual morphometric differentiation in pinnipeds (*Otaria byronia* - Tarnawski et al., 2014), and for ecotype characterizations based in dental alterations related to use, as in the case of *Orcinus orca* (Ford et al., 2011). Furthermore, the dentine and cement from aquatic mammals' teeth are classified as recording structures, being shaped by growth layers with continuous deposition which allows accessing the exact age of individuals (Scheffer and Myrick, 1980; Hohn et al., 1989; Klevezal, 1996). These layers, named growth layer groups (GLGs), are cyclically deposited in parallel to the tissue formation's surface, creating a pattern that can be counted. The validation of 1 year – 1 GLG is needed in order to confirm this chronological correspondence, and has been widely demonstrated for several species (Hohn et al., 1989; Da Silva, 1994; Ramos et al., 2000; Read et al., 2018). This research aimed to identify teeth from *Sotalia* spp. found in handicrafts sold at traditional markets of Northern and Northeastern Brazil, and assess the more frequent age classes used in their production, and therefore, affected by the trading of dolphin's body parts.

MATERIALS AND METHODS

Sampling

Handicrafts, purchased by authors REL, AFC, and SS for this study, at five traditional or public markets from major cities of Northern and Northeastern Brazil were analyzed in addition to

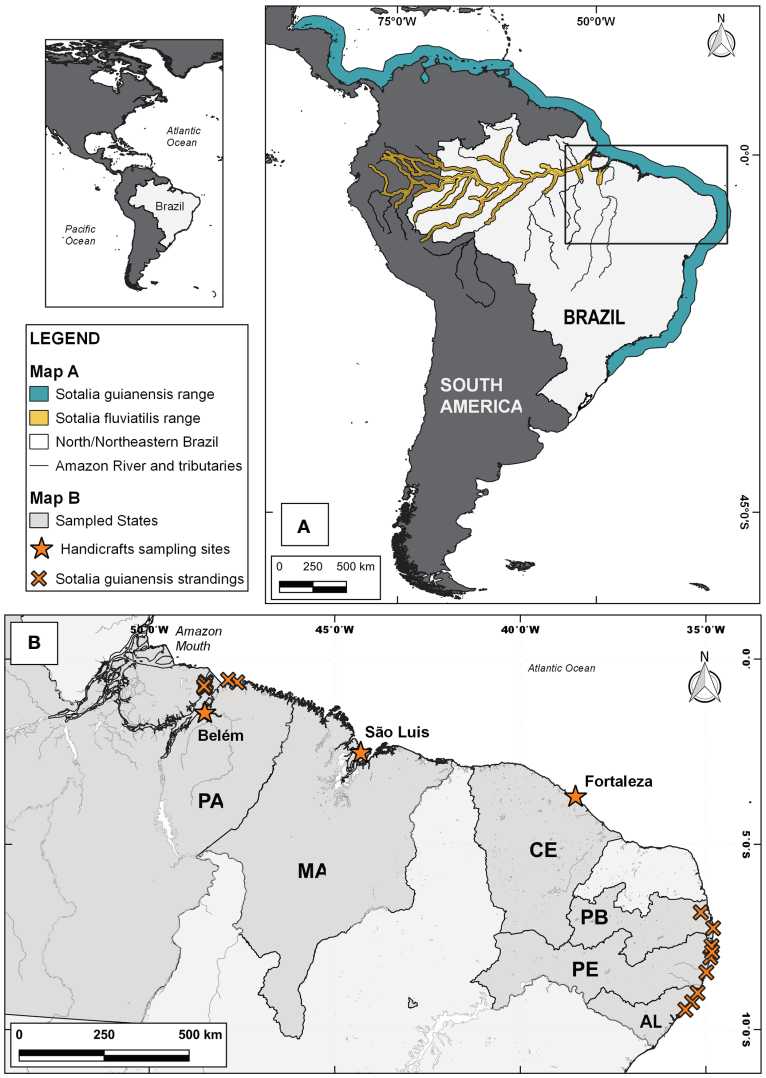

FIGURE 1 | Distribution range of the species from the genus *Sotalia* in South America (Map **A**) and sampling points in the Northern (Map **B**: PA - Pará State), and Northeastern (Map **B**: MA - Maranhão State, and CE - Ceará State, PB - Paraíba State, PE - Pernambuco State and AL - Alagoas State) regions of Brazil were handicrafts and stranded animals were collected. Distribution range data were extracted from NatureServe and IUCN (2021).

teeth obtained from stranded dolphins, used for comparison purposes only (**Figure 1B**). From these handicrafts, 118 teeth, being 99 acquired in Belém (Pará State), 4 in São Luís (Maranhão State), and 15 in Fortaleza (Ceará State), were used to assess the *Sotalia* spp. dolphins and the frequent ages commercialized in the trade of dolphin-derived artisanal objects (**Table 1**).

Data Classification

External characteristics of the teeth adorning necklaces, bracelets, and earrings (**Figures 2A–C**), were used to confirm the genus/species to which they belonged. Each tooth was visually compared with teeth from small cetaceans preserved in the mammal collection at the Museu Paraense Emílio Goeldi, in Pará, Northern Brazil. This collection includes ~600 specimens of Delphinidae recovered in Pará, same geographical region where teeth are commercialized. Based on visual inspection all teeth used in this study were similar to *Sotalia* sp. specimens from the referred collection. Furthermore, since two different sizes of teeth from adult specimens were detected in the sample (**Figures 2D, E**), four external measurements were used to assess possible differences in teeth morphology that indicated different species of *Sotalia* genus being used in the handicrafts (Ramos et al., 2000): tooth total length (TL), measured from the apical extremity of the crow to the end of the root, root diameter (RD) measured as the maximum width of the root, crown length (CL), measured from the apical extremity of the crown to the root cingulum, and cingulum diameter (CID), measured as a diameter of the tooth at the intersection area of the crown with the root. In addition, teeth from 10 *S. guianensis* specimens stranded at the coast of Pará State (PA) and 10 *S. guianensis*

TABLE 1 | Handicrafts collected in three major cities from the Brazil Northern and Northeastern regions.

Object	Place	City	State	Number of Teeth
Earrings 1	Mercado Central	São Luís	Maranhão	2
Earrings 2	Mercado Central	São Luís	Maranhão	2
Bracelet 1	Loja Artesanato da Amazônia	Belém	Pará	10
Bracelet 2	Loja Artesanato da Amazônia	Belém	Pará	10
Bracelet 3	Loja Artesanato da Amazônia	Belém	Pará	10
Bracelet 4	Loja Artesanato da Amazônia	Belém	Pará	11
Bracelet 5	Feira da Praça da República	Belém	Pará	9
Necklet 1	Loja Artesanato da Amazônia	Belém	Pará	26
Necklet 2	Mercado Ver-o-Peso	Belém	Pará	23
Necklet 3	Loja Praia de Iracema	Fortaleza	Ceará	15

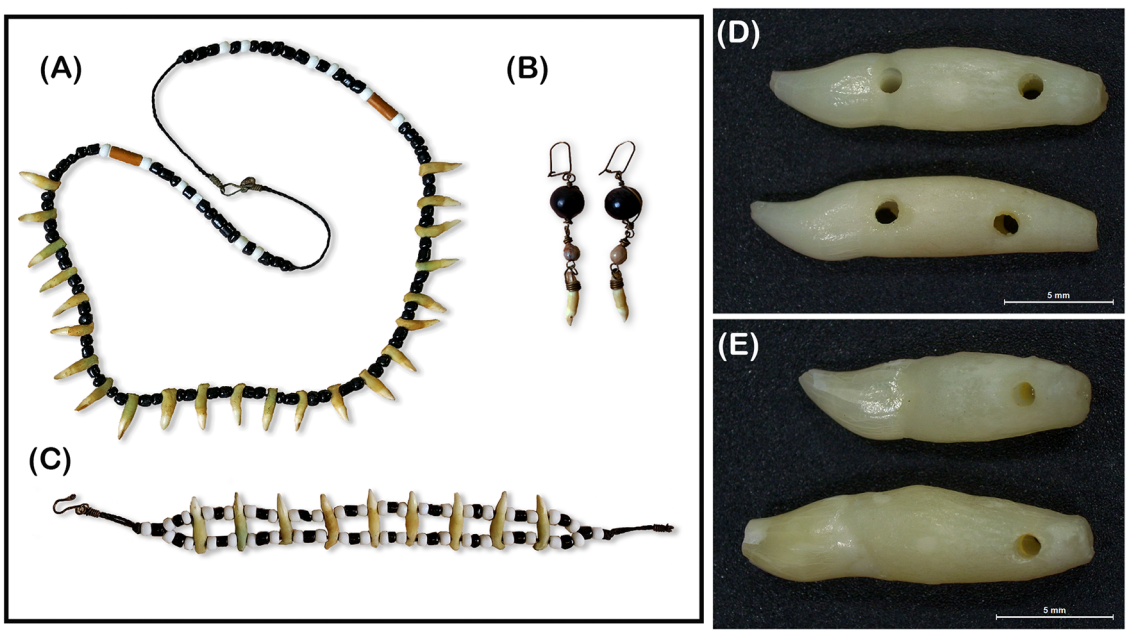

FIGURE 2 | Sample of handicrafts manufactured with teeth of *Sotalia* dolphins and commercialized in the trade of artisanal objects in the North-Northeastern of Brazil: **(A)** necklace, **(B)** earrings, **(C)** bracelet, **(D)** *Sotalia guianensis* teeth from a bracelet, **(E)** Two different types of *Sotalia* sp. teeth detected in the necklaces.

specimens stranded in the Northeastern region of Brazil (NE) (**Figure 1B**) were measured and used for further comparisons as two different control groups (see statistical analyses section). Measurements were taken with a caliper to the nearest 0.05 mm and only teeth relatively straight and with no notable wear of the crow were analyzed.

Age Determination

The age frequency of the handicrafts was assessed by counting the growth layer groups (GLGs) deposited in the teeth' dentine (**Figure 3**), and considering the described by Rosas et al. (2003). Each tooth was subsequently wearing, decalcified with RDO®, and cut with a manual freezing microtome in thin sections of 20-30 μm. Posteriorly, the thin sections were stained with Mayer's hematoxylin, blued in Ammonia 2%, and finally, mounted in glycerin 100% (Rosas et al., 2003; Conversani et al., 2020). After being mounted, thin sections were photographed using a Leica S9i Stereomicroscope with an attached digital camera, and the images were processed by Leica Application Software v4.12 (Leica Microsystems, 2018) with a maximum magnification of 55x at Mammal Ecology Laboratory, Universidade do Vale do Rio dos Sinos, Brazil. Readings were made in the images three different times by two researchers separately to determine the chronological age of each tooth. The GLGs were counted in the dentine, having in consideration a correspondence of 1 GLG and 1 year of life described previously for the genus *Sotalia* (Da Silva, 1994; Rosas et al., 2003; Di Beneditto and Ramos, 2004). In some cases, the final age was classified as minimum age because the perforations made for the craft construction coincided with the center of the pulp cavity (**Figures 2** and **3**), making it impossible to read the most recently deposited GLGs. The best age estimate was defined as the coincidental readings of at least two from the three times. In the case of a discrepancy ≤ 2 GLGs between readings, final age was considered as the mean of the three

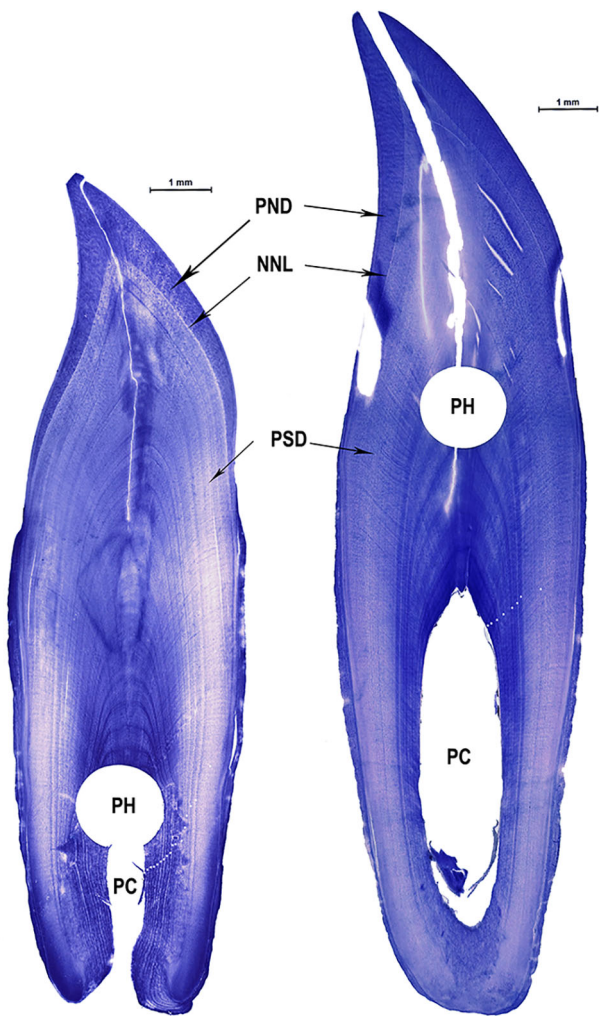

FIGURE 3 | Thin sections from two *Sotalia* sp. teeth collected from handicrafts (left: tooth N1-10 with TL= 13.16 mm; right: tooth B2-5 with TL=17.4 mm). PND, Prenatal dentine, NNL, Neonatal line; PSD, Postnatal dentine; PC, Pulp cavity; PH, Holes made for craft construction. White dots indicate the GLGs counts in the dentine corresponding to the age.

different ages. If a discrepancy between readers occurs, final ages were obtained by a consensus reading involving the two researchers. Readings with differences ≥ 3 GLGs were not considered in the analysis.

Finally, based on the age readings, teeth were classified as belonging to three different age categories: juveniles (age ≤ 6), young adults (6 < age ≤ 12), and adults (13 ≥ ages), taking into account that a) the age of 7 years as the age of sexual maturity for *Sotalia guianensis* (Rosas and Monteiro-Filho, 2002; Rosas et al., 2003) and b) the ages between 7 and 12 years as the range when occurs the complete fusion of the sutures of the occipital complex, concurring with the age of sexual maturity in *S. guianensis* and *S. fluviatilis* (Fettuccia et al., 2009; Novais et al., 2020).

Statistical Analyses

Since two species from the genus *Sotalia* are described in the literature (*S. guianensis* and *S. fluviatilis*), we intend to answer three questions through the statistical analysis. First, if two different groups of *Sotalia* sp. teeth were present in the collected handicrafts. Secondly, if some of these groups were more similar to teeth from *S. guianensis* specimens stranded in two different coastal localities of the sampling area (**Figure 1B**), and if these differences were influenced by age or if they were exclusively morphological differences. To this end, we included data from stranded specimens as two control groups (PA and NE), assuming that they adequately represented the morphological variation of the species *S. guianensis*, since individuals from populations with the smallest body size (PA region, Novais et al., 2020) and populations with the largest body size (NE region, Monteiro-Filho et al., 2002) described for the species were added. Finally, as a third question, we wanted to know what the most frequent ages in these products were for the detected groups.

Teeth from handicrafts were initially grouped through a cluster analysis using the four external measurements (TL, RD, RC, and CID) in order to find potential groups related to the tooth external characteristics of the two species of *Sotalia*. The dendrogram was constructed based on the unweighted pair group method using arithmetic means, UPGMA (Hale and Dougherty, 1988), and Euclidean distances (Podani, 2000) as a measure of dissimilarity. To evaluate the stability of the cluster, indicated by the representativeness of the structure of the data, a bootstrap resampling with 1000 iterations was performed and obtained the Jaccard coefficient of similarity between sets (Hennig, 2007). Cluster results were visualized including also the sampling localities to check if the geographical distribution of teeth was also related to the grouping.

Resulting groups from cluster analysis and teeth groups of stranded specimens (Tooth Type factor), the age category of teeth (Age class factor), and as dependent variables the four morphological measurements, were combined in a two-way multivariate analysis of variance (MANOVA). Also, the interaction between factors was evaluated in this analysis. Box-Cox transformation was used for two dependent variables (RC, and CID), to fulfill the MANOVA assumptions of homogeneity of the variance-covariance matrices and multivariate normality. Since multivariate significant differences were detected, we performed analyses of variance (ANOVAs) for each dependent variable. We used an *a posteriori* Tukey test to find out where the significant differences were in the ANOVA results. Likewise, to determine which morphological variables most influenced the composition of the teeth groups, a principal component analysis (PCA) was performed using also the four external measurements from teeth. Critical value for coefficients of correlation between variables and components, *i.e.* loading, was set to 0.5. In the graphical visualization of the PCA results, data were identified using the classification from the clusters, the two control groups, and the growth categories of teeth as a way to visualize the MANOVA results. Thus, to see if the distribution of the scores

had some relation with the age or were only influenced by the morphological variables indicating similarities in teeth characteristics in the groups, and therefore, a high probability to belong to the same species.

We used $p < 0.05$ as the critical p-value of statistically significant. Descriptive statistics such as Range, Mean, Median, Standard deviation (SD), and 95% confidence interval (CI) were also acquired. Finally, the age distribution of the sample was obtained using the age estimates, and differences in the age distribution between age classes and groups of teeth from handicrafts were described.

All statistical analyses and graphics were conducted in the free software RStudio v.1.4.1717 (RStudio Team, 2020). The packages used were *stat*, from software R v.4.0.1 (R Core Team, 2020), *car* v.3.0-12 (Fox and Weisberg, 2019), *ggdendro* v. 0.1.22 (De Vries and Ripley, 2020), *fpc* v. 2.2-9 (Hennig, 2020), and *factorextra* v. 1.0.7 (Kassambara and Mundt, 2020). Graphics were created using the packages *ggplot2* v.3.3.5 (Wickham, 2016) and *gridExtra* v.2.3 (Auguie, 2017).

RESULTS

Two clusters were generated by the analyses (Euclidean distance for separation = 3.843), indicating two different morphotypes of teeth from dolphins of the genus *Sotalia* in the sample (**Figure 4**). The Jaccard coefficients of the two clusters, 0.94 and 0.90, indicated good representability of the data structure by the dendrogram. The morphotype named as Type 1 included 67 teeth from handicrafts, while the morphotype named as Type 2 included 42 teeth. Related to geographical distribution, Type 1 morphotype was found in all sampled markets of Northern and Northeastern Brazil, while Type 2 was found only in two markets of Belém, the capital city of Pará State, placed at the southeastern Amazon Estuary (**Figure 4**).

Type 1 morphotype of teeth shows more similar dimensions to PA and NE control groups than Type 2 morphotype (**Figure 5**). The two-way MANOVA results indicated that measurements differed between the four groups of teeth (Type 1, Type 2, NE and PA) (df = 3, Pillai Trace = 1.080, approximate F = 15.900, p < 2.2e-16), age classes (juveniles, young adults, and adults) (df = 2, Pillai Trace = 0.283, approximate F = 4.600, p = 3.178e-05) and also between teeth morphotypes in relation to the ages classes (df = 6, Pillai Trace = 0.3698, approximate F = 1.900, p = 0.005). Individual ANOVAs detected statistical differences between groups of teeth for the measurements total length (TL), root diameter (RD), crown length (CL), and cingulum diameter (CID), while differences between the age classes were only related to root diameter (RD) (**Supplementary Table S1**). Type 1 morphotype were similar to PA control group in TL (Mean differences = -0.80, p = 0.152) and CID (Mean differences = 0.28, p = 0.971), and similar to NE control group in CID (Mean differences = 1.417, p = 0.109), and CL (Mean differences = -1.92, p = 0.052). Type 2 shows the smallest dimensions for the four measurements, being statistically different from the others groups of teeth in TL, CID and CL (**Figure 5** and **Supplementary Table S2**). Type 2 morphotype were also different from Type 1 and NE control group in RD, but similar to PA teeth (**Figure 5** and **Supplementary Table S2**). Related to the ages classes, juveniles (Mean differences = -0.35, p = 0.0001) and young adults (Mean differences = - 0.27, p = 0.0032) show smaller RD dimensions, being statistically different from adults (**Supplementary Table S2**).

Interaction between factors influenced the CID, with some age categories being different between the types of teeth, but always

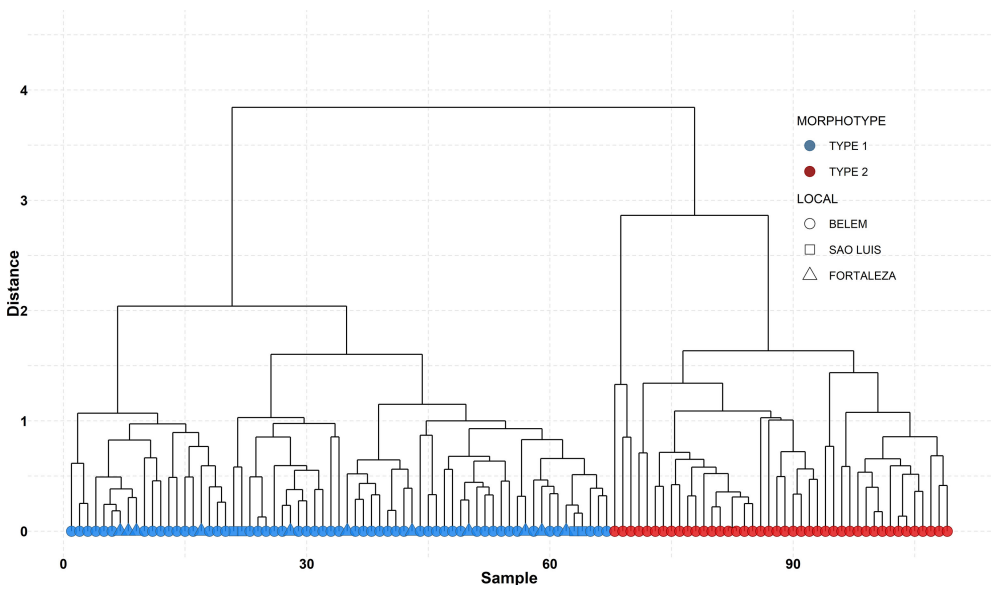

FIGURE 4 | Dendrogram from the clustering analysis showing the two principal clusters separating tooth types. Colors indicate the teeth morphotype groups and symbols indicate the sample's locality in the North and Northeast regions of Brazil.

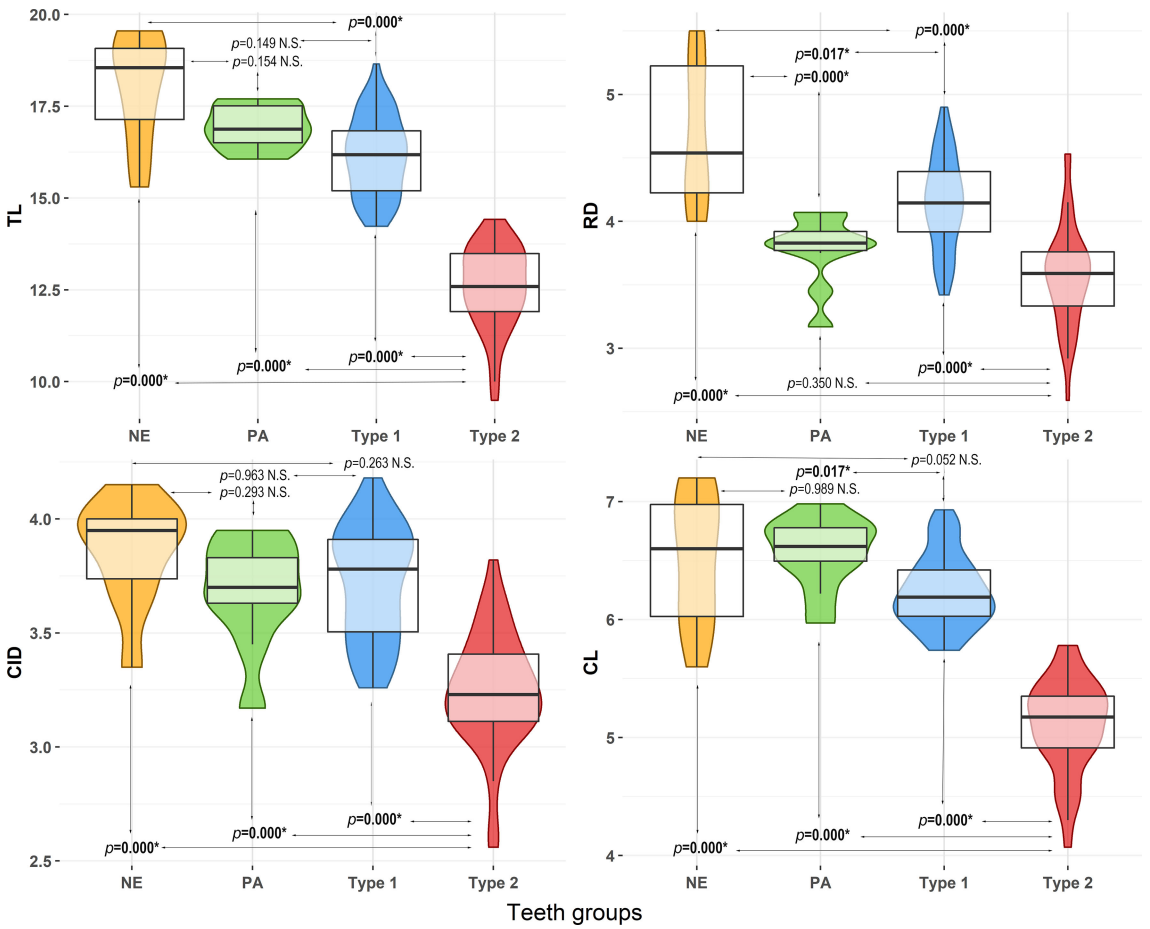

FIGURE 5 | Violin data representation and pairwise comparison between groups of teeth for the measurements of total length (TL), root diameter (RD), cingulum diameter (CID), and crown length (CL). Type 1 and Type 2 comprised groups of teeth resulting from cluster analysis and the localities of Pará coast (PA) and Northeast Brazil (NE) comprised only stranded specimens of *Sotalia guianensis* used as control groups. Box represents values between the first and third quartile, the horizontal dark line represents the median, and vertical lines inside the violin shapes represent the lowers and upper values. Bold and asterisk in p-values indicate significant differences from pairwise comparisons for p<0.05; N.S., indicates non-significant differences.

related with Type 2 morphotype having smaller dimensions (**Supplementary Table S2**). Thus, juveniles, young adults and adults teeth from Type 1 morphotype had, respectively, significant higher dimensions than juveniles (Mean differences = 2.14, p=0.00), young adults (Mean differences = 1.68, p=2.862E-03) and adults (Mean differences = 1.68, p=1.040E-05) from Type 2 morphotype. Juveniles teeth from Type 2 morphotype were also smaller than juveniles from PA (Mean differences = -2.42, p=8.267E-04) and NE (Mean differences = -2.46, p=1.210E-05) control groups, and smaller than adults from Type 1 morphotype (Mean differences =-2.19, p=0.00), PA (Mean differences= -1.90, p= 0.0162), and NE (Mean differences = -2.55, p=0.00) control groups. Young adult teeth from Type 2 were smaller than juveniles (Mean differences = -1.74, p= 0.0001) and adults (Mean differences = -1.81, p= 2.870E-05) from Type 1 morphotye, and juveniles (Mean differences = -2.13, p= 0.0038) and adults (Mean differences = -2.23, p= 1.660E-05) from NE control group. Finally, adults from Type 2 were smaller than juveniles (Mean differences = -1.61, p=5.600E-05) and young adults (Mean differences = -1.55, p=0.004) from Type 1 morphotype (Mean differences = -2.55, p=0.00), and juveniles (Mean differences = -2.02 p=0.008) from NE control group.

The PCA grouping shows a similar data structure to the clustering analysis with the first two dimensions explaining 87.1% of the data ordination (**Figure 6**; **Supplementary Table S3**). The first principal component (PC1) explained 72.9% ± 0.22 SD of the total variability. The measurement with higher loading for this PC was the TL (Loading = 0.6405), describing the size difference between the groups, followed by the CL but with a smaller correlation (Loading = 0.4818). The PC2 explained only 13.8% ± 0.09 SD of the variance, being more related to the DR (Loading = 0.8172), indicating an increase in diameter through the age categories (**Figure 6**) and supporting the differences between adults and the others ages classes founded in the ANOVA analysis.

Ages of 116 teeth adorning the handicrafts were possible to obtain, with 64 teeth bellowing to Type 1, 42 bellowing to Type 2,

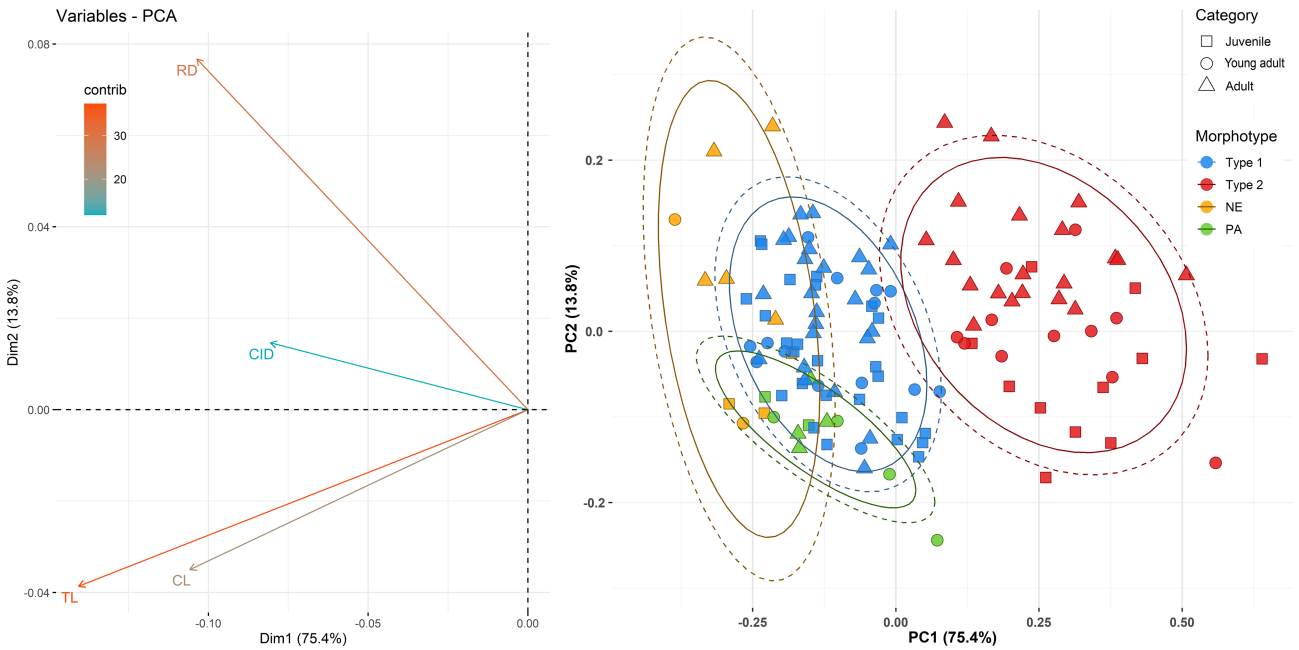

FIGURE 6 | Principal component analysis for teeth measurements obtained from samples of *Sotalia* dolphins from North and Northeastern Brazil. Left: Contribution of the variables total length (TL), root diameter (RD), crown length (CL), and cingulum diameter (CID) to the principal component dimensions (PCs). Right: Scores distribution in the two dimensions of the PCs. The category indicates juveniles, young adults, and adults in the sample, and colors indicate the teeth morphotypes or species. The localities of Pará coast (PA) and Northeast Brazil (NE) included only stranded specimens of *Sotalia guianensis*.

and 10 teeth with no classification. The total sample showed a diverse age composition, ranging between 2 and 30 years (Mean =11 ± 7 SD, Median = 10, Mode = 3, 4, 8, and 19 years). Some teeth from the ages 25, 28, and 29 years were aged as minimal age because of the loss of part of the pulp cavity in the construction of handicraft (see **Figure 2**), thus probably being older than was detected. Juveniles dolphins (ages ≤ 6) were 34% of the teeth sample, while young adults (6 < ages ≤ 12) corresponded for 27% and adults (ages ≥ 13) for 41%. Where the sample was split by teeth morphotype, Type 1 was composed of 40% juveniles, 22% by young adults, and 38% by adults. The type 2 sample was divided into 24% of juveniles, 25% of young adults, and 49% of adults. Age composition for the teeth classified as Type 1 ranged between 2 and 30 years (Mean =10 ± 7 SD), with the age class of 3 years being the most frequent in this group (17% of the sample), followed by the ages of 4 and 13 years which represented in both cases 11% of the sample. The ages of teeth classified as Type 2 ranged between 4 and 28 years (Mean=14 ± 6 SD), with the ages of 5 (12%) and 16 years (10%) being the most frequently observed. Adults, between the ages of 7 and 20 years, represent, respectively, 52% and 63% of Type 1 and Type 2 morphotypes. The distribution of ages for both morphotypes of teeth showed a multimodal frequency distribution (**Figure 7**; **Supplementary Table S4**).

DISCUSSION

Two morphotypes of teeth, presumably belonging to both species from the genus *Sotalia*, *S. guianensis*, and *S. fluviatilis* were found on handmade necklaces, bracelets, and earrings sold in public markets of three main cities of the North and Northeast coast of Brazil. Age distribution of teeth indicated that juveniles and young adult and adult dolphins, between the age of 2 and 8 years, and between the age of 11 and 20 years, are more used in these trades since were the most frequent ages observed in the different handicraft objects.

Some aspects of geographical variation in morphology of the genus *Sotalia* have been more frequently investigated, mainly related to general body size and osteology (Borobia, 1989; Monteiro-Filho et al., 2002; Fettuccia et al., 2009; Ramos et al., 2010; Fettuccia, 2010; Fettuccia et al., 2012; Arcoverde et al., 2014f). For these results, it is confirmed a variation in body length and cranial size for *S. guianensis* on the Brazilian coast, with the smallest individuals habiting the Amazon estuarine region, and a gradual increase in size for populations inhabiting coastal areas from more Northeastern, Southeastern and Southern regions (Fettuccia, 2010; Emin-Lima, 2012; Arcoverde et al., 2014). Additionally, *S. fluviatilis*, the riverine species of the genus, is described as significantly smaller in body dimensions compared to *S. guianensis*, with slower body development and with the preservation of some pedomorphic characters (Fettuccia et al., 2009; Fettuccia, 2010). In our study, it was possible to detect this geographic variation in size in the teeth collected from the handicrafts and stranded animals. Thus, teeth of stranded specimens from the Northeastern Brazilian region (NE), had the highest dimensions, while teeth from specimens stranded in the coastline of Pará state (PA), North

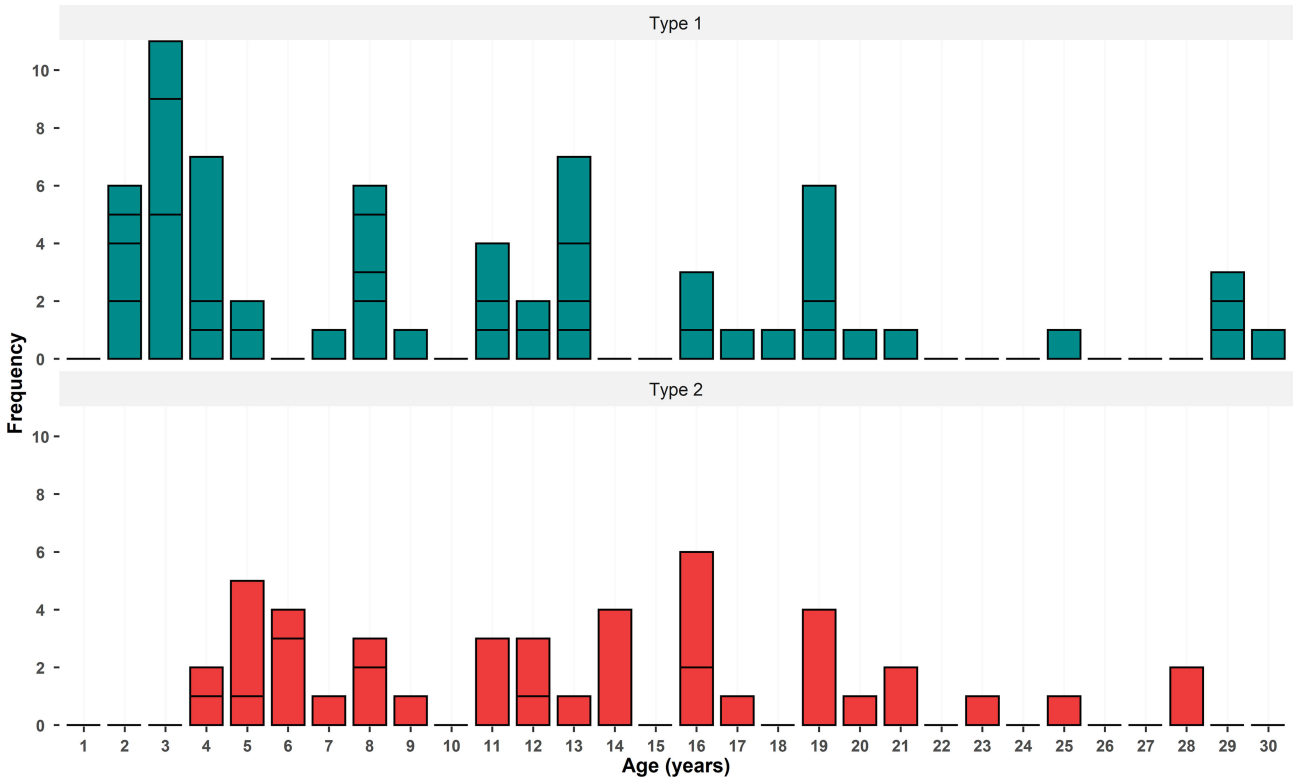

FIGURE 7 | Age distributions for the two morphotypes of *Sotalia* spp. dolphin teeth detected in the trade of handcrafted objects in the North and Northeast regions of Brazil. Branches indicated the frequency of each age class in the handicraft objects.

region, were relatively smaller in size, but with no statistical difference between the two regions for the majority of measurements. Overlapping with these two groups, the teeth classified as Type 1 showed a similar interval of size, although being statistically different from those collected in Northeastern Brazil in total length and root diameter. On the other hand, the Type 2 morphotype, detected in two of the handicrafts, showed evident smaller dimensions being separated from the other three groups in the ordination analysis and with a statistically significant difference in almost all measurement comparisons. Furthermore, in the only variable where the interaction of age classes and tooth type was identified (CID), the results indicated that all age classes of the Type 2 morphotype were mostly smaller than the age classes of other tooth groups, and no differences were found within any group. Thus, suggesting that the differences found are related to the morphological characteristics of the groups and not to possible modifications throughout growth. In addition, the Type 2 morphotype was found only in the Southeastern Amazon Estuary region, while Type 1 was found in all North-Northeastern localities. Based on these results it is suggested that the Type 1 teeth morphotype belongs to the species *S. guianensis*, while the Type 2 morphotype of teeth belongs to the riverine species *S. fluviatilis*.

The use of body parts of *Sotalia* dolphins has been widely reported in South America. In Brazil and Venezuela, estuarine dolphins *S. guianensis* accidentally captured in gillnet are commonly used for human consumption and bait in longline-fishing or shark-related-fishing (Siciliano et al., 2008; Briceño et al., 2021). In addition, some practices related to Amazon traditional culture from northern Brazil, *i.e.*, the use of dolphin parts as love's charms (Siciliano et al., 2018), have been linked to the increased trade of genitals, eyes, dorsal fin, skin, and teeth of Amazon dolphin species in traditional markets (Siciliano, 1994; Da Silva and Best, 1996; Siciliano et al., 2008; Sholl et al., 2008). More recently, forensic genetics results have indicated that *S. guianensis* is the most used cetacean in the majority of dolphin's love charms sold in traditional Brazilian markets, including the markets in the cities of Belém, São Luís and Fortaleza sampled in our research (Gravena et al., 2008; Sholl et al., 2008; Siciliano et al., 2018). Thus, our results are consistent with the geographical distribution of this trade in Northern and Northeastern Brazil and reveal specific segments of *S. guianensis* populations being more frequently used.

For the riverine species *S. fluviatilis*, its use as bait principally for the piracatinga (*Calophysus macropterus*) fishery, has been documented in the Amazonian regions of Peru and Brazil (Brum et al., 2015; Campbell et al., 2020), and medicinal and magical religious use has been also reported in Brazilian, Peruvian and Bolivian Amazon region (Alves et al., 2013). Based on the analysis of genetic markers on the tissue samples from love's charms acquired in Brazilian traditional markets, Siciliano et al. (2018) did not detect the use of *S. fluviatilis* individuals in this

kind of trade. However, handicraftsmen who were carrying a notable quantity of *Sotalia* teeth, interviewed near Belém, Pará, reported to the authors that they had acquired the teeth from several fishing communities in their trip through the Amazon. Therefore, it is possible the presence of *S. fluviatilis* teeth brought from riverine regions in handicrafts sold at public markets of Belém or another coastal region of the southeastern Amazon. Siciliano et al. (2018) also described those necklaces and bracelets made with *Sotalia* teeth were found in traditional open-air market squares from Belém and were the only charm found in street markets from Fortaleza, Ceará State. In this context, it could be possible that the trading of teeth and handicrafts made with them, would take a different path to these sites, more related to these travelers selling handicrafts at squares and street markets, and not with the traditional trading of other dolphin body parts. This fact could also explain the absence of *S. fluviatilis* in the love's charm samples from the study conducted by Siciliano et al. (2018).

The age distribution obtained in our study indicated that teeth from juvenile individuals, between the ages of 2 and 6 years, and young adults and adults, between 8 and 19 years, are more frequently used in handicrafts. Both *Sotalia* species show this pattern of age distribution, being more evident in *S. guianensis*. Although some of the teeth may be from the same individuals, we believe that the repetition of ages on the different objects collected indicates a recurrence of animals of these age classes as a source of teeth for handicrafts. In addition, no teeth of specimens younger than two years old were found, maybe because crafts with smaller or finer teeth can be more difficult to manufacture and/or because teeth providing this trade could be collected from specimens accidentally caught in fishing gillnets, being newborns and yearlings probably discarded or less affected by this activity. This last hypothesis needs further confirmation. However, some inferences are possible to be made. In marine mammal populations under a stable age distribution, natural mortality rates are expected with the greatest frequency of yearlings (animals < 1year), followed by juveniles and adults with much less frequency (Reilly and Barlow, 1986; Barlow and Boveng, 1991; Mannocci et al., 2012). When mortality is affected by the action of bycatch (where animals are accidentally caught in gillnets during fisheries), the expected age distribution changes because not all ages are equally affected in the process (Moore and Read, 2008; Mannocci et al., 2012). Thus, an increase in juveniles' and young adults' mortalities relative to the other age classes is observed. This had been detected in different dolphin populations, such as striped dolphin (*Stenella coeruleoalba*) and common dolphin (*Delphinus delphis*) in the Northeast Atlantic Ocean (Mannocci et al., 2012; Brown et al., 2014), and common bottlenose dolphins (*Tursiops truncatus*) in North Carolina coast (Byrd and Hohn, 2017).

On the North Pará coast, *S. guianensis* is the most frequent marine mammal found stranded, as a result of its interactions with local fishing activity (Siciliano, 1994; Siciliano et al., 2008; Costa et al., 2017). The use of dolphin carcasses, stranded and/or incidentally captured in fisheries as a source of products found in regional markets of Northern Brazil has been previously suggested (Siciliano, 1994; Da Silva and Best, 1996; Sholl et al., 2008; Siciliano et al., 2018), as well as its use as bait or for human consumption (Siciliano, 1994; Tosi et al., 2009; Zappes et al., 2009; Dos Santos-Filgueira et al., 2021). Moreover, studies on *S. guianensis* bycatch for populations of Northeastern, Southeastern, and Southern Brazil indicated that juveniles and adult individuals, with body length superior to 160 cm (> 6 years old), have more vulnerability to by caught in fishing nets representing until 80% of the captures (Rosas et al., 2003; Di Beneditto and Ramos, 2004; De Moura et al., 2009; Meirelles et al., 2010; Lima et al., 2016). For *S. fluviatilis*, despite interactions with fishing activities are extremely difficult to record, punctual data of bycatch and intentional catches in Western Brazilian Amazon indicated a high incidence of juveniles (of ~138 cm of body length) and adults (reproductively matures animals) captured in tambaqui (*Colossoma macropomum*) and pirapitinga (*Piaractus brachypomus*) fishing gillnets (Da Silva and Best, 1996; Iriarte and Marmontel, 2013a). Furthermore, Iriarte and Marmontel (2013b), raised concern about the use of Amazonian River dolphin carcasses as a productive cost-effective means of obtaining bait for fishermen. The cost, viability, and effectiveness as drivers for the use of dolphins as bait by fishermen have been recently confirmed on the Peruvian coast, where similar human-related pressures increased the mortality of coastal small cetaceans (Campbell et al., 2020).

Whether the origin of our sample is from stranded or accidental capture, remains unknown. However, based on our results, and having into consideration the most frequent age categories observed in our sample, juveniles and adults younger than 20 years, it is possible to suggest that handicrafts commercialized as part of dolphin charm trade in the North and Northeast of Brazil and *Sotalia* dolphins' bycatch seems to affect the same population segment, and they are probably related in this region. Public policies and conservation actions have been encouraged in Brazil to reduce dolphin bycatch. However, the real impact of this activity on populations of *Sotalia* spp. dolphins from Northern-Northeastern Brazil are still unknown. Ethnoecological studies with fishermen's communities indicated a high incidence of this anthropic action in the region, with animals being mostly discarded and finally stranding at the coast (Siciliano, 1994; Brito, 2012; Dos Santos-Figueira et al., 2021). The overlapping in the occurrence of the dolphin population's groups with preferential areas for gillnet placements has been suggested as one of the principal causes of the high incidence of bycatch (Martins et al., 2004; Beltrán-Pedreros and Petrere, 2010). In this scenario, the sale of body parts could be both, indicative of the high mortality of these species in the region, and a pressure that can influence this mortality, increasing the vulnerability of the local dolphin populations.

In summary, our study attempted to detect different cetaceans teeth used in the confection of handicrafts traded as artisanal dolphin-derived products in North and Northeastern Brazil using linear morphometry as a tool. We detected two different morphotypes of teeth from the genus *Sotalia* in the handicrafts,

presumably belonging to the species *S guianensis* and *S. fluviatilis*. However further studies are needed to confirm this inference. In addition, we see the necessity of future researchers related to cetaceans' tooth morphology, that confirm the use of teeth as an effective tool for distinguishing species where other data is unknown. We also attempted to access the segments within the *Sotalia* spp. populations more used in this trade by determining the age of the teeth. We found that juveniles and adults younger than 20 years are more frequently used in these handicrafts ornamented with teeth, coinciding with the age categories that have the highest incidence of cetaceans mortality related to bycatch in this region. Thus, the technic of age determination showed its applicability as a tool in gathering information about human actions, directly and indirectly, affecting specific groups of coastal populations of *Sotalia* dolphins in Northern and Northeastern Brazil. In addition, this result raises concerns about the increased vulnerability of the two species, but especially related to the threatened species *S. fluviatilis*, as scarce data have been reported on their life-history traits and only one age-related research was found available. Finally, we believe that this improved data could allow for more realistic management policies to help in the conservation of the two *Sotalia* species in the Amazon region, guiding enforcement actions, and improving environmental education with traders and local fishermen. However, it is imperative to assess the real impact of fisheries and bycatch on the *S. guianensis* population of the Northern Brazilian coast, as a way to understand how the population has been affected over the years and how this trade is supplied.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because teeth used in this study were collected from stranded carcasses or from handicrafts sold at local markets. Sampling permits were granted under licenses numbers No. 30327-1 and No. 54305-1.

AUTHOR CONTRIBUTIONS

GFR, LRO, and SS: study design, data analysis, and writing the manuscript. RE-L, AC, FA, FL, and SS: fieldwork. GFR: Methodology and data curation. GFR, LRO, and SS: Supply reagents, materials, and analysis tools. GFR, LRO, RE-L, AC, FA, FL, and SS: reviewing and editing the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.867068/full#supplementary-material

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