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Tooth wear and dental pathology in Amazon River dolphins (*Inia geoffrensis*) and tucuxis (*Sotalia fluviatilis*)

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Abstract

The investigation of tooth wear and pathology in freshwater dolphins can increase our understanding of their life history, including interactions with the environment and impacts of disease and morbidity. This paper evaluated the occurrence, prevalence and discussed the putative etiology of dental wear and pathology in freshwater dolphins - tucuxi (*Sotalia fluviatilis*) and the Amazon River dolphin (*Inia geoffrensis*) - from the central Amazon basin. Teeth of 29 Amazon River dolphins and 14 tucuxis were visually inspected to identify wear facets and the presence of pathology. Dental wear was observed in 55% (n = 16) of the Amazon River dolphin and 79% (n = 11) of the tucuxi specimens. For both species, superficial wear restricted to the tooth crown was more frequent. Calculus deposits were observed in both species, occurring in 14% of tucuxi (n = 2) and 41% of Amazon River dolphin (n = 12) specimens. Caries-like lesions were observed in a third of Amazon River dolphin specimens (n = 10), affecting 10% of the teeth (n = 197). Gross caries was the most commonly caries type observed. Only one tucuxi had caries-like lesions, affecting 1.6% of the teeth (n = 13). While conditions such as tooth wear arise from normal physiological processes, severe wear and pathology such as caries and calculus can contribute to further disease development and morbidity, impacting the health of the animals. Further studies using materials from museum collections in other regions from the Amazon Basin will help elucidate the occurrence, etiology, and health impact of tooth wear and dental pathology in freshwater cetaceans, contributing to our growing understanding of their life history.

Keywords:
abrasion, calculus, caries, dental wear, pigmentation, river dolphins

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Introduction

Two endemic species of cetaceans inhabit the Amazon basin: the tucuxi (*Sotalia fluviatilis*) and the Amazon River dolphin or boto (*Inia geoffrensis*). These species occur in sympatry along most of their distribution (Best & da Silva, 1989). Tucuxis and Amazon River dolphins are found in channels, lakes and rivers; however, Amazon River dolphins can also be observed within flooded forests and river rapids (Best & da Silva, 1989; Borobia et al., 1991). Amazon River dolphins and tucuxis are listed as Endangered by the IUCN Red List, with main threats related to hunting for bait, entanglement in fishing gear and modifications to their natural habitats such as damming of rivers and environmental pollution (da Silva et al., 2018, 2020, 2023). Density and abundance estimates for both species vary greatly between areas studied, and there are no estimates of total population size range-wide for either species (da Silva et al., 2018, 2020).

Due to increase in fishing activities in the Brazilian Amazon region, accidental entanglement in fishing gear (da Silva & Best, 1996; Iriarte & Marmontel, 2013b), direct conflict and competition with fishermen (da Silva & Best, 1996; Loch et al., 2009), and use of dolphin carcasses as bait in fisheries (Iriarte & Marmontel, 2013a; Brum et al., 2015) have contributed to increased mortality of freshwater dolphins in recent years. Eventually, some of deceased animals end up being collected, prepared, and curated by local museums and other institutions, becoming an important research resource for these elusive species.

As most living cetaceans, tucuxis and Amazon River dolphins have simplified, monophyodont and numerous dentitions. Tucuxi dolphins have homodont, conical and peg-like teeth, as common with many small delphinids. Tucuxi dolphins have 29 - 35 teeth per quadrant (Fettuccia, 2006). The Amazon River dolphin has 24 - 34 teeth per quadrant, which are covered by a rugose layer of

enamel (Flower, 1867; Ness, 1966). Different from other cetaceans, the posterior teeth of Amazon River dolphins have a broad lingual lobe at the base of the crown while anterior teeth are conical (Flower, 1867). Some authors interpret this as a form of pseudo-heterodonty (Flower, 1867; Ness, 1966). For both species, as in most cetaceans, the upper and lower teeth fit closely into the interspaces of the opposite series, but generally do not occlude to masticate food as in most terrestrial mammals. This means their teeth are important in hunting and holding onto prey but have limited function in food processing (Ungar, 2010). Amazon River dolphins and tucuxis are generalist species, preying mostly on freshwater fish. Remains of 43 species of fish have been found on Amazon River dolphin stomachs, including armoured catfishes. Other hard prey such as river turtles and crabs are also consumed, likely aided by their specialised posterior teeth (Best & da Silva, 1993). Tucuxis have been found to consume up to 28 species of fish, with greater diet diversity during the low water season (da Silva & Best, 1996).

Many pathological and physiological conditions can affect the teeth of dolphins and other mammals. Dental wear is a multifactorial process involving four synergistic components: attrition (*i.e.* tooth-to-tooth contact), abrasion (*i.e.* friction against exogenous materials), erosion (*i.e.* chemical acid dissolution) and abfraction (*i.e.* microstructural loss of dentine due to stress) (Grippo et al., 2004). Dental wear increases as age progresses and its occurrence is affected by the consistency of the diet and other parafunctional uses of teeth (Grippo et al., 2004; Ungar, 2010). In addition to dental wear, other conditions such as dental pathology and developmental abnormalities can be observed in the teeth of dolphins and other wild animals. These conditions are often result of endogenous or systemic causes, such as infections and nutritional deficiencies, or are related to exogenous agents, such as bacterial and non-bacterial hard tissue loss by acid dissolution and mineral deposition following dental plaque formation (Brooks & Anderson, 1998; Loch et al., 2011).

The study of dental wear and pathology in the teeth of dolphins is relevant because it can increase our understanding of their life history, including interactions with the environment and impacts of disease and morbidity. Due to their elusive behavior and logistic difficulties with studies in the wild, the assessment of deceased-stranded specimens of Amazon River dolphins and tucuxis can provide much needed information on conditions of health and disease of these species. Such opportunistic observations can complement more thorough capture-release programmes, which in turn are much more expensive and logistically challenging to conduct. This paper evaluated the occurrence, prevalence and discussed the putative etiology of dental wear and pathology in freshwater dolphins from the Amazon basin.

Material and Methods

Teeth of 29 Amazon River dolphins and 14 tucuxis were analysed (n = 43; Appendix). Specimens were deposited in the Instituto de Desenvolvimento Sustentável Mamirauá (IDSM) Aquatic Mammals' collection (Tefé, Amazonas, Brazil), and consisted of skeletons, skulls, and teeth of deceased-stranded or accidentally entangled animals. Specimens were opportunistically collected from 1991 to 2005 in the middle Solimões River area, mainly in the Mamirauá and Amanã Sustainable Development Reserves near the Tefé township. Upon collection, carcasses were measured, sexed and identified at species level. Specimens were labelled with collection number, species, and collection year. Collected specimens were prepared via water maceration and teeth were preserved either dry or immersed in 70% alcohol.

Figure 1. Tooth wear in the Amazon River dolphin *Inia geoffrensis*. Superficial wear (Index 1; left) and Moderate wear (Index 2; right).

Individual teeth were visually inspected by a trained dental researcher to identify wear facets and the presence of pathology. Dental wear intensity was scored based on an estimated percentage of loss of the tooth crown area, following Loch & Simões-Lopes (2013). In brief, superficial wear (Index 1) concerned wear restricted to the enamel and superficial dentine, with less than 10% of crown area loss. Moderate wear (Index 2) resulted in exposure of deeper layers of dentine, with 20 - 50% of the crown area lost. Severe wear (Index 3) resulted in more than 50% of crown area worn, sometimes reaching the cingulum and root and potentially resulting in pulp exposure. The location of wear facets (apical, lateral, or apical/lateral wear facets combined), anatomical extent (wear restricted to the crown, or extending to cingulum or root) and the presence of fractured teeth were also recorded. The identification of abnormalities and dental pathology followed Loch et al. (2011) and included the presence of calculus deposits, caries-like lesions and exogenous pigmentation of the tooth crown.

Results

Dental wear

Dental wear was observed in 55% (n = 16) of the Amazon River dolphin specimens analysed, affecting 37% (n = 737) of the teeth evaluated (Fig. 1). For the tucuxi, 79% (n = 11) of the animals had worn teeth, which represented 65% (n = 521) of the teeth examined. Superficial wear (Index 1 *sensu* Loch & Simões-Lopes, 2013) was more frequent than moderate and severe wear for both species (Table 1). Although both species had a similar proportion of teeth worn moderately (Index 2 *sensu* Loch & Simões-Lopes, 2013), the Amazon River dolphin had more severe wear than the tucuxi (14 vs. 7%). For both species, wear restricted to the tooth crown was more frequent than wear down to the cingulum or root

Table 1. Total number of teeth of Amazon River and tucuxi dolphins analysed, number and percentage of teeth worn and number and percentage of teeth in the different wear categories (following Loch & Simões-Lopes, 2013).

Species	Total number of teeth analysed	Number of teeth worn	Wear intensity			Location			Anatomical extent			Fractured teeth
			Superficial	Moderate	Severe	Apex	Lateral	Apex/Lat	Crown	Cingulum	Root	
Inia geoffrensis (n= 29)	1988	737 (37%)	569 (77%)	66 (9%)	66 (9%)	371 (50%)	75 (10%)	291 (39%)	556 (75%)	64 (9%)	117 (16%)	29 (1%)
Sotalia fluviatilis (n= 14)	800	521 (65%)	434 (83%)	49 (9%)	49 (9%)	63 (12%)	227 (44%)	231 (44%)	403 (77%)	52 (10%)	66 (13%)	5 (1%)

Figure 2. Calculus deposits. a) Subgingival calculus in the cingulum of Amazon River dolphin *Inia geoffrensis* (IDSM Ig 9404). b) Subgingival calculus in the root of tucuxi *Sotalia fluviatilis* (IDSM Sf 2005-02).

(Table 1). Lateral and apical/lateral wear were more frequent for the tucuxi, while apical and apical/lateral wear more common for the Amazon River dolphin (Table 1). Fractured teeth were uncommon in the sample, being observed in about 1% of the teeth examined for both species (n = 29 for the Amazon River dolphin and n = 5 for the tucuxi).

Dental pathology

The main pathologies observed among the specimens studied involved cases of calculus deposits, caries-like lesions and exogenous pigmentation. Calculus deposits were observed in both species, occurring in 14% of the teeth of the tucuxi (n = 113) and 22% of the teeth of the Amazon River dolphin (n = 428) (Table 2). In the tucuxi, most calculus deposits were in the cingulum region (65%; n = 73), followed by root deposits (25%; n = 28) (Fig. 2b). Almost all teeth with calculus in the Amazon River dolphin had cingulum deposits (99%; n = 426) (Fig. 2a). Caries-like lesions were observed in a third of Amazon River dolphin specimens analysed (n = 10), affecting 10% of the teeth (n = 197) (Table 2). Caries-like lesions were either small to moderate in size (< 5 mm in diameter), or gross (> 5 mm, often occupying the whole crown surface) (Fig. 3a - c). Gross caries represented 69% of the carious teeth in Amazon River dolphins, while small to moderate caries were less frequent. Only one tucuxi individual had caries-like lesions, affecting 1.6% of the teeth analysed (Fig. 3d). Exogenous pigmentation of the tooth crown was common in the tucuxi, being observed in 78% of the teeth examined (n = 626) (Table 2). The tooth crowns of the Amazon River dolphin are already naturally dark coloured; thus, no further pigmentation was observed.

Discussion

Dental wear was common in Amazon River dolphins and tucuxis, occurring in most of the specimens analysed for both species. Despite being common, most dental wear recorded was

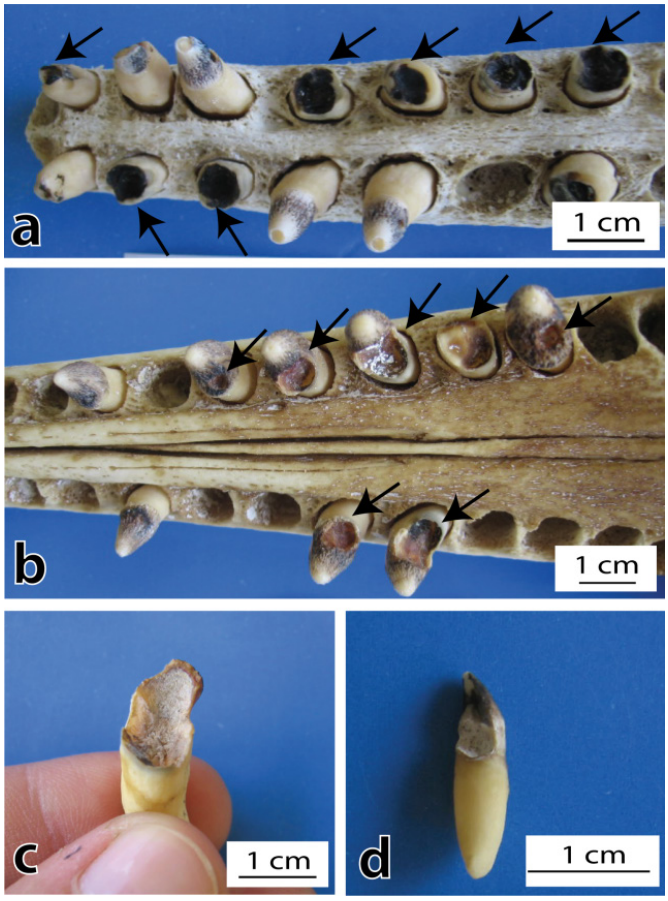

Figure 3. Caries-like lesions in Amazon River dolphin *Inia geoffrensis*. a) Gross caries (arrows) in the mandible of specimen ISDM 2005-06. b) Gross caries (arrows) in the maxilla of specimen ISDM 2005-05. c) Close-up view of gross caries in specimen ISDM Ig 9505, showing evidence of destruction and softening of dental tissues. d) Interproximal caries in tucuxi *Sotalia fluviatilis* (specimen IDSM Sf 2005-02).

superficial, affecting the enamel and superficial layers of dentine. Dental pathology such as calculus deposits, caries-like lesions and exogenous pigmentation were also recorded, with varied prevalence in both species.

Tooth wear is the loss of dental hard tissues which is not caused by trauma or due to acids of bacterial origin (Grippo et al., 2004). Dental wear has been recorded in a range of marine dolphins, with causes commonly linked to attrition and abrasion (Ford et al., 2011; Loch & Simões-Lopes, 2013). Chemical wear by acid erosion has also been reported in marine odontocetes, including orca *Orcinus orca*, bottlenose dolphins *Tursiops truncatus*, and Guiana dolphins *Sotalia guianensis* (Loch et al., 2013). Alongside mechanical wear by attrition and abrasion, chemical wear by erosion also contributes to tooth tissue loss in mammals, including odontocetes. In this study, tooth wear was prevalent in both species, being recorded in more than a third of Amazon River dolphin and more than half of tucuxi teeth analysed. However, for most specimens, wear was superficial and located in apical and lateral faces of teeth, similar to what has been reported in other odontocetes (Loch & Simões-Lopes, 2013). Superficial apical and lateral wear as observed in Amazon River dolphins and tucuxis is related to tooth-to-tooth contact during jaw closure and abrasion from piercing and grasping food items. Although tooth wear is a common physiological process in humans and other mammals, severe wear can result in pulp exposure which can lead to pain, inflammation and periapical abscesses, generating morbidity which impacts the health of the animals (Kaidonis, 2008; Kierdorf et al., 2019). Extreme examples of such severe levels of wear are known from captive orcas which often need tooth drilling to remove infected pulp tissues (Jett et al., 2017).

Calculus deposits, caries-like lesions and exogenous pigmentation of the crown were also diagnosed in both species. Calculus deposits are a mineralised evidence of periodontal disease, a chronic inflammatory condition leading to recession of the gums, bone loss, and eventually edentulism, both in humans and other mammals (Niemiec, 2008; Oz & Puleo, 2011; Nazir, 2017). Periodontal disease is preceded by gum inflammation from the accumulation of plaque biofilms; plaque mineralised over time forms hardened dental calculus (Oz & Puleo, 2011; Akcalı & Lang, 2018). In this study, calculus deposits were observed in less than a quarter of analysed teeth for the Amazon River dolphin and tucuxi. Dental calculus has been described in marine odontocetes (Van Bressem et al., 2007; Loch et al., 2011), including the Guiana dolphin, closely related to the tucuxi. In her age estimation study, da Silva (1996) mentioned the occurrence of calculus rings under the gum line for the Amazon River dolphin; calculus was also observed by Ness (1966) in Amazon River dolphin specimens from the British Museum of Natural History. Interestingly, Flynn et al. (2013) described mineral deposits consistent with dental calculus which provided an indication of localized periodontal disease in a Miocene odontocete, suggesting periodontal disease also occurred in fossil cetaceans. In humans and other mammals, the formation of calculus deposits is dependent on the mineralisation of dental plaque via calcium and phosphate from the saliva; however, little is known on the formation of dental plaque in odontocetes. Similarly, the source of minerals for calculus formation is also unknown since saliva production is interpreted as negligible due to rudimentary salivary glands in odontocetes (Cozzi et al., 2016).

Due to the lack of confirmed carbohydrate sources in the diet of cetaceans, this research has adopted the term "caries-like" to refer to the apparent carious lesions (Miles & Grigson, 2003). While caries-like lesions had been described in other odontocetes such as the bottlenose, common (*Delphinus capensis*) and Guiana dolphins (Loch et al., 2011), their frequency of occurrence was low. Caries-like lesions were observed in one third of Amazon River dolphin specimens analysed and in one tucuxi individual. In most Amazon River dolphins analysed, gross caries was the most common, with complete destruction of the tooth crown. Caries-like lesions had already been reported in Amazon River dolphins (Ness, 1966; Pilleri & Gihr, 1969), in which gross lesions were common in the anterior portion of the tooth rows. Reasons behind the apparent high incidence of severe caries in Amazon River dolphins are still unknown. Whether the nutrient rich waters of the Amazon basin (see Gonsior et al., 2016) contribute to the development of caries in the absence of a carbohydrate-rich diet deserve further investigation.

Exogenous pigmentation of the crown was observed in most of the tucuxi teeth examined, seen as a dark brown/black staining on enamel surface. In the Amazon River dolphin, the rugose enamel layer is naturally dark coloured, hindering the observation of exogenous pigmentation. Exogenous pigmentation was also previously reported in marine delphinids, including a quarter of Guiana, Atlantic spotted (*Stenella frontalis*), and bottlenose dolphin specimens examined (Loch et al., 2011). Extrinsic tooth pigmentation is linked to dietary items being adsorbed into dental plaque and/or the acquired pellicle, with potential involvement of chromogenic bacteria and proteins (Watts & Addy, 2001). Darker metallic stains can also result from exposure to environmental metallic salts such as iron and copper (Watts & Addy, 2001). It is plausible that both dietary items and environmental metals dissolved in the waters of the Amazon basin could contribute to the exogenous pigmentation of the crown in tucuxis.

Table 2. Total number of teeth of Amazon River dolphin and tucuxi analysed, number and percentage of teeth affected by pathology (following Loch et al., 2011).

Species	Total number of teeth analysed	Number with teeth with calculus	Calculus deposits			Number of teeth with caries-like lesions	Caries-like lesions		Exogenous pigmentation
			Crown	Cingulum	Root		Small	Gross	
Inia geoffrensis (n= 29)	1988	428 (22%)	0 (0%)	426 (99%)	2 (1%)	197 (10%)	61 (31%)	136 (69%)	?
Sotalia fluviatilis (n= 14)	800	113 (14%)	0 (0%)	85 (75%)	28 (25%)	13 (1.6%)	13 (100%)	0 (0%)	626 (78%)

Recent capture-release programmes have provided reference health parameters for Amazon River dolphins and tucuxis, including haematological chemistry and microbiological and parasitic infections (Mello et al., 2009, 2021; da Silva et al., 2023). However, those programmes are expensive to run and logistically challenging, particularly in the Amazon region. Analysis of pathological findings from opportunistic deceased-stranded animals can provide valuable complementary information on health conditions affecting freshwater cetaceans, especially in hard tissues such as bones and teeth. This study investigated the prevalence and severity of tooth wear and dental pathology in Amazon River dolphins and tucuxis. Despite some of these conditions arising from normal physiological processes (such as tooth wear), progressive tooth wear and dental pathology can contribute to further disease development and morbidity, impacting the health of the animals. Further studies using materials from museum collections in other regions from the Amazon Basin will help elucidate the occurrence, etiology, and impact of tooth wear and dental pathology in freshwater cetaceans, contributing to our growing understanding of their life history and providing an additional snapshot of the health of freshwater ecosystems.

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Appendix

Material examined: *Inia geoffrensis* (IDSM Ig 2005-01, IDSM Ig 2005-03, IDSM Ig 2005-04, IDSM Ig 2005-05, IDSM Ig 2005-06, IDSM Ig 2201, RDSM Ig 21001, RDSM Ig 9301, RDSM Ig 9303, RDSM Ig 9403, RDSM Ig 9404, RDSM Ig 9501, RDSM Ig 9502, RDSM Ig 9505, RDSM Ig 9507, RDSM Ig 9508, RDSM Ig 9509, RDSM Ig 9511, RDSM Ig 9512, RDSM Ig 9515, RDSM Ig 9516, RDSM Ig 9601, RDSM Ig 9601, RDSM Ig 9602, RDSM Ig 9603, RDSM Ig 9605, RDSM Sf 9301* (*Inia*), RDSM Sf 9503* (*Inia*), Helder 1991. *Sotalia fluviatilis*: IDSM Sf 2005-02, IDSM Sf 2005-03, IDSM Sf 2005-04, IDSM Sf 2005-05, IDSM Sf 2005-06, IDSM Sf 2005-07, IDSM Sf 2005-08, IDSM Sf 2005-12, RDSM Sf 9501, RDSM Sf 9502, RDSM Sf 9503, RDSM Sf 9507, RDSM Sf 9702, RDSM Sf 9801).

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