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Dental erosion in South Atlantic dolphins (Cetacea: Delphinidae): A macro and microscopic approach

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Dental erosion is the loss of dental hard tissue through a chemical process without action of bacteria, with both enamel and dentine demineralized because of acidification in the oral cavity (Nunn 1996, Addy and Shellis 2006). It is uncertain whether the acids involved in chemical demineralization are of extrinsic or intrinsic origin. Intrinsic acids might relate to disorders of the gastroesophageal tract leading to regurgitation of gastric acid. Extrinsic acids are linked to diets rich in acidic substances or acids related to environmental factors (Addy and Shellis 2006).

Erosion of dental tissues has been widely studied and documented in humans, through case reports to population-based surveys. The wider availability and consumption of soft drinks and acidic fruit juices by Western populations (mainly in the Americas and Europe) was first assumed to be the main etiological factor for dental erosion (Grando *et al.* 1996). However, gastric regurgitation provoked by gastroesophageal reflux disease (GERD) or vomiting induced by psychological disturbances has also been proposed as causative agents (Nunn 1996).

The loss of dental hard tissues such as enamel and dentine in humans has major implications to dental health status because the normal thickness and shape of the tissues lost will not be regenerated (Bell *et al.* 1998). As well as having obvious

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aesthetic and clinical consequences in humans, loss of dental tissue can generate increased sensitivity and pain, which can affect the feeding behavior and welfare of the individual (Nunn 1996).

Although attention has focused on the understanding of the etiology and implications of dental erosion in humans, the same is not true for other mammalian species. Few studies have addressed erosive processes in the teeth of mammals, with most being directed to primates. Lukas (1999) reviewed the occurrence of "regurgitation/reingestion" behavior in captive gorillas—behavior is also considered to cause erosion in humans. Cuozzo *et al.* (2008) investigated the salivary pH and buffering capacity of saliva in a population of wild lemurs, relating dental erosion to the acidic diet of some species. The same approach was earlier adopted by Dumont (1997) using insectivorous and frugivorous bats.

Studies of pathological conditions in teeth of cetaceans are scanty (*e.g.*, Ness 1966, Brooks and Anderson 1998, Loch *et al.* 2011). Dental erosion was unreported in cetaceans until we documented this condition in dolphins from southern Brazil and commented on the possible diagnosis and etiology of this pathology (Loch *et al.* 2011). In this study, we aim to evaluate the prevalence and macro- and microstructural characteristics of dental erosion in dolphins from the South Atlantic which show similar physical attributes of erosion in humans. Microscopic analyses involved light microscopy, scanning electron microscopy, and micro-computed tomography (micro-CT). We also discuss possible etiological factors and implication of this condition to the animals.

Teeth derived from 350 individuals of 10 species of marine delphinid cetaceans collected along the southern coast of Brazil were inspected (Table 1). Specimens were collected independently and opportunistically from 1977 to 2007. Individuals of most of the age classes (calves, juveniles, subadults, and adults) were evaluated. Teeth were prepared normally by water maceration to remove residues of soft tissues, and stored dry or immersed in ethanol in individual containers. Most of the specimens had their teeth preserved out of the skulls, and position in the tooth row and quadrants were generally not recorded. Before examination under a stereoscopic microscope, teeth were surface cleaned with a soft brush or a cotton swab immersed in ethanol.

Dental erosion was diagnosed as smooth, silky-shining, glazed tooth surfaces, showing loss of surface luster and less-reflective enamel. Eroded areas were observed as shallow concavities or grooving and cupping of tooth surface coronal to the enamel-cement junction (Ganss 2006). These surfaces are normally nonocclusal and lack the directional wear attributed to food abrasion or tooth-to-tooth attrition. Alterations observed in areas of occlusal contact or that show clinical characteristics of mechanical wear (*e.g.* flat surfaces) were not included in our study.

Macroscopic deviations from the original tooth morphology were described using the anatomical terminology of Smith and Dodson (2003). For histological analysis, eroded teeth were sectioned longitudinally into 700 μm slices using a slow-speed saw (Isomet 1000, Buehler, IL) with a diamond wafering blade (Buehler 15HC, Buehler, IL) at 250 rpm. Tooth slices were polished with carborundum paper discs (300, 500, and 1,000 grit). Final polishing was obtained manually using fine and extra-fine powdered pumice stone and water. Thin slices were mounted on glass slides

Table 1. Species, number of individuals and number of teeth analyzed, body length range of the specimens, prevalence and percentage of erosion in delphinid cetaceans.

Species	Number of individuals	Body length range (cm)	Number of teeth analyzed	Prevalence erosion	Percentage individuals
Delphinus capensis (long-beaked common dolphin)	18	178–222	1,690	–	–
Lagenodelphis hosei (Fraser's dolphin)	9	216–258	1,032	–	–
Orcinus orca (killer whale)	3	378–397	68	1	33
Pseudorca crassidens (false killer whale)	4	370–523	104	1	25
Sotalia guianensis (Guiana dolphin)	205	83–203	17,867	10	5
Stenella coeruleoalba (striped dolphin)	8	212–243	869	–	–
Stenella clymene (Clymene dolphin)	2	198–200	188	–	–
Stenella frontalis (Atlantic spotted dolphin)	23	157–204	2,033	–	–
Steno bredanensis (rough-toothed dolphin)	17	200–277	1,055	4	24
Tursiops truncatus (bottlenose dolphin)	61	150–358	3,071	2	3
Total	350	–	27,977	18	–

and examined in a Zeiss Axiostar Plus microscope (Carl Zeiss, Göttingen, Germany) using 40× magnification. Images were captured with a Canon A80 camera (Canon USA Inc., NY).

To characterize the ultrastructural details of the eroded tooth surfaces, scanning electron microscope (SEM) images were taken using a JEOL JSM-6700F Field Emission SEM (JEOL Ltd., Tokyo, Japan), operating at 1.5 kV and 10 μA. Natural specimen surfaces were examined without gold or carbon coating.

Some specimens were also analyzed using micro-CT. A Skyscan 1172 micro-CT desktop system (Skyscan, Kontich, Belgium) was used. X-rays were generated at 100 kV and 100 μA, with 0.5-mm-thick aluminum and copper filters placed in the beam. Cross-sectional slices were then reconstructed using NRecon software (NRecon, version 1.4.4, Skyscan). Examination of cross sections and creation of profiles were performed using ImageJ software.

Cases of dental erosion were diagnosed in 5 of 10 species. Whereas cases were less frequent in *Sotalia guianensis* and *Tursiops truncatus*, the prevalence of dental erosion was moderate in *Steno bredanensis*, *Pseudorca crassidens*, and *Orcinus orca* (Table 1).

Although the lingual side of the teeth appeared to be more heavily affected, the mesial and distal surfaces sometimes were clearly eroded. Buccal faces were rarely affected. Erosion on the cervix resulted in collaring of teeth, altering the tooth

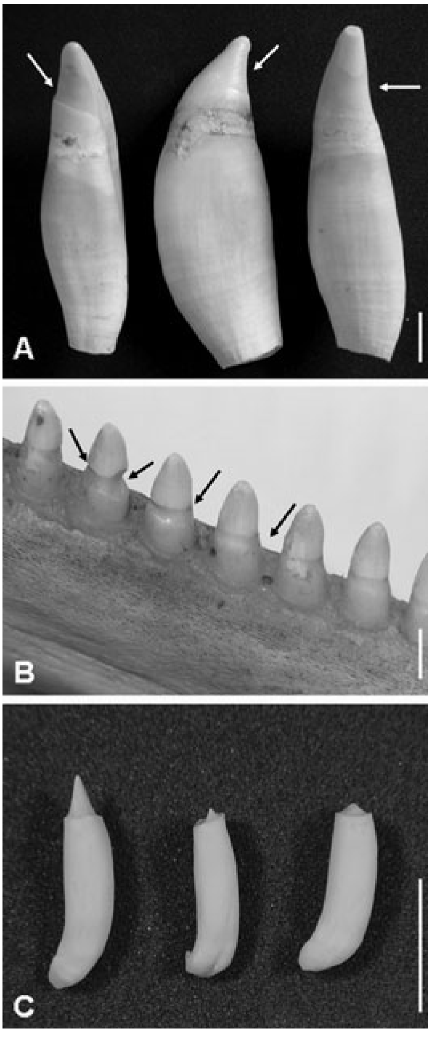

Figure 1. Macroscopic assessment of dental erosion. (A) Mild erosion in *O. orca* (GEMARS 0366), restricted to the enamel and superficial layers of dentine (arrows). (B) Moderate erosion in *Steno bredanensis* (UFSC 1001) evidenced by the narrowing of the teeth cervix (arrows). (C) Severe erosion in *Sotalia guianensis* (MCN 301) resulting in loss of tooth morphology and reduction of crown height. Scale bar = 1 cm.

morphology (Fig. 1B). Eroded surfaces were also common toward the apical region of the crowns of affected teeth.

Areas of erosion showed loss of luster, with a glazed appearance. Shallow concavities were observed in the crowns of affected teeth, with well-defined enamel borders along the margins. In some cases, a cap of preserved enamel was observed apical to the eroded area and commonly close to the tooth tip. However, intact enamel was also seen as a band basal to the erosion site, close to the cementum–enamel junction (CEJ).

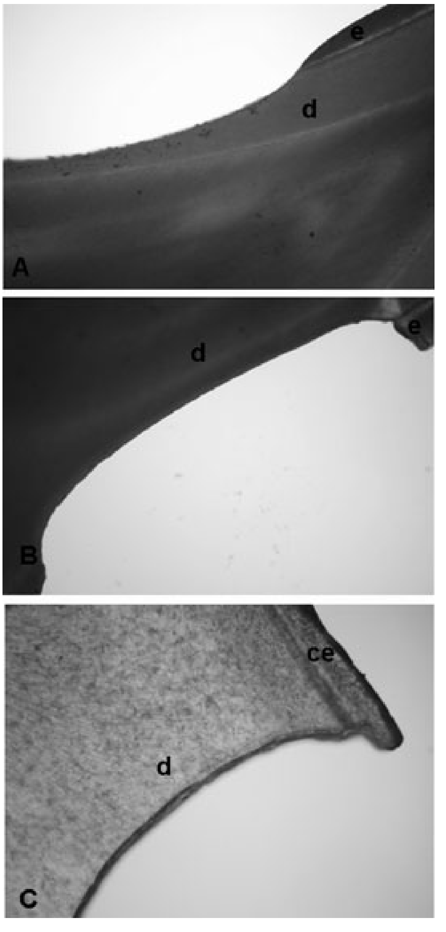

Figure 2. Light microscopy sections in *S. guianensis*, 40× magnification. (A) Loss of enamel and moderate loss of dentine in the eroded area (MCN 06). (B) Smooth basal area of erosion site, with reparative dentine underneath (IPeC 269). (C) Complete loss of enamel and severe dentine cupping (MCN 228) (e = enamel; d = dentine; ce = cementum).

Erosion varied in terms of severity and extent of hard tissue loss, from mild loss of enamel and dentine (Fig. 1A), to moderate erosion resulting in narrowing of the tooth cervix (Fig. 1B), and to severe erosion where the tooth shape and morphology were heavily affected (Fig. 1C). In the latter cases the crown height was much reduced. All these stages of erosion were observed in samples of the Guiana dolphin, *S. guianensis*, suggesting that dental erosion is a progressive process with cumulative effects.

Light microscopy sections revealed areas of complete loss of enamel and distinct areas of dentine cupping (Fig. 2A, C). Eroded areas were seen as concavities of varying depths, with well-defined margins set at oblique angles to the enamel or cement surface. The basal areas of lesions were smooth and even. In some cases reparative dentine was observed basal to the eroded area (Fig. 2B).

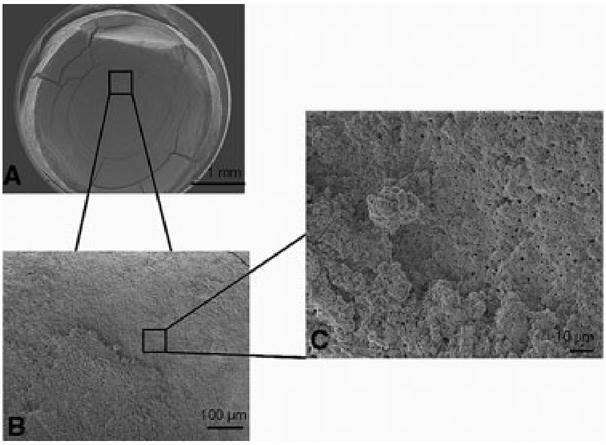

Figure 3. SEM images of eroded tooth of *S. guianensis* (MCN 228). (A) Low-power apical view (25×) of severely eroded tooth. (B) Higher-power view (170×) of cupped and exposed dentine. (C) High-power view (1,000×) of denatured dentine, showing a rough and irregular surface characteristic of acid etching.

SEM images of a specimen with severe erosion revealed a rough and uneven surface in the area where dentine was cupped (Fig. 3A, B). The enamel covering was completely absent in this region. Higher magnification images (170–1,000×) showed details of a dentine surface with projections and irregularities characteristic of denaturation (Fig. 3B). Openings of dentinal tubules were visible at the surface (Fig. 3C).

Reconstructed micro-CT cross-sections showed varying amounts of enamel and dentine loss. A specimen with dental erosion restricted to the lingual surface revealed distinctly different mineral concentration profiles to pristine teeth. Noneroded regions showed pristine enamel (gray-scale voxel values about 250) and dentine (values ranging from about 125 to 175), with an abrupt density change from enamel to dentine (Fig. 4A). A section through the eroded area revealed loss of enamel and moderate loss of adjacent dentine (Fig. 4B). Gray-scale values/distance profiles along the lines shown in Figure 4(B) reveal the absence of the first of the two peaks in gray-scale values corresponding to enamel. The profile starts with gray-scale values that are relatively homogenous ranging from about 125 to 175, which correspond to dentine, followed by an abrupt change to values similar to enamel, at around 250.

The physiological processes of tooth wear (*sensu latu*) are related to the action of physical and chemical mechanisms. Whereas attrition and abrasion correspond to physical or mechanical wear, dental erosion is basically a chemical process (Ganss 2006). It is widely known that attrition, abrasion, and erosion interact and contribute to different extents in causing tooth surface loss, varying in relative importance during the life cycle (Addy and Shellis 2006).

Morphological characteristics of dental erosion allow acid-induced tissue loss to be distinguished from mechanically derived dental wear. These characteristics include

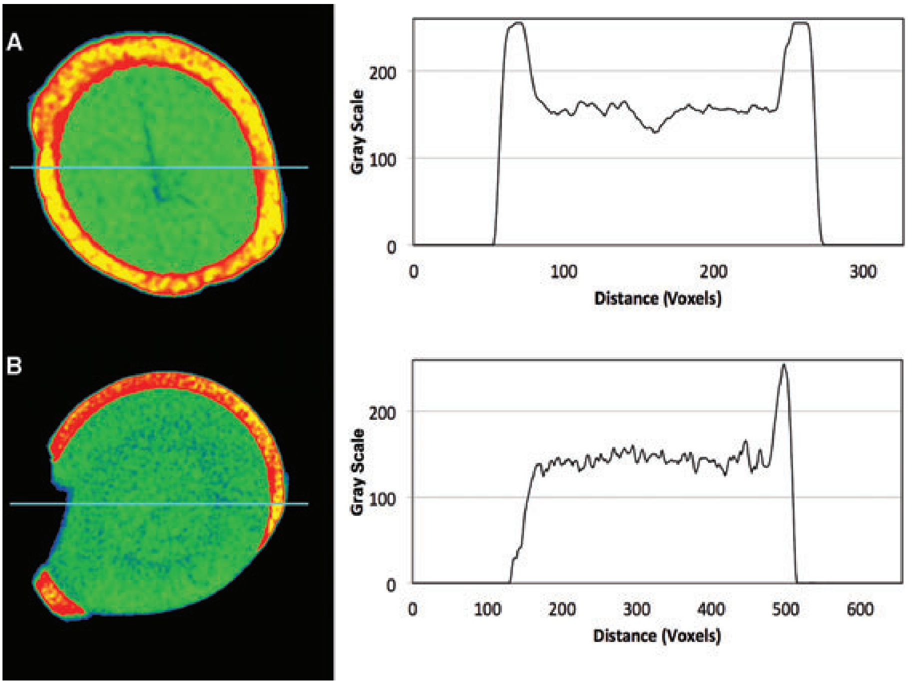

Figure 4. Reconstructed micro-CT cross-sections of an eroded tooth of *S. guianensis* (MCN 060). (A) Projected reconstructed image and gray-scale value/distance profile of noneroded area. (B) Projected reconstructed image and gray-scale value/distance profile of eroded area.

changes in luster and morphology of dental surfaces, which were observed most commonly affecting nonocclusal surfaces. In contrast, mechanical wear is normally recognized as glossy and flat occlusal surfaces with corresponding features seen in antagonistic teeth (Ganss and Lussi 2006). These features, together with the characteristic cupping of dentine (Kieser *et al.* 2001), allowed us to diagnose dental erosion in dolphins from abrasive or attritional wear.

Of the 10 species analyzed, half were diagnosed with dental erosion. The prevalence was low in *S. guianensis* and *T. truncatus*, two species with large sample sizes. On the other hand, the prevalence was moderate in *S. bredanensis*, *P. crassidens*, and *O. orca*, species with smaller sample sizes, which could have biased our estimations. The occurrence of erosion in animals from distinct geographic locations and collected in different years suggests this was not a regional or time frame-restricted event.

Dental erosion in dolphins presented similar features to those seen in humans in relation to appearance and formation of cavities (Ganss and Lussi 2006). Similarly to what has been documented in humans (Ganss 2006), in severe cases tooth morphology was affected and crown height was significantly reduced. Light microscopy revealed features compatible with demineralization of hard tissue as seen in humans, such as smooth surfaces and formation of reparative dentine as a biological response to compensate for loss suffered (Ganss 2006). SEM images allowed qualitative assessment of ultrastructural changes in eroded dentine, commonly observed as surface roughness and opening of dentinal tubules (Attin 2006). Finally, micro-CT provided high-resolution images that contributed to qualitative characterization of dental erosion, having the distinct advantage of being nondestructive. This technique has been proven useful and a powerful tool in human dental research (Swain and Xue 2009), with clear potential to be applied in other mammals.

Although the occurrence of dental erosion in dolphins has been documented and confirmed by this study, establishing its etiology is a challenge. Extrinsic acids could be available by environmental exposure or due to acidic diets. Aquatic pollution is a worldwide phenomenon (Reijnders and Aguilar 2002), but a lack of reports indicating water acidification in the sampled area and the low prevalence of erosion in species with larger sample size weakens the cause–effect relationship expected. Dolphins feed mostly on fish and squid (Pauly *et al.* 1998, Santos *et al.* 2002). Considering the high content of protein in their diet, it would be expected that dolphins have an alkaline oral environment due to metabolism of amino acids and urea into ammonia (Hillson 1979). For these reasons, it is unlikely that normal oral dietary acids caused dental erosion in these animals.

It is known that not only extrinsic acids cause dental erosion, but also that intrinsic factors may be involved. Gastric acid reaching the oral cavity as a result both of vomiting or gastroesophageal reflux can cause dental erosion. However, acid exposure must occur regularly over a long period of time for dental hard tissues to become demineralized (Scheutzel 1996). In humans, this is observed in disorders associated with chronic vomiting (*e.g.*, anorexia and bulimia) and persistent regurgitation or GERD. Upper gastrointestinal tract disorders such as peptic ulcers and chronic gastritis are often associated with vomiting and reflux, and secondarily with dental erosion. In these cases, palatal (or lingual) surfaces are frequently more heavily affected (Scheutzel 1996, Bartlett 2006).

The higher prevalence of erosion on the lingual surfaces observed in dolphins in this study may be explained by intrinsic factors, as seen in humans due to disturbances of the gastroesophageal tract. Although we cannot completely discount the potential influence of dietary acids and environmental exposure within the aquatic environment, regurgitation of gastric secretions seems to be a more plausible explanation for the dental erosion of cetacean teeth.

Gastric ulcers have been reported both in wild and captive cetaceans, frequently associated with parasitic infections by anisakine nematodes such as species of the genus *Anisakis* (Abollo *et al.* 1998, Motta *et al.* 2008). Nonparasitic gastric ulcers have been reported in dolphins, and a causal connection to a diagnosis of *Helicobacter* sp. has been proposed, as observed in humans with gastritis (Harper *et al.* 2000). *Helicobacter* spp. has been diagnosed both in the oral cavity and digestive tract of captive and wild cetaceans (Suárez *et al.* 2010, Goldman *et al.* 2011). Suárez *et al.* (2010) reported positive stomach biopsies for *Helicobacter* in a dolphin that also presented macroscopic ulcerations and inflammatory alterations, conditions normally present in cases of gastrointestinal disease.

We conclude that cetaceans can develop upper gastrointestinal disorders with potential to promote persistent gastric acid regurgitation into their oral environment. Besides, other biological factors may also contribute to susceptibility for tooth erosion, such as a lack of salivary buffering, neutralizing, and remineralization potentials in cetaceans, due to their rudimentary and less effective salivary glands (Rommel and Lowestine 2001). However, a clear connection between gastric lesions, low salivary flow and dental erosion in dolphins has not been established yet. Further studies are desirable and necessary to clarify the etiology, physical characteristics, prevalence, and distribution of this condition in these mammals.

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