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Antigone Uzunidis, Antonio Pineda, Sergio Jiménez-Manchón, Alexandros Xafis, Vincent Ollivier, et al.. The impact of sediment abrasion on tooth microwear analysis: an experimental study. *Archaeological and Anthropological Sciences*, 2021, 13 (8), pp.134. <10.1007/s12520-021-01382-5>. <hal-04751662>

HAL Id: hal-04751662

<https://hal.science/hal-04751662v1>

Submitted on 24 Oct 2024

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The impact of sediment abrasion on tooth microwear analysis: an experimental study

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Abstract

Dental microwear analysis is a proxy for analysing the diet in extinct and extant vertebrates, especially mammals. The limits of these approaches are still rather poorly known, especially in terms of taphonomic impacts. Indeed, several physical or chemical phenomena may have altered the microscopic features linked to the diet and compromised their study. In this article, we evaluate the effect of sediment abrasion on teeth on low-magnification tooth wear studies. We used a tumbling machine in order to reproduce abrasion marks on 57 molars and premolars of *Equus* sp., *Capra hircus* and *Sus scrofa* employing two types of sediments: a mixture of clay and sand sediment with small (150-200 µm) and rounded particles and a sandy one with larger (350-500 µm) and sub-angular particles. The teeth underwent up to two hours of tumbling simulation and casts were made at regular intervals in order to evaluate the evolution of the taphonomic impact over time. Our experiment shows that 1) both sediments strongly alter the teeth after a certain time; 2) the fine particles contained in the mix of sand and clay sediment have a much stronger impact on the enamel than the sand; 3) the mix of clay and sand sediment tends to increase the number of pits and reduce the number of scratches, vice versa for the sand; 4) sedimentary and dietary marks do not have the same morphology and can be distinguished. The abrasion marks (compared to dietary scratches) tend to be wider, shorter, with an isotropic distribution, more frequent on the most exposed parts of the teeth (such as the cusps or the edges). The pits resulting from sediment tumbling present an irregular morphology in comparison with dietary pits, which are rounder. Both sediments have an impact on the enamel surfaces. Thus, when signs of taphonomic alteration (e.g. presence of abrasion marks, taphonomic pits, notches in the edges of enamel) are documented, we recommend avoiding studying the tips of the cups of the Suidae (and probably other bunodont teeth) and the portions of enamel at the edge of equid teeth which are more affected by taphonomic processes, especially in the mix of sand and clay sediment. This work has important implications for microwear studies applied to fossil samples. It makes it possible to recognise some taphonomic features linked to mechanical abrasion of the enamel, to consider with more caution the teeth that have been preserved in fine sediment, and to choose, in order to characterise the diet, the areas least impacted by taphonomic alterations.

Keywords:

Tooth wear; Taphonomy; Tumbling; Sequential experimentation; mechanical alteration

Declarations

Funding

This research was funded by the International Research Network (IRN 0871 CNRS-INEE): Taphonomy European Network (TaphEN). It has also been supported by LabEx ARCHIMEDE from the “Investissement d’Avenir” programme ANR-11-LABX-0032-01. The research of FR is supported by the Spanish Ministry of Science and Innovation through the PID2019-103987GB-C31project and the “María de Maeztu” excellence accreditation (CEX2019-000945-M) and by the Generalitat de Catalunya through the 2017 SGR 836 project and the CERCA Program/Generalitat de Catalunya. A. X. was funded by the Austrian Science Fund (FWF, project number P29501-B25). A. P. is supported by the Spanish Ministry of Science and Innovation (FJC2019-040804-I, Subprograma Juan de la Cierva-Formación).

Conflicts of interest/Competing interests

None

Availability of data and material

All raw data are sent with this manuscript

Code availability

Not applicable

Authors' contributions

Antigone Uzunidis: conception of the work; acquisition, analysis and interpretation of the data; writing of the manuscript

Antonio Pineda: conception of the work; analysis and interpretation of the data; writing and revision of the manuscript

Sergio Jiménez-Manchón: conception of the work; acquisition, analysis, and interpretation of the data; revision of the manuscript

Alexandros Xafis: analysis of the data; revision of the manuscript

Vincent Ollivier: acquisition of the data

Florent Rivals: conception of the work; acquisition, analysis and interpretation of the data; writing and revision of the manuscript

1. Introduction

For a long time, many works have been dedicated to reconstructing ecological parameters, and in particular the diet of fossil taxa. Many proxies have been developed for this purpose, such as the shape analysis of the maxilla (e.g. Solounias et al. 1988; Solounias and Moelleken, 1993a; Solounias and Moelleken 1993b), the study of the of the hypsodonty index of the teeth (e.g. Fortelius 1985; Janis 1988; 1995; Mendoza et al. 2002), tooth mesowear (Fortelius and Solounias 2000) and dental microwear, both stereomicroscopic analysis (Walker et al. 1978; Solounias and Hayek 1993; Solounias and Semprebon 2002; Semprebon et al. 2004a) and dental microwear texture analysis (Calandra and Merceron 2016; Scott et al. 2006). Dental microwear is an approach with a high temporal resolution which allows the characterisation of an individual's diet on a short temporal scale corresponding to the time of death (Grine, 1986; Davis and Pineda Munoz 2016; Sánchez-Hernández et al. 2016). The other methods

correspond rather to a longer temporal scale, the morphology-based ones reflecting the evolutionary trend of a lineage and of the tooth mesowear, the animal life-time tendency (Ackermans et al. 2020).

The first studies of dental microwear date back to the 1950s when Butler (1952) and Mills (1955) noticed that the orientation of the scratches observed on the wear facets of the tooth enamel could reflect the directions of movement of the jaws and, most probably, the diet. In 1959, Baker and colleagues correlated tooth wear in sheep populations with diet and the composition of quartz-rich soils. Subsequently, new evidence of the abrasive action of phytoliths was revealed by their discovery at the end of scratches on the surface of human tooth enamel (Fox et al. 1994 1996). Dental microwear is, since Grine (1977), widely used to study the palaeodietary flexibility of wild taxa (e.g. Teaford 1988; Semprebon and Rivals 2007) and to reconstruct the habitats in which herbivores lived (e.g. Semprebon et al. 2004a, 2004b; Merceron et al. 2004; Rivals 2012). To a lesser extent, this tool has also been used to analyse the palaeodiet in domestic animals in order to better understand the management strategies employed by herders/farmers and landscape use (e.g. Mainland 2003, 2006; Gallego et al. 2017; Jiménez-Manchón et al. 2019).

Dental microwear studies refer to several techniques of analysis: scanning electron microscopy (e.g. Walker et al. 1978), microwear texture analysis (Scott et al. 2005), and low-magnification microwear analysis (Solounias and Semprebon 2002; Semprebon et al. 2004). Although these techniques are widely used, their limitations are still rather poorly defined. Various authors have highlighted problems concerning inconsistencies in the teeth or facets selected (Krueger et al. 2008; Ungar et al. 2010; Xafis et al. 2017), variations in moulding and casting methods (Galbany et al. 2006; Williams and Doyle 2010; Mihlbachler et al. 2019), or inter-observer variability (e.g. Mihlbachler et al. 2012). Among the possible biases for defining an individual's diet from tooth wear, taphonomic problems are largely underestimated and poorly investigated. Although teeth are often much better preserved than bones (Lyman 1994), chemical or physical alterations can destroy or alter diet-related information (King et al. 1999; El-Zaatari 2010; Dauphin et al. 2018; Böhm et al. 2019; Weber et al. 2020).

So far, very few experiments have tested the effects of tumbling on tooth enamel. Those experiments were designed to mimic the effect of sediment abrasion (Gaudzinski-Windheuser et al. 2010). Gordon (1983, 1984) tumbled human teeth in four types of sediment (dry sediment and in aqueous mixtures of these sediments) and showed that tumbling only seems to erase feeding features (scratches and pits) rather than adding new ones. King and colleagues (1999) carried out the same type of experiment on human teeth and with three types of sediment (quartz pebbles, particles size: 2,000–11,000 μm ; coarse sand, particles size: 500–1,000 μm ; medium sand, particles size: 250 and 500 μm). This experiment showed similar results to that of Gordon (1984) - tumbling would remove the traces and not add to them. Puech and colleagues (1985) tested the impact of projection and friction of sand grains (between 50 and 200 μm) on human tooth surfaces. This experiment allows the authors to describe specific marks related to the diet which can be partially or completely erased due to taphonomic alterations. Thus, when taphonomic alterations are observed on the teeth, several authors indicate that chemical and physical alterations can be differentiated from the preserved areas to avoid discarding specimens and reducing the sample size (Puech et al. 1985; Teaford 1988; King et al. 1999; Martínez and Pérez-Pérez 2004).

For now, only one experimentation has been conducted on non-human mammal teeth (Böhm et al. 2019). This experiment sought to evaluate the impact of tumbling on studies based on microwear texture analysis using confocal microscopy. The impact of three types of sediment (fine sand: particles size: 51–168 μm ; fine sand particles size: 112–292 μm ; medium sand: particles size: 221–513 μm) on the teeth of three different taxa (*Equus* sp., *Capreolus*

capreolus and *Otomys* sp.) was tested. This study showed that some parameters were strongly affected by tumbling and others were not and that the results depend on the taxon and the sediment. *Otomys* sp. teeth were hardly affected by tumbling and the differences between browsers and grazers among large mammals still persisted after tumbling.

Low-magnification microwear analysis involves direct observation through a stereomicroscope at 35x of the micro-features (pits and scratches) caused by food and grit particles on the occlusal surface of dental enamel. Compared to microwear texture analysis, this method allows a direct observation of the sample by the observer. Although this is often considered a limitation due to interobserver bias (e.g. DeSantis et al. 2013; Mihlbachler et al. 2012), it also offers the possibility of distinguishing taphonomic (abrasion marks) and non-taphonomic (dietary) traces on the basis of their morphology as it is commonly applied to the study of bones (e.g. Behrensmeyer et al. 1986).

In this study, our goal is to establish the impact of tumbling on the teeth of several ungulate taxa, depending on the type of sediment. Tumbling experiments were already used in several taphonomic studies in order to mimic the effect of water abrasion on faunal remains (e.g. Gaudzinski-Windheuser et al. 2010; Pineda et al. 2019). Pits and scratches are the main features studied in dental microwear studies. The abrasion marks and pits that originated during the tumbling process were identified, characterised, and compared to the traces resulting from the diet. For that purpose, we selected three taxa according to their dental morphology: hypsodont teeth with thick enamel (*Equus* sp., enamel thickness: 1.5-2 mm, Kozawa et al. 1988), hypsodont teeth with thin enamel (*Capra hircus*, enamel thickness: 0.53 mm, Grine et al. 1987), and brachydont teeth (*Sus scrofa*). The teeth were separated into four groups, two of which were tumbled with a Miocene sand rich in quartz and the other two with a mix of sand and clay sediment. The abrasion was simulated with a tumbling machine in which they spent a total of two hours. At regular intervals, the teeth were moulded in order to follow the evolution of the taphonomic alterations over time. We hypothesised that Miocene sand would have a greater effect on the enamel due to its abrasive properties, compared to the mixed one (Rozada et al. 2018). Also, we expected a difference between taxa, i.e. that equid teeth would be more resistant to alterations due to their thicker enamel. Comparison of these features with non-taphonomic ones would help to avoid bias and improve dental microwear studies.

2. Material and methods

2.1 Sediments

Two types of sediment were selected because of their composition in order to evaluate the significance of their possible impact. The sediments were not sieved for the experiment in order to compare the effects of sediments that can be found on teeth from archaeological sites. We therefore chose sediments with opposite characteristics: Miocene sand and a mixed sediment with clay and sand. The particles that make up the Miocene sand are larger and sub-angular, those that make up the mix of sand and clay sediment are smaller and rounder. The determination of the size of the grains was carried out by sieving that was limited to the extraction of the sedimentary matrix (mesh 250 µm) and measurements were carried out directly on the grains themselves via dinolite software (measurements of axes). The angularity of the grains was qualitatively determined: the sub-angular grains have sharp or right-angled edges while the rounded grains have no edges.

The Miocene sand comes from La Motte d'Aigues (Vaucluse, France). This sediment is composed of medium to fine grains and is rich in translucent sub-angular quartz (60% of the

sediment) and blunt to rolled green quartz (30% of the sediment). The particles are quite large in size (350-500 μm).

The mixed clay-sand sediment comes from a cave in the town of La Bouilladisse (Bouches-du-Rhône, France), dug into Jurassic limestone. The sediment is sandy-silty to gravelly, mainly composed of relatively heterogeneous limestone and quartz elements. The quartz particles are quite small in size (150-200 μm), several varieties can be observed (pink quartz and white quartz), all are blunt. Limestone ceiling elements are visible (heterometric and sub-angular), as well as some fragments of white calcitic and conglomerate cements. Aeolian feldspar (pink to almost red), with the quartz, represents about 20% of the observed fractions.

2.2 Experimental protocol

Teeth from extant animals were selected for this experiment. Taxa used for the purpose of this study included equids (*Equus* sp), goats (*Capra hircus*) and wild boars (*Sus scrofa*). Teeth were separated into four groups: Group 1 included two equid teeth and 10 goat teeth; Group 2 was composed of 15 wild boar teeth; Group 3 included three equids and nine goat teeth; and, finally, Group 4 was composed of fourteen teeth belonging to wild boars. The teeth were separated in order to limit tooth-to-tooth impacts between them during the experiment and to facilitate their distribution and study afterwards. Groups and variables included in each one of them are summarised in **Table 1**.

All the teeth were subjected to the abrasion process in a tumbling machine (KT-3010 SUPER-TUMBLER, size: 300x240 mm) located at the *Catalan Institute of Human Paleoecology and Social Evolution* (IPHES) in Tarragona. Groups 1 and 2 were exposed to the mix of sand and clay sediment mixed with water (mix sand-clay: 60%, water: 40%, 2 kg in total) whereas Groups 3 and 4 were exposed to the sand sediment mixed with water (sand: 60%; water: 40%, 2 kg in total). The time of exposition was the same for all four groups. Six cumulative cycles of exposure to tumbling were reproduced: cycle 1 (2'), cycle 2 (3'), cycle 3 (10'), cycle 4 (15'), cycle 5 (30') and cycle 6 (60'). The total time at the end of the experimentation was two hours. The tumbling machine was set to 83.3 revolutions per minute (i.e. 5000 revolutions per hour) and the rotations were uninterrupted and unidirectional in each cycle.

Moulds of the occlusal surface were produced before the beginning of the experiment and after each cycle. Before moulding, the occlusal surface of each tooth was cleaned using acetone and then 96% ethanol. Then the surface was moulded with a high-resolution silicone (vinylpolysiloxane; Provil novo light, regular set) and casts were made using clear epoxy resin (EPO 150). The transparent casts were then observed with a stereomicroscope at magnifications of $\times 35$ and microphotographs were taken using a SMZ1500 stereomicroscope (AU), Zeiss Stemi 2000C (FR), Leica MZ16 (SJM), Leica MZ12 stereomicroscope and Leica CLS 100 oblique lighting source (AX). Observations were restricted to a standard surface of 0.16 mm^2 (using an ocular reticule). The variables chosen to observe and quantify are those established by Solounias and Semprebon (2002) and Semprebon et al (2004a), which are traditionally used in a large number of studies (e.g. Xafis et al. 2017; Rivals et al. 2017; 2019; Uzunidis 2020): pits (small and large), scratches (fine, coarse and hypercoarse), cross scratches and gouges. These features were quantified on the protoconid and/or metaconid on the lower teeth and the paracone and/or metacone on the upper teeth. The variable "cross scratches" has not been recorded for the suids because, due to the conformation of their teeth (bunodonts), the scratches are always crossed. It is therefore not possible to observe a preferential orientation to which to refer to count the exact number of cross scratches. The same area was observed on the subsequent moulds of the same tooth (with the previous-experiment mould) through the six cycles of the experiment. We used reference features on

the surface, such as obvious pits or scratches or the topography of the surface, to locate the reticule precisely at the same place on the successive casts.

The four different groups were analysed by different observers. All observers have previous experience in dental microwear analysis, although one of them (FR) is more experienced than the others (AU, SJM and AX). Group 1 was analysed by SJM, Group 2 by FR, group 3 by AU, group 4 by AX.

2.3 Morphometrical study of the taphonomic alterations

In order to characterise abrasion marks compared to dietary micro-features we have selected and described the evolution of a sample of six teeth. These teeth belong to the three taxa studied (wild boar, goat, and equid), taking into account the two types of sediments used (mix of sand and clay and sand). The teeth were randomly selected but they are representative of the wear pattern for the group in question. The specific elements studied are:

- 1: Tooth #II.1. Right M3 of *Sus scrofa* exposed to tumbling in the mix of sand and clay sediment.
- 2: Tooth # IV. 8. Right M1 of *Sus scrofa* exposed to tumbling in sands.
- 3: Tooth #I.6. Right M2 of *Capra hircus* exposed to tumbling in the mix of sand and clay sediment.
- 4: Tooth #III. 10. Right M1 of *Capra hircus* exposed to tumbling in sands.
- 5: Tooth #I.7. Right PM4 of *Equus* sp. exposed to tumbling in the mix of sand and clay sediment.
- 6: Tooth #III.6. Left M1 of *Equus* sp. exposed to tumbling in sands.

The weathering stage of these remains was established according to Behrensmeyer's (1978) stages. The occlusal surfaces of the teeth were observed and described (see supplementary data 2). The nomenclature used to describe the alterations closely resembles the terms used for dental microwear. To avoid misunderstandings, the term "abrasion marks" will be used for scratches of taphonomic origin produced in the tumbling machine, while the term "scratch" will refer exclusively to dietary features. For other features, we will use the adjective "taphonomic" when necessary (e.g. "taphonomic pit").

2.4 Statistical analysis

In order to fully understand the changes in each of the variables considered as a function of the time spent in the tumbling machine, we used two statistical analyses: principal component analyses (PCA) and the Friedman test.

The PCA allowed us to observe how each of the variables changed over the cycles, and the Friedman test allowed us to determine whether any differences were significant ($p = 0.95$). The minimum number of individuals per sample to perform those statistical analyses is five. Both were realised using the software Xlstat v. 2014.5.03. The principal component analysis was built thanks to the Spearman rank correlation coefficient, which is recommended for small datasets (Vásquez-Correa and Laniado Rodas 2019). Friedman's ANOVA is a non-parametric test which allows testing if paired samples come from the same population or from populations with identical properties. It can be applied to small samples and is more likely to be dependent on the nature of sample variances, so it allows a parsimonious interpretation of the data (Rigdon 1999). In order to artificially increase the number of samples and to reduce the errors due to the small size of the sample we used the Monte-Carlo method and simulated the distribution of the variables 10,000 times.

3. Results

3.1 *Capra hircus* in the mix of sand and clay sediment.

From the ten teeth available at the beginning (**Tab. 1**), one was immediately (after 0 min) discarded because of its alteration, probably in relation to a health issue with the animal, and only two samples (belonging to other animals) were suitable for microwear analysis one hour later, and none two hours later. Because of the experiment, the “non-suitable teeth” showed enamel surface alterations that were too pronounced to recognize dental wear parameters (e.g. pit, scratches, gouges) used in the reconstitution of the diet. The statistical analyses will focus on a tumbling period between 0 and 30 minutes where sufficient teeth, five, are available (**Tab. 2**). The number of pits, coarse scratches and cross scratches remains very similar, from 0 to two minutes (**Fig. 1**). However, the number of coarse scratches and cross scratches increases a little between two and five minutes of tumbling, as do the number of large pits after 30 minutes. The number of gouges never changes, regardless of the time spent in the tumbling machine, and always remains equal to zero. The differences between the different cycles are still statistically insignificant in every variable (cf. supplementary data 1).

3.2 *Equus* sp. in the mix of sand and clay sediment.

Only two teeth were available to study the impact of tumbling on *Equus* enamel surfaces (**Tab. 1**). No statistical tests were carried out due to the small number of teeth. On the two teeth (**Tab. 3**), quantification of tooth microwear was possible up to 30 minutes of tumbling for the first one (tooth # I.1), and one hour for the second (tooth # I.7). The comparison of the evolution of the variables from the beginning to 30 or 60 minutes of tumbling (according to the teeth) shows that the friction of the mix of sand and clay sediment against the enamel seems to increase the number of small and large pits and erases the fine scratches. These alterations remain quite limited since there is a maximum of seven extra pits in one case and five fine scratches erased in another.

3.3 *Sus scrofa* in the mix of sand and clay sediment.

From the fifteen teeth available at the beginning (**Tab. 1**), five were not suitable for the experiment (cf 3.1) and only two still preserved microwear features after one hour of tumbling and none after two hours. The statistical analyses will focus on the samples from the period from the beginning to 15 minutes of tumbling, where sufficient sample size is available. The other features we quantified increase regularly through time (**Fig. 1**). While the microwear patterns remain similar between the start and two minutes of tumbling, the number of fine and coarse scratches, small and large pits increases between five and 10 minutes of tumbling. The differences between the cycles are very small and never statistically significant except for the number of small pits between the beginning and five minutes of experiment ($p= 0.0267$) (cf. supplementary data 1). This variable increases from a mean of 7.33 pits at cycle 0 to 9.17 pits at cycle 3 (**Tab. 4**).

3.4 *Capra hircus* in sand sediment.

From the nine teeth available at the start (**Tab. 1**), five were observable after two hours, which allows statistical analyses on every cycle (**Tab. 2**). The number of coarse and hyper coarse scratches never changes regardless of the time spent in the tumbling machine and always

remains equal to zero. On the first axis (43.24% of the variance) the teeth that were tumbled for up to 30 minutes remained quite similar, while the number of gouges and cross scratches increased after one and two hours of experiment (**Fig. 2**). Nevertheless, the differences between the different cycles are still statistically insignificant in every variable (cf. supplementary data 1).

3.5 *Equus* sp. in sand sediment.

Only three teeth were available at the beginning of the experiment (**Tab. 1**). No statistical tests were carried out due to the small number of teeth. For all the teeth (**Tab. 3**), the observations were possible up to two hours of experiment. On two teeth (teeth # III.1 and III.6), the number of small pits decreased and the number of fine scratches increased. On the last one (tooth # III.2), the number of fine scratches remained mostly the same while the number of small pits increased. These alterations remained quite limited since there was a maximum of six extra pits, 10 erased small pits, and up to seven fine scratches added and five erased.

3.6 *Sus scrofa* in sand sediment.

From the fourteen teeth available at the start (**Tab. 1**), one was discarded at the beginning of the experiment (cf. 3.2) and eight were suitable two hours later, which allows statistical analyses on every cycle. The teeth from the beginning, and two minutes later, remained quite similar, while the number of gouges, coarse scratches and large pits increased from five minutes of tumbling up to two hours (**Fig. 2**). The differences between the cycles are very small and never statistically significant except for the number of coarse scratches between the beginning (mean = 4.38) and 10 minutes of tumbling (mean = 6.88) ($p = 0.0204$), and the beginning and two hours of tumbling (mean = 7.13) ($p = 0.0093$), which increased during the experiment (**Tab. 4**). It is also significant for the number of small pits between the beginning (mean = 20.75) and 10 minutes of tumbling (mean = 15.13) ($p = 0.0040$), and the beginning and one hour of tumbling (mean = 15.88) ($p = 0.0169$), which decreased during the experiment (cf. supplementary data 1).

In groups 1 and 2, after one hour of tumbling, one out of two equid teeth is observable and only 22.2% of the *S. scrofa* and 25% of the *C. hircus*. After two hours, the surface of all the teeth regardless of the species was too badly altered and none of them could be studied. For the sandy sediment, after one and two hours of experimentation, the three equid teeth were still observable, about 60% of the suid teeth (cycle 5: 69.23%, cycle 6: 61.53%), and 55.55% (for the two cycles) of the caprid teeth.

3.7 Morphological description of the occlusal surface of the six selected teeth

During the observation, it has been possible to document that the main problem when carrying out microwear analyses on teeth affected by tumbling processes is that this process may have altered or erased the dietary microwear features. Since dietary inferences are made from quantification of these scratches and pits, their partial or total disappearance can lead to erroneous dietary interpretations. For this reason, we have proceeded to describe the moment in which the number of microwear scratches or pits differs from the original, as well as to identify criteria that allow the identification of abrasion marks.

After the microscopic analysis, two trends have been observed (see supplementary material 2): 1) appearance of abrasion marks produced during tumbling; 2) disappearance of microwear scratches and pits as a result of tooth enamel abrasion after tumbling.

Regarding the first point the appearance of new abrasion marks and taphonomic pits it is noteworthy that they are more likely to be distinguishable from dietary microwear scratches in all phases of the observation (**Fig. 3**). The abrasion marks tend to be wider, shorter, with a chaotic random distribution, being more frequent on the most exposed parts of the teeth (such as the cusps or the edges) and producing changes in the texture of the enamel. The taphonomic pits present an irregular morphology and changes in relation to the enamel texture (**Fig. 3**). Regarding the second point, relative to the disappearance of microwear scratches and pits, we can determine that it is a consequence of the abrasion or erosion produced by the sedimentary particles.

This evidence appeared earlier in the case of wild boar and goat (during cycle 2), while in the case of equids these can be documented up to cycle 4.

According to the type of sediment, in the case of wild boar and equids, the mix of sand and clay sediment produced more abrasion marks and taphonomic pits than sand. The mix of sand and clay sediment began to obliterate the microwear pattern after five minutes (wild boar) and 30 minutes (equid), while the sands did so after 15 minutes and 60 minutes respectively. In the case of the goat the two sediments erased the microwear features starting at 30 minutes in both sedimentary contexts. Polishing the enamel surface implied the progressive disappearance of at least part of the scratches. It has been observed that polishing appeared at different times in the case of equid and boar teeth. In the case of wild boar, the tips of the cusps are the first place where abrasion is documented, while at the base of the cusps there are no signs of alteration. In the case of the equid, the abrasion appears first at the edges of the tooth, while the enamel in the middle of the occlusal surface remains unaltered. This does not occur in the case of the goat, probably as a consequence of the specific morphology of selenodont teeth.

4. Discussion

4.1 Differences between sand and the mix of sand and clay sediments.

Taphonomic analysis revealed a tendency towards an increase in the numbers of abrasion marks, taphonomic pits and gouges over time, both in sands and in the mix of sand and clay sediment. Some previous experimental work on bone tumbling had determined that the creation of striae is a consequence of the abrasion process, regardless of the content of the sedimentary matrix employed (Behrensmeyer et al. 1986; Domínguez-Rodrigo et al. 2009, 2017; Gaudzinski-Windheuser et al. 2010; Rabinovich et al. 2012; Pineda et al. 2019). In fact, the first works of Behrensmeyer established that abrasion is caused by the sedimentary particles in suspension (Behrensmeyer et al. 1986). These works had not analysed the impact of this process on teeth; however, our results suggest that it also occurs on teeth in a similar form.

The appearance of these new features (abrasion marks, taphonomic pits) is exponential to the time of exposure of the materials to the experimental process. However, a difference in relation to the types of sediment used was detected: while in the mix of sand and clay sediment most abrasion marks, taphonomic pits and gouges appear in intermediate cycles (2 and 3, which imply a maximum of 15 minutes of abrasion), in the case of sands, a notable increase is detected from more advanced cycles (more than 30 minutes of exposure). This fits with the observations made by Fernández-Jalvo and Andrews (2003), which suggested that sands have a high abrasive power when experimental processes of abrasion are conducted. Through these observations, in our experiment the sand sediment was expected to be more

abrasive than the mix of sand and clay sediment due to the abundant presence of sub-angular quartz particles (60% of the sediment). In the mix of sand and clay sediment, the quartz grains were scarcer (20% of the sediment) and blunt.

Miocene sand particles are larger (350-500 μm) than the ones in the mixed sand and clay sediment (150-200 μm). As we used the same weight of sediment in the tumbling machine, this means that more particles were present in the mixture of sand and clay sediment. It is possible that the abrasion potential of each particle has a lower impact than the number of times a particle comes into contact with the enamel. Since more grains are present in the mix of sand and clay sediment, the probability of impacting the enamel is higher than in the sandy sediment, which contains fewer particles. In their experiment, King et al. (1999) also observed that the “smallest particles caused the most damage to microwear features and enamel” (p. 367).

On the other hand, in some cases we have documented a decrease in scratches and pits. This phenomenon occurs in the fine scratches documented in equid teeth subjected to tumbling with the mix of sand and clay sediment and pitting in equid and wild boar teeth in sands. Previous experimental research focused on the effect of the abrasion processes on anthropogenic (cut marks) and natural (abrasion marks) modification has shown the power of this process to alter and obliterate bone surface modifications (Shipman and Rose 1983; Behrensmeyer et al. 1989; Gaudzinski-Windheuser et al. 2010; Rabinovich et al. 2012; Pineda et al. 2019). After a prolonged exposure of the materials to tumbling (more than one hour) the main characteristic is the total or almost total disappearance of scratches and pits from the enamel surfaces in both types of sediment (**Tab. 5; Fig. 4**) In the case of microwear analysis, the enamel surfaces of the teeth were too altered by the experiment (often after one hour of tumbling) and the original microwear pattern was completely unreadable. The dietary microfeatures were completely erased and replaced by abrasion marks. In a regular dietary microwear study, these teeth would have been discarded. For the teeth that still preserve some original (dietary) microwear pattern, the identification of features of taphonomic origin is necessary to recognize enamel areas that are suitable for microwear analysis and to produce reliable and accurate results.

Tumbling experiments on bones with more or less quartz-rich sediment show that the mix of sand and clay sediment has a similar impact to sandy sediment (Rozada et al. 2018). Several leads can explain the importance of the impact of the mix of sand and clay sediment compared to sand sediment, but this issue needs to be more carefully examined and other types of sediment need to be considered with a larger sample of teeth.

Before the microwear pattern was completely erased, we were able to observe that the friction of the sediment against the enamel slightly altered the diet-related features. For all the species, the mix of sand and clay sediment appears to increase the number of pits and reduce the number of scratches while the sand appears to have the opposite effect. However, those impacts are minimal and never significant except in some cases for *Sus scrofa* (cf. *infra*). This experiment highlights the different impact on the teeth depending on the sediment. Studies conducted on archaeological and paleontological material should be more cautious and look out for taphonomic alterations, especially when the sediment is fine (like in the mix of sand and clay sediment).

4.2 Differences between *Equus* sp., *Capra hircus* and *Sus scrofa*

A larger proportion of equid teeth than those of goats or wild boars can be studied in cycles 5 or 6 in the two types of sediments i.e. after 60 to 120 minutes of tumbling (**Tab. 5**). This could be due to the different morphology of the teeth. Equid teeth, being larger, elongated, and heavier than those of wild boar or goat, must roll in the tumbler on their lateral faces (of

the crown of the tooth) and have little impact with the occlusal surface (which we have observed). On the contrary, the teeth of wild boar or goat, being smaller and lighter, must have had a similar impact on all their faces. It could also suggest that equid enamel is stronger than the other two species. The hardness of enamel depends on its mineral density, which permits its resistance to abrasion (Waters 1980). This property also makes it more fragile and easily breakable, especially at the interface of the crystallites that compose it (Waters 1980; Currey 1999). Our experiment did not result in macroscopic fractures but in an intensive abrasion of the occlusal surface. Previous works already underlined the hardness of equid enamel because of its adaption to grazing a large amount of low-quality fodder, which leads to high abrasion patterns (Janis 1976), although the hardness of the enamel in horses seems to vary between domestic breeds and increases with increasing age (Muylle et al. 1999). We could not find any direct data evaluating the hardness of *Capra* enamel, but it is probably less hard than that of horses (Popowics and Herring 2006). Studies have shown that the enamel of Suidae is weak, especially compared to humans (Popowics et al. 2004; Popowics and Herring 2006). This property allows suid teeth to remove the tip of the tooth and preserve what remains, which would improve the durability of the crown for a varied diet (Popowics *op. cit.*; Popowics and Herring *op. cit.*). However, in our study, we only considered very slightly worn suid teeth with intact cusp tips. Thus, we considered the most fragile portions of the suid teeth.

We have only included a few equid teeth for comparison but these preliminary observations would indicate that the strength of their enamel preserves them better than other species from alterations due to tumbling. Goat teeth are more fragile. It is likely that this is also due to the thinner enamel of the buccal and lingual walls compared to those of equids. Nevertheless, as long as the enamel remained in good enough condition to allow microscopic observation, the variations observed between cycles were always minimal and not significant. Due to the 'rounded' shape of the wild boar teeth, i.e. brachydont and bunodont teeth, they tumble on all surfaces. Equid and goat teeth are hypsodont and their elongated shape probably tumbled around their main axis, and the contact with the sediment affected the tooth crown more than the occlusal surface. In wild boar the tips of the cusps were altered before the deeper 'valleys' in between the cusps (more protected areas). In equid and goat teeth, the tumbling affected the crown, and not so much the occlusal surface. For the equids, the edges of the surface at the limit with the crown are impacted by the tumbling, while for the goat the potential alterations on the occlusal surface are rather homogenous, probably due to the smaller occlusal surface of the teeth.

4.3 Effect of taphonomic processes on microwear studies

The observation of qualitative microscopic features on the sample of six selected teeth highlights two trends: 1) the appearance of abrasion marks and pits produced during tumbling; 2) the disappearance of microwear scratches and pits as a result of tooth enamel abrasion from tumbling; 3) the appearance of polished surfaces, in some cases, at the tip of the cusps in wild boar teeth and at the edges of the occlusal surface in equid teeth.

The effects of abrasion include changes in the texture of the enamel, loss of tissue (mostly inferred though documentation of notches on the edges of the teeth) and changes in the surface of the teeth, including the appearance of new striae and the obliteration of dietary-related ones.

Microscopically, more abrasion marks and taphonomic pits are likely to be distinguished from those produced by diet, one reason why its identification is not a problem for microwear studies (Teaford 1988; King et al. 1999; El-Zaatari 2010). The abrasion marks tend to be thicker, shorter, with an isotropic distribution, more frequent on the most exposed parts of the

teeth (such as the cusps or the edges) and produce changes in the texture of the tooth enamel. The pits present an irregular morphology and also changes in relation to the enamel texture (Fig. 3).

Also, in previous experimental works, the presence of polished surfaces on the enamel surface of domestic caprines has been related to a higher intake of abrasive diets (e.g. Walker et al. 1978; Mainland 1997; Jiménez-Manchón et al. 2020). This work shows that taphonomic enamel polishing appears on precise locations on the teeth according to the shape of the teeth (and so according to the species). Thus, the study of diet-related polished surfaces must be limited to the areas least sensitive to taphonomic alterations.

Loss of dietary features, scratches and pits could represent a limitation for microwear research. Dental microwear analyses are based on the quantitative analysis of these features. If they disappear due to taphonomic alterations, then it is no longer possible to correctly characterise an individual's diet. Ignoring the presence of alterations in an archaeological or paleontological assemblage could lead to misinterpretation and errors. For this reason, microwear studies should include a taphonomic analysis of the occlusal surface before observing and analysing the microwear features. The taphonomic study should include identification of the features described in the paper, with consideration of their location on the surface and their quantity. This would allow the location of areas suitable for microwear analysis. Areas with polished surfaces are not suitable for microwear as the original microwear patterns have been completely erased. The microwear analysis should be limited to teeth presenting as being well-preserved, non-altered surfaces of enamel. In this sense, our experimental work has shown that polishing did not affect all the occlusal surfaces in the same way. In general, equid enamel is more robust than that of suids and caprid. In equids, the evidence of abrasion first appears at the edges of the occlusal surface. For wild boar, the tips of the cusps are the more fragile part of the teeth and the first area where abrasion is documented. However, we have observed that other areas less exposed to abrasion (such as the base of the cusps of the wild boar tooth, or the enamel areas in the middle of the occlusal surface of the equid), remain unaltered over a much longer time of exposure to abrasion. Statistical analyses show that the increase in the number of features is generally low and does not have a significant impact on the interpretation of an individual's diet until the texture of the enamel is completely altered. Nevertheless, as a precaution, observers should reserve their studies for the best-preserved portions of teeth and discard those which present alterations on the whole occlusal surface. This does not occur in the case of goats, probably as a consequence of the specific morphology of selenodont teeth.

5. Conclusions

Our study aimed to evaluate the impact of a taphonomic alteration corresponding to sediment abrasion, tumbling, on the dental microwear patterns from occlusal enamel surfaces. Our analyses showed that these alterations had a different impact according to the sediment used and the taxa. Indeed, fine particles such as the mix of sand and clay sediment polish the enamel surfaces more, thus producing more alterations than the more abrasive but larger particles of sand. Polishing the enamel surface implies the progressive disappearance of part of the microwear features, thus leading to a quantitative error in the analysis, because the counting of scratches and pits would be biased by this alteration and would entail erroneous interpretations about the diet of the individuals studied. Therefore, we can conclude that in the areas where evidence of polishing of the enamel is documented, microwear studies cannot be carried out. However, it has been observed that polishing appears at different times during the experiment in the case of equid and wild boar teeth. In the case of wild boar, the tips of the cusps are the first where abrasion is documented, while at the base of the cusps there is no

evidence of alteration. In the case of the equid, the abrasion appears first at the edges of the occlusal surface, while the enamel in the centre of the surface remains unaltered. For this reason, we propose that the study of these preserved areas is valid even if there is evidence of abrasion in the most exposed areas. In any case, the abrasion marks that were produced during tumbling are different from the original dietary pits and scratches. It is possible, with some training by experienced researchers, to identify and discard them from the microwear quantification.

Acknowledgements

The authors would like to thank the International Research Network (IRN 0871 CNRS-INEE): Taphonomy European Network (TaphEN) which supported this project. It has also been supported by LabEx ARCHIMEDE from the “Investissement d’Avenir” programme ANR-11-LABX-0032-01. The research of F.R. is supported by the Spanish Ministry of Science and Innovation through the PID2019-103987GB-C31 project and the “María de Maeztu” excellence accreditation (CEX2019-000945-M) and by the Generalitat de Catalunya through the 2017-SGR-836 projects, and the CERCA Program. A.X. was funded by the Austrian Science Fund (FWF, project number P29501-B25). A.P. is supported by the Spanish Ministry of Science and Innovation (FJC2019-040804-I, Subprograma Juan de la Cierva-Formación). The authors would also like to thank two anonymous reviewers for their very helpful comments.

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Table captions

Group	Number of teeth				Type of sediment	Time of exposition
	<i>Equus</i> sp.	<i>Capra hircus</i>	<i>Sus scrofa</i>	Total		
G1	2	10		12	Mix of sand and clay	2h (6 cycles)
G2			15	15		
G3	3	9		12	Sand	
G4			14	14		

Table 1: Groups used in the tumbling experiment: taxa, number of teeth, sediment type and time of exposition.

Sediment	Cycle	Time in the tumbler	Total time	N	Large pits	Small pits	Fine scratches	Coarse scratches	Hyper-coarse scratches	Cross scratches	Gouges
Sand-clay	0	0 min	0 min	5 m s	1.60 1.34	17.60 6.19	4.20 0.84	0.40 0.89	0.00 0.00	0.20 0.45	0.00 0.00
Sand-clay	1	2 min	2 min	5 m s	1.80 1.30	18.00 4.36	5.00 2.83	0.40 0.89	0.00 0.00	0.20 0.45	0.00 0.00
Sand-clay	2	3 min	5 min	5 m s	2.40 1.67	20.40 3.71	6.00 2.00	1.00 1.73	0.00 0.00	0.80 1.10	0.00 0.00
Sand-clay	3	10 min	15 min	5 m s	1.80 1.30	23.00 7.11	7.60 2.41	0.40 0.55	0.00 0.00	1.00 1.41	0.00 0.00
Sand-clay	4	15 min	30 min	5 m s	3.00 2.12	28.40 15.53	6.80 3.11	0.20 0.45	0.20 0.45	0.00 0.00	0.00 0.00
Sand	0	0 min	0 min	5 m s	0.80 1.79	25.40 8.93	10.20 4.66	0.00 0.00	0.00 0.00	1.00 0.71	0.00 0.00
Sand	1	2 min	2 min	5 m s	0.60 0.89	24.60 9.45	9.60 3.05	0.00 0.00	0.00 0.00	1.00 0.71	0.00 0.00
Sand	2	3 min	5 min	5 m s	1.40 1.34	24.60 9.69	9.80 3.11	0.00 0.00	0.00 0.00	1.00 0.71	0.00 0.00
Sand	3	10 min	15 min	5 m s	1.40 1.34	20.60 4.04	9.00 3.08	0.00 0.00	0.00 0.00	1.00 0.71	0.00 0.00
Sand	4	15 min	30 min	5 m s	0.20 0.45	23.20 6.83	10.60 2.07	0.00 0.00	0.00 0.00	1.20 0.84	0.00 0.00
Sand	5	30 min	60 min	5 m s	0.40 0.89	19.80 5.07	8.20 2.49	0.00 0.00	0.00 0.00	1.80 0.45	0.00 0.00
Sand	6	60 min	120 min	5 m s	0.00 0.00	22.20 8.41	7.00 2.12	0.00 0.00	0.00 0.00	1.40 0.55	0.40 0.89
Sediment	Cycle	Time in the tumbler	Total time	N	Large pits	Small pits	Fine scratches	Coarse scratches	Hyper-coarse scratches	Cross scratches	Gouges

Table 2: Summary of the data for the teeth of *Capra hircus* in sand and in the mix of sand and clay sediment. N = number of teeth; m = mean; s = standard deviation.

Sand-clay	0	0 min	0 min	1	n°I.7	7	24	9	4	0	1	0
Sand-clay	1	2 min	2 min	1	n°I.7	6	24	10	4	0	2	0
Sand-clay	2	3 min	5 min	1	n°I.7	4	22	12	2	0	2	0
Sand-clay	3	10 min	15 min	1	n°I.7	4	23	12	3	0	2	0
Sand-clay	4	15 min	30 min	1	n°I.7	3	23	14	2	0	0	3
Sand-clay	5	30 min	60 min	1	n°I.7	4	26	10	4	0	4	0
Sand	0	0 min	0 min	3	m s	0.00 0.00	11.33 5.51	18.00 6.08	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
Sand	1	2 min	2 min	3	m s	0.00 0.00	12.67 3.06	17.33 6.66	0.00 0.00	0.00 0.00	0.67 0.58	0.00 0.00
Sand	2	3 min	5 min	3	m s	0.00 0.00	15.00 3.61	17.00 4.36	0.00 0.00	0.00 0.00	0.67 0.58	0.00 0.00
Sand	3	10 min	15 min	3	m s	0.00 0.00	12.33 0.58	16.33 5.86	0.00 0.00	0.00 0.00	2.00 1.00	0.00 0.00
Sand	4	15 min	30 min	3	m s	0.67 1.15	10.67 2.52	16.33 2.31	0.00 0.00	0.00 0.00	1.00 1.00	0.00 0.00
Sand	5	30 min	60 min	3	m s	3.00 3.00	12.00 2.65	15.33 3.06	0.67 0.58	0.00 0.00	1.67 0.58	0.00 0.00
Sand	6	60 min	120 min	3	m s	4.00 3.61	15.33 1.15	13.33 6.51	0.33 0.58	0.00 0.00	1.33 0.58	0.33 0.58
Sediment	Cycle	Time in the tumbler	Total time	N		Large pits	Small pits	Fine scratches	Coarse scratches	Hyper- coarse scratches	Cross scratches	Gouges

Table 3: Summary of the data for the teeth of *Equus* sp. in sand and in the mix of sand and clay sediment. N = number of teeth; m = mean; s = standard deviation.

Sand-Clay	0	0 min	0 min	6 m	3.50	7.33	9.00	4.83	1.17	1.00	0.33
				s	3.08	2.16	2.53	1.72	1.17	0.00	0.82
Sand-Clay	1	2 min	2 min	6 m	3.33	8.17	8.83	4.83	1.17	1.00	0.33
				s	2.80	2.48	2.48	1.72	1.17	0.00	0.82
Sand-Clay	2	3 min	5 min	6 m	4.00	9.17	9.17	5.33	1.50	1.00	0.67
				s	3.41	2.23	3.31	2.16	1.64	0.00	1.03
Sand-Clay	3	10 min	15 min	6 m	5.83	9.17	9.33	6.67	2.33	1.00	3.50
				s	5.49	3.43	6.35	2.42	2.25	0.00	3.89
Sand	0	0 min	0 min	8 m	6.50	20.75	22.38	4.38	1.00	6.63	0.38
				s	1.85	3.49	3.38	0.92	0.76	1.06	0.52
Sand	1	2 min	2 min	8 m	6.63	18.75	23.13	5.25	1.00	8.13	0.13
				s	2.07	1.98	2.59	1.04	0.53	1.64	0.35
Sand	2	3 min	5 min	8 m	8.88	16.38	23.25	6.25	0.88	7.00	0.38
				s	2.10	2.20	1.67	1.67	0.64	0.93	0.52
Sand	3	10 min	15 min	8 m	7.13	15.13	21.38	6.88	0.75	6.63	0.25
				s	1.64	2.36	2.07	1.46	0.71	1.51	0.46
Sand	4	15 min	30 min	8 m	6.25	16.38	20.25	6.38	0.88	6.38	0.63
				s	1.49	2.45	2.96	1.30	0.64	2.92	0.52
Sand	5	30 min	60 min	8 m	6.88	15.88	21.38	6.38	1.25	7.00	0.38
				s	2.23	2.95	2.07	1.85	0.46	2.33	0.52
Sand	6	60 min	120 min	8 m	6.75	19.25	22.00	7.13	1.25	7.25	0.13
				s	1.58	2.60	2.98	1.25	0.46	1.83	0.35

Table 4: Summary of the data for the teeth of *Sus scrofa* in sand and in the mix of sand and clay sediment. N = number of teeth; m = mean; s = standard deviation.

	Cycle	Duration of tumbing	<i>Equus</i> sp.		<i>Sus scrofa</i>		<i>Capra hircus</i>	
			N	%	N	%	N	%
Sand-Clay sediment	0	0 min	2	100	9	100	8	100
	1	2 min	2	100	9	100	8	100
	2	3 min	2	100	9	100	7	87.5
	3	10 min	2	100	6	66.7	6	75
	4	15 min	2	100	3	33.3	6	75
	5	30 min	1	50	2	22.2	2	25
	6	60 min	0	0	0	0	0	0
Sand sediment	0	0 min	3	100	13	100	9	100
	1	2 min	3	100	11	84.6	9	100
	2	3 min	3	100	9	69.2	7	77.8
	3	10 min	3	100	9	69.2	7	77.8
	4	15 min	3	100	9	69.2	7	77.8
	5	30 min	3	100	9	69.2	5	55.5
	6	60 min	3	100	8	61.5	5	55.5

Table 5: Number of teeth analysed for microwear after each cycle of tumbling in the mix of sand and clay sediment and sand sediments and percentage of teeth still suitable for observation for each cycle compared to cycle 0.

Figure captions

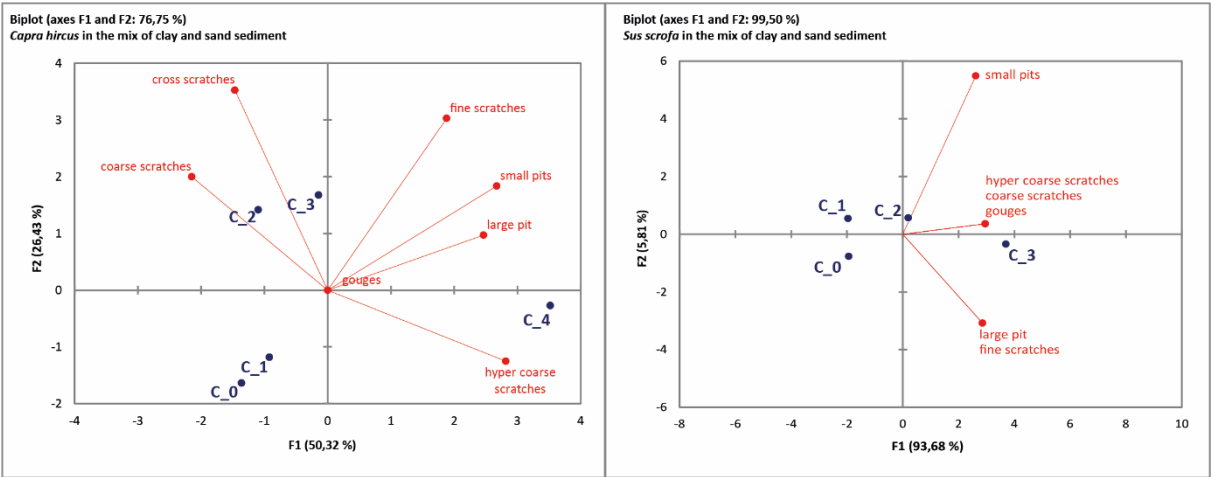


Figure 1: Principal Component Analysis (PCA) based on the microwear variables traditionally employed to characterise the dietary traits of herbivores on the tumbled teeth of *Sus scrofa* and *Capra hircus* in the mix of sand and clay sediment. The variables used are the number of fine, coarse, and hyper coarse scratches, the number of small and large pits, the number of gouges and cross scratches (except for *Sus scrofa*). Duration of the cycles of tumbling: C0 = 0 min; C1 = 2 min; C2 = 3 min; C3 = 10 min; C4 = 15 min.

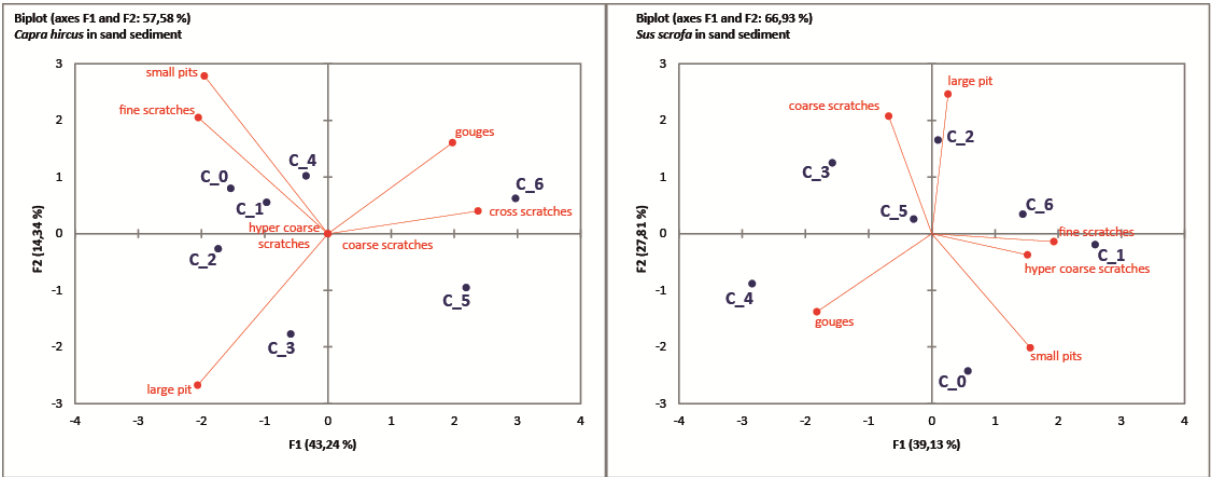


Figure 2: Principal Component Analysis (PCA) based on the microwear variables traditionally employed to characterise the dietary traits of herbivores on the tumbled teeth of *Sus scrofa* and *Capra hircus* in sand. The variables used are the number of fine, coarse, and hyper coarse scratches, the number of small and large pits, the number of gouges and cross scratches (except for *Sus scrofa*). Duration of the cycles of tumbling. C0 = 0 min; C1 = 2 min; C2 = 3 min; C3 = 10 min; C4 = 15 min; C5 = 30 min; C6 = 60 min.

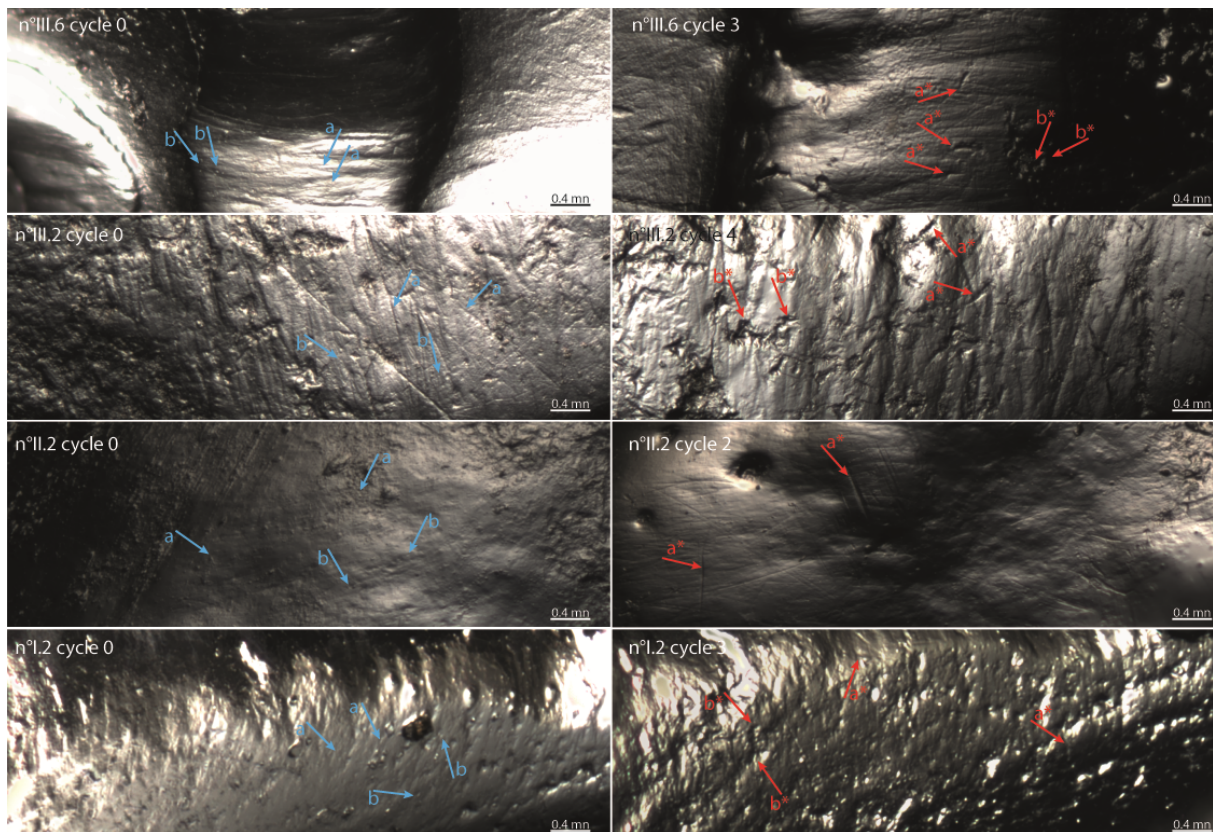


Figure 3: Examples of the morphology of taphonomic features compared to dietary features: a: dietary scratches; b: dietary pits; a*: trampling marks; b*: taphonomic pits. N°III.6 = *Equus* sp.; n°III.2 = *Equus* sp.; n°II.2 = *Sus scrofa*; n°I.2 = *Capra hircus*.

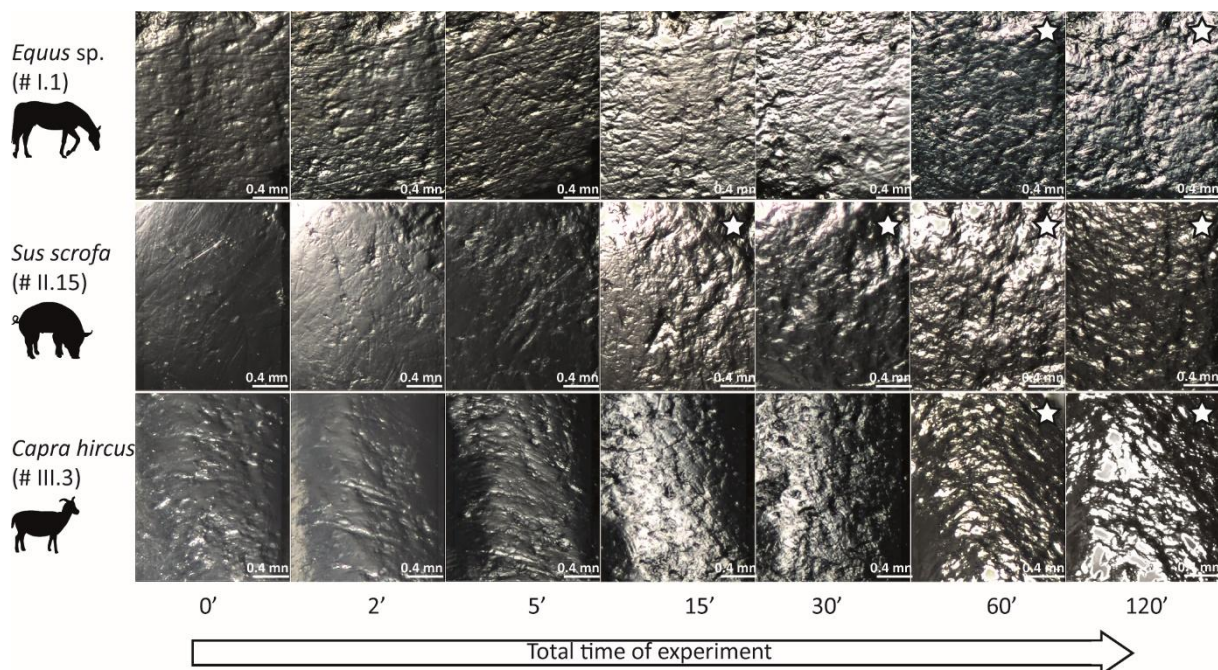


Figure 4: Example of the evolution of the alterations over time (from the beginning to two hours of tumbling). The white stars indicate the moment when the dietary observations

986 become impossible. N°I.1 = *Equus* sp.; n°II.15 = *Sus scrofa*; n°III.3 = *Capra hircus*. Animal
987 silhouettes downloaded from all-free-download.com.
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