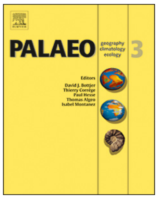
Palaeogeography, Palaeoclimatology, Palaeoecology 556 (2020) 109886

Contents lists available at ScienceDirect

Palaeogeography, Palaeoclimatology, Palaeoecology

journal homepage: www.elsevier.com/locate/palaeo



# Dental wear at macro- and microscopic scale in rabbits fed diets of different abrasiveness: A pilot investigation

Louise F. Martin[a,*], Lisa Krause[b], Arlett Ulbricht[c], Daniela E. Winkler[b,d], Daryl Codron[e], Thomas M. Kaiser[b], Jacqueline Müller[a], Jürgen Hummel[f], Marcus Clauss[a], Jean-Michel Hatt[a], Ellen Schulz-Kornas[b,c,g]

[a] *Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, 8057 Zurich, Switzerland*
[b] *Center of Natural History, University of Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany*
[c] *Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany*
[d] *Institute for Geosciences, Johannes Gutenberg University Mainz, J.-J.-Becher-Weg 21, 55128 Mainz, Germany*
[e] *Department of Zoology and Entomology, University of the Free State, PO Box 339, 9300 Bloemfontein, South Africa*
[f] *Ruminant Nutrition, Department of Animal Sciences, University of Goettingen, Kellnerweg 6, 37077 Goettingen, Germany*
[g] *Department of Cariology, Endodontics and Periodontology, University of Leipzig, Liebigstrasse 12, 04103 Leipzig, Germany*

## ARTICLE INFO

*Keywords:*
Lagomorph
Tooth wear
Mesowear
Texture analysis
Grit
Phytoliths

## ABSTRACT

To differentiate the effects of internal and external abrasives on tooth wear, we performed a controlled feeding experiment in rabbits fed diets of varying phytolith content as an internal abrasive and with addition of sand as an external abrasive. 13 rabbits were each fed one of the following four pelleted diets with different abrasive characteristics (no phytoliths: lucerne L; phytoliths: grass G; more phytoliths: grass and rice hulls GR; phytoliths plus external abrasives: grass, rice hulls and sand GRS) for two weeks. At the end the feeding period, three tooth wear proxies were applied to quantify wear on the cheek teeth at macroscopic and microscopic wear scales: CT scans were obtained to quantify tooth height. Mesowear was scored adapted to this species, and 3D dental microwear texture analysis (DMTA) was performed on four antagonistic teeth. Both external and internal abrasives resulted in increased wear in all proxies compared to the phytolith and sand-free diet (L). The wear effect was more prominent on the maxillary than on the mandibular teeth. On the GRS diet, the upper third premolar had the largest decline in relative tooth height compared to others in the same tooth row. The impact of diet abrasiveness on the mesowear signal was only clearly visible for the most abrasive diet, most likely due to the limited sample size. DMTA was especially sensitive to phytolith changes in the diet, and surface roughness generally increased with increasing amounts of abrasive agents (L < G < GR < GRS) as expressed in an increase of most height and volume parameters. The fast pace of dental wear in this species led to some expected correlations between tooth height, mesowear and DMTA parameters, creating a distinct wear pattern for each diet. Animal models with high wear rates may be particularly suitable for investigations on functional inter-relationships of different wear proxies.

## 1. Introduction

Due to the prominent use of dental wear proxies in palaeobiology, but also because of the high incidence of dental diseases in pet animals, research is directed at understanding the mechanical processes and sequences of tooth wear. Dental wear in general occurs whenever a tooth comes into contact with another tooth (‘attrition’) or ingesta (‘abrasion’; Kaiser et al. (2016)). One of the major questions in tooth wear research is what substances are responsible for abrasive wear.

The abrasive elements in an herbivore diet are customarily separated by origin into external (e.g. grit, dust, sand) and internal (e.g. plant phytoliths) abrasives. Their respective effects have been debated based on outcomes of both theoretical and experimental studies, and are key for palaeo-ecological reconstruction (Kaiser et al., 2018; Lucas et al., 2014; Merceron et al., 2016; Sanson et al., 2017). The estimated range for soil intake in pronghorns (*Antilocapra americana*) is on

---

* Corresponding author.
*E-mail addresses:* lfmartin@vetclinics.uzh.ch (L.F. Martin), daniela.winkler@uni-mainz.de (D.E. Winkler), CodronD@ufs.ac.za (D. Codron), thomas.kaiser@uni-hamburg.de (T.M. Kaiser), jhummel@gwdg.de (J. Hummel), mclauss@vetclinics.uzh.ch (M. Clauss), jmhatt@vetclinics.uzh.ch (J.-M. Hatt), Ellen.Schulz-Kornas@medizin.uni-leipzig.de (E. Schulz-Kornas).

https://doi.org/10.1016/j.palaeo.2020.109886
Received 27 January 2020; Received in revised form 6 May 2020; Accepted 11 May 2020

average 5.4% of daily dry matter intake and 6.3% in wild jack rabbits (*Lepus californicus,* Arthur and Gates (1988)). These examples illustrate that external inorganic abrasives are unavoidable for herbivorous animals whilst feeding, as wind and rain drops distribute dust particles from the ground onto plant material, and have been shown to cause a considerable amount of tooth wear (Damuth and Janis, 2011; Hummel et al., 2011; Karme et al., 2016; Müller et al., 2014; Müller et al., 2015). On the other side, several in vivo experiments have demonstrated characteristic differences in tooth wear patterns when animals were fed with experimental diets varying exclusively in phytolith concentration (Martin et al., 2019; Müller et al., 2014; Schulz et al., 2013; Winkler et al., 2019).

Tooth wear can be assessed using a variety of proxies at different scales of resolution. Scott (1979) introduced the term ‘macrowear’ in humans to define the loss of enamel at the occlusal surface with a ranking system. Enamel loss of 2 mm in aging people is designated with the maximum score of macrowear (Smith and Knight, 1984). In comparison, tooth wear rates in wild rabbits are estimated at around 60 mm per year alone (Damuth and Janis, 2014)! The tooth anatomy of hypselodont mammals differs fundamentally from human teeth. Enamel initially covers the entire occlusal surface of the tooth, but with wear eventually forms only the rim surrounding one or two dentin basins (Ungar, 2010). Therefore, macroscopic dental wear in rabbits can be defined as actual loss of crown tissue which can be measured either by quantifying the tooth dimensions (Meredith et al., 2015; Müller et al., 2014), or mesowear scoring (Ulbricht et al., 2015).

The mesowear method classifies wear by the cusp shape and the occlusal relief of the ectoloph of premolars and molars. It was developed to differentiate dietary preferences in large mammals, mostly ungulates (Fortelius and Solounias, 2000) but has recently been validated for small mammals, including *Leporinae* (Ulbricht et al., 2015). The microscopic wear caused by attrition and abrasion have traditionally been interpreted via manual or automated inspection of the tooth surface under a microscope (Gomes Rodrigues et al., 2009; Nelson et al., 2005). Non-contact automated methods have become more popular and allow for an interpretation of the microwear beyond 2D (Calandra et al., 2016; Merceron et al., 2010; Schulz et al., 2010; Schulz et al., 2013a; Scott et al., 2005; Ungar et al., 2003). 3D methods using interferometric or confocal laser- surface scanning provide a comprehensive image of the microscopic landscape created on the enamel surface. The current standard is dental microwear texture analysis (DMTA), using standardized parameter sets from industrial applications to quantify surface characteristics such as roughness, (an)isotropy, and complexity (Calandra and Merceron, 2016; Scott et al., 2006). Fortelius and Solounias (2000) note that each level of resolution answers somewhat different questions on the functionality of tooth wear, and simultaneously reflects different time signals. Typically, DMTA is considered a short-term signal in comparison to mesowear (Davis and Pineda Munoz, 2016). By contrast, there is no clear concept on the sequence and relationship between macroscopic wear and mesowear: a loss of cusp tissue leading to a change in cusp shape is evidently macroscopic wear. Yet, it has also been suggested that macroscopic wear can occur (by proportional loss of all aspects of the occlusal surface) without changing occlusal relief or cusp shape reflected in the mesowear score (Kaiser et al., 2013).

Whether dental wear differs systematically across the cheek tooth row, resulting in a ‘wear gradient’, has been investigated repeatedly. In several species differing in dental morphology and digestive physiology, it was shown that not every tooth is affected by wear to the same extent (Clauss et al., 2007; Martin et al., 2019; Müller et al., 2014; Müller et al., 2015; Schulz et al., 2010; Taylor et al., 2016). In terms of macroscopic wear (tooth height and mesowear), this may be an effect of sequential eruption, where older teeth simply show more wear. In hypselodont species like rabbits, however, a wear gradient cannot be explained by the eruption sequence. Other causes for a gradient might include systematic differences in the specific anatomy of the cheek teeth, such as differences in the enamel to dentine ratio along the ungulate cheek tooth row (Winkler and Kaiser, 2015); differences in enamel hardness along the tooth row in rabbits (Shakila et al., 2015); differences in enamel decussation patterns (Koenigswald et al., 2010) and their orientation relative to the chewing stroke; changes in chewing force along the tooth row or a higher amplitude of the chewing movement in laterally chewing mammals with increasing distance to the mandibular joint; or a systematic change in the exposure to external abrasives that are suggested to be less mixed into the ingesta bolus at ingestion (Schulz et al., 2010; Taylor et al., 2013).

The interaction of dental morphology, function and wear in lagomorphs has been explored paleontologically (Fraser, 2010; Koenigswald et al., 2010) and experimentally at different levels of resolution, ranging from macroscopic tissue loss (Meredith et al., 2015; Müller et al., 2014; Ness and Brown, 1956) down to microscopic wear traces (Schulz et al., 2013). The rabbit's (*Oryctolagus cuniculus*) hypselodont cheek teeth are more susceptible to wear than the hypsodont dentition of ungulates (Damuth and Janis, 2014) whilst growing at a flexible rate to compensate for crown loss (Wyss et al., 2016). Since this compensatory mechanism is not exact (Müller et al., 2014), differences in tooth wear can be detected at short-term intervals, and the expeditious wear and regrowth allows the use of these animals in a sequential experiment design.

The aim of this study was to quantify the wear effect of internal and external abrasives on lagomorph teeth at different levels of resolution in the same animals in which previously macroscopic wear measurements had been performed (Müller et al., 2014), and to test for the correlations between the three wear proxies. Following the results from the macroscopic wear measurements (Müller et al., 2014), we expect to detect corresponding wear effects in mesowear and microwear surface texture analysis. In addition, we aim to explore the wear gradient and the difference in wear pattern between maxillary and mandibular teeth at all resolutions.

## 2. Methods

The experiment was performed with the approval of the Cantonal Veterinary Office in Zurich, Switzerland (no.80/2012). As previously described in Müller et al. (2014), thirteen female New Zealand White rabbits (*Oryctolagus cuniculus)* were housed individually for the duration of a serial feeding experiments. The rabbits received the four pelleted experimental diets for two weeks per diet in a random order. The diets were identical in size and fed for ad libitum consumption. They consisted of a pelleted lucerne hay (L), grass hay pellets (G), G with incorporated rice hulls (GR) or GR with incorporated sand (GRS, added sand for playgrounds, grain size 0–1 mm, REDSUN garden products B.V., Heijen, Denmark; mean particle size measured by sieve analysis according to Fritz et al. (2012) of 0.233 ± (SD) 0.002 mm; proportion of material retained on sieve size 0.125 mm: 72 ± 2%; on sieve size 0.250 mm: 25 ± 1%; on sieve size 0.500 mm: 0%). The content of acid insoluble ash, a proxy for the silica and hence abrasives content of the diets, analysed according to Hummel et al. (2011), was 5, 16, 24 and 77 g/kg dry matter for diets L, G, GR and GRS, respectively. There was no difference in food intake (g/day), food intake rate (min per g), body mass development or health between the four diets (see also Müller et al. (2014)). After every feeding period, CT scans (Philips Brilliance 16, Philips Healthcare, Zurich, Switzerland; Images were acquired at 120 KV, 117 mA, a 10 cm FOV with a slice thickness of 1 mm.) were performed to quantify tooth height for each individual. At the end of the experiment, the animals were killed by bolt gun stunning and exsanguination, before a final CT scan was acquired. This study reports only the results from the last feeding period with the following sample numbers: L $n = 3$, G n = 3, GR $n = 4$, GRS $n = 3$. Only the cheek teeth of the left lower and upper jaw were used for analyses in this experiment. Dental nomenclature was applied as follows: mandibular cheek teeth (tm) were labelled with lower case letters (p3 to m3) and

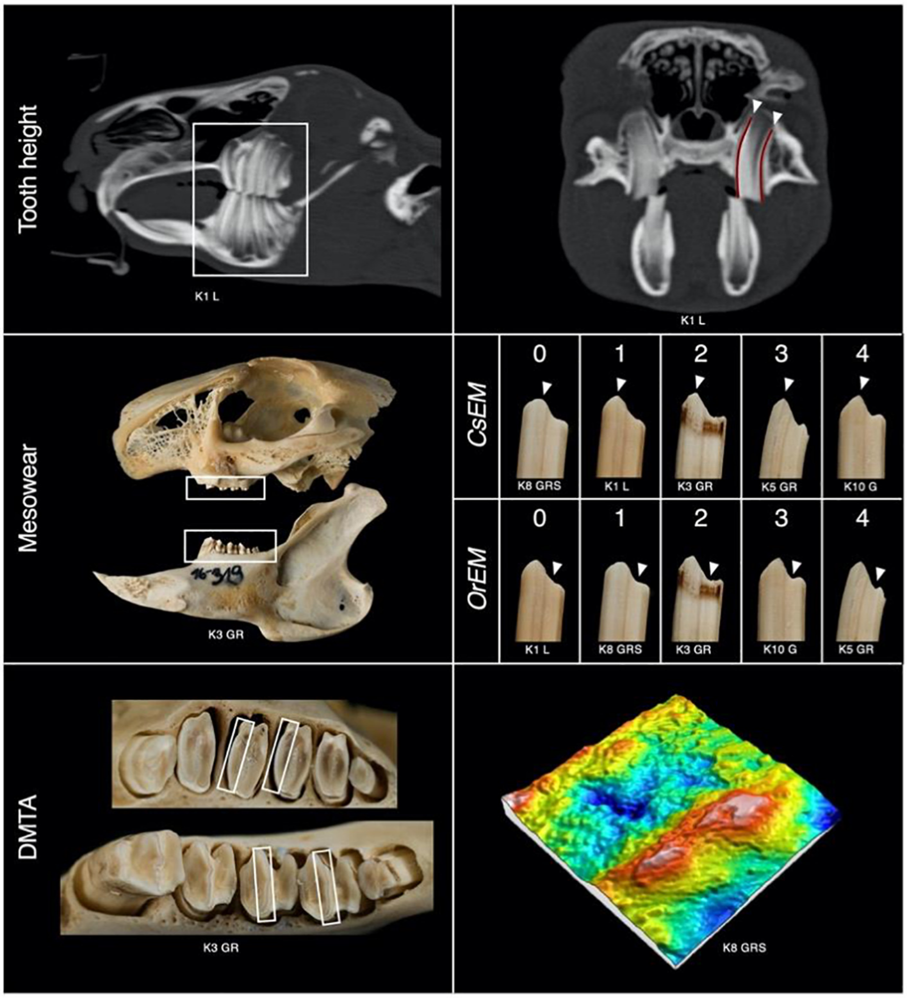


**Fig. 1.** Explanatory illustration of measurements for macrowear and DMTA parameters on the left cheek teeth of rabbits (*Oryctolagus cuniculus*, *n* = 13) fed four different pelleted diets for 14 days. Tooth height was measured on multiplanar CT scans by tracing the lingual and buccal enamel borders with an open polygon function (panel upper right). Mesowear scoring was performed on the buccal projection (panel middle left) with the extended scoring for cusp shape (CsEM) and occlusal relief (OrEM). Pictured are the scores for the anteroconid cusp and the occlusal relief of selected m1 (panel middle right; K designates the individual, followed by the pelleted diet; L lucerne, G, grass, GR grass and rice hulls, GRS grass, rice hulls and sand). DMTA analysis was performed on the anterior band of m1 and m2 as well as P4 and M1 (panel lower left). Pictured is a graphical representation of a scan (160 μm x160 μm) on m1 (panel lower right). For detailed information see methods.

maxillary cheek teeth (tx) with capital letters (P2 to M3). The recording of each wear proxy was exclusively done by one person (macroscopic wear: JM, mesowear: LFM, DMTA: LK) to avoid inter-observer error and was performed blind to the respective diet groups. Fig. 1 offers an illustrative description of applied methods.

### 2.1. Tooth height analysis

In ever-growing teeth, tooth height can vary systematically with diet because growth does not compensate wear completely (Martin et al., 2019; Müller et al., 2014; Müller et al., 2015). On rendered DICOM images, absolute tooth height was averaged for the measured tracings of the buccal and lingual border of each tooth with an open polygon function using OsiriX® software (PixmeoSarL, Bernex, Switzerland) (Fig. 1, upper panel). Relative tooth height was calculated with the tooth height on Lucerne (L) for each animal (from CT scans taken in previous stages of the serial feeding experiment) set as 100%. Note that due to the lower sample size used in the present evaluation (with only one diet per animal), the macroscopic wear results differ quantitatively from those given by Müller et al. (2014) from the same experiment, where data for all diets in all animals was reported.

### 2.2. Mesowear analysis

Cusp shape and occlusal relief were used to describe mesowear on buccal projections of the lower and upper jaw under a stereo microscope (Leica Wild M3B, Leica Microsystems Wetzlar Germany). The original scoring system developed by Ulbricht et al. (2015), with a possible score of 1 (blunt) to 3 (sharp) on p3, M2 and M1, was adapted to include an extended scoring system (Winkler and Kaiser, 2011) (Fig. 1, middle panel). Additional teeth were also included to recover as much information as possible, scoring p3, p4, m1, m2, P3, P4, M1 and M2. The lower last molar m3 and the upper first premolar P2 were excluded, because neither cusps nor a relief could be clearly identified in any specimen. On p3, the anteroconid (Cs_a), the protoconid (Cs_b) and the hypoconid (Cs_c) as well as both reliefs (Or_a, Or_b) in between were scored. The other cheek teeth were scored only on the anterior (Cs_a) and posterior cusp (Cs_b) as well as the relief (Or_a) in between. Cusp shape was classified as sharp (Cs4), round-sharp (Cs3), round (Cs2), round-round (Cs1) or blunt (Cs0). Occlusal relief was classified as high-high (Or4), high (Or3), low (Or2), flat (Or1) or flat-negative (Or0). Scores were averaged to summarize the scores for each tooth.

### 2.3. Dental microwear texture analysis (DMTA)

Three dimensional surface texture measurements were taken with a high-resolution confocal disc-scanning measurement system μsurf custom (NanoFocus AG, Oberhausen, Germany) with a blue LED (470 nm) and high-speed progressive-scan digital camera (984 × 984 pixel), set to a 100× long distance objective (numerical aperture 0.8, resolution in x, y = 0.16 μm, step size in z = 0.06 μm) according to Schulz et al. (2013). The mean of four non-overlapping scans was obtained from the anterior band of m1 and m2 as well as P4 and M1 (Fig. 1, lower panel). Only complete and clean surfaces were included in the study. Due to the narrow geometry of the enamel bands, a 40 × 40 μm measurement square had to be manually cut out of the

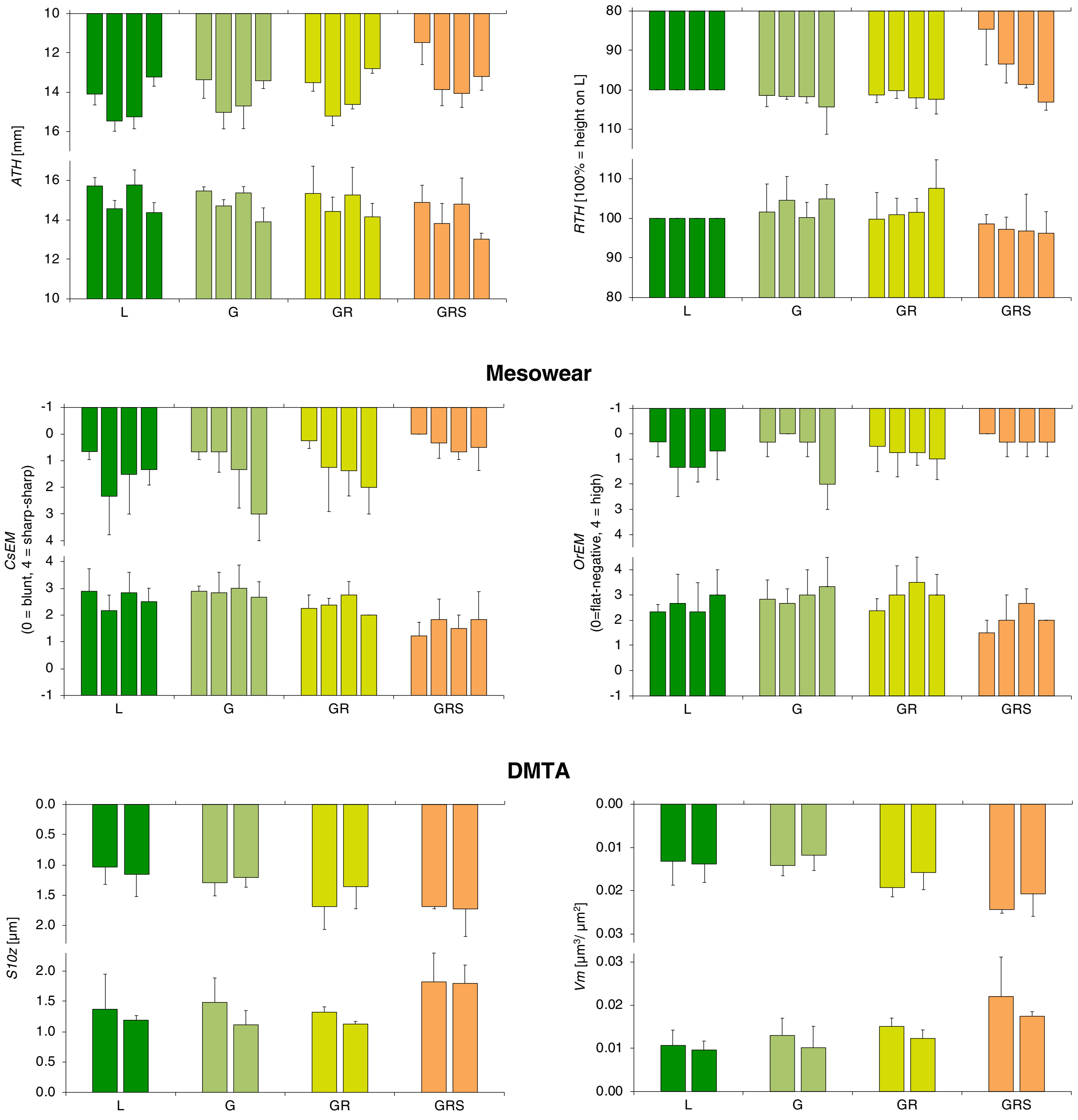


**Fig. 2.** Results ( ± SD) of macrowear (tooth height and mesowear) and DMTA parameter (*S10z* and *Vm*) measurements for selected mandibular (lower x-axis) and maxillary (upper x-axis) cheek teeth in rabbits (*Oryctolagus cuniculus*, n = 13) fed four different pelleted diets (L lucerne, G grass, GR grass and rice hulls, GRS grass, rice hulls and sand) for 14 days. Tooth height is given as absolute tooth height *(ATH)* and relative tooth height *(RTH)*, mesowear as extended score for cusp shape (*CsEM*) and occlusal relief (*OrEM*) on p3 to m2 and P3 to M2. *S10z* and *Vm* are presented on m1/m2 and P4/M1. For parameter descriptions see Table S1 and for statistics see Table S2, for figure explanation see Fig. S1.

original 160 × 160 µm frame using MountainsMap Premium v. 7.4.8803 Software (DigitalSurf, Besançon, France, www.digitalsurf.com). The processing of the surface texture data was conducted according to Schulz et al. (2010) and Schulz et al. (2013a). Subsequently we applied the following tools of the µsoft analysis premium software to the original 3D surface data: levelling (least square plane by subtraction) and spatial filtering (denoising median 5 × 5 filter size and Gaussian 3 × 3 filter size; default cut-offs are used). Therefore, we excluded outliers in the measurement, which are related to measurement errors based on wrong reflections typical for the optical principle used here. The default operator was the set of S-Filters. As default, the areal Gaussian filter (one of the S-Filters) was applied because it excludes the smallest scale elements (i.e., random noise) from the surface, resulting in the primary surface. To suppress form alterations (e.g., the curvature of the enamel), the F-operator was applied, which results in the S-F surface. In general, a second-order polynomial was sufficient to remove the form alterations from the surfaces. Subsequently, the L-Filter removes the low frequency alterations (= waviness). Forty-six surface texture parameters were quantified using the ISO 25178 (roughness), motif, furrow, isotropy, ISO 12871 (flatness), and Scale-sensitive fractal analysis (SSFA). Parameters were grouped according to their main characterising feature in the following categories: area, complexity, density, direction, height, peak sharpness, plateau size, slope, and volume (Table S1).

### 2.4. Statistics

In order to facilitate different statistical approaches to data analysis by other researchers, the original data is provided as a supplementary file. The supplementary material also includes descriptive statistics for all measurements (Tables S3-S6) and the results of statistical tests (Tables S2, S7–10). Data charts are prepared according to the system used in Fig. S1 in order to illustrate the jaws and individual teeth for the different diets.

The sample size was very limited for all measurements, leading to low overall statistical power. This also precluded the use of complex analytical models, leading to a very large number of individual, low-powered statistical tests. We consider our study, therefore, an exploratory pilot investigation. Data was analysed using a General Linear Model (GLM) with Tukey's post hoc tests for multiple comparisons where necessary. This was done for each jaw separately. The inclusion of individual as a random factor (to account for repeated measurements of the different teeth in one individual) was not possible because of a lack of degrees of freedom due to the small sample size. If residuals were not distributed normally, ranked data was used to perform the GLM (making it a nonparametric test). Correlations between measurements were assessed nonparametrically by Spearman's rho. Those analyses were performed in SPSS 25.0.0.1 (IBM, Armonk, NY, USA). Additional statistics on DMTA were carried out using the R software (R Core Team, 2016) with the packages xlsx (Dragulescu, 2014), rJava (Urbanek, 2016), doBy (Højsgaard and Halekoh, 2016), and R.utils (Bengtsson, 2016). All R scripts are available from Calandra (2011). For significance testing Calandra et al. (2012) and Schulz et al. (2013) was followed using a combination of statistical tests. As DMT data is generally non-normally distributed, the procedure of Wilcox (Wilcox, 2012) was used, applying a robust one-way heteroscedastic Welch-Yuen omnibus test (Welch, 1938; Yuen, 1974), coupled with a heteroscedastic pairwise "Dunnett's T3 test" (Dunnett, 1980). For individual teeth, we assessed the effect of diet in this way. Within a diet, we assessed differences between teeth. The significance level was set to 0.05 for all statistical test.

## 3. Results

### 3.1. Tooth height measurements

Absolute tooth height was different across diets and the added sand (GRS) consistently resulted in lower tooth height in the lower and especially in the upper jaw (Fig. 2; lower $p = .022$, upper $p < .001$; GLM results in Table S2, descriptive statistics in Table S3). The effect was homologous for the relative tooth height (Fig. 2; lower $p = .017$, upper $p < .001$; Table S2). Comparison of the relative tooth height revealed that for the upper jaw, the anterior teeth were more affected by the sand diet than the posterior teeth (Fig. 2, Table S2). Note that the significant differences between other diets reported by Müller et al. (2014) were not detectable in the reduced sample of the present study.

### 3.2. Mesowear

Mesowear scores were lowest on the sand diet (GRS) i.e. the cusp shape was more rounded and the occlusal relief less pronounced (Fig. 2, GLM results in Table S2, descriptive statistics in Table S4). On the upper jaw, cusp shape was less sharp across all diets compared to the lower jaw and P3 more affected than M2 (Fig. 2; $p = .001$; Table S2) suggesting a wear gradient similar to the results for relative tooth height. The occlusal relief on the upper jaw was in general very low and no effect of diet and tooth position was discernible (Fig. 2, Table S2).

### 3.3. Dental microwear texture analysis (DMTA)

Area, complexity, density, direction, peak sharpness and slope parameters showed a prominent effect of diet (Fig. S2) although in many cases differences were not statistically different (GLM results in Table S2, descriptive and further statistics in Tables S5-S7). An obvious diet effect was present in most height and volume parameters (Table S2, Fig. 2). The sand diet (GRS) was characterised by higher height and volume parameters compared to the other diets (Tables S2, S5–7). Differences of DMTA between different teeth were only detectable in the non-sand diets (Table S7): for some height parameters (*S10z, meh, madf, FLTv*), m1 was more affected than m2, especially on the phytolith enriched grass rice hull diet (GR; Tables S2, S5). Similarly, P4 showed more microwear traces than M1 represented by several DMTA parameters, but again only on the non-sand diets. Comparing the effect on the antagonistic teeth (m1 and P4, m2 and M1), some DMTA parameters were significantly different between m1 and P4 (Table S5, Fig. 2) on the phytolith enriched grass rice hull diet (GR).

### 3.4. Correlation between macroscopic wear scales (tooth height and mesowear)

Absolute tooth height correlated with mesowear scores on a single tooth (P4: rho = 0.60, $p = .031$; Table S9). Relative tooth height correlated positively with cusp shape on p4 (rho = 0.57, $p = .041$; Table S10, Fig. 3), M1 (rho = 0.60, $p = .029$; Table S9, Fig. 3), and showed a similar trend on P3 (rho = 0.49, $p = .087$; Table S10; Fig. 3).

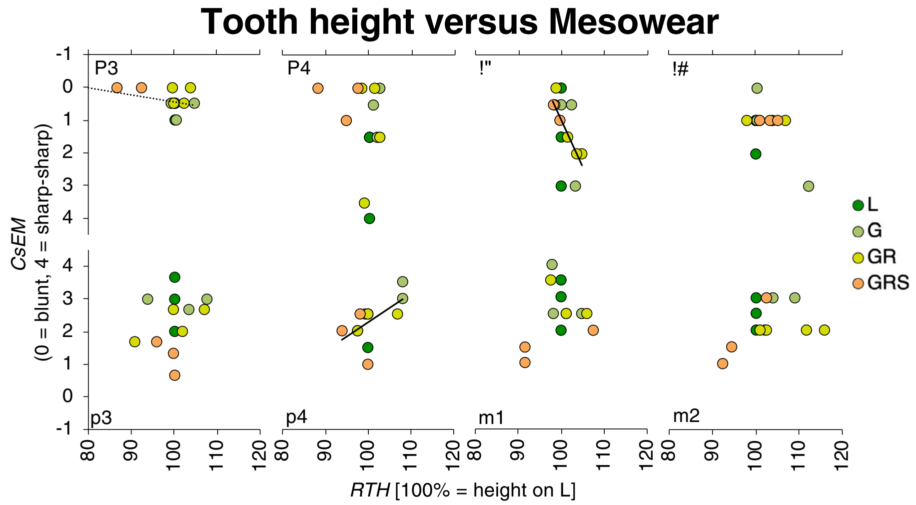


**Fig. 3.** Correlation of relative tooth height (*RTH*) and extended mesowear score for cusp shape (*CsEM*) of selected mandibular (lower x-axis, p3 to m2) and maxillary (upper x-axis, p3 to m2) cheek teeth in rabbits (*Oryctolagus cuniculus*, n = 13) fed four different pelleted diets (L lucerne, G grass, GR grass and rice hulls, GRS grass, rice hulls and sand) for 14 days. For parameter descriptions see Table S1 and statistics see Tables S8–10. (Bold black line represents correlation with $p < .05$. Dotted line represents correlation trend with $p < .1$).

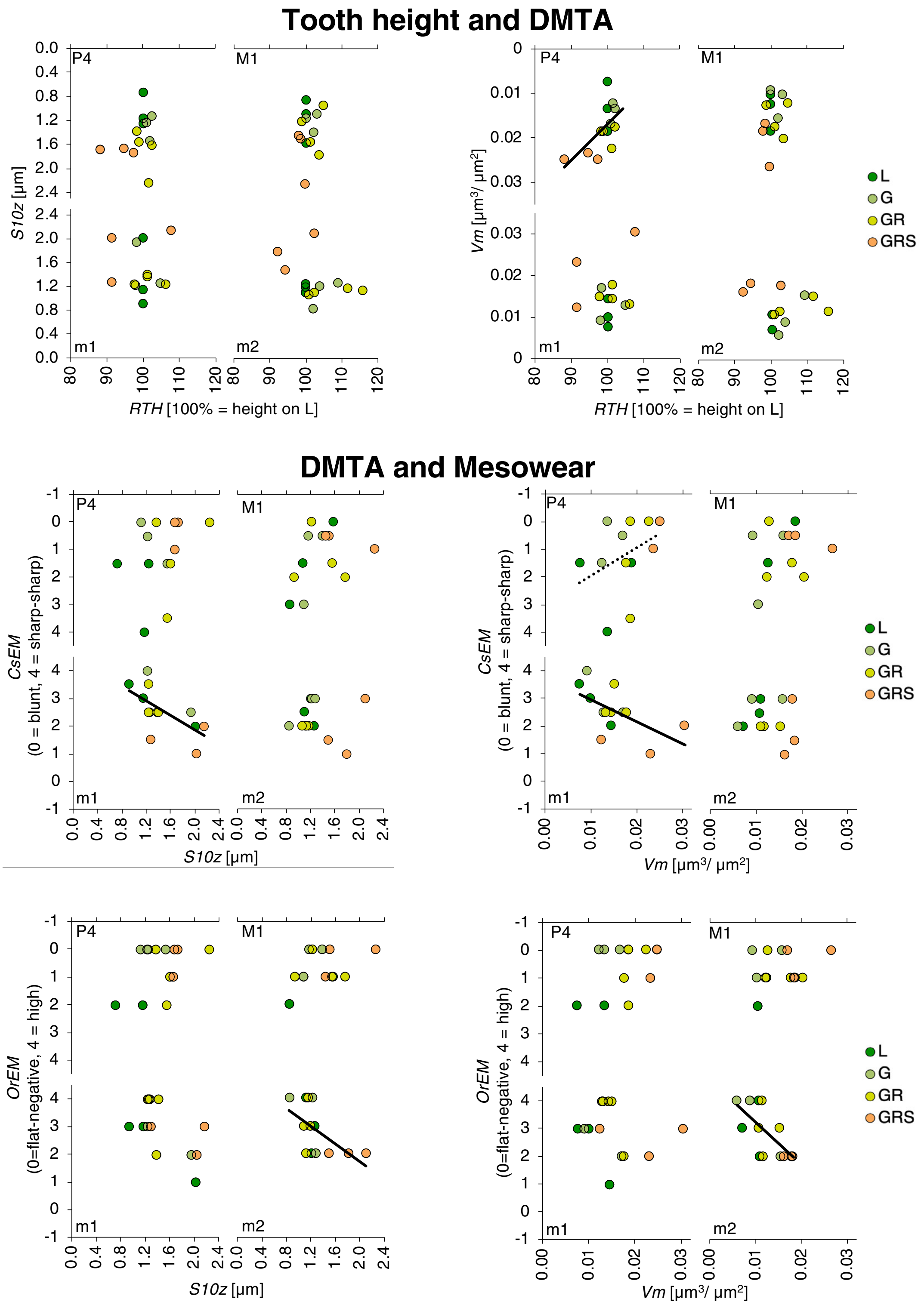


**Fig. 4.** Correlation of relative tooth height (RTH) with DMTA parameters (*S10z* and *Vm*), extended mesowear score for Cusp Shape (*CsEM*) and occlusal relief (*OrEM*) respectively with same DMTA parameters (*S10z* and *Vm*). Results are presented for selected mandibular (lower x-axis, m1 and m2) and maxillary (upper x-axis, P4 and M1) cheek teeth in rabbits (*Oryctolagus cuniculus*, n = 13) fed four different pelleted diets (L lucerne, G grass, GR grass and rice hulls, GRS grass, rice hulls and sand) for 14 days. For parameter descriptions see Table S1 and statistics see Tables S8 and S9. (Bold black line represents correlation with $p < .05$. Dotted line represents correlation trend with $p < .1$).

The occlusal relief correlated with cusp shape in all upper teeth, and in p3 and p4 (Tables S9–10), but not in m1 and m2 (Table S8). There was also no statistical correlation between occlusal relief scores and either tooth height parameter for any of the analysed teeth (Tables S8, S9, Fig. S3).

### 3.5. Tooth height versus microscopic wear scale (DMTA)

There were hardly any correlations of DMTA parameters with absolute and relative tooth height for M1 (Table S9). For the other cheek teeth, tooth height and DMTA parameters did not consistently correlate in the categories of area, complexity, density, direction, peak sharpness and slope (Tables S8-S9). Most height and volume parameters, however, were negatively correlated with absolute or relative tooth height, especially on m2 and P4 (Tables S8-S9). For example, volume parameter *Vm* on P4 increased with decreasing tooth height ($\text{rho} = -0.70$, $p = .007$; Fig. 4).

### 3.6. Mesowear versus DMTA

Again, here wereonly few correlations of mesowear and DMTA parameters for M1 (Table S9) and, as mentioned previously, the occlusal relief was generally very low in the upper jaw, resulting in no correlation with any DMTA parameter (Table S9). For m1 and P4, cusp shape was negatively correlated with height and volume parameters (Tables S8-S9, Fig. 4). For m2, the occlusal relief correlated better with the DMTA parameters than cusp shape (Table S8, Fig. 4).

## 4. Discussion

The results reported here show that large external abrasives (sand) can change tooth morphology in rabbits in less than fourteen days, which can consequently be detected not only as microscopic traces on the occlusal enamel, but also as changes to the occlusal relief and actual macroscopic tissue loss. This abrasive wear was not homogenously distributed across all teeth, but was more pronounced on the anterior and maxillary cheek teeth. On a microscopic level, endogenous phytoliths also caused a distinctive wear gradient. The functional connection between the different dental wear proxies was established through several directional correlations in spite of the limited sample size. Ideally, a serial design should be the ethical choice for such a study to establish tooth height, mesowear and DMTA parameters after each diet before using the same animals as their own control on further diets, but the narrow mouth gape and body size of rabbits represent a logistical challenge so far unsolved for the moulding procedures needed prior to mesowear and DMTA measurements on living animals. The lack of serial data is a major constraint in the present study. In order to keep the number of experimental animals low, the original study design – planned for macroscopic tooth wear measurements only – was based on repeated measurements per animal (Müller et al., 2014). When additionally measuring mesowear and DMTA in the animals on their terminal diet, this left us with a small sample size of either 3 or 4 individuals per diet group for the current study, which decreased statistical power of the tests, and the results therefore need to be treated with caution.

Still rabbits appear as ideal model animals to assess the functional interplay of wear at different levels of resolution compared to larger herbivore species with non-hypselodont teeth. They are easy in upkeep and develop their permanent dentition as early as 4 weeks of age (Michaeli et al., 1980). They are herbivorous and accept diets in a pelleted form as long as it is smaller than 3–4 mm in diameter (de Blas and Wiseman, 2010). Coprophagy, although a natural physiological behaviour, is sometimes argued to interfere with standardized feeding experiments, but in the context of dental wear only has a negligible effect because rabbits swallow their caecotrophs without chewing (Gidenne, 2010).

### 4.1. External and internal abrasives

The dental wear signature of the sand diet (GRS) was clearly different, with more macroscopic tissue loss, lower mesowear scores for cups shape and occlusal relief, and, among other DMTA changes, especially higher peaks (*Sa, Sp, Sxp*), deeper furrows (*metf*) and a higher mean density of furrows (*medf*) on a microscopic scale. Regardless of parameter, GRS lead to most extreme parameter values in DMTA, highlighting that ingestion of external abrasives results in more pronounced microscopic wear traces than high concentrations of internal abrasives (phytoliths) alone. This signature seen on both a macroscopic and microscopic resolution matches the one from similar in vivo and field studies in small and large herbivores on macroscopic dental wear (Müller et al., 2015; Schulz et al., 2013a), field observations and DMTA in rodents (Winkler et al., 2016), as well as other microscopic enamel features (Hoffman et al., 2015). By contrast, other studies suggest a limited effect of external abrasives on mesowear and on DMTA (Burgman et al., 2016; Merceron et al., 2016), and even an increased molar height in voles fed a diet containing both internal and external abrasives as compared to animals on a less abrasive diet (Kropacheva et al., 2017).

In the present study, no difference in DMTA parameters between the dicot-based diet L and the simple monocot-based diet G was evident. Similarly, Winkler et al. (2019) found only limited differences between a lucerne and a temperate zone grass diet, whereas a grass richer in phytoliths (bamboo) lead to distinct signals. Also in the present study, the phytolith-rich diet (GR) caused significant differences for several DMTA parameters in comparison to the other non-sand diets (*Sda, Sal, Str, S10z, Sp, Ssk, Sv, Sxp, Vm, Vvv*), which could theoretically also be interpreted as mere microscopic enamel deformation (Lucas et al., 2013). But the results from the serial feeding experiment confirmed an actual wear effect of GR compared to the grass only (G) and lucerne diet (L), reflected in changes of relative and absolute tooth height (Müller et al., 2014). Thus, a direct link between macroscopic tissue loss due to abrasion and dental microwear textures characterised by high surface roughness (large peaks and deep valleys) is possible. Increasing sample size and phytolith content could possibly reveal the effect of the internal abrasives on hypselodont teeth more clearly, both at macroscopic (Martin et al., 2019) and microscopic resolution (Calandra et al., 2016; Winkler et al., 2019).

In large herbivores, mesowear scores are traditionally used to differentiate between phytolith-rich (i.e., monocot or grass) and phytolith-poor (i.e., dicot or browse) diets (Fortelius and Solounias, 2000; Kaiser et al., 2013; Taylor et al., 2013). The principle has been successfully applied in intraspecific comparisons of different populations, for example in sika deer (*Cervus nippon*) (Kubo and Yamada, 2014). A controlled feeding experiment in goats (*Capra aegagrus hircus*) also yielded different mesowear scores for phytolith-poor and phytolith-rich diets, albeit at much lower magnitudes than expected (Ackermans et al., 2018). Previous analyses of hypselodont teeth presumed that differences in dietary phytoliths and external abrasives correspond to mesowear scores (Ulbricht et al., 2015), similar to the current study. Nevertheless, the contribution of both phytoliths and external abrasives to dental wear in free-ranging populations remains contentious (Sanson et al., 2017).

Several reasons may account for differences in the observed effects of abrasives between studies: The size of the abrasives themselves is probably important (e.g. Hoffman et al., 2015; Ackermans et al., 2020), their concentration, as well as the size relationship between the field of measurements (esp. microscopic) and the abrasive components (Ramdarshan et al., 2017). Taxon-specific differences most likely also apply. For example, one would expect animals with ever-growing teeth and high wear rates, such as lagomorphs and rodents, to have softer enamel compared to animals with non-growing teeth – a hypothesis that has, to our knowledge, not been studied systematically. Support for this hypothesis can be drawn from data on enamel hardness collected in Berkovitz and Shellis (2018), and preliminary data from microhardness testing of rabbit enamel point in the same direction (Shakila et al., 2015) when compared, for example, to enamel hardness measurements in ungulates (Kaiser et al., 2018). Here, one has to mention the high variability between methods for enamel hardness measurements and consequent results, as well as whether measurements are conducted under wet or dry conditions (Kaiser et al., 2018). Another example for taxon-specificity of abrasive effects on teeth is the washing mechanism in ruminants, which should, on the one hand, make ruminants less susceptible to effects of external abrasives that are removed preceding rumination, but possibly more susceptible to effects of internal abrasives that are concentrated in the material regurgitated for rumination (Hatt et al., 2019; Hatt et al., 2020).

### 4.2. Wear gradient between/along jaws

The existence of differences in wear measurements between jaws or along the tooth row are currently under debate. Ramdarshan et al. (2017) compared DMTA measurements from opposing facets as well as facets along the tooth row in sheep and found no differences within a single dietary category.

The chewing movement of rabbits is proposed to function like an inverted-pestle mortar system (Müller et al., 2014), where food is stationary on the mandibular teeth and ground against the maxillary teeth (Lucas, 1979). Dental wear should thus be more pronounced in the maxillary teeth, increasing macroscopic wear and DMTA signals while decreasing the sensitivity of mesowear. Furthermore, inhomogeneous signals along the tooth row, could be caused by differences in enamel hardness between premolars and molars (Shakila et al., 2015).

Both the wear gradient between the jaws and along the tooth row were documented in the current study: the GRS diet macroscopically affected all lower cheek teeth identically, whereas it caused a distinctive wear gradient along the upper tooth row, with P3 being the most affected. Mesowear scoring could not pick up a dietary wear effect along the maxillary cheek teeth, due to the consistently low relief, but the cusp shape parameters point towards a gradient similar to that detected by Taylor et al. (2016) in equids. DMTA analysis was only applied to m1/m2 and P4/M1, limiting the interpretation forof a possible gradient. On the GRS diet, the DMTA parameters did not show any differences between the individual teeth except for *Tr1R*, whereas on GR, G, and L many parameters showed differences between the teeth indicative of the proposed wear gradient. The GR diet, for example, caused higher *S10z* (ten-point height) on m1 compared to m2 and larger *Vm* (material volume at a given material ratio) on P4 compared to M1 (Fig. 2) – both results are representative of increased wear. The smaller phytoliths seem to cause more subtle traces than the large sand grains, with the former allowing a wear gradient to form, which the latter possibly overwrite too quickly for detection. This explanation might appear intuitive also because the sand particles, at a mean particle size of 233 μm, outsized the scanned area of 40 μm × 40 μm by a factor of six. Apart from our low sample size, the size of the scanned area itself can influence the discriminating power of DMTA; Ramdarshan et al. (2017) showed in sheep that for a diet with very low abrasiveness, different sizes of the scanned area led to different results, whereas this was not the case for more abrasive diets.

### 4.3. Correlations

At present, our functional understanding of dental wear measures is still limited. One way to address this research quandary is to ascertain the nature of the relationships between wear proxies – those that correlate are likely reacting to the same wear effect. Generally, corresponding analyses are scarce, even though tooth height, mesowear and DMTA have been used simultaneously to extrapolate diet at presumably different time scales (Davis and Pineda Munoz, 2016). For example, Mihlbachler et al. (2018) found significant correlations between mesowear scores and microwear features across extant rhinoceros species. Arguably, for a functional understanding of the wear process, intraspecific comparisons that exclude putative taxon-specific signatures are more valuable.

Different opinions on the lack of correlations can be found in the literature, and also among the authors of the present study: On the one hand, it is thought that this lack can be explained by the different timescales at which the wear proxies are thought to develop, where mesowear reflects a long-term signal yet microwear suggests that in a shorter period before death, a specific signal was effective (Davis and Pineda Munoz, 2016). On the other hand, a consistent absence of correlations across studies of wild populations reminds us that we still do not understand the wear process in full; otherwise, by chance alone, we would expect congruence between wear proxies as we understand them more often than not, particularly in species that are not intermediate feeders. Yet, in artiodactyls (*Bison, Camelopus*) and equids (Smith, 2019), no significant correlations between mesowear scores and DMTA measures were detected. The absence of such correlations is even more challenging for our understanding of wear if it occurs within the same species of animals on controlled diets fed for prolonged periods of time, like in experimental goats (Schulz-Kornas et al., 2020). We are not aware of functional hypotheses that explain these findings. One possible reason could be that different diet items that lead, for example, to the same mesowear signal, could nevertheless trigger different mastication patterns (and shown for different feeds in horses by Bonin et al., 2007; as postulated for different feeds in rabbits by Crossley, 2003) and hence different DMTA signals. At the moment, this is, however, purely speculative.

By contrast, the present study – albeit with its very restricted sample size – found several correlations between different wear proxies that match an intuitive functional understanding of wear. Lower scores for cusp shape describing blunt cusps were reflected in lower relative tooth height for several teeth. Volume DMTA parameters increase with higher microscopic tissue loss and in turn correlated with lower absolute and relative tooth height as well as lower scores for cusp shape and occlusal relief. The significant correlations cannot be consistently found for every tooth or each measurement resolution, but when significant, the correlation always followed the expected direction. The sample size of the current study severely limits the assertion on the universality of such a pattern and warrants further research. It restricted our analysis of relationships between variables to nonparametric correlations. The inevitable lack of regression models fit to these data means we cannot evaluate any differences in the rates by which the different variables changed under the different diet conditions. The optimal initial experimental approach would possibly be a highly standardized in vitro protocol to test whether a specific macroscopic wear effect only results in one specific DMTA pattern, and whether different DMTA patterns can lead to the same macroscopic wear effect.

Until such results are available, correlations between wear proxies as shown here for rabbits must be considered specific for this species. The reported macroscopic wear rates in wild (Damuth and Janis, 2014) and experimental rabbits (Müller et al., 2014) point towards a high turnover rate in their hypselodont dentition, changing the timeframe to record a dietary wear signal profoundly as compared to animals whose teeth do not grow continuously. The dietary tooth height loss and mesowear signals developed in less than two weeks, and the DMTA showed a reliable signal in the same timeframe. Such a rapid development of diet-specific wear patterns could be a potential prerequisite to find the intuitive correlations between macroscopic wear and DMTA measurements, whereas in the more durable, non-hypselodont teeth of larger herbivores such correlations may be more prone to interference of other, putatively chance factors.

## 5. Conclusions

We assessed a variety of wear proxies in rabbits fed diets containing varying concentrations of phytoliths and/or sand. Dental wear and corresponding wear gradients between and along jaws show a dietary signal when assessed with tooth height, mesowear, and DMTA in rabbits. Depending on the abrasive agent, a specific wear proxy allows for better differentiation of the diet signal than another proxy. When analysing different teeth from animals of unknown diets for dietary reconstructions, caution is mandated due to the different wear effect along the tooth row and between jaws. The presumably soft enamel of hypselodont teeth possibly facilitates a fast, simultaneous development of different wear proxies that are proposed to reflect different levels of temporal resolution in species with more durable dental tissue. Under these conditions, correlations between the different wear proxies that correspond to a functional understanding of the wear process may be found.

## Authors' contributions

DEW, TMK, MC, JMH and ESK designed the study, JM and MC performed the animal experiment, LK performed the surface texture measurements, AU and ESK developed the mesowear score, LFM performed the mesowear analyses, JM performed the tooth height measurements, JH provided the nutritional analyses of the diets, ESK, DEW, LFM, DC and MC analysed the data, LFM and MC wrote the first draft of the manuscript that then received input from all co-authors.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This study was part of project 31003A_163300/1 funded by the Swiss National Science Foundation to JMH. The work of L.F.M. was funded by the Candoc Forschungskredit of the University of Zurich. The work of DEW was funded by the European Research Council (ERC) under the European Union's Horizon 2020 Research and Innovation Programme (Grant Agreement 681450) (ERC Consolidator Grant Agreement to Thomas Tütken).

## Data

The original data measured in this study are available via the Dryad Data repository linked to this article.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

We thank Anja Tschudin and Dietmar Ranz for support in pelleted diet formulation and Michelle Aimee Oesch for providing the photographs of the skull and teeth.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.palaeo.2020.109886.

## References

Ackermans, N.L., Winkler, D.E., Martin, L.F., Kaiser, T.M., Clauss, M., Hatt, J.-M., 2020. Dust and grit matter: abrasives of different size lead to opposing dental microwear textures in experimentally fed sheep (*Ovis aries*). J Exp Biol 223.

Ackermans, N.L., Winkler, D.E., Schulz-Kornas, E., Kaiser, T.M., Müller, D.W.H., Kircher, P.R., Hummel, J., Clauss, M., Hatt, J.-M., 2018. Controlled feeding experiments with diets of different abrasiveness reveal slow development of mesowear signal in goats (*Capra aegagrus hircus*). J. Exp. Biol. 221, jeb186411.

Arthur, W.J., Gates, R.J., 1988. Trace element intake via soil Ingestion in pronghorns and in black-tailed jackrabbits. J. Range Manag. 41, 162–166.

Bengtsson, H, 2016. R.utils: Various programming utilities. R package, version 2.4.0. https://CRAN.Rproject.org/package=R.utils.

Berkovitz, B., Shellis, P., 2018. Chapter 3 - Herbivory. In: Berkovitz, B., Shellis, P. (Eds.), The Teeth of Mammalian Vertebrates. Academic Press, pp. 47–56.

Bonin, S.J., Clayton, H.M., Lanovaz, J.L., Johnston, T., 2007. Comparison of mandibular motion in horses chewing hay and pellets. Equine Vet. J. 39, 258–262.

Burgman, J.H., Leichliter, J., Avenant, N.L., Ungar, P.S., 2016. Dental microwear of sympatric rodent species sampled across habitats in southern Africa: Implications for environmental influence. Integr. Zool. 11, 111–127.

Calandra, I., 2011. Tribology of Dental Enamel Facets of Ungulates and Primates (Mammalia): Tracing Tooth-Food Interaction through 3D Enamel Microtexture Analyses. University of Hamburg.

Calandra, I., Merceron, G., 2016. Dental microwear texture analysis in mammalian ecology. Mammal Rev. 46, 215–228.

Calandra, I., Schulz, E., Pinnow, M., Krohn, S., Kaiser, T.M., 2012. Teasing apart the contributions of hard dietary items on 3D dental microtextures in primates. J. Hum. Evol. 63, 85–98.

Calandra, I., Zub, K., Szafrańska, P.A., Zalewski, A., Merceron, G., 2016. Silicon-based plant defences, tooth wear and voles. J. Exp. Biol. 219, 501–507.

Clauss, M., Franz-Odendaal, T.A., Brasch, J., Castell, J.C., Kaiser, T., 2007. Tooth wear in captive giraffes (*Giraffa camelopardalis*): Mesowear analysis classifies free-ranging specimens as browsers but captive ones as grazers. J. Zoo Wildl. Med. 38, 433–445.

Crossley, D.A., 2003. Oral biology and disorders of lagomorphs. Vet. Clin. N. Am. Exotic Anim. Pract. 6, 629–659.

Damuth, J., Janis, C.M., 2011. On the relationship between hypsodonty and feeding ecology in ungulate mammals, and its utility in palaeoecology. Biol. Rev. 86, 733–758.

Damuth, J., Janis, C.M., 2014. A comparison of observed molar wear rates in extant herbivorous mammals. Ann. Zool. Fenn. 51, 188–200.

Davis, M., Pineda Munoz, S., 2016. The temporal scale of diet and dietary proxies. Ecol. Evol. 6, 1883–1897.

de Blas, C., Wiseman, J., 2010. Nutrition of the Rabbit. CAB International, Oxfordshire.

Dragulescu, A., 2014. Xlsx: Read, Write, Format Excel 2007 and Excel 97/2000/XP/2003 Files. R Package, Version 0.5.7.

Dunnett, C.W., 1980. Pairwise multiple comparisons in theunequal variance case. J. Am. Stat. Assoc. 75, 796–800.

Fortelius, M., Solounias, N., 2000. Functional characterization of ungulate molars using the abrasion-attrition wear gradient: a new method for reconstructing paleodiets. Am. Mus. Novit. 3301, 1–36.

Fraser, D., 2010. The use of gross dental wear in dietary studies of extinct lagomorphs. J. Paleontol. 84, 720–729.

Fritz, J., Streich, W.J., Schwarm, A., Clauss, M., 2012. Condensing results of wet sieving analyses into a single data: a comparison of methods for particle size description. J. Anim. Physiol. Anim. Nutr. 96, 783–797.

Gidenne, T., 2010. Feeding behaviour of the rabbit. In: De Blas, C., Wiseman, J. (Eds.), Nutrition of the Rabbit. CAB International, Oxfordshire, pp. 233–252.

Gomes Rodrigues, H., Merceron, G., Viriot, L., 2009. Dental microwear patterns of extant and extinct Muridae (Rodentia, Mammalia): ecological implications. Naturwissenschaften 96, 537–542.

Hatt, J.-M., Codron, D., Müller, D.W.H., Ackermans, N.L., Martin, L.F., Kircher, P.R., Hummel, J., Clauss, M., 2019. The rumen washes off abrasives before heavy-duty chewing in ruminants. Mamm. Biol. 97, 104–111.

Hatt, J.-M., Codron, D., Ackermans, N.L., Martin, L.F., Richter, H., Kircher, P., Gerspach, C., Hummel, J., Clauss, M., 2020. The effect of the rumen washing mechanism in sheep differs with concentration and size of abrasive particles. Paleogeogr. Paleoclimatol. Paleoecol. 550, 109728.

Hoffman, J.M., Fraser, D., Clementz, M.T., 2015. Controlled feeding trials with ungulates: a new application of in vivo dental molding to assess the abrasive factors of microwear. J. Exp. Zool. 218, 1538–1547.

Højsgaard, S., Halekoh, U., 2016. doBy: Groupwise Statistics, LSmeans, Linear Contrasts, Utilities. R Package, Version 4.5–15.

Hummel, J., Findeisen, E., Südekum, K.-H., Ruf, I., Kaiser, T.M., Bucher, M., Clauss, M., Codron, D., 2011. Another one bites the dust: faecal silica levels in large herbivores correlate with high-crowned teeth. Proc. R. Soc. B 278, 1742–1747.

Kaiser, T.M., Muller, D.W.H., Fortelius, M., Schulz, E., Codron, D., Clauss, M., 2013. Hypsodonty and tooth facet development in relation to diet and habitat in herbivorous ungulates: implications for understanding tooth wear. Mammal Rev. 43, 34–46.

Kaiser, T.M., Clauss, M., Schulz-Kornas, E., 2016. A set of hypotheses on tribology of mammalian herbivore teeth. Surf. Topogr. Metrol. 4.

Kaiser, T.M., Braune, C., Kalinka, G., Schulz-Kornas, E., 2018. Nano-indentation of native phytoliths and dental tissues: implications for herbivore-plant combat and dental wear proxies. Evol. Syst. 2, 55–63.

Karme, A., Rannikko, J., Kallonen, A., Clauss, M., Fortelius, M., 2016. Mechanical modelling of tooth wear. J. R. Soc. Interface 13, 20160399.

Koenigswald, W.v., Anders, U., Engels, S., Schultz, J.A., Ruf, I., 2010. Tooth morphology in fossil and extant lagomorpha (Mammalia) reflects different mastication patterns. J. Mamm. Evol. 17, 275–299.

Kropacheva, Y.E., Sibiryakov, P.A., Smirnov, N.G., Zykov, S.V., 2017. Variants of tooth mesowear in Microtus voles as indicators of food hardness and abrasiveness. Russ. J. Ecol. 48, 73–80.

Kubo, M.O., Yamada, E., 2014. The inter-relationship between dietary and environmental properties and tooth wear: comparisons of mesowear, molar wear rate, and hypsodonty index of extant Sika deer populations. PLoS One 9, e90745.

Lucas, P.W., 1979. The dental-dietary adaptations of mammals. N. Jb. Geol. Paläont. 8, 486–512.

Lucas, P.W., Omar, R., Al-Fadhalah, K., Almusallam, A.S., Henry, A.G., Michael, S., Thai, L.A., Watzke, J., Strait, D.S., Atkins, A.G., 2013. Mechanisms and causes of wear in tooth enamel: implications for hominin diets. J. R. Soc. Interface 10, 20120923.

Lucas, P.W., Casteren, A.v., Al-Fadhalah, K., Almusallam, A.S., Henry, A.G., Michael, S., Watzke, J., Reed, D.A., Diekwisch, T.G.H., Strait, D.S., Atkins, A.G., 2014. The role of dust, grit and phytoliths in tooth wear. Ann. Zool. Fenn. 51, 143–152.

Martin, L.F., Winkler, D.E., Tütken, T., Codron, D., De Cuyper, A., Hatt, J.-M., Clauss, M.,

2019. The way wear goes: phytolith-based wear on the dentine-enamel system in Guinea pigs (*Cavia porcellus*). Proceed. Royal Soc. B-Biol. Sci. 286, 20191921.

Merceron, G., Escarguel, G., Angibault, J.-M., Verheyden-Tixier, H., 2010. Can dental microwear textures record inter-individual dietary variations? PLoS One 5, e9542.

Merceron, G., Ramdarshan, A., Blondel, C., Boisserie, J.-R., Brunetiere, N., Francisco, A., Gautier, D., Milhet, X., Novello, A., Pret, D., 2016. Untangling the environmental from the dietary: dust does not matter. Proc. R. Soc. B Biol. Sci. 283, 20161032.

Meredith, A.L., Prebble, J.L., Shaw, D.J., 2015. Impact of diet on incisor growth and attrition and the development of dental disease in pet rabbits. J. Small Anim. Pract. 56, 377–382.

Michaeli, Y., Hirschfeld, Z., Weinrub, M.M., 1980. The cheek teeth of the rabbit: morphology, histology and development. Acta Anat. 106, 223–239.

Mihlbachler, M.C., Campbell, D., Chen, C., Ayoub, M., Kaur, P., 2018. Microwear-mesowear congruence and mortality bias in rhinoceros mass-death assemblages. Paleobiology 44, 131–154.

Müller, J., Clauss, M., Codron, D., Schulz, E., Hummel, J., Fortelius, M., Kircher, P., Hatt, J.M., 2014. Growth and wear of incisor and cheek teeth in domestic rabbits (*Oryctolagus cuniculus*) fed diets of different abrasiveness. J. Exp. Zool. 321, 283–298.

Müller, J., Clauss, M., Codron, D., Schulz, E., Hummel, J., Kircher, P., Hatt, J.M., 2015. Tooth length and incisal wear and growth in Guinea pigs (*Cavia porcellus*) fed diets of different abrasiveness. J. Anim. Physiol. Anim. Nutr. 99, 591–604.

Nelson, S., Badgley, C., Zakem, E., 2005. Microwear in modern squirrels in relation to diet. Palaeontol. Electron. 8, 15.

Ness, A.R., Brown, G.L., 1956. The response of the rabbit mandibular incisor to experimental shortening and to the prevention of its eruption. Proc. R. Soc. B Biol. Sci. 146, 129–154.

Yuen, KK, 1974. The two-sample trimmed t for unequal population variances. Biometrika (61), 165–170.

R Core Team, 2016. R: A Language and Environment for Statistical Computing, Version 3. 3.1, in: Computing, R.F.f.S, (Vienna).

Ramdarshan, A., Blondel, C., Gautier, D., Surault, J., Merceron, G., 2017. Overcoming sampling issues in dental tribology: Insights from an experimentation on sheep. Palaeontol. Electron. 20, 1–19.

Sanson, G.D., Kerr, S., Read, J., 2017. Dietary exogenous and endogenous abrasives and tooth wear in African buffalo. Biosurf. Biotribol. 3, 211–223.

Schulz, E., Calandra, I., Kaiser, T., 2010. Applying tribology to teeth of hoofed mammals. Scanning 32, 162–182.

Schulz, E., Calandra, I., Kaiser, T.M., 2013a. Feeding ecology and chewing mechanics in hoofed mammals: 3D tribology of enamel wear. Wear 300, 169–179.

Schulz, E., Piotrowski, V., Clauss, M., Mau, M., Merceron, G., Kaiser, T.M., 2013. Dietary abrasiveness is associated with variability of microwear and dental surface texture in rabbits. PLoS One 8, e56167.

Schulz-Kornas, E., Winkler, D.E., Clauss, M., Carlsson, J., Ackermans, N.L., Martin, L.F., Hummel, J., Müller, D., Hatt, J.-M., Kaiser, T., 2020. Everything matters: molar microwear texture in goats fed diets of different abrasiveness. Palaeogeogr. Palaeoclimatol. Palaeoecol. 552.

Scott, E.C., 1979. Dental wear scoring technique. Am. J. Phys. Anthropol. 51, 213–217.

Scott, R.S., Ungar, P.S., Bergstrom, T.S., Brown, C.A., Grine, F.E., Teaford, M.F., Walker, A., 2005. Dental microwear texture analysis shows within-species diet variability in fossil hominins. Nature 436, 693–695.

Scott, R.S., Ungar, P.S., Bergstrom, T.S., Brown, C.A., Childs, B.E., Teaford, M.F., Walker, A., 2006. Dental microwear texture analysis: technical considerations. J. Hum. Evol. 51, 339–349.

Shakila, N., Ali, A., Zaidi, S., 2015. Micro hardness of dental tissues influenced by administration of aspirin during pregnancy. Int. J. Morphol. 33, 586–593.

Smith, G.J., 2019. Coupling Paleoecological Proxies to Infer Competition, Niche Partitioning and Ecosystem Structure in Extinct Mammalian Communities, Earth and Environmental Sciences. Vanderbilt University, Nashville, Tennessee, pp. 203.

Smith, B.G., Knight, J.K., 1984. An index for measuring the wear of teeth. Br. Dent. J. 156, 435–438.

Taylor, L.A., Kaiser, T.M., Schwitzer, C., Müller, D.W.H., Codron, D., Clauss, M., Schulz, E., 2013. Detecting inter-cusp and inter-tooth wear patterns in rhinocerotids. PLoS One 8, e80921.

Taylor, L.A., Müller, D.W.H., Schwitzer, C., Kaiser, T.M., Castell, J.C., Clauss, M., Schulz-Kornas, E., 2016. Comparative analyses of tooth wear in free-ranging and captive wild equids. Equine Vet. J. 48, 240–245.

Ulbricht, A., Maul, L.C., Schulz, E., 2015. Can mesowear analysis be applied to small mammals? A pilot-study on leporines and murines. Mamm. Biol. Zeitschrift für Säugetierkunde 80, 14–20.

Ungar, P.S., 2010. Mammal Teeth - Origin, Evolution and Diversity. John Hopkins University Press, Baltimore Maryland USA.

Ungar, P.S., Brown, C.A., Bergstrom, T.S., Walkers, A., 2003. Quantification of dental microwear by tandem scanning confocal microscopy and scale-sensitive fractal analyses. Scanning 25, 185–193.

Urbanek, S., 2016. rJava: Low-Level R to Java Interface. R Package, Version 0.9-8.

Winkler, D.E., Kaiser, T., 2011. A Case Study of Seasonal, Sexual and Ontogenetic Divergence in the Feeding Behaviour of the Moose (*Alces alces,* Linné, 1758). 46. Verhandlungen des Naturwissenschaftlichen Vereins Hamburg, pp. 331–348.

Winkler, D.E., Kaiser, T., 2015. Structural morphology of molars in large mammalian herbivores: enamel content varies between tooth positions. PLoS One 10, e0135716.

Welch, BL, 1938. The significance of the difference between two means when thepopulation variances are unequal. Biometrika (29), 350–362.

Wilcox, R.R., 2012. Introduction to robust estimation and hypothesis testing. Elsevier Academic Press, London.

Winkler, D.E., Andrianasolo, T.H., Andriamandimbiarisoa, L., Ganzhorn, J.U., Rakotondranary, S.J., Kaiser, T.M., Schulz-Kornas, E., 2016. Tooth wear patterns in black rats (*Rattus rattus*) of Madagascar differ more in relation to human impact than to differences in natural habitats. Ecology and Evolution 6, 2205–2215.

Winkler, D.E., Schulz-Kornas, E., Kaiser, T.M., De Cuyper, A., Clauss, M., Tütken, T., 2019. Forage silica and water content control dental surface texture in Guinea pigs and provide implications for dietary reconstruction. Proc. Natl. Acad. Sci. U. S. A. 116 (4), 1325–1330.

Wyss, F., Müller, J., Clauss, M., Kircher, P., Geyer, H., von Rechenberg, B., Hatt, J.-M., 2016. Measuring rabbit (*Oryctolagus cuniculus*) tooth growth and eruption by fluorescence markers and bur marks. J. Vet. Dent. 33, 39–46.