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Surface texture analyses complement scale sensitive fractal analyses in an *in vivo* human dental microwear study

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ABSTRACT

The study of dental microwear, the microscopic patterns left on teeth from interactions with food, has become instrumental in examining the diets of past societies. This approach gained prominence with the advent of dental microwear texture analysis (DMTA), an automated method that minimises observer error. Nevertheless, interpreting microwear patterns remains challenging due to limited knowledge about which foods and processing methods produce specific markings. Given the subtle variations in human diets compared to other species, there is a pressing need for more comprehensive *in vivo* data on microwear production.

In this study, we improved our understanding of DMTA by employing multivariate analyses to combine parameters from surface texture analyses (STA) with the more common parameters derived from scale sensitive fractal analyses (SSFA). We collected dental impressions from five Kenyan communities: El Molo, Turkana (Kerio), Luhya (Webuye), Luhya (Port Victoria), and Luo (Port Victoria), representing a range of subsistence strategies – fishing, pastoralism, and agriculture. Regrettably, the presence of oral biofilm – a bacterial layer covering teeth in living individuals – often hampers the accurate moulding of dental microwear *in vivo*. Despite the constraint imposed by the presence of biofilm, which limited our sample to only 37 usable surfaces, we found that while SSFA variables failed to distinguish between populations, combining them with STA parameters in multivariate analyses successfully differentiated the El Molo from the other populations, as well as the groups from Port Victoria.

Our findings suggest that this approach offers a more comprehensive understanding of microwear variation. To ensure the continued relevance of dental microwear studies in understanding the diets of past societies, we must improve our understanding of the relationship between dental microwear patterns and the complex, mixed diets of humans, and overcome the current limitations of the technique. Consistently incorporating ISO 25178 in our analyses represents a promising avenue for achieving this objective.

1. Introduction

Dental microwear refers to the microscopic patterns of wear on teeth resulting from interactions between teeth, abrasives, and food. These patterns allow us to reverse-engineer an individual's diet, and they have found extensive use in neo- and paleoecology studies, as diet represents a primary link between the organism and its environment (Calandra and Merceron, 2016; Schmidt et al., 2020; Ungar, 2015).

Dental microwear research draws inspiration from biotribology, a scientific discipline studying wear, friction, lubrication, and bearing design, as applied to biological systems (Schmidt and Ungar, 2023; Zhou et al., 2013). While dental microwear analysis has a history dating back nearly a century, it gained traction in the 1970s when researchers employed light and scanning electron microscopy to manually measure and count wear features on two-dimensional representations of three-dimensional (3D) tooth surfaces (Calandra et al., 2019; Calandra and Merceron, 2016). However, these approaches proved costly and time-consuming, lost relevant 3D information, and suffered from high observer error rates (DeSantis et al., 2013; Grine et al., 2002). Efforts to standardise data collection (Mihlbachler and Beatty, 2012; Teaford, 1988) yielded limited success until the emergence of dental microwear texture analysis (DMTA) in the early 2000 s (Scott et al., 2006, 2005; Ungar et al., 2003). DMTA employs confocal microscopy to generate a data cloud representing the tooth surface, utilising automated

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techniques to quantify 3D surface characteristics rather than relying on feature counts. This approach yields more realistic representations of tooth surfaces, is non-destructive, less subjective, and minimises observer errors. Texture analysis revolutionised dental microwear studies, sparking a resurgence of research interest (Ungar, 2015; Watson and Schmidt, 2020). Nevertheless, ongoing standardisation efforts grapple with variations introduced by different impression materials (Goodall et al., 2015; Sawaura et al., 2022) and microscopes (Arman et al., 2016).

DMTA has employed two approaches to analyse microwear surfaces obtained through confocal microscopy: scale-sensitive fractal analyses (SSFA) and surface texture analyses (STA) (Calandra and Merceron, 2016). SSFA, developed at the onset of DMTA, recognizes that surface texture appears different at varying observation scales, such that it may appear smooth at lower resolution and become rougher with increasing resolution. This approach produces different parameters that characterise the surface, such as complexity (e.g. *Asfc* – area scale fractal complexity) and anisotropy (e.g. *epLsar* – length-scale anisotropy of relief) (Scott et al., 2006, 2005; Ungar et al., 2003). In contrast, STA employs established industrial parameters from the International Organization of Standardization (ISO) to characterise a microwear surface, particularly ISO 25178, which describes the texture of 3D areal surfaces. These ISO 25178 parameters are further grouped according to the surface characteristics on which they are based. Thus, height parameters encode the distribution of height values along the surface, spatial parameters focus on the spatial periodicity of the surface, particularly direction, hybrid parameters relate to the spatial shape of the surface, function parameters are calculated from the material ratio curve, and feature parameters encode the dales and hills on the surface (Schulz et al., 2010; ISO, 2021). (Paleo)ecological studies were the first to apply STA to dental microwear data (Purnell et al., 2012; Schulz et al., 2010), but its use is expanding to (bio)archaeological studies (e.g., Peterson et al., 2018; Ungar et al., 2019). Although SSFA variables have a slightly more intuitive interpretation, including STA variables provides a more complete description of the microwear surface (Calandra et al., 2012; Purnell et al., 2013, 2012; Schulz et al., 2010). Thus, DMTA stands at the intersection between biology and engineering, being one of the few methods that assesses consumed diet, unlike other proxies (e.g. dental morphology) which indicate what diet an organism is adapted to eat, as opposed to what they actually ate. Together with stable isotopic analyses, DMTA can be applied to both present and past specimens, facilitating the study of longitudinal and inter- and intra-species variation (Calandra and Merceron, 2016; Grine et al., 2012; Lee-Thorp and Sponheimer, 2006; Ungar, 2015).

Most often, dental microwear patterns are interpreted based on the material properties of food. Hard foods, such as nuts, create complex surfaces characterised by numerous pits, whereas tough foods, such as leaves, create anisotropic surfaces with aligned scratches (Scott et al., 2006; Ungar, 2015). However, recent research suggests that the mechanical properties of food, like shape, can also impact microwear patterns. For instance, isodiametric foods, i.e. roughly spherical foods, such as nuts, can produce complex pitted surfaces, whereas thin food, such as leaves, can produce anisotropic surfaces with aligned scratches (Lucas et al., 2014; van Casteren et al., 2020, 2018). Although in part complementary, the second approach argues that microwear will be produced only when abrasive particles, such as grit or phytoliths, is present in the oral cavity, as the key variable producing microwear is the predictable or unpredictable loading produced during contact between teeth and grit particles as food is moved around the mouth. In other words, grit embedded regularly along a leaf will leave aligned scratches through opposing tooth surfaces in close proximity sliding in parallel to each other (anisotropic surfaces) while isodiametric food breaks at their weakest points, producing unpredictable contact points between abrasives and teeth (complex surfaces). This explanation seems to address previous research suggesting that hard food items did not leave markings on teeth, and hence that seeds from grasses and sedges could have been consumed by past hominins (van Casteren et al., 2020). Clearly, more research is needed to reach full understanding of dental microwear production.

Although the mechanics of microwear production are not yet completely understood, DMTA has been successfully used to shed light on the diets of past and present mammal groups, including humans and other hominids (e.g. El Zaatari, 2010; Grine et al., 2012; Peterson et al., 2018; Schulz et al., 2010; Scott et al., 2005; Ungar et al., 2017). Human diets, in particular, have received substantial attention since the inception of DMTA (Scott et al., 2005). However, interpreting human microwear patterns is challenging, as they can also be influenced by environmental and cultural factors, such as climate, subsistence strategies, food processing, non-masticatory tooth use, oral hygiene, and demographics (e.g., age and sex) (Díaz-Navarro et al., 2023; El Zaatari, 2010; Estalrrich and Rosas, 2015; Krueger et al., 2017; Schmidt et al., 2019; Ungar et al., 2019; Watson and Schmidt, 2020). Most often, determining the specific food items consumed based on dental microwear remains challenging; instead, diets are characterised as being hard, soft, abrasive, or some combination of these. Other lines of evidence, such as written records or stable isotope analyses, are then used to produce a model of consumed diet (El Zaatari, 2010; Hernando et al., 2021; Schmidt et al., 2020, 2019). Yet, precisely because human microwear is impacted by so many different factors, its study is also a potential tool to interpret human adaptation (Krueger et al., 2017; Puech and Pinilla, 2014; Schmidt et al., 2019; Watson and Schmidt, 2020). To unlock the full potential of DMTA, further research is needed to understand the relationship between diet and resulting textures, notably through *in vitro* studies simulating mastication in controlled environments, *in vivo* controlled-feeding experiments involving individuals with varied diets, and *in vivo* natural studies examining the impact of measurable dietary diversity (Calandra and Merceron, 2016; Krueger et al., 2021; Ungar, 2015).

Despite offering a promising path forward, an important element has received little attention in current discussions: oral biofilm, the lubrication aspect within the biotribological context of the mouth. Oral biofilm consists of the dental pellicle, a protein layer that forms rapidly on any solid surface exposed to the oral environment, and the bacterial layer, a concentration of oral microorganisms and their products that inhabit the dental pellicle. Together, these components act as lubricant between the hard enamel and the soft mucosal tissues, providing protection against wear and acidic erosion, and contributing to remineralization (Armstrong, 1967; Hannig, 2002; Hillson, 2023, p. 254). Indeed, absence of oral biofilm, such as in cases of xerostomia or irradiation therapy, leads to rapid tooth wear (Łysik et al., 2019; Zhou et al., 2013). This has posed two problems for recent studies: *in vitro* studies have faced added challenges in replicating the oral environment, although progress has been made in using artificial saliva as a substitute (Krueger et al., 2021; Lewis and Dwyer-Joyce, 2005; Zhou et al., 2013); and, *in vivo* studies have struggled to remove oral biofilm obscuring microwear surfaces prior to moulding (Correia et al., 2021; Gordon and Walker, 1983; Kay and Covert, 1983; Teaford et al., 2020, 2017; Teaford and Oyen, 1989).

In this study, we attempted to analyse dental microwear patterns in five Kenyan communities practising different subsistence strategies – fishing, pastoralism, and agriculture. These communities are not “living fossils” nor are they insulated from change, and hence their livelihoods cannot be interpreted as a direct proxy for past behaviours (Gosselain, 2016). However, studying diverse livelihoods enriches our understanding of the world (Cunningham and MacEachern, 2016; Lyons and David, 2019). In the specific case of dental microwear, studies trying to address the gap in *in vivo* microwear studies outlined above must contend with the fact that over the past 50 years, dietary diversity decreased substantially under the pressures of globalisation and urbanisation, leading to an increase in the consumption of animal foods and of ultra-processed foods high in sugar, fat, and salt – a dietary change termed the nutritional transition (Bird et al., 2021; FAO et al., 2022; Kearney, 2010; Khoury et al., 2014; Popkin et al., 2020). This diet is unlikely to be very abrasive and indeed it is well known that dental macrowear decreased first during the transition from hunting gathering to agro-pastoralism and then to industrialization (Deter, 2009; Godinho et al., 2023; Lavelle, 1970; Mays, 2002; Smith, 1984). It is less well understood how these changes translate to dental microwear although Schmidt et al. (2019) studied archaeological populations to suggest that the microwear surfaces of foragers had higher complexity and lower anisotropy whereas those of farmers and pastoralists had lower complexity and higher anisotropy, corresponding to less abrasive diets for the later. We do not expect the populations studied here to be isolated from the ongoing nutritional transition but suggest that any ongoing change is worth of study in and of itself. In line with this, and although most microwear research centres around interpretations of past diets, the study of dental microwear in living populations can also contribute to current dentistry, as our understanding of tooth wear is still limited and tooth wear has become an increasing clinical problem as life expectancy increases (Lee et al., 2012; Wang et al., 2022).

In this context, our aim is to contribute to the research gap in *in vivo* microwear studies, but our sample size has been substantially affected by the presence of oral biofilm in dental impressions. A prior publication provided preliminary analyses of SSFA variables and methodological considerations in oral biofilm removal (Correia et al., 2021). In this report, we delve deeper into the DMTA results, incorporating STA and employing multivariate analyses to combine SSFA and STA. We explore how such an integrated approach may enhance the interpretation of microwear textures, particularly in contexts with limited sample sizes.

2. Material and methods

2.1. *Groups studied*

Subjects in this study represented individuals from five communities from four ethnic groups in Kenya: (a) El Molo fishers from the south-eastern margin of Lake Turkana; (b) Turkana pastoralists from the Kerio Valley in Turkana County; (c) Luhya farmers from Webuye; and (d) Luhya and (e) Luo fishers from the northern margin of Lake Victoria (Fig. 1).

The El Molo are a fisher-forager community of circa six hundred people living on the south-eastern shore of Lake Turkana, an arid to semi-arid environment (Marsabit County, Eastern Province, Kenya). Their diet is mostly consistent of fish from the lake although they also keep some livestock, such as goats and chickens, and acquire other foodstuffs through commerce or relief food programmes, such as yellow maize, maize and wheat flour, cooking oil, sugar, and tea (Correia, 2018; Heine, 1980; Kiura, 2005; Scherrer, 1978; Tishkoff et al., 2009). In turn, the Turkana make up most of the Turkana County population, numbering over nine hundred thousand. Historically, among African pastoralist populations, the Turkana had the highest proportion of animal products in their diet, mainly in the form of milk and blood, which were supplemented by some cultivated goods, such as sorghum, maize flour, and sugar, mostly obtained through purchase, barter, or relief programmes (Akall, 2021; Campbell et al., 1999; Galvin, 1985; Galvin and Little, 1999; Turkana County Government, 2020). The Turkana have long been exposed to droughts, conflict, and famine, to which they have developed strategic adaptations. For instance, the Turkana only consume fish as a backup resource, seeking refuge in communities along the lakeshore (like the El Molo) in times of shortage, until they can re-enter pastoralim (Akall, 2021; Campbell et al., 1999; Kiura, 2005). Development interventions, however, have focused on irrigated farming and settlement, leading to the breakdown of traditional coping mechanisms and dietary changes, namely a higher consumption of maize flour. The Turkana resisted these changes and nomadic livestock keeping remains the main economic activity in the region, although it faces accumulating challenges to mobile pastoralism (Akall, 2021; Correia, 2018; Turkana County Government, 2020). Then, the Luhya are the second largest group in Kenya (after the Kikuyu) with over five million people. Concentrated in the Bungoma County in a subhumid environment, the Luhya live in more densely populated areas and are more market integrated than the two previous groups. Most people in the Bungoma County engage in some form of agricultural activity, with a smaller proportion being formally employed. Main crops include maize, beans, finger millet, sweet potatoes, and bananas, while cash crops (sold for profit) are sugar cane, cotton, palm oil, coffee, sunflower, and tobacco (Bungoma County Government, 2013; Kishino et al., 2022; MacArthur, 2013; Wagner, 1949). Finally, the Luo are the third largest ethnic group in Kenya, numbering over four million. They live in the north-eastern margins of Lake Victoria, mostly in the Counties of Nyanza Province, with a few pockets located in Busia County, where fieldwork was conducted and where they live together with the Luhya. Close to Lake Victoria, Luo's diet is highly reliant on fish, although they also keep goats and sheep and consume agricultural produce, mostly maize, sorghum, and beans (Andika et al., 2011; Correia, 2018; DuPré, 2004). Considering the large size of the last two groups, we do not expect them to have homogenous diets, but these group affiliations and their associated location for the most part predate colonisation and are likely to be more reliable predictors than colonial state formation (Paine et al., 2024).

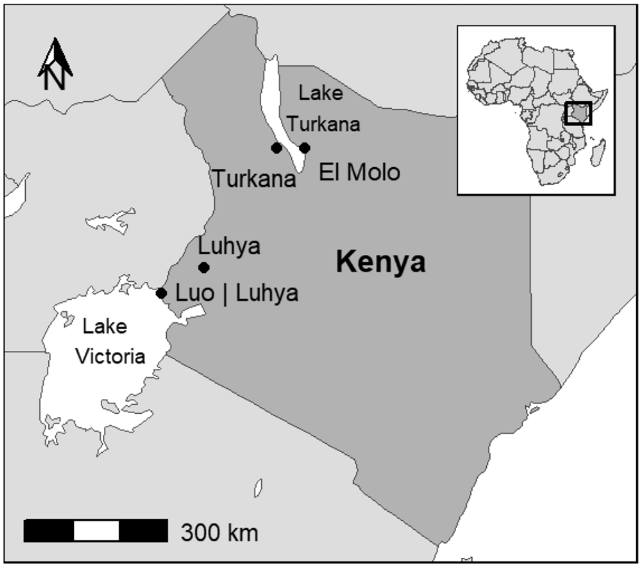

Fig. 1. Location of fieldwork sites in Kenya. Circle icons identify sites, labelled in bold with the name of the studied group. Map produced in R (2023) using packages tidyverse (Wickham et al., 2019) and rnaturalearth (v. 0.3.4, Massicotte et al., 2023). The full reproducible code is available on GitHub (Correia, 2024).

Within the rich cultural and environmental diversity of Kenya, these communities represent three different subsistence strategies from both semiarid and subhumid environments (and access to fish from two different lakes) and diverse cultural background, although their livelihoods have changed due to globalisation and development interventions over the last 50 or more years (Akall, 2021; Bungoma County Government, 2013; Correia et al., 2019; Korir et al., 2023; Turkana County Government, 2020).

2.2. *Health and safety*

Mould collection took place between April and September 2015, under the IN-AFRICA Project permit granted to Prof. Marta Mirazón Lahr by the Government of the Republic of Kenya (NACOSTI/P/15/2669/4758). The project met the ethical criteria set out in the Department of Archaeology and Anthropology guidelines in 2015 when the research was carried out, and were signed off by the Head of

Department. Essential to that approval was governmental approval, competent implementation, and informed consent of all participants. Prior to fieldwork, training was provided by a local UK dentist and the procedure was extensively practiced in volunteer colleagues at the Department. During fieldwork, if it was not possible to communicate with the participant in English, a local translator was employed. Informed consent was obtained by joint reading of the consent form, written in English and Swahili (Kenya's two official languages), of which participants kept the Swahili version. If participants had low literacy levels, the consent form was read out to them, followed by the collection of a thumbprint in lieu of a signature. The informed consent form explained the purpose of the study, what was involved for the volunteer participants, and the nature of the data collected. We maintained active consent throughout the participants' involvement in the study, with particular attention to health and safety during the dental moulding process. In detail, dental moulding only took place if the subject (a) fully consented to the procedure – if available, the procedure was first demonstrated on a research team member, or if that was not possible, bilingual posters were employed to illustrate the procedure; (b) the individual had good dental health (no cavities or periodontal disease); and (c) reported no complaints of tooth ache or sensitivity, including no discomfort when having an air compressor directed on teeth for a minimum of 5 seconds (prior to the beginning of the dental moulding). Both the air compressor and the irrigator (see below) were demonstrated on participant's fingers prior to their use, so that participants could become familiar with the sensation and reaffirm their consent. Participants were continuously monitored for signs of discomfort and oral continued consent was obtained between each step. If at any point, the participant showed uneasiness (verbal or in other ways), the procedure was interrupted and discontinued. Finally, the researcher used vinyl gloves, replaced between each participant; mixing tips and oral tips used in the dispenser gun and in the air compressor were also discarded after each single use; and participants wore a disposable rain coat to protect their clothes.

2.3. Fieldwork procedure

Dental microwear data were collected using established protocols (Goodall et al., 2015; Sawaura et al., 2022) by moulding teeth with a polyvinylsiloxane silicone putty-wash system. In detail, the protocol was specifically tailored for dental moulding in living people in fieldwork conditions, as developed by Ungar et al. (2019) based on previous research in a dental office setting (Janal et al., 2007; Raphael et al., 2003). Subjects were given one cup of popcorn to chew on one side of their mouth of their choosing, followed by rinsing with half a cup of bottled or filtered water. Then, teeth were brushed using an individually wrapped, unflavoured and pre-pasted toothbrush (MediInn® Disposable Toothbrushes) for 2 min, again rinsing with half a cup of water. Afterward, the surface of teeth was again cleaned with 165 ml of water using an oral irrigation device (Panasonic® EW-DJ10 Travel Oral Irrigator). Subsequently, a clean cotton roll was inserted under the tongue to absorb any excess saliva and an individually wrapped tongue depressor placed lightly over the tongue. Teeth were dried by wiping them with a cotton roll and having air from an air compressor directed at them for at least 2 min. Then, a dental mould was taken using Affinis light body wash (Coltene Whaledent) directly applied to teeth using a dispenser gun, and left to set in place for 5 min..

In addition to dental microwear data, we collected other sources of dietary information, including 24-hour recall and diet questionnaires and hair and nail samples for carbon and nitrogen isotopic analyses (Correia, 2018; Correia et al., 2019). The 24-hour recall and diet questionnaires were completed in an interview with the researcher, where all participants were asked to list all foods they remembered eating the previous day, as well as food preferences and dislikes. In addition, participants were also queried on their consumption regarding a set list of foods, in order to complement the 24-hour recall (Baranowski, 2012; Galvin, 1985; Gibson, 1993, 1990; Murphy et al., 2004, 1991; Willett and Lenart, 2012). The form used to collect this information is available as a Supplementary file. Hair samples of at least 2 cm in length were cut at the nape of the head, while nail samples were collected by having participants clip the distal edge of a fingernail of their choosing (Correia et al., 2019; O'Connell et al., 2001; O'Connell and Hedges, 1999).

2.4. Laboratory procedure

At the Dorothy Garrod Laboratory (University of Cambridge), Epotek 301 was used for casting following established protocols (Galbany et al., 2004; Goodall et al., 2015; Scott et al., 2006). Epoxy casts were scanned using a Sensofar Plμ Neox white-light scanning confocal microscope fitted with a × 100 objective at the Ungar Laboratory (University of Arkansas). Each individual was represented by one crushing facet (Phase II) from a lower molar (first, second, or third) with the best microwear preservation. Scanning generated a cloud of points for each surface, with a lateral sampling interval of 0.17 μm (μm), a vertical resolution of 0.005 μm, and a field of view of 138 × 102 μm. Data were collected from four adjoining fields and stitched together to create an area of approximately 242 × 181 μm. Using the SolarMap software, scans were levelled and cleaned of any small identifiable defects, such as dust particles. In addition, we applied a form removal algorithm, removed data spikes by thresholding out the upper and lower 0.1 % of data, and applied a single spline filter (2.5 μm cut off point). Missing data points as a result of this treatment were filled in using a nearest neighbour algorithm. Scale-sensitive fractal analysis was conducted using the Toothfrax and Sfrax softwares (SurFract Corp, Worcester, MA), while surface texture analyses were conducted using the Mountains Map software (Digital Surf Corp, Besançon, France).

The intake of nutrients (g/day) from dietary questionnaires was calculated using food composition tables. Not all foods reported by participants were found on a single food composition table, and data was collated from different composition tables; a common practice as the original tables also glean data from different analyses and reports. As a rule, the main food composition table used was the International Minilist (IML), developed specifically for foods consumed in rural Kenya (Murphy et al., 2004, 1991), supplemented by the Food and Agriculture Organization of the United Nations Database (FAO, 2016) and the United States Department of Agriculture National Nutrient Database for Standard Reference (USDA, 2015).The nutrient contents of blood could not be found in any of the above tables, and this information was obtained from Galvin (1985), the only study so far, to the best of our knowledge, to have analysed blood for its potential as food. The isotopic analyses of hair and nail were carried out at the Godwin Laboratory, Department of Earth Sciences, University of Cambridge (Cambridge, UK), using a Costech (Valencia, CA, USA) elemental analyser coupled in continuous-flow mode to a ThermoFinnigan (Bremen, Germany) Delta V isotope ratio mass spectrometer.

2.5. Statistical analyses

Statistical analyses of microwear data were performed using R (version 4.3.1) (R Core Team, 2023). To investigate whether group affiliation explains the variance observed in multiple dependent variables, we initially employed a multivariate analysis of variance (MANOVA). However, the data violated some of the MANOVA assumptions, particularly multivariate normality and heteroscedasticity (Correia, 2024), which can be explained by the difference in scales of measurement between variables. Hence, we opted for a permutational version of the test (PERMANOVA), which assesses differences between groups based on distances between objects using distribution-free permutation techniques (Anderson, 2017; v. 0.9–83-7, Herve, 2023).

To comprehensively explore the data, we followed the PERMANOVA with a three-part approach: 1) to assess multivariate distance between group pairs, we used pairwise PERMANOVA models (Anderson, 2017; v.

0.9–83-7, Herve, 2023); 2) to determine univariate variation between groups, we conducted ANOVAs on rank-transformed data (i.e., Kruskal-Wallis tests), followed by pairwise Dunn's tests for significant variables, a non-parametric version of the more common post hoc Tukey HSD (Agbangba et al., 2024; v. 0.7.2, Kassambara, 2023); 3) to identify linear combinations of variables characterising the groups, we performed canonical discriminant analysis (CDA); in other words, CDA identifies orthogonal vectors in the dependent variable space that explain the greatest possible between-group variation (v. 0.8–6, Friendly and Fox, 2021; Friendly and Sigal, 2017). While the first approach is common in PERMANOVA, the second and third are recommended as follow-up methods for standard MANOVA. ANOVA detects individual variables driving data variation, while CDA adds exploratory detail to multivariate analyses by maximising group differences (Field et al., 2012, p. 719). Additionally, we reported effect sizes of tests as a measure of the strength of the relationship between two variables or groups, and when necessary, we applied a false-discovery rate (FDR) correction to control for multiple tests (Cohen, 1988; Field et al., 2012; Wilcox, 2012). Given the sample size limitations in this study (see below), this statistical framework sufficiently explores the variation present in the data, while employing adequate measures to ensure the conservative interpretation of data and reduce the chances of false positives – i.e. assumption testing, effect size evaluation, Type I/II error control, and conservative tests, such as Kruskal Wallis and Dunn's tests (Agbangba et al., 2024; Field et al., 2012). The complete, reproducible code is available on GitHub (Correia, 2024) and as a Supplementary file.

3. Results

3.1. Diet characterization

The results from the 24-hour recall, diet questionnaires, and stable isotopic analyses partly corroborated the ethnographic dietary profiles, with the Turkana as an exception, who appear to be transitioning toward a more sedentary lifestyle and associated diet characterised by reduced animal food consumption and an increased reliance on maize (Akall, 2021; Correia, 2018; Correia et al., 2019). Dietary intake varies substantially between individuals of the same group, but, across the entire sample, plant foods formed the bulk of the diet for all groups. Maize flour was a staple for most, with some consumption of sorghum and other cultivated vegetables like cassava and sweet potatoes, particularly among the Luhya in Webuye. While the maize flour was predominantly industrially processed, the El Molo community also engaged in manually grinding maize using a metal tube to produce their flour. Fish was a significant component of the diet for all fishing communities, and it was prepared through various methods such as boiling, smoking, or sun-drying. Animal foods, primarily in the form of milk, were consumed in relatively small quantities across all communities. Notably, the pastoralist Turkana group consumed even less animal foods than the El Molo or the Luhya (Webuye), despite being ethnographically described as having a diet rich in blood and milk (Galvin, 1985). Interestingly, as mentioned before, nomadic pastoralism remains the main activity in the region (Akall, 2021; Turkana County Government, 2020), and study participants still kept herds, self-identified as pastoralists, and reported their preference for consuming blood, milk, and meat (Correia, 2018). However, they seldom reported having consumed animal products in the previous 24 h, meat consumption was restricted to celebratory events, such as weddings, and livestock was viewed as wealth and a key cultural cornerstone. Indeed, the stable isotope results of this group were the most variable, suggesting that individuals are undergoing a dietary change and could be moving between consumption of animal products and heavier reliance on plant foods, particularly maize flour (Correia et al., 2019). As expected,the Luhya from Webuye were the most market-integrated, with access to a variety of ultra-processed foods (Correia, 2018). The aim of the present report does not include the in-depth scrutiny of the results from 24-hour recall, diet questionnaires, and stable isotopic analyses, whose analyses and discussion would not fit within the constraints of a journal publication and which are reported elsewhere (Correia, 2018; Correia et al., 2019). Notwithstanding, the summarised results of this section are key to the interpretation of the microwear results addressed in the Discussion. As mentioned above, the El Molo and the Turkana inhabit semiarid regions, while the Luhya and Luo reside in subhumid areas. This environmental distinction may influence the presence of dust in their food, particularly in sun-dried fish. As for dental hygiene practices, the El Molo and the Turkana often chew on *Salvadora persica*, a natural toothbrush, while the Luhya and Luo are more familiar with conventional toothbrushing, despite having limited access to dental care (Correia, 2018; Halawany, 2012).

3.2. DMTA results

In this study, we generated SSFA and STA variables for a total of 37 microwear surfaces, each representing an individual, with 9 surfaces for the El Molo group, 8 for the Luo (Port Victoria), 7 for both the Turkana and the Luhya (Webuye), and 6 for the Luhya (Port Victoria). The success rate for moulding human teeth under our fieldwork conditions was only 25 % – most of this data loss resulted from oral biofilm obscuring microwear surfaces as extensively discussed in Correia et al. (2021).

From the computed DMTA variables, we used the SSFA variables and the STA variables prioritised in a previous microwear study on human living groups (Ungar et al., 2019). We also excluded 3 STA variables (*Sdr*, *Vvv*, and *S5v*) prior to statistical analyses due to their high correlation (Correia, 2024), because such collinearity is detrimental to multivariate analyses (Field et al., 2012). Table 1 lists the remaining

Table 1
Description of the 11 variables studied. Parameters from scale sensitive fractal analyses (SSFA) according to Scott et al. (2006), and parameters from surface texture analyses according to ISO 2 (2021).

Parameter	Type	Group	Name	Description
Asfc	SSFA	–	area-scale fractal complexity	change of roughness with scale
HAsfc$_{81}$	SSFA	–	heterogeneity of area-scale fractal complexity	variation of Asfc across the surface in a 9 × 9 grid
epLsar	SSFA	–	length-scale anisotropy of relief	measure of feature orientation
Tfv	SSFA	–	textural fill volume	function between the shape and texture of the surface
Ssk	STA	Height	skewness of height distribution along the *z* axis	degree of bias of the roughness shape
Sp	STA	Height	maximum peak height of surface	height of the highest peak
Sz	STA	Height	maximum height of the surface	distance from highest peak and deepest pit
Sdq	STA	Hybrid	root mean square gradient of the surface	root mean square of slopes at all points
Sxp	STA	Functional	extreme peak height	height difference between the average plane and the peak plane
Sda	STA	Feature	mean closed dale area	average area of dales connected to the edge
Sdv	STA	Feature	mean closed dale volume	average volume of dales connected to the edge

Table 2
Summary of DMTA variables, with number of surfaces (*n*), mean, standard deviation (SD), median and interquartile range (IQR).

		El Molo	Turkana	Luhya (Webuye)	Luhya (Port Victoria)	Luo (Port Victoria)
	n	9	7	7	6	8
Asfc	mean (SD)	3.38 (1.56)	3.32 (0.98)	3.02 (1.82)	2.21 (0.97)	2.47 (0.86)
	median (IQR)	3.78 (2.49)	3.23 (1.16)	2.50 (1.72)	2.16 (1.45)	2.75 (1.30)
$HAsfc_{81}$	mean (SD)	0.45 (0.20)	0.26 (0.12)	0.50 (0.13)	0.56 (0.44)	0.44 (0.12)
	median (IQR)	0.46 (0.28)	0.21 (0.06)	0.52 (0.16)	0.43 (0.25)	0.44 (0.15)
epLsar	mean (SD)	0.004 (0.002)	0.002 (0.001)	0.002 (0.001)	0.003 (0.001)	0.003 (0.002)
	median (IQR)	0.003 (0.002)	0.001 (0.001)	0.002 (0.002)	0.002 (0.001)	0.003 (0.001)
Tfv	mean (SD)	53,682 (7782)	41,618 (8024)	49,741 (8328)	51,667 (5783)	41,223 (10192)
	median (IQR)	52,229 (10723)	44,927 (5903)	53,447 (11367)	50,000 (4299)	45,092 (8974)
Ssk	mean (SD)	−0.03 (0.23)	−0.32 (0.20)	−0.30 (0.26)	−0.54 (0.35)	−0.88 (0.43)
	median (IQR)	−0.03 (0.21)	−0.34 (0.25)	−0.28 (0.32)	−0.49 (0.33)	−0.64 (0.46)
Sp	mean (SD)	3.40 (2.25)	2.38 (1.07)	2.50 (1.61)	2.47 (1.36)	2.24 (1.44)
	median (IQR)	2.58 (3.71)	2.04 (0.79)	1.73 (1.45)	2.02 (1.67)	1.92 (0.51)
Sz	mean (SD)	5.88 (3.16)	7.16 (7.64)	4.82 (1.24)	5.02 (1.46)	5.10 (1.94)
	median (IQR)	4.67 (4.55)	3.77 (2.75)	4.22 (2.24)	5.08 (1.59)	4.57 (1.00)
Sxp	mean (SD)	0.86 (0.17)	0.71 (0.17)	0.79 (0.20)	0.70 (0.19)	1.06 (0.26)
	median (IQR)	0.80 (0.31)	0.77 (0.16)	0.79 (0.26)	0.70 (0.21)	1.04 (0.30)
Sdq	mean (SD)	0.21 (0.05)	0.23 (0.09)	0.21 (0.10)	0.18 (0.04)	0.20 (0.04)
	median (IQR)	0.21 (0.07)	0.20 (0.07)	0.18 (0.08)	0.19 (0.07)	0.20 (0.06)
Sda	mean (SD)	316.52 (159.67)	234.190 (122.79)	306.476 (124.43)	502.664 (196.92)	333.072 (101.10)
	median (IQR)	308.72 (135.16)	207.12 (126.19)	274.60 (154.34)	519.20 (298.24)	314.12 (70.99)
Sdv	mean (SD)	7.35 (5.56)	4.693 (3.332)	7.309 (4.474)	12.467 (8.062)	10.347 (7.417)
	median (IQR)	5.717 (5.787)	3.380 (3.998)	7.491 (6.915)	12.226 (12.700)	8.368 (5.473)

variables consisting of 4 SSFA variables and 7 STA variables.

Table 2 provides a summary of standard and robust descriptive statistics for all variables. Notably, the measures of central tendency, such as the mean and median, exhibit minimal differences, while measures of dispersion, such as the standard deviation and interquartile range, are relatively large.

A Type II PERMANOVA using Euclidean distances found a significant and large effect of group diet on the DMTA variables, $F(4,36) = 3.81$, $p = 0.01$, $\eta^2 = 0.32$.

As described above, to further investigate this significant outcome, we conducted pairwise PERMANOVAS, univariate ANOVA's on rank-transformed data, and CDA. Table 3 summarises the results of the pairwise PERMANOVAS, which identified two statistically significant comparisons. Specifically, there were significant differences between the El Molo and both the Turkana and the Luo (Port Victoria). Additionally, there were two other comparisons with large effect sizes: Turkana versus Luhya (Port Victoria) and Luhya (Port Victoria) versus Luo (Port Victoria).

Next, the Kruskal-Wallis tests examined whether variation was driven by a single variable. Among all the variables examined, only *Ssk* was significantly different between groups, $H(4) = 23.07$, $p = 0.001$, $\eta^2 = 0.60$. Additionally, three other variables – $Hasfc_{81}$, *Sxp*, and *Tfv* – had large effect sizes, but did not reach statistical significance (Correia, 2024). For pairwise comparisons of the *Ssk* variable, we employed Dunn's tests, as reported in Table 4, which detected four statistically significant comparisons. Specifically, we observe that:

1) the Luo (Port Victoria) were statistically different from all other populations except for the Luhya (Port Victoria);
2) the El Molo were also significantly different from the Luhya (Port Victoria);
3) all comparisons, except the one between the Turkana and the Luhya from Webuye, have moderate or large effect sizes.

Table 3
Results of pairwise PERMANOVA with FDR correction for the multivariate space. Interpretation of R^2: small = 0.01, *medium* = 0.09, and **large** = 0.25 (Cohen, 1988). Underline identify statistically significant results at $p < 0.05$.

Group 1	Group 2	Statistic (*pseudo-F*)	*p*-value	R^2
El Molo	Turkana	9.21	0.045	**0.38**
El Molo	Luhya (Webuye)	0.95	0.473	0.06
El Molo	Luhya (Port Vict.)	0.29	0.719	0.02
El Molo	Luo (Port Vict.)	8.14	0.045	**0.35**
Turkana	Luhya (Webuye)	3.45	0.170	*0.22*
Turkana	Luhya (Port Vict.)	6.48	0.050	**0.37**
Turkana	Luo (Port Vict.)	0.01	0.959	0.001
Luhya (Webuye)	Luhya (Port Vict.)	0.23	0.719	0.02
Luhya (Webuye)	Luo (Port Vict.)	3.08	0.170	*0.19*
Luhya (Port Vict.)	Luo (Port Vict.)	5.02	0.095	**0.30**

Table 4
Results of pairwise Dunn's tests with FDR correction for the Ssk variable. Interpretation of Hedge's g: small = 0.20, *medium* = 0.50, and **large** = 0.80 (Cohen, 1988). Underline identify statistically significant results at $p < 0.05$.

Group 1	Group 2	Mean difference	Statistic (*z*)	*p*-value	Hedge's *g*
El Molo	Turkana	−9.05	−1.66	0.162	**1.24**
El Molo	Luhya (Webuye)	−9.33	−1.71	0.162	**1.04**
El Molo	Luhya (Port Vict.)	−16.00	−2.80	0.019	**1.61**
El Molo	Luo (Port Vict.)	−24.33	−4.63	0.001	**2.33**
Turkana	Luhya (Webuye)	−0.29	−0.05	0.961	−0.05
Turkana	Luhya (Port Vict.)	−6.95	1.15	0.298	*0.74*
Turkana	Luo (Port Vict.)	15.29	2.73	0.019	**1.59**
Luhya (Webuye)	Luhya (Port Vict.)	−6.67	−1.11	0.298	*0.72*
Luhya (Webuye)	Luo (Port Vict.)	−15.00	−2.68	0.019	**1.53**
Luhya (Port Vict.)	Luo (Port Vict.)	−8.33	−1.43	0.220	**0.81**

CDA identified four discriminant dimensions, of which two exhibited significantly different group means; function 1: $F(44, 86) = 2.12$, $p = 0.002$; function 2: $F(30, 68) = 1.87$, $p = 0.02$. As illustrated in Fig. 2, the first function explained 41.2 % of the variance, while the second explained 33.9 %, totalling 75.1 % of all variance in the data. Notably, the first canonical dimension was more strongly associated with *Ssk*, and separated the El Molo from the two Port Victoria populations. The second canonical dimension was more strongly associated with *Tfv*, *Sda*,

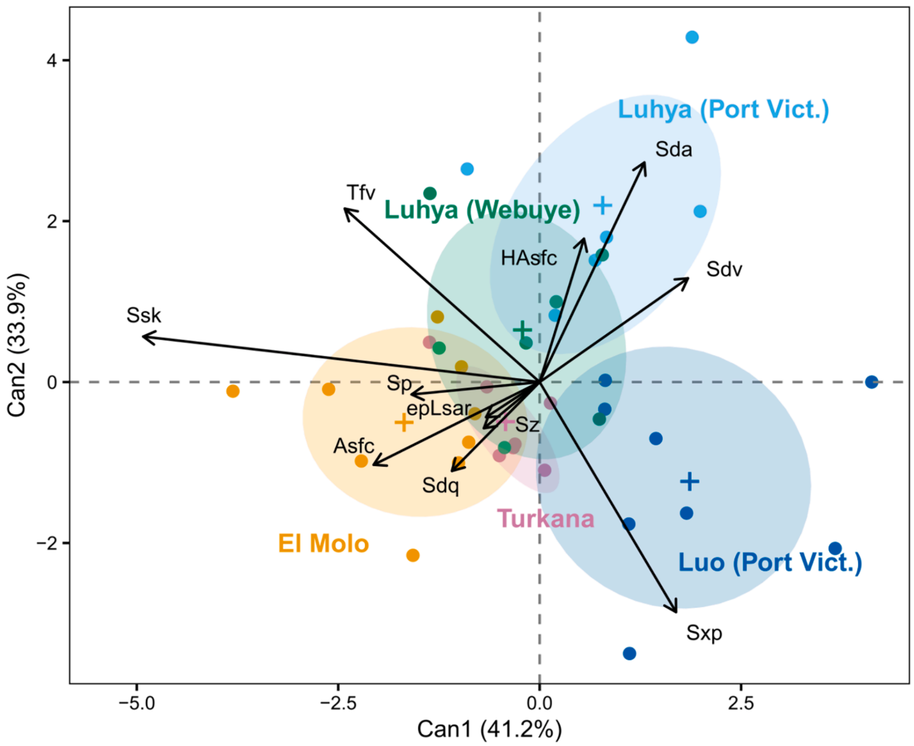

Fig. 2. Biplot of the first two functions from the canonical discriminant analysis (CDA) of DMTA variables. Data ellipses show the covariance structure for each group, while the vectors show the projection of the original variables in the CDA space.

and *Sxp*, and separated the Luhya and Luo from Port Victoria. The remaining variables are less strongly associated with the discriminant functions, and these functions did not distinctly separate the two remaining groups, the Turkana and the Luhya (Webuye) (Correia, 2024).

Collectively, the statistical analyses consistently indicate that the Luhya (Webuye) and the Turkana cannot be distinguished from each other. In turn, the El Molo can be separated to different extents from all other groups in the multivariate space, particularly from the Port Victoria populations, although this separation is largely driven by the *Ssk* variable. Finally, the two populations from Port Victoria are distinguishable only through the CDA, under a combination of the variables *Tfv*, *Sda*, and *Sxp*.

4. Discussion

When analysing the DMTA results within the dietary context of the studied populations, several key findings emerge:

1) The El Molo have the most distinctive microwear pattern, likely resulting from the inclusion of grit in their diets through sun drying fish and manually grinding maize in a dry environment.

Supporting this interpretation, grit has long been considered an important factor in microwear production (Kay and Covert, 1983; Lucas et al., 2014), and experimental data found that including stone-ground maize in human diets substantially alters microwear (Teaford and Lytle, 1996). However, more recent research suggests that the impact of grit on microwear also depends on the material properties of the foods themselves (Hua et al., 2020; Merceron et al., 2016). In our case, at the time of fieldwork, the El Molo sun dried fish for commerce and consumption and grounded their maize using a metal tube. On their own, neither process introduces grit into the food, but the El Molo inhabit a semiarid environment undergoing erosion (Akall, 2021; Turkana County Government, 2020) and grit from the environment could have been introduced into food during both preparation processes.

2) The El Molo microwear pattern is partly driven by the *Ssk* variable, which might suggest a soft, but abrasive diet.

This variable quantifies the asymmetry of the height distribution along the surface, and has been proposed as an indicator of “soft abrasive diets” (Francisco et al., 2018). Notably, the *Ssk* variable separates the El Molo from the two other fishing populations, the Luhya and Luo from Port Victoria. And indeed, fish is a soft food that may become abrasive through the inclusion of grit. While the Port Victoria groups also sun-dry their fish, the arid and under erosion environment of the El Molo may result in more grit being incorporated into the food than in Port Victoria (Akall, 2021; Turkana County Government, 2020). Indeed, previous research found environmental grit can impact dental microwear, although it must be incorporated into food and that drier environments will not always correspond to higher grit accumulation, and thus, that interpretations must be environment specific (Geissler et al., 2018; Merceron et al., 2016; Ungar et al., 1995). Additionally, the Port Victoria groups consume fine-ground industrially processed maize flour rather than manually-ground maize, which may also contribute to this microwear pattern.

3) The Luhya (Port Victoria) diet is closer to that of the Luo, both groups inhabiting the same location, than to the Luhya from Webuye.

The same pattern was true for the other sources of dietary information collected, most notably stable isotopic analyses (Correia, 2018; Correia et al., 2019), suggesting that, among these groups, environmental factors and food availability play a more substantial role in shaping dietary habits than cultural affiliation. Alternatively, the Luhya (Webuye) had a diet mostly based on C$_4$ foods, compatible with a higher market integration. As the Luhya and Luo are two very large groups, it is reasonable to assume that the ongoing nutrition transition is impacting their diets in different ways in different regions.

4) Yet, the multivariate microwear data separates the two Port Victoria groups to some extent.

This difference indicates that these groups' diets differ slightly in material or mechanical properties, possibly due to different preparation techniques, since other sources of dietary information found no substantial differences between the two groups in Port Victoria (Correia, 2018; Correia et al., 2019). Alternatively, consumption of different types of fresh plants could also drive this distinction, since such food items often have differing material and mechanical properties but share isotopic signatures (e.g. most fresh foods are C_3 plants) and were not exhaustively discriminated in the dietary questionnaires. Either way, this finding supports the idea that DMTA are more powerful when employed alongside other dietary inference methods, particularly stable isotopic analyses – a concept often endorsed in reviews on the subject (e. g., Calandra and Merceron, 2016; Lee-Thorp and Sponheimer, 2006; Schmidt et al., 2020).

5) The distinction between the two Port Victoria groups is driven by the variables *Tfv*, *Sda*, and *Sxp*, where the first two may indicate pitted surfaces, while the last may indicate deep features.

Based on Table 1, *Tfv* is a function between the shape and texture of the surface, and an increasing value may indicate a pitted surface with many valleys and hills (Scott et al., 2006). Similarly, the *Sda* quantifies the area of the valleys, and an increasing value suggests a pitted surface. Then, *Sxp* represents the height difference between the average plane and the peak plane of the surface, and an increasing value suggests deep features (ISO, 2021). Here, we could infer that the Luhya (Port Victoria) have shallowly pitted surfaces, while the Luo (Port Victoria) have less pitted surfaces with deep features. The first may be associated with a harder diet while the second may be associated with a tougher diet with incorporated grit that creates deep features.

6) The Turkana and Lhuya (Webuye) have similar microwear patterns.

Previous archaeological studies suggest that, because of food processing methods and the preponderance of animal products, pastoralists have the softest diets, followed by agriculturalists, with both groups being characterised by anisotropic microwear surfaces with many parallel scratches. In contrast, foragers have harder diets, resulting in complex microwear surfaces, with dense concentrations of microwear features (Schmidt et al., 2019, 2015; Watson and Schmidt, 2020). The present study did not include a forager group, but our results indicate that the fisher populations have more distinct microwear patterns, probably resulting from a combination of grit introduced during processing methods and the food properties themselves. In turn, the agriculturalist Luhya (Webuye) and the pastoralist Turkana have indistinguishable soft diets that do not produce particular microwear features in comparison with the other groups. Notably, these two groups have distinct isotopic signatures (Correia, 2018; Correia et al., 2019), mainly resulting from the high consumption of C_4 staples by the Luhya (Webuye). Furthermore, the isotopic results suggest that the Turkana no longer rely on a pastoralist subsistence and are shifting towards a more maize-based diet, which might explain why their microwear pattern is closer that of the agriculturalist Luhya.

7) Complexity (*Asfc*) and anisotropy (*epLsar*) were not informative.

These two SSFA variables are sometimes favoured in human DMTA studies (e. g., Schmidt et al., 2019, 2015); however, in this study, other variables, particularly STA variables, were more efficient in distinguishing diets. Using a modern ruminant model, Francisco et al. (2018) demonstrate that *Ssk* and another STA variable (*Rmax*) produce better classifications than the traditional *epLsar* and *Asfc* pairing, further enlightening the role that *Ssk* played in our data.

8) Many non-significant comparisons had a high effect size, suggesting that differences in microwear are present but that the sample size was still insufficient to demonstrate the associations.

Thus, although we maintain that more studies into contemporary human microwear are needed, we still need to improve our ability to remove oral biofilm (Correia et al., 2021). We also recognise that although using a greater number of relevant parameters improved our ability to capture subtle differences in diets, this approach poses different issues, producing, for instance, high dimensional data, where the number of variables is close to the number of observations. Alternatives include developing procedures to identify and prioritise the most discriminative parameters (Francisco et al., 2018).

9) We partly overcame the sample size restrictions by combining SSFA and STA variables within a multivariate approach, which improved DMTA interpretation in relation to our previous study (Correia et al., 2021).

And yet, few DMTA human studies have combined these two types of parameters so far (e.g., Peterson et al., 2018; Ungar et al., 2019). We urge the community to explore the usefulness of including STA variables in their microwear studies.

As a final point, and as already pointed out by Ungar (Ungar, 2015, p. 37), "We may be approaching the point […] where our abilities to characterise dental surface textures at micro-scales are beginning to outpace our abilities to interpret those characterizations". Thus, we need to invest in more research into the realities of human microwear variation. Of key importance to this goal, we require the development of standardised and validated dietary assessment tools tailored around food processing techniques and putative microwear-producing foods as common dietary assessment tools do not target these points explicitly. Understandably, the variables these tools produce are targeted towards the evaluation of adequate nutrition intake and are difficult to relate to microwear variables, other than in the general contextualisation employed here (Baranowski, 2012; Gibson, 1990; Willett and Lenart, 2012). The same is true of bioarchaeological studies that employ general written records of the studied populations to interpret the microwear data (e.g. El Zaatari, 2010; Schmidt et al., 2019), and so, both approaches could benefit from a more standardised approach to the quantification of consumed food through the lens of microwear production. This endeavour, however, is likely to prove a challenge as stable isotope research has long struggled to effectively link information from diet assessment tools and stable isotope results, as the later have low specificity, i.e. a clear link to particular foods (Correia et al., 2019; Hülsemann et al., 2017; Patel et al., 2014; Ryman et al., 2014).

5. Conclusion

Combining SSFA and STA data provides a more complete picture of human microwear, but multivariate analyses require high sample sizes, which are often difficult to obtain within human DMTA studies. In this study, we found that the fisher El Molo have soft abrasive diets, possibly resulting from grit being incorporated into their sun-dried fish and manually-processed maize flour. In addition, the Luhya (Port Victoria) have harder diets than the Luo (Port Victoria), possibly due to unrecorded differences in processing techniques or fresh food consumption. Finally, the agriculturalist Luhya (Webuye) and the pastoralist Turkana have soft diets lacking distinctive microwear patterns when compared to the other groups. Going forward, human DMTA studies would benefit from including both SSFA and STA parameters, but we still need more research on the link between human diets and resulting microwear parameters.

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CRediT authorship contribution statement

Maria Ana Correia: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Robert Foley:** Writing – review & editing, Supervision, Conceptualization. **Marta Mirazón Lahr:** Supervision, Funding acquisition, Conceptualization.

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.

Data availability

All materials and code are available from GitHub repository (https://macorreia42.github.io/JASR2024_supplementary/JASR2023) and archived on Zenodo (DOI: 10.5281/zenodo.10518855).

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jasrep.2024.104718.

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