Enamel daily secretion rates of deciduous molars from a global sample of children.

Gina McFarlane*[1], Carolina Loch[2], Debbie Guatelli-Steinberg[3], Priscilla Bayle[4], Mona Le Luyer[4], Nina Sabel[5], Alessia Nava[1], Bruce Floyd[6], Mark Skinner[7], Sophie White[2], Rosie Pitfield[1], Patrick Mahoney[1].

[1]Human Osteology Lab, School of Anthropology and Conservation, University of Kent, Canterbury, UK.
[2]Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin, New Zealand
[3]Department of Anthropology, The Ohio State University, Columbus, Ohio, USA.
[4]Univ. Bordeaux, CNRS, MC, UMR 5199 PACEA, Pessac, France.
[5]Department of pedodontics, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Sweden.
[6]School of Social Sciences, University of Auckland, New Zealand.
[7] Department of Archaeology, Simon Fraser University, Burnaby, Canada

ABSTRACT

Objective: To investigate and describe the variation in enamel daily secretion rates (DSRs) of naturally exfoliated deciduous molars (n = 345) from five modern-day populations (Aotearoa New Zealand, Britain, Canada, France, and Sweden).

Design: Each tooth was thin sectioned and examined using a high-powered Olympus BX51 microscope and DP25 digital microscope camera. Mean DSRs were recorded for the inner, mid, and outer regions of cuspal and lateral enamel, excluding enamel nearest the enamel-dentin junction and at the outermost crown surface.

Results: Mean DSRs did not vary significantly between populations, or by sex. Cuspal enamel grew slightly faster than lateral enamel (mean difference 0.16 μm per day; $p < 0.001$). The trajectory of DSRs remained relatively constant from inner to outer cuspal enamel and increased slightly in lateral enamel ($p = 0.003$).

Conclusions: The DSRs of deciduous molars from modern-day children are remarkably consistent when compared among populations. While growth rates are faster in cuspal than lateral enamel, the trajectory of enamel formation changes only slightly from inner to outer regions. The trajectory of DSRs for deciduous molars differs to that of permanent molar enamel, which typically display a steep increase in matrix deposition from inner to outer enamel.

KEY WORDS: daily secretion rates, dental development, enamel formation, cross striations, deciduous molars, incremental growth.

1 | INTRODUCTION

Human teeth retain histological evidence of enamel growth processes as incremental markings. One of these markings is a daily constriction or cross striation that is present along enamel prisms, or rods, and forms as secretory ameloblasts periodically alter deposition of new matrix (Boyde, 1989; Zheng et al., 2013). The distance between adjacent cross striations is the amount of enamel deposited by ameloblasts in a 24-hour period, which can be expressed as a daily secretion rate (DSR) (Reid et al., 1998). Thus, enamel appositional growth results from the daily elongation of enamel prisms as secretory ameloblasts move away from the enamel-dentine junction (EDJ). Studies of DSRs from permanent teeth provide key insights into the evolution of hominin life history (Dean et al., 2001; Macchiarelli et al., 2006) and underpin histological reconstructions of tooth crown formation time (Hillson, 2014; Reid & Dean, 2006). In a forensic and anthropological context, DSRs of permanent teeth have been incorporated into age-at-death estimations (Boyde, 1964; Dean et al., 1993) and the timing of mineralization related to chemical signatures of diet and stress (Humphrey, 2014; Humphrey et al., 2008; Li et al., 2020; Nava et al., 2020).

There is a good understanding of some aspects of deciduous dental development. Studies have explored the timing of tooth development and eruption (AlQahtani et al., 2010; Liversidge, 2016), crown tissue proportions (Mahoney, 2010, 2013) and the rate that crowns and roots extend in height (Deutsch et al., 1985; Mahoney, 2015, 2019; Shellis, 1984; Stack, 1967). Histologically derived enamel formation times are known for molar crowns (FitzGerald & Hillson, 2009; Mahoney, 2011, 2015). Yet, compared to permanent teeth, much less is known about secretion rates from deciduous teeth of modern-day children. Schour and Poncher (1937) described the secretion rate of one deciduous molar that was between 3.6 to 4.3 μm per day. Shellis (1984) presented summary data for different deciduous tooth types with rates that ranged between 4.5 to 5.3 μm per day. More recently, DSRs have been presented for a total of

seven deciduous molars (Birch & Dean, 2009, 2014; Macchiarelli et al., 2006), and six deciduous teeth (incisors, canines, and premolars) from a Syrian archaeological context that ranged from 3.36 to 4.02µm per day (Witzel, 2014). Several other studies of DRSs are based upon Greek or British archaeological samples (FitzGerald & Hillson, 2009; Mahoney, 2011, 2012, 2015). Recent work suggests that archaeologically derived DSRs might not represent those of modern-day children (Aris et al., 2020; Dean et al., 2020; Nava et al., 2017). No study has compared DSRs between modern-day children from different geographic regions so the degree of variation that might exist is unknown. Given the role that deciduous DSRs play in establishing the pace of life history variables including dental development, birth, weaning, as well as the timing of stress events and age-at-death of juveniles (Aris et al., 2020; Birch & Dean, 2009, 2014; Dean et al., 2020; FitzGerald & Hillson, 2009; FitzGerald et al., 2006; Huda & Bowman, 1995; Kierdorf et al., 2021; Lorentz et al., 2019; Macchiarelli et al., 2006; Mahoney, 2011, 2012, 2015; Nava et al., 2017, 2019, 2020; Witzel, 2014) understanding the degree of variation present amongst modern-day children from different populations is critical.

Daily enamel secretion rates are typically presented for cuspal or lateral enamel that has been subdivided into regions or thirds (inner, mid, outer) (Beynon et al., 1991). In permanent teeth, secretion rates can vary considerably between these regions (e.g., Smith et al.,2007), because ameloblasts typically secrete less new matrix near the EDJ and significantly more matrix in outer enamel regions. This leads to a typical trajectory of permanent enamel secretion (see review in Mahoney, 2008). In studies of hominoid taxonomy, it makes sense therefore to restrict comparisons of DRSs to specific regions of permanent enamel (e.g., Mahoney et al., 2007), or to incorporate local DSRs into formation times of different enamel regions (Reid & Dean, 2006). It is not clear whether deciduous enamel of modern-day children displays the same growth trajectory as permanent enamel. Shellis (1998) noted that rates increased by 106% from the inner to outer enamel of permanent teeth, but that these rates changed by 20% through the same regions of deciduous teeth. While there was some variation

in the trajectory of DSRs of four deciduous molars of modern-day children (Birch & Dean, 2009; Macchiarelli et al., 2006), these studies included the first formed enamel near the enamel-dentine junction, which is typically slow relative to mid and outer enamel regions (Mahoney, 2015).

The aim of this study is to report DSRs for a global sample of deciduous molars (n=345) from 285 modern-day children. Our main objective is to understand if the daily rate that enamel is secreted in deciduous molars varies amongst children from different geographic regions. To ensure comparability between the samples, rates are measured and compared between equivalent enamel regions. We excluded DSRs from the first 100 μm of enamel nearest the enamel-dentin junction because of pronounced localised changes in the pace of secretion (e.g., Mahoney, 2015). We did not record cross striations in the 100 μm of enamel nearest the outermost crown surface, as rates can also vary considerably in this region. Comparative secretion rates for permanent teeth are taken from the published literature.

2 | SAMPLES AND METHODS

2.1 |Dental Samples

Samples are part of the biorhythm of childhood growth project that is a longitudinal study of deciduous enamel biorhythms in modern-day children. Samples comprised first and second deciduous molars (Table 1). Sex was known for some, but not all samples.

The British dental samples were from the Kent-UCL collection, and the Percy Butler collection. Samples were fully anonymised, and were either dental extractions or naturally exfoliated teeth that were collected with consent during routine dental treatment during the 1960's and 1970's. These samples are held in the Human Osteology Lab of the University of Kent with ethical approval for histological analysis obtained from the UK National Health Service research ethics committee (REC reference: 16/SC/0166, ID 203541; REC reference: 19/REM/0126, ID 261173).

The Canadian dental samples were collected during the 1970s at the Simon Fraser University. These samples are held in the Human Osteology Lab of the University of Kent with ethical approval for histological research obtained from the UK National Health Service research ethics committee (REC reference: 19/REM/0126, ID 261173).

The French dental samples are part of the Tooth Fairy collection (Le Luyer and Bayle, in review) and consisted of naturally exfoliated deciduous teeth collected in 2014. The collection is held in the University of Bordeaux, France. Samples were fully anonymised and written consent was obtained from the participants prior to conduct histological analyses. Data collection and exploitation was approved by the French authority "Commission Nationale de l'Informatique et des Libertés" (CNIL).

New Zealand samples consisted of exfoliated deciduous molars collected from children aged between 9-10 years residing in the city of Dunedin, New Zealand, that were mostly of New Zealand European, Māori, or Pacific Islander ethnicity. Additional samples were collected by dental therapists from the School Dental service of the Whanganui and Hawkes Bay District Health Boards. Ethical approval for histological research on these samples was obtained from the University of Otago Human Ethics Committee (approval number H19/030).

The dental samples from Sweden comprised of second deciduous molars, were collected during 2008 at the Clinic of Pediatric Dentistry, Public Dental Care, Västra Götaland, Sweden. Teeth were extracted for odontological reasons. The age of the patients varied from 3 to 16.5 years. Research ethical approval obtained from the Ethical Committee at the Sahlgrenska Academy at the University of Gothenburg, Göteborg, Sweden, registered 432-08.

2.2 | Methods

Teeth were embedded in epoxy resin (Buehler EpoxiCure®) and sectioned through the tip of the cusp and dentine horn of molar mesial cusps using a Buehler Isomet 1000 precision saw. Sections were fixed to glass microscope slides, ground with a series of grinding pads (P400,

P600, P1200), and polished with 0.3µm aluminium oxide powder to a 60 – 90 µm-thick section. Each section was cleaned in an ultrasonic bath, dehydrated in 95-100% ethanol, cleared (Histoclear®), and mounted with a coverslip using a xylene-based mounting medium (DPX®). The dental thin sections were examined using a high-powered microscope (Olympus® BX53) with a mounted microscope camera (Olympus® DP25). Images of the thin sections were obtained and examined in CELL® Live Biology imaging software.

To ensure DSRs were recorded within equilivent enamel regions of each tooth, the enamel was first divided into three main regions: cuspal, lateral and cervical, by dividing the length of the enamel dentine junction into three equal lengths (Fig. 1). The cervical third was not assessed because the enamel was typically very thin, frequently damaged, and cross striations can sometimes be poorly preserved in this region. Following Beynon et al. (1991), cuspal and lateral enamel areas were further separated into inner, mid, and outer regions by dividing the linear enamel thickness into thirds. The regions of interest in the cuspal enamel were located just below the dentine horn, as most of these exfoliated teeth had some degree of occlusal wear. In the lateral enamel, the regions of interest were in the half nearest the cuspal division, where the enamel is typically thicker.

Daily secretion rates were calculated using standard methods (e.g., Reid et al., 1998). Rates were measured along the long axis of an enamel prism within each region (following Mahoney, 2008). Rates were calculated by measuring the length of prisms between four to six adjacent cross striations (representing three to five days of enamel secretion respectively). Multiple (minimum of ten) assessments of DSRs were taken across the region where cross striations were visible, and then the mean was calculated.

2.3 | Analyses

First and second deciduous molars were analysed collectively. The data were organised in two ways depending on the type of analysis required. One approach utilised the dependent nature

of our data and grouped DSRs by individual tooth. Matched or paired sample testing is generally more powerful than independent sample tests, as the ability to detect differences between groups is increased and inter-subject variability is controlled (Fradette et al., 2003). Therefore, matched samples tests, such as paired *t* tests and repeated measures or two-way mixed ANOVA, were used to test for differences in DSRs within teeth, e.g., cuspal versus lateral DSRs and variation from inner to outer enamel regions. If the assumption of sphericity was violated for ANOVA, a Greenhouse-Geisser correction was used. Mean cuspal and lateral DSRs were calculated using the pooled DSRs for each region (inner, mid, and outer), while mean inner, mid, or outer rates used pooled cuspal and lateral DSRs. Individual mean DSRs were calculated as the mean of all available DSRs for each tooth.

The second approach treated DSRs as independent data, allowing all individual DSRs to be collectively analysed. Specifically, this approach is used when it is inappropriate to use matched data, such as testing mean DSRs between populations, and is used for independent *t* tests and one-way ANOVA.

Where multiple separate tests were carried out, Bonferroni adjustments are applied to the p-values using Dunn's (1964) procedure. Otherwise, an alpha level of 0.05 was used to establish statistical significance. All statistical testing was undertaken using SPSS V.25 (IBM).

3 | RESULTS

3.1 | Mean daily secretion rates

Daily secretion rates did not differ between boys and girls (Table 2). Independent *t* tests detected no significant difference between the sexes in overall DSRs, or when DSRs of either cuspal or lateral enamel regions were compared between the sexes. From here on, teeth from males and females were grouped for all analyses.

Figure 2 displays all DSRs measurements (n = 1396) for the entire sample including the inner, mid, and outer regions of cuspal and lateral enamel. The overall mean DSR is 3.82

μm per day (standard error of mean = 0.013). The distribution is approximately normal with some slight skewing to the right (Skewness = 0.622, Kurtosis = 0.939). DSR values ranged from 2.43 to 5.96 μm per day with a median value of 3.78 μm per day. When analysed in terms of the mean DSRs per tooth, the overall rate remained the same (3.82 μm per day, standard deviation = 0.39, n = 345).

3.2 | Daily secretion rates compared between populations.

The average DSRs of individual teeth were similar when compared between the populations (Fig. 3, Appendix A: Supplementary Table S1 for DSRs subdivided by cuspal and lateral regions for each population). No significant differences were found between populations in their overall mean DSRs per tooth, or when DSRs were compared between equivalent enamel regions. Also apparent in Table S.1. is that the range of DSRs within each population is not uniform across populations. When values from all enamel regions are considered collectively, populations vary in their dispersion of maximum and minimum DSRs. For example, New Zealand showed the greatest variation in maximum values, which ranged from 4.85 to 5.96 μm (a difference of 1.11 μm per day), while the French sample showed a lower and more limited range of maximum values from 4.10 μm to 4.31 μm per day (a difference of 0.21 μm).

Samples sizes varied considerably between some populations, so caution is needed when interpreting these tests. The Swedish sample is particularly small (n = 7). However, even considering the maximum mean variation in cuspal and lateral enamel, the differences were minor. The greatest mean difference in cuspal enamel was between Sweden's unusually low mid region and the French outer region with a difference of 0.59 μm per day. In the lateral enamel, the greatest difference was between Sweden's mid region and the French outer region (mean difference = 0.61 μm per day).

3.3 | The trajectory of DSRs from inner to outer enamel regions

When DSRs from all populations were pooled and treated as independent data (i.e., DSRs were not grouped by individual teeth), mean outer enamel DSRs showed a slight but non-significant increase in pace of 1.3% relative to inner DSRs (Fig. 4).

When regional (inner, mid, and outer) DSRs were matched within each tooth, outer DSRs showed a slight but significant mean increase of 2.7% relative inner DSRs: Repeated measures ANOVA inner – outer mean difference = 0.070 μm per day, Mauchly's sphericity test ($\chi 2(2) = 11.502$, p = 0.003), Greenhouse-Geisser correction ($\varepsilon = 0.952$), $F(1.904, 426.557) = 4.603$, $p = 0.012$).

Little variation was evident between the populations in their general growth rates from inner to outer enamel regions (Fig. 5). Using One-way ANOVA, the French sample showed a slight increase in mean DSRs from inner to outer regions in both cuspal (mean difference = 0.25 μm per day) and lateral enamel (mean difference = 0.26 μm per day), but once a Bonferroni adjustment for multiple testing was applied (Dunn, 1964, alpha level = 0.001) the change in rates was not significant. Sweden could only be tested for differences in cuspal enamel (due to insufficient data for lateral enamel), which showed no significant variation.

When DSRs from inner, mid, and outer regions were matched within individual teeth, no significant differences in the trajectory of DSRs from inner to outer regions in cuspal enamel was found between populations (Fig. 6). Variation was observed in lateral enamel (two-way mixed ANOVA $F(3,167) = 7.05$, $p < 0.001$), with New Zealand children displaying slightly slower DSRs compared with British (mean difference = 0.22 μm per day, $p = 0.002$), Canadian (mean difference = 0.22 μm per day, $p = 0.024$) and French children (mean difference = 0.35 μm per day, $p = 0.008$).

3.4 | Cuspal versus Lateral enamel growth rates

Overall, when DSRs were treated as independent, cuspal enamel grew at a mean rate that was 5.1% faster than lateral enamel (Table 3), with a mean difference of 0.186 μm per day, t = 7.537, df = 1393, $p < 0.001$. Within the enamel areas, however, DSRs did not significantly vary between inner, mid, and outer regions.

Cuspal and lateral DSRs were matched within individual teeth to test for within tooth differences in growth rates from inner to outer enamel regions. The same growth pattern evident in the independent data still held within individual teeth. Overall, cuspal enamel grew at a rate that was 5.63% faster than lateral enamel (Mean difference = 0.16 μm per day, Paired t = 6.055, df = 253, $p < 0.001$). Within cuspal enamel, growth rates increased by 2.2% as ameloblasts moved from the inner to the outer enamel, while in the lateral enamel rates increased by 2.7% (Fig. 7). However, unlike the independent DSRs, when matched within individual teeth lateral enamel showed a statistically significant increase in the outer enamel: Lateral inner – outer mean difference = 0. 097 μm per day, two-way mixed ANOVA Mauchly's test ($\chi^2(2) = 10.246$, $p = 0.006$), Greenhouse-Geisser applied ($\varepsilon = 0.944$), $F(1.889, 321.110) = 6.123$, $p = 0.003$. The difference in results between the independent and matched DSRs was slight and reflects the ability of paired testing to focus only on variation within each tooth rather than between teeth.

The pattern of faster cuspal growth relative to lateral enamel was present in most of the populations (Table 4). Of the groups tested, only the French sample showed no significant difference in DSRs between the two enamel areas. However, due to the low sample size this result should be treated with caution. The Swedish sample was not tested due to insufficient data.

4 | DISCUSSION

The overall mean DSR for the entire sample of modern human deciduous molars was 3.82 μm per day. The mean value that we report is slightly slower than the 4.10 μm per day reported by

Mahoney (2011) for medieval and Bronze age deciduous molars, and slightly faster than the overall mean DSR predicted for deciduous molars using the regression equation of Birch and Dean (2014) (their dm1 = 3.88 μm per day and dm2 = 3.53 μm per day). The range of DSRs in our entire sample (cuspal and lateral combined) lay between 2.43 to 5.96 μm per day and encompass the rates of 2.5 to 5.3 μm per day reported by Schour and Poncher (1937), Shellis (1984), and Birch and Dean (2009, 2014). There was no clear reduction in DSRs after birth in our sample, which is consistent with findings for DSRs from deciduous canines (Dean et al., 2020). The molar cross striations sometimes became less visible or less defined for a period after birth (see Mahoney, 2011: Fig 6a-d), which might tie into the short period after birth in which the circadian system matures and melatonin production develops (Kennaway et al.,1992; Rivkees & Hao, 2000). We found no evidence that DSRs differ significantly, or consistently, when compared between boys and girls.

Daily enamel secretion rates varied only slightly when equivalent enamel regions were compared between populations. Rates remained relatively consistent in comparisons of cuspal enamel, though slightly more variation was present when comparisons were undertaken between populations using lateral enamel. New Zealand molars displayed slightly faster DSRs in the outer region compared to British, Canadian and French samples, but the mean difference in rates was minor, ranging from 0.22 to 0.35 μm per day. Although the Swedish sample is represented by a limited number of teeth, overall mean DSRs were similar to the others, suggesting that if variation does exist between these populations the difference is minor.

Despite mean DSRs showing little inter-population variation, the range and dispersion of values, such as maximum and minimum DSRs, was not completely uniform across the populations. This variation may reflect real differences in the dispersion of DSRs within the population groups, but may also be partly influenced by sample sizes, particularly as the New Zealand sample had the greatest variation in maximum values and the largest sample size (174

teeth), while the French sample was relatively smaller in size (14 teeth) and showed a lower and more limited range of maximum DSRs.

The trajectory of enamel secretion remained relatively consistent within cuspal enamel from inner to outer regions. The trajectory was slightly more marked in lateral enamel as older secretory ameloblasts deposited an additional 2.7% of new matrix nearer the outer enamel surface relative to the amount deposited by younger ameloblasts nearer the EDJ. Shellis (1984) described a more pronounced gradient of enamel secretion whereby rates increased by 20% from the inner to outer enamel but this was for a mixed sample of deciduous teeth. Birch and Dean (2014) noted increased DSRs in cuspal (their occlusal) enamel of four deciduous second molars, but this included rates that were adjacent to the enamel-dentine junction which we did not include in our methodology. Secretion rates immediately adjacent to the EDJ are typically slow relative to mid and outer enamel regions (Mahoney, 2015), but are necessary for studies that calculate enamel extension rates (e.g., Birch & Dean, 2014; Dean et al., 2020; Mahoney, 2015; Nava et al., 2017; Shellis, 1984). As none of our DSRs were measured within the 100 μm of the EDJ (to avoid abrupt localised changes in pace), it might be expected that our inner DSRs will reflect a slightly faster pace of growth than reported in studies that include DSRs from the region that is adjacent to the EDJ.

Researchers have drawn attention to a much steeper increase in the rate that enamel is deposited in outer compared to inner enamel regions of permanent teeth relative to deciduous teeth (Birch & Dean 2009; Shellis, 1998). The growth trajectory we describe for deciduous molars is consistent with this observation when our data are compared to previously published values for permanent teeth. Within individual deciduous molars, the average increase in rate from inner to outer enamel was 2.7%. This is substantially less than the 113% increase in DSRs from 2.55 μm in inner to 5.45 μm per day in outer enamel of 21 permanent molars from several different populations (Smith et al., 2007). The percentage of enamel deposited by ameloblasts in outer compared to inner enamel regions lies between 85% to 14% in other studies of

permanent molars (Aris et al. 2020; Lacruz & Bromage, 2006), but this is still much greater than the rise of 2.7% that we report for deciduous molars (Fig. 8).

The flatter trajectory of secretion rates produced by deciduous molar ameloblasts, compared to permanent molars, may partly reflect methodological differences in how DSRs were assessed, but might also result from the thinner enamel and the greater amount of prenatal enamel formed in deciduous crowns (Grine, 2005; Mahoney, 2008, 2011). On average, 100% of the cuspal enamel in deciduous first molars (protoconid) and 76% of the deciduous second molar (protoconid) are formed *in utero* (Mahoney, 2011). The buffered prenatal environment may allow newly recruited ameloblasts to reach their average daily secretion rate more quickly than ameloblasts in permanent teeth. In permanent molars, almost all cuspal enamel is typically formed after birth (Mahoney, 2008). As such, new ameloblasts secrete matrix in a postnatal environment that is far less protected relative to the prenatal environment, evidenced by the increase of accentuated, or pathological, lines in enamel that forms after birth (FitzGerald & Saunders, 2005). Ameloblasts in permanent teeth begin to secrete enamel matrix at the EDJ at a slow rate and continue to do so for a prolonged period of time. The marked increase in the enamel secretion rate towards the outer permanent enamel surface might therefore provide a period of 'catch-up' growth, so that enamel formation is completed within a required timeframe. This would also explain why the lateral enamel of our deciduous molars showed a slightly steeper trajectory compared to cuspal enamel, as a greater proportion of lateral enamel formation occurs in the early postnatal period (Birch & Dean 2014; Mahoney, 2011). Another possible source of variation between studies is the size of area over which mean DSRs are calculated. We calculated mean DSRs for each inner, mid, and outer third but studies that calculate mean DSRs for narrower areas (e.g., Mahoney, 2015) will likely detect finer detail in variation across the breath of enamel.

The overall mean DSR for deciduous molars is similar to the overall mean DSR for permanent molars. Mean overall rates recalculated from those reported for inner, mid, and outer

cuspal enamel of permanent molars rates lie between 4.16 to 3.93 μm per day (Beynon et al., 1991; Lacruz & Bromage 2006; Smith et al., 2007). The rate for cuspal enamel of permanent molars is similar to the overall mean rate of 3.92 μm per day that we report for cuspal enamel of deciduous molars. The mean overall rate for lateral enamel of permanent molars was 3.93 μm per day (Beynon et al., 1991; Lacruz & Bromage 2006), which is also similar to the 3.73 μm per day that we report for lateral enamel of deciduous molars. Thus, both deciduous and permanent molar enamel thickness is attained with a similar overall rate of enamel secretion, even though the trajectory of enamel secretion is very different for each tooth type.

Cuspal enamel of deciduous molars grew, on average, 5% faster than lateral enamel. The same general pattern of faster cuspal enamel growth compared to lateral is evident within most of our populations, except for the French children, who displayed no change in mean rates. Our findings are similar to those of Birch and Dean (2014, their Table 1b) who reported paired differences between occlusal and lateral (labial/buccal aspects) DSRs that increase along the tooth row, with the deciduous second molar displaying the greatest mean difference. This contrasts with DSRs measured close to the EDJ that tend to show little variation from the dentine horn to the cervix (Birch & Dean, 2014; Dean et al., 2020; Nava et al., 2017). The difference in these findings likely reflects the gradient in daily growth rates created as ameloblasts move away from the EDJ towards the outer surface (Birch & Dean, 2014; Dean et al., 2020; Macchiarelli et al., 2006; Mahoney, 2015).

Amongst the populations we investigated, deciduous molar enamel formed at similar rates and variation, when it did occur, was relatively minor. Our findings suggest that amongst the samples we investigated, enamel growth rates and trajectories are comparable. However, it is unknown if molar enamel growth rates are essentially uniform across all geographic regions. Our approach was to avoid the more variable regions within the first and last formed enamel allowing us to observe variation in DSRs in the bulk of the crown. Our

findings provide a sound basis from which to compare deciduous molar enamel growth rates in children from other geographic regions, as well as in archaeological and fossil teeth.

Conclusion

There was little to no significant variation in the pace of enamel secretion in deciduous molars from populations from Britain, Canada, France, New Zealand, and Sweden. The daily rate of enamel secretion for these populations was similar when compared between equivalent regions of cuspal and lateral enamel. Cuspal enamel DSRs were faster than lateral DSRs. DSRs also showed a slight increase in pace towards the outer enamel, especially in lateral enamel. The trajectory of deciduous molar DSRs differs to that observed in permanent molars.

ACKNOWLEDGEMENTS

This study was undertaken as part of The Biorhythm of Childhood Growth project funded by The Leverhulme Trust (grant number RPG-2018-226). The Royal Society provided an equipment grant (grant number RG110435). The European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie (Grant H2020-MSCA-IF-2018-842812 – WEAN_IT) awarded to AN.

We are extremely grateful to all children who took part in this study.

New Zealand schools: Tainui School, Carisbrook School, Tahuna Intermediate, St Bernadette's School, Kavanagh college, George St Normal School, Dunedin North Intermediate, Balmacewen school, Columba college, John McGlashen college, St Clair Primary school, Wakari School, Bathgate Park School, Elmgrove School, Taieri College, Fairfield School, East Taieri School, Arthur St School, Grant's Braes School.

We thank community dental clinics in Hawkes Bay and Whanganui, especially Noeline Barrow and Lerlene Wright for coordination and logistic support.

APPENDIX A: Supplementary information

Table S1 Mean DSRs for enamel regions by population (independent data)

			Daily secretion rates (DSR) µm per day					
Population	**Enamel**	**Region**	**Mean**	**Std. Dev.**	**Median**	**Minimum**	**Maximum**	***n***
New Zealand	**Cuspal**	**Inner**	3.88	0.49	3.79	2.81	5.30	114
		Mid	3.87	0.58	3.73	2.85	5.96	135
		Outer	3.88	0.48	3.81	2.43	5.50	114
		Total	3.88	0.52	3.79	2.43	5.96	363
	Lateral	**Inner**	3.63	0.50	3.58	2.64	4.85	92
		Mid	3.66	0.53	3.54	2.66	5.24	138
		Outer	3.69	0.47	3.67	2.89	5.83	116
		Total	3.66	0.50	3.60	2.64	5.83	346
	Total	**Inner**	3.77	0.51	3.75	2.64	5.30	206
		Mid	3.76	0.56	3.63	2.66	5.96	273
		Outer	3.78	0.48	3.74	2.43	5.83	230
		Total	3.77	0.52	3.71	2.43	5.96	709
British	**Cuspal**	**Inner**	3.91	0.44	3.87	3.09	5.04	67
		Mid	4.01	0.51	3.90	3.27	5.15	84
		Outer	4.03	0.47	3.93	3.25	5.20	67
		Total	3.98	0.48	3.90	3.09	5.20	218
	Lateral	**Inner**	3.75	0.39	3.62	2.85	4.62	65
		Mid	3.74	0.42	3.69	2.89	5.02	102
		Outer	3.84	0.39	3.77	3.15	5.44	75
		Total	3.77	0.40	3.71	2.85	5.44	242
	Total	**Inner**	3.83	0.42	3.81	2.85	5.04	132
		Mid	3.86	0.48	3.76	2.89	5.15	186
		Outer	3.93	0.44	3.87	3.15	5.44	142
		Total	3.87	0.45	3.79	2.85	5.44	460
Canadian	**Cuspal**	**Inner**	3.96	0.24	3.99	3.46	4.37	22
		Mid	3.97	0.29	3.92	3.48	4.49	21
		Outer	3.99	0.28	3.97	3.58	4.43	11
		Total	3.97	0.26	3.97	3.46	4.49	54

	Lateral	**Inner**	3.81	0.32	3.90	2.97	4.22	29
		Mid	3.81	0.30	3.94	3.16	4.29	30
		Outer	3.82	0.30	3.87	3.13	4.27	30
		Total	3.81	0.30	3.90	2.97	4.29	89
	Total	**Inner**	3.88	0.30	3.95	2.97	4.37	51
		Mid	3.87	0.31	3.94	3.16	4.49	51
		Outer	3.86	0.30	3.88	3.13	4.43	41
		Total	3.87	0.30	3.94	2.97	4.49	143
French	**Cuspal**	**Inner**	3.83	0.19	3.92	3.55	4.10	12
		Mid	3.93	0.17	3.96	3.68	4.22	12
		Outer	4.08	0.20	4.17	3.74	4.29	9
		Total	3.93	0.21	3.95	3.55	4.29	33
	Lateral	**Inner**	3.78	0.25	3.80	3.33	4.10	12
		Mid	3.98	0.20	4.06	3.64	4.27	13
		Outer	4.04	0.14	4.02	3.84	4.31	13
		Total	3.94	0.22	3.98	3.33	4.31	38
	Total	**Inner**	3.81	0.22	3.88	3.33	4.10	24
		Mid	3.95	0.19	3.97	3.64	4.27	25
		Outer	4.06	0.16	4.07	3.74	4.31	22
		Total	3.94	0.21	3.95	3.33	4.31	71
Swedish	**Cuspal**	**Inner**	3.63	0.06	3.63	3.59	3.67	2
		Mid	3.49	0.15	3.51	3.28	3.64	4
		Outer	3.74	0.26	3.72	3.45	4.07	4
		Total	3.62	0.21	3.61	3.28	4.07	10
	Lateral	**Mid**	3.43	0.18	3.43	3.3	3.56	2
		Total	3.43	0.18	3.43	3.3	3.56	2
	Total	**Inner**	3.63	0.06	3.63	3.59	3.67	2
		Mid	3.47	0.14	3.51	3.28	3.64	6
		Outer	3.74	0.26	3.72	3.45	4.07	4
		Total	3.58	0.21	3.57	3.28	4.07	12

REFERENCES

AlQahtani, S. J., Hector, M., & Liversidge, H. (2010). Brief communication: the London atlas of human tooth development and eruption. *American Journal of Physical Anthropology,* 142(3), 481-490.

Aris, C., Mahoney, P., & Deter, C. (2020). Enamel growth rates of anterior teeth in males and females from modern and ancient British populations. *American Journal of Physical Anthropology,* 173(3), 236-249.

Beynon, A. D., Dean, M. C., & Reid, D. J. (1991). On thick and thin enamel in hominoids. *American Journal of Physical Anthropology,* 86(2), 295-309.

Birch, W., & Dean, M. C. (2009). Rates of enamel formation in human deciduous teeth. In T. Koppe, G. Meyer, K., Alt K (Eds.) *Comparative Dental Morphology* (pp. 116-120): Karger Publishers.

Birch, W., & Dean, M. C. (2014). A method of calculating human deciduous crown formation times and of estimating the chronological ages of stressful events occurring during deciduous enamel formation. *Journal of Forensic and Legal Medicine,* 22, 127-144.

Boyde, A. (1963). Estimation of age at death of young human skeletal remains from incremental lines in the dental enamel. *Third International Meeting in Forensic Immunology, Medicine, Pathology and Toxicology* (Vol. 1, pp. 36-37). Wiley-Liss

Boyde, A. (1989). Enamel. In B. K. B., Berkovitz, A., Boyde, R. M., Frank, H. J., Hohling, B. J., Moxham, J., Nalbandian, C. H., Tonge (Eds.) *Teeth. Handbook of Microscopic Anatomy (Continuation of Handbuch der mikroskopischen Anatomie des Menschen)* (pp. 309-473). Springer.

Dean, M. C. (1998). A comparative study of cross striation spacings in cuspal enamel and of four methods of estimating the time taken to grow molar cuspal enamel in *Pan, Pongo* and *Homo*. *Journal of Human Evolution,* 35(4), 449-462.

Dean, M. C., Beynon, A., Thackeray, J., & Macho, G. (1993). Histological reconstruction of dental development and age at death of a juvenile *Paranthropus robustus* specimen, SK 63, from Swartkrans, South Africa. *American Journal of Physical Anthropology,* 91(4), 401-419.

Dean, M. C., Humphrey, L., Groom, A., & Hassett, B. (2020). Variation in the timing of enamel formation in modern human deciduous canines. A*rchives of Oral Biology*, 104719.

Dean, M. C., Leakey, M. G., Reid, D., & Schrenk, F. (2001). Growth processes in teeth distinguish modern humans from *Homo erectus* and earlier hominins. *Nature,* 414(6864), 628.

Deutsch, D., Tam, O., & Stack, M. (1984). Postnatal changes in size, morphology and weight of developing postnatal deciduous anterior teeth. *Growth,* 49(2), 207-217.

Dunn, O. J. (1964). Multiple comparisons using rank sums. *Technometrics,* 6(3), 241-252.

FitzGerald, C., & Hillson, S. (2009). Deciduous tooth growth in an ancient Greek infant cemetery. In: T., Koppe, G., Meyer, K., Alt, (Eds). In *Comparative Dental Morphology* (pp. 178-183): Karger Publishers.

FitzGerald, C., Saunders, S., Bondioli, L., & Macchiarelli, R. (2006). Health of infants in an Imperial Roman skeletal sample: perspective from dental microstructure. *American Journal of Physical Anthropology,* 130(2), 179-189.

FitzGerald, C., & Saunders, S. R. (2005). Test of histological methods of determining chronology of accentuated striae in deciduous teeth. *American Journal of Physical Anthropology,* 127(3), 277-290.

Fradette, K., Keselman, H., Lix, L., Algina, J., & Wilcox, R. R. (2003). Conventional and robust paired and independent-samples t tests: Type I error and power rates. *Journal of Modern Applied Statistical Methods,* 2(2), 22.

Grine, F. (2005). Enamel thickness of deciduous and permanent molars in modern *Homo sapiens*. *American Journal of Physical Anthropology,* 126(1), 14-31.

Hillson, S. (2014). *Tooth development in human evolution and bioarchaeology*. Cambridge University Press.

Huda, T. F., & Bowman, J. (1995). Age determination from dental microstructure in juveniles. *American Journal of Physical Anthropology,* 97(2), 135-150.

Humphrey, L. T. (2014). Isotopic and trace element evidence of dietary transitions in early life. *Annals of Human Biology,* 41(4), 348-357.

Humphrey, L. T., Dean, M. C., Jeffries, T. E., & Penn, M. (2008). Unlocking evidence of early diet from tooth enamel. *Proceedings of the National Academy of Sciences of the United States of America,* 105(19), 6834-6839.

Kennaway, D. J., Stamp, G. E., & Goble, F. C. (1992). Development of melatonin production in infants and the impact of prematurity. *The Journal of Clinical Endocrinology & Metabolism,* 75(2), 367-369.

Kierdorf, H., Witzel, C., Bocaege, E., Richter, T., & Kierdorf, U. (2021). Assessment of physiological disturbances during pre-and early postnatal development based on microscopic analysis of human deciduous teeth from the Late Epipaleolithic site of Shubayqa 1 (Jordan). *American Journal of Physical Anthropology,* 174(1), 20-34.

Lacruz, R. S., & Bromage, T. G. (2006). Appositional enamel growth in molars of South African fossil hominids. *Journal of Anatomy,* 209(1), 13-20.

Le Luyer, M., & Bayle, P. (in review). The Tooth Fairy collection (la collection Petite souris), a sample of documented human deciduous teeth at the University of Bordeaux, France. *American Journal of Physical Anthropology*.

Li, Q., Nava, A., Reynard, L. M., Thirlwall, M., Bondioli, L., & Müller, W. (2020). Spatially-Resolved Ca Isotopic and Trace Element Variations in Human Deciduous Teeth Record Diet and Physiological Change. *Environmental Archaeology*, 1-10.

Liversidge, H. M. (2016). *Dentition*. In L. Scheur & S. Black (Eds.), *Developmental Juvenile Osteology* (pp. 149-176): Academic Press.

Lorentz, K., Lemmers, S., Chrysostomou, C., Dirks, W., Zaruri, M., Foruzanfar, F., & Sajjadi, S. (2019). Use of dental microstructure to investigate the role of prenatal and early life physiological stress in age at death. *Journal of Archaeological Science,* 104, 85-96.

Macchiarelli, R., Bondioli, L., Debénath, A., Mazurier, A., Tournepiche, J.-F., Birch, W., & Dean, M. C. (2006). How Neanderthal molar teeth grew. *Nature,* 444(7120), 748.

Mahoney, P. (2008). Intraspecific variation in M 1 enamel development in modern humans: implications for human evolution. *Journal of Human Evolution,* 55(1), 131-147.

Mahoney, P. (2010). Two-dimensional patterns of human enamel thickness on deciduous (dm1, dm2) and permanent first (M1) mandibular molars. *Archives of Oral Biology,* 55(2), 115-126.

Mahoney, P. (2011). Human deciduous mandibular molar incremental enamel development. *American Journal of Physical Anthropology,* 144(2), 204-214.

Mahoney, P. (2012). Incremental enamel development in modern human deciduous anterior teeth. *American Journal of Physical Anthropology,* 147(4), 637-651.

Mahoney, P. (2013). Testing functional and morphological interpretations of enamel thickness along the deciduous tooth row in human children. *American Journal of Physical Anthropology,* 151(4), 518-525.

Mahoney, P. (2015). Dental fast track: prenatal enamel growth, incisor eruption, and weaning in human infants. *American Journal of Physical Anthropology,* 156(3), 407-421.

Mahoney, P. (2019). Root growth and dental eruption in modern human deciduous teeth with preliminary observations on great apes. *Journal of Human Evolution,* 129, 46-53.

Mahoney, P., McFarlane, G., & Nava, A. (in preparation). The histology of dental age estimation for medieval and modern children.

Mahoney, P., Smith, T. M., Schwartz, G. T., Dean, C., & Kelley, J. (2007). Molar crown formation in the Late Miocene Asian hominoids, *Sivapithecus parvada* and *Sivapithecus indicus*. *Journal of Human Evolution,* 53(1), 61-68.

Nava, A., Bondioli, L., Coppa, A., Dean, M. C., Rossi, P. F., & Zanolli, C. (2017). New Regression Formula to Estimate the Prenatal Crown Formation Time of Human Deciduous Central Incisors Derived from a Roman Imperial Sample (Velia, Salerno, I-II cent. CE). *PloS one,* 12(7), e0180104.

Nava, A., Frayer, D. W., & Bondioli, L. (2019). Longitudinal analysis of the microscopic dental enamel defects of children in the Imperial Roman community of Portus Romae (necropolis of Isola Sacra, 2nd to 4th century CE, Italy). *Journal of Archaeological Science: Reports,* 23, 406-415.

Nava, A., Lugli, F., Romandini, M., Badino, F., Evans, D., H, H. A., . . . Benazzi, S. (2020). Early life of Neanderthals. *Proceedings of the National Academy of Sciences Sciences of the United States of America,* 117 (46) 28719-28726.

Reid, D. J., Beynon, A. D., & Ramirez Rozzi, F. (1998). Histological reconstruction of dental development in four individuals from a medieval site in Picardie, France. *Journal of Human Evolution,* 35(4-5), 463-477.

Reid, D. J., & Dean, M. C. (2006). Variation in modern human enamel formation times. *Journal of Human Evolution,* 50(3), 329-346.

Rivkees, S. A., & Hao, H. (2000). Developing circadian rhythmicity. *Seminars in Perinatology* (Vol. 24, pp. 232-242): Elsevier.

Schour, I., & Poncher, H. G. (1937). Rate of apposition of enamel and dentin, measured by the effect of acute fluorosis. *American Journal of Diseases of Children,* 54(4), 757-776.

Shellis, R. (1984). Variations in growth of the enamel crown in human teeth and a possible relationship between growth and enamel structure. *Archives of Oral Biology,* 29(9), 697-705.

Shellis, R. (1998). Utilization of periodic markings in enamel to obtain information on tooth growth. *Journal of Human Evolution,* 35(4), 387-400.

Smith, T. M., Reid, D. J., Dean, M. C., Olejniczak, A. J., & Martin, L. (2007). New perspectives on chimpanzee and human molar crown development. In S. Bailey & J.-J. Hublin (Eds.), *Dental perspectives on human evolution: state of the art research in dental paleoanthropology* (pp. 177-192): Springer. https://doi.org/10.1007/978-1-4020-5845-5_12

Stack, M. (1967). Vertical growth rates of the deciduous teeth. *Journal of Dental Research,* 46(5), 879-882.

Witzel, C. (2014). Echoes from birth–Mutual benefits for physical and forensic anthropology by applying increment counts in enamel of deciduous teeth for aging. *Anthropologischer Anzeiger,* 71(1-2), 87-103.

Zheng, L., Seon, Y. J., Mourão, M. A., Schnell, S., Kim, D., Harada, H., . . . Papagerakis, P. (2013). Circadian rhythms regulate amelogenesis. *Bone,* 55(1), 158-165.

FIGURE CAPTIONS

Fig. 1 Diagram of buccal enamel showing assessment areas for inner, mid, and outer regions of cuspal and lateral enamel.

Fig. 2 Distribution of DSRs (n = 1396) measured across enamel regions of 345 deciduous molars of modern human children from 5 populations (New Zealand, Britain, Canada, France, and Sweden). Black vertical line marks the mean (3.82 μm per day, standard deviation = 0.470, standard error of the mean = 0.0126), curve marks a normal distribution.

Fig. 3 Mean DSRs (μm per day) for each population. Error bars +/- 1 standard deviation

Fig. 4 Overall mean DSRs for inner, mid, and outer enamel regions (cuspal and lateral DSRs combined) Error bars +/- 1 standard deviation.

Fig. 5 Mean DSRs (μm per day) for inner, mid, and outer regions of cuspal (upper) and lateral enamel (lower) by population. Error bars +/- 1 standard deviation. Note only lateral DSRs from the mid enamel region were available for the Swedish sample.

Fig. 6 Mean DSRs for inner, mid, and outer regions by population for cuspal (upper) and lateral enamel (lower). Error bars +/- 1 standard deviation.

Fig. 7 Mean DSRs from inner to outer regions within cuspal and lateral enamel (dependent samples). Error bars +/- 1 standard deviation.

Fig. 8 Illustrates the enamel secretion trajectories of permanent molars compared to deciduous molars from modern-day children. Upper chart shows the collective mean DSRs for deciduous molars from each of the five modern populations (this study). Lower chart shows collective mean DSRs for modern permanent molars; Dean 1998, n = 1; Beynon et al., 1991 n = 9; Lacruz and Bromage 2006, n = 10; Macchiarelli et al. 2006: Fig.2, n = 2; Aris et al. 2020, n = 15)

TABLES

Table 1 Number of teeth, by tooth type and population.

Populations	Number of individuals	Deciduous 1st molar	Deciduous 2nd molar	Total teeth
British	120	53	65	118
Canadian	19	17	15	32
French	10	10	4	14
New Zealand	129	84	90	174
Swedish	7	0	7	7
Total	285	164	181	345

Table 2 Descriptive statistics for DSRs by sex.

	Mean	**Std. Dev.**	**Median**	**Minimum**	**Maximum**	***n***
Boys	3.83	.40	3.82	3.00	5.26	93
Girls	3.81	.38	3.75	3.04	5.30	120
Total	3.82	.39	3.76	3.00	5.30	213

Table 3 Descriptive statistics for all DSRs (μm per day) grouped by inner, mid, and outer regions for cuspal and lateral enamel.

Enamel	Region	Mean	Std. Dev.	Median	Minimum	Maximum	N
Cuspal	**Inner**	3.89	.44	3.87	2.81	5.30	217
	Mid	3.92	.53	3.82	2.85	5.96	256
	Outer	3.94	.46	3.87	2.43	5.50	205
	Total	3.92	.48	3.86	2.43	5.96	678
Lateral	**Inner**	3.70	.44	3.68	2.64	4.85	198
	Mid	3.72	.46	3.65	2.66	5.24	285
	Outer	3.77	.42	3.74	2.89	5.83	234
	Total	3.73	.44	3.70	2.64	5.83	717
Total	**Inner**	3.80	.45	3.79	2.64	5.30	415
	Mid	3.81	.50	3.73	2.66	5.96	541
	Outer	3.85	.45	3.81	2.43	5.83	439
	Total	3.82	.47	3.78	2.43	5.96	1395

Table 4 Results of Paired t tests for differences between mean cuspal and lateral DSRs.

Population	**Mean**	**SD**	***t***	***df***	***P*[1]**
New Zealand	.18135	.50642	4.342	146	**.000**
British	.18473	.34966	4.575	74	**.000**
Canadian	.07700	.11640	2.958	19	**.008**
French	-.00803	.19448	-.137	10	.894

[1]An alpha level of 0.013 is used for multiple testing (four groups) using Dunn's (1964) procedure.