Quantifying prehistoric physiological stress using the TCA method: preliminary results from the Central Balkans

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ABSTRACT – *The Neolithic way of life was accompanied by an increase in various forms of physiological stress (*e.g. *disease, malnutrition). Here we use the method of tooth cementum annulation (TCA) analysis in order to detect physiological stress that is probably related to calcium metabolism. The TCA method is applied to a sample of teeth from three Mesolithic and five Neolithic individuals from the Central Balkans. The average number of physiological stress episodes is higher in the Neolithic group – but the statistical significance of this result cannot be evaluated due to the small sample size, therefore these results should be taken as preliminary.*

KEY WORDS – *stress-layers; tooth cementum annulation (TCA); Mesolithic; Neolithic; Central Balkans*

Kvantificiranje fiziološkega stresa v prazgodovini s pomočjo metode anulacije zobnega cementa (TCA): preliminarni rezultati iz osrednjega Balkana

IZVLEČEK – *Življenje v neolitiku je spremljal porast različnih oblik fiziološkega stresa (npr. bolezni, podhranjenost). Predstavljamo uporabo analitske metode anulacije zobnega cementa (TCA), s katero lahko odkrivamo fiziološki stres, ki je verjetno povezan s presnovo kalcija. Metodo smo uporabili pri analizah vzorcev treh mezolitskih in petih neolitskih posameznikov iz osrednjega Balkana. Povprečno število fizioloških epizodnih stresov je v neolitski skupini večje – vendar zaradi majhnega števila vzorcev tega rezultata statistično ne moremo ovrednotiti in ga predstavljamo kot preliminarnega.*

KLJUČNE BESEDE – *plasti-stresov; metoda anulacije zobnega cementa (angl. TCA); mezolitik; neolitik; osrednji Balkan*

Introduction

One of the major events in human prehistory was the transition from hunter-gatherer lifestyle to agricultural food production in the Holocene, which significantly influenced the way of life in this era. This transition was followed by the beginning of a fully sedentary way of living, the cultivation of domestic plants and breeding of animals. Many scholars have hypothesized that these changes had a dramatic impact on population size and structure, resulting in a significant increase of the world population, the demographic process known as the Neolithic demographic transition (*Bocquet-Appel 2008; 2011*).

Moreover, with an increase in population size an overall decline in health has also been documented worldwide (*Cohen, Armelagos 1984; Cohen, Crane-Kramer 2007*). Usually named among the main causes of this decline are changes in diet, a limited food

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range, and the low level of food quality (*Cohen 2008*). Besides changes in diet, an increase in fertility with narrow birth spacing, increased sedentism and life in villages close to domestic animals, resulted in poor hygienic conditions and higher rates of zoonotic disease (*Bocquet-Appel 2008; Stock, Pinhasi 2011*).

Studies across Europe based on human skeletal remains document a general decline in health status (*Jarošova, Dočkalova 2008; Wittwer-Backofen, Tomo 2008; Papathanasiou 2011; Stock, Pinhasi 2011; Ash* et al. *2016; Jovanović 2017*). These show that around 50% of the individuals examined had some kind of growth disruption as a consequence of the new lifestyle in the Neolithic period, while in the Mesolithic only 20% of individuals were affected by growth risk factors during childhood (*Jarošova, Dočkalova 2008; Wittwer-Backofen, Tomo 2008; Papathanasiou 2011*). A recent study with a focus on the diet and health of Mesolithic-Neolithic inhabitants of the central Balkan region also showed that Early Neolithic people had limited nutritional resources and a greater prevalence of various dental and skeletal pathological conditions, as well as growth disturbances (*Jovanović 2017*). Stable isotope values show that, at the beginning of the 7th millennium, hunter-fisher-gatherers from the central Balkans, mainly dependent on aquatic resources, increased their consumption of terrestrial resources (*Bonsall* et al. *1997; Grupe* et al. *2003; Borić* et al. *2004; Nehlich* et al. *2010; de Becdelievre* et al. *2015; Jovanović 2017*). At the same time, the frequency of caries increased, possibly due to a diet rich in carbohydrates (*Turner 1979; Powell 1979; Larsen, Griffin 1991; Larsen 1995*). Furthermore, analysis of micro-plant fossils (starch grains) found in dental calculus lends weight to the argument that Neolithic people in the Central Balkans started to consume more terrestrial resources, and probably significant amounts of carbohydrates (*Jovanović 2017*). This dietary shift and poor hygienic conditions in the Neolithic Central Balkans resulted in the higher incidence of non-specific stress markers such as enamel hypoplasia, cribra orbitalia, and porotic hyperostosis (*Jovanović 2017*).

In this paper, we address the aspects of the Mesolithic-Neolithic transition related to health by looking at the changes caused by physiological stress at the microscopic level. We apply the method of tooth cementum annulation analysis to a sample of Mesolithic and Neolithic individuals from the Central Balkans. We expect the frequency of variation in the cementum layers as indicators of physiological stress to be higher in the Neolithic than in the Mesolithic sample, as predicted by theory and previous empirical studies.

Archaeological context

Mesolithic and Early Neolithic sites have been discovered on the territory of the Central Balkans (Fig. 1). One of the key areas is the Danube Gorges, where a series of settlements yielded well-preserved archaeological remains which document the chronological continuity of occupation along the Danube River from the Mesolithic to the Neolithic (10 000–5500 cal BC) (*Radovanović 1996; Roksandić 2000; Borić 1999; 2002a; 2002b; Borić, Miracle 2004; Borić, Stefanović 2004; Borić, Dimitrijević 2009*).

The Mesolithic-Neolithic sequence in the Danube Gorges is characterized by a specific material culture, including complex settlement architecture (trapezoidal buildings), sculpted sandstone boulders, and specific mortuary rites (*Srejović 1972; Radovanović 1996; Jovanović 2008; Borić 2011; 2016*). Archaeological excavations of these sites uncovered more than 500 human skeletons (*Roksandić 2000; Borić* et al. *2004; Stefanović in press*).

The inhabitants of these sites were semi-sedentary hunter-fisher gatherers, who settled in the vicinity of natural whirlpools which provided good hunting and fishing spots (*Borić 2002; Živaljević 2012*). During the Mesolithic and transitional Mesolithic-Neolithic phases the economy was mainly based on aquatic resources (*Clason 1980; Bartosiewicz* et al. *1995; 2001; 2008; Dinu 2010; Borić 2011; Dimitrijević* et al. *2016*) and wild game (*Bökönyi 1972; 1978; Dimitrijević 2000; 2008*). In the Early Neolithic (post *c.* 5900 cal BC) domesticated animals (cattle, ovicaprid, pig) started to appear (*Borić, Dimitrijević 2007*). In addition, in the Neolithic period people included more plants in their diet, possibly cereals (*Filipović* et al. *2017*). The only domesticated animal that appeared before the Neolithic is the dog, locally domesticated during the Mesolithic (*Bökönyi 1978; Dimitrijević, Vuković 2015*).

During the Neolithic phase, hunter-fisher-gatherer communities in the Danube Gorges began to have intensive contacts with first farmers in the region (*Borić 2002; Borić, Dimitrijević 2007; Borić, Price 2013*). The beginning of the sixth millennium BC in the western central Balkan region is associated with the Early Neolithic Starčevo culture (6200–5200 cal BC) (*Whittle* et al. *2002*). Aquatic resources and wild game still played a significant role in the diet of Neolithic inhabitants of the Danube Gorges, as indicated by stable isotope and archaeozoological analyses (*Borić* et al. *2004; Borić, Dimitrijević 2006; Borić 2008; 2011*). Animal husbandry and stock-breeding played a major role in subsistence, but wild game remains (red deer, roe deer, wild boar, and aurochs) were also found (*Bökönyi 1974; 1984; 1988; Clason 1980; Vörös 1980; Greenfield 1993; Blažić 1985; 1992; 2005; Arnold, Greenfield 2006*). Cultivated cereals (such as wheat and barley) and pulses (lentils, peas), were also identified at some Early Neolithic sites (*Filipović, Obradović 2013*). Although there is a large number of excavated sites, burials are very rare (*Borić 2014*). They are mostly found as single inhumations in a flexed position, located within the settlement.

Tooth cementum

Tooth cementum has a principal function of anchoring the tooth in the jaw, attaching the fibre of the periodontal membrane to the tooth root surface (*Condon* et al. *1986; Liebermann 1994*). Tooth cementum surrounds the dentine and forms in annual layers, with the first deposited layer defining the cemento-dentine junction. The cementum extends from the enamel-dentine junction to the apex of the root, varying from a thin layer close to the tooth crown up to 0.5mm thickness at the apex at older age (*Schröder 2000*).

Incremental bands, annual layers, or cementum growth layers (*Klevezal', Myrick 1984*) are rhythmic depositions of the tooth cementum. They consist of alternating dark and bright lines, differing in mineralization as seen under transmitting light microscopy (*Wittwer-Backofen 2012*). These depositions are seasonal, and are visible in a broad variety of mammalian species (*Grue, Jansen 1976, 1979; Lieberman 1993, 1994; Grupe* et al. *2012*). In humans, structured appositional growth of the tooth cementum can be seen in the acellular extrinsic fibre cementum concentrated in the middle third section of the root (*Wittwer-Backofen 2012*).

Compared to morphological traits correlated to age, the advantage of this method is the often better preservation of teeth compared to bones. Tooth cementum is less vulnerable to decomposition processes than osteological remains (*Grupe* et al. *2012*). For adults, this age estimation method resulted in more precise ages than estimates based on standard macroscopic indicators of age (*Grosskopf 1990; Wittwer-Backofen* et al. *2004; Naji* et al. *2016*). An individual's chronological age is estimated by adding the average age of tooth root formation by tooth type and sex to the mean number of counted incremental layers, or by applying a mathematical algorithm which comes close to this procedure (*Wittwer-Backofen* et al. *2004; Grupe* et al. *2012; Gupta* et al. *2014*). Under optimal conditions, TCA provides a highly precise age at death estimate with an error margin of ±2.5 years (*Wittwer-Backofen* et al. *2004*), or additionally a determination of the season of death (*Klevezal', Shishlina 2001; Wedel 2007*).

Due to its strict appositional growth, the acellular extrinsic fibre cementum is a valuable tool for the reconstruction of certain life-history parameters (*Kagerer, Grupe 2001*). More specifically, TCA layers differ from each other in width and appearance, and it is assumed that these irregularities are formed as a response to life-events of physiological stress related to the sensitive calcium metabolism.

Further clinical studies into the origin of these patterns showed that surgery performed on the spine and/or bones, and other orthopaedic interventions, renal disease, tuberculosis, and pregnancies leave a visible mark in the tooth cementum (*Kagerer 2000; Kagerer, Grupe 2001; Caplazi 2004*), suggesting that stress layers could be interpreted as reflecting specific life-events. However, diabetes, thyroid disorders, metabolic bone diseases such as osteoporosis, malnutrition, rachitis, periodontal disease, or leprosy do not leave visible traits in the dental cementum (*Kagerer 2000; Kagerer, Grupe 2001; Bertrand* et al. *2016; Broucker* et al. *2016*). Another study on captive great apes showed that extreme weather leaves marks, too (*Cipriano 2002*). This was explained by the lack of sunlight, caused during a long cold winter, leading to reduced vitamin D levels. What all these occurrences have in common is their impact on the calcium metabolism. Conditions such as kidney diseases and traumas mobilize calcium in the body and influence the concentration of available calcium (*Kagerer, Grupe 2001*). Pregnancy and lactation are processes that are energetically costly (*Medill* et al. *2010*), and these physiological demands as well as increased hormone activity also cause alterations in the cementum layers. An increased thickness of cementum layers is also connected with weaning or menarche, as well as with dry and rainy seasons in baboons (*Dirks* et al. *2002*). Even in periods of extreme calcium demand, such as pregnancy or lactation, the growth process of the incremental layers is not interrupted, leading to the fact that the number of AEFC layers is closely correlated to chronological age and does not depend on major life events or living conditions. Correlation of stress markers and pregnancies has been documented in humans (*Kagerer, Grupe 2001; Künzie, Wittwer-Backofen 2008*). During pregnancies, due to low levels of metabolically available calcium, the cementum layers are still produced but appear differently mineralized, broader, and with higher or lower translucency than other layers (*Kagerer, Grupe 2001*). Peter Kagerer and Gisela Grupe (*2001.79*) showed that in all cases of pregnant women the "*translucent layers corresponded exactly with the age when the female had been pregnant, interbirth intervals were maintained and exactly datable*". Besides humans, these changes in cementum have been detected in polar bears (*Medill* et al. *2010*), dolphins (genus Stenella) (*Klevezal', Myrick 1984*), great apes (*Cipriano 2002*) and black bears (*Carrel 1994*).

These layers are described as "*hypomineralized incremental lines*", "*conspicuous incremental lines*" or "*broad and translucent layers*" (*Kagerer, Grupe 2001*), "*irregularities in terms of hypomineralized bands*", and "*influence on the quality of incremental lines*", and "*stress-related variation in line quality*" (*Cipriano 2002*). As these lines do refer to a certain stress-related life-events, in this study they will be referred to as stress layers. However, despite the vast evidence of the occurrence of cementum layers that correspond to life-events, a standardized methodological approach for the determination of such stress layers is not available yet.

The variation of these layers involves two features: (1) disparities in width of the layers, with stress events supposed to result in broader layers, and (2) difference of optical appearance under transmitting light microscopy, with a greater contrast between dark and bright lines, *i.e.* the stress-related layers are broader and appear darker. To count the pairs of light and dark lines that represent one year in age determination, we use the dark lines as markers as they are easier to determine visually. The first line, the eruption line, is also a dark one.

When it comes to the darker appearance of the layers, there are no strict criteria for the definition of a layer being darker or lighter, whereas the width of the respective cementum layer can be evaluated by measurements. It thus rather depends on the subjective impression of the observer. This leads to highly subjective determinations of potential stress layers. Galina A. Klevezal' and Albert C. Myrick (*1984. 104*) described in their research the dentine of toothed whales and dolphins that consist of numerous layers having different optical densities. The variations in optical appearance are described as subjective: "*DSLs (deep stained layers) in males were subtly different in character from those observed in females. Nevertheless, clear distinctions between DSLs in males and in females were difficult to describe, and we have here used the same definition for both sexes.*" In their study the presence of 'doubtful' layers is noted, emphasizing that the criterion for that description is subjective (*Klevezal', Myrick 1984*).

As an indicator for a determination of a stress layer the presence and visibility of striking incremental layers through all sections of the same tooth (*Kagerer, Grupe 2001*) is suggested.

Materials, methods and results

Sample description

Eight archaeological specimens (currently investigated at the Laboratory for Bioarchaeology, Department of Archaeology, Faculty of Philosophy, University of Belgrade) from the Mesolithic and Neolithic periods were analysed for tooth cementum stress layers (Tab. 1). All samples are from individuals without visible traumata and from secure archaeological contexts. They originate from excavated archaeological sites that have clear prehistoric contexts (Fig. 1). For some directly dated individuals a radiocarbon date is available, whereas others are assigned to a period (Mesolithic or Neolithic) based on the dating of the entire site and the burial position. In the Danube Gorges, Mesolithic individuals are buried in supine position, whereas Neolithic individuals are buried in flexed position lying on their sides (*Borić 2011*). As a preparatory first step, all samples were photographed, 3D scanned, and a cast was made of each tooth before the sample preparation took place.

TCA sample preparation

Only single-rooted teeth were investigated. The general protocol for the preparation of samples was based on the work of Ursula Wittwer-Backofen (2012). Teeth were embedded into Biodur epoxy resin (Biodur E12 resin with hardener E1 in the ratio 100:28) and the middle third of the tooth was cut cross-sectionally with a slice thickness of 80μm using a Leica 1600 rotating diamond microtome. This resulted in 7 to 17 sections per tooth. Each section was observed visually and individually by

Archaeological site	Grave	Sex	Tooth (FDI)	Macroscopic age estimate	TCA age estimate	Period	Absolute date 95% CI (reference)
Vlasac	38	Female	42	30–59	70 ± 2.5	Mesolithic	7514–7351 cal BC (this study)
Vlasac	24	Male	21	25–29	44 ± 2.5	Mesolithic	6640–6220 cal BC (*Borić 2011*)
Padina	18b	Female	35	>30	65 ± 2.5	Mesolithic	9115–8555 cal BC (*Mathieson* et al. *2018*)
Vinča–Belo brdo	VII	Female	43	15–18	23 ± 2.5	Neolithic	5565–5470 cal BC (*Tasić* et al. *2015*)
Lepenski Vir	66	Male	22	25–30	34 ± 2.5	Neolithic	5995–5848 cal BC (this study)
Lepenski Vir	8	Female	44	30–49	36 ± 2.5	Neolithic	5990–5790 cal BC (*Bonsall* et al. *2015*)
Ajmana	11	Female	41	>30	60 ± 2.5	Neolithic	/
Lepenski Vir	9	Female	13	>15	55 ± 2.5	Neolithic	5980–5740 cal BC (*Bonsall* et al. *2015*)

Tab. 1. Samples analysed for tooth cementum stress layers (where no date for the specific individual was available field was marked with "/", this individual was assigned to a period according to burial position and the dating of the site).

the first author using the transmission light microscope Leica DM RXA 2 with magnifications of 20x and 40x. Photographs of all regions of interest were taken using a digital tubus camera Leica DC 250 and saved in a database. Each pair of light and dark cementum layers was counted for the age at death estimation (SFig. 1–SFig. 8 at http://dx.doi.org/10.4312/dp.46.17). Three sections from each tooth were selected for stress layer evaluation. The average number of layers was calculated by averaging the number of layers counted covering all sections (total number of sections for each tooth). One representative photo from each section was analysed. Age at death is calculated by adding the sex-specific average age of tooth root eruption for the respective tooth type, as noted in Peter Adler (1967), to the average number of cementum layers counted on all sections.

Methods for stress layer determination

We used two different methods for the determination of stress layers according to their width and colour of appearance. Both methods are based on the assumption that cementum layers influenced by physiological stress show a significantly broader extension compared to regular cementum layers. The verification and counting of stress layers was a blind procedure in the sense that the researcher making the count did not know which particular tooth was being analysed. This measure was taken in order to avoid preconceptions about the sex and the period that the samples come from (Neolithic or Mesolithic), and to avoid these expectations influencing the results.

Method 1 consists of measuring each pair of dark and bright layers (Fig. 2) by using the Leica software Image measurement tool. The detailed measurements (*i.e.* the thickness of the pair of lines) were taken from three selected sections of the same tooth. For each section the average width of layers and the corresponding standard deviation was calculated. All layers with values greater than the average +1 standard deviation value were defined as stress layers. This method indicated stress layers based on their differing width, independent of their visual appearance or observer determination.

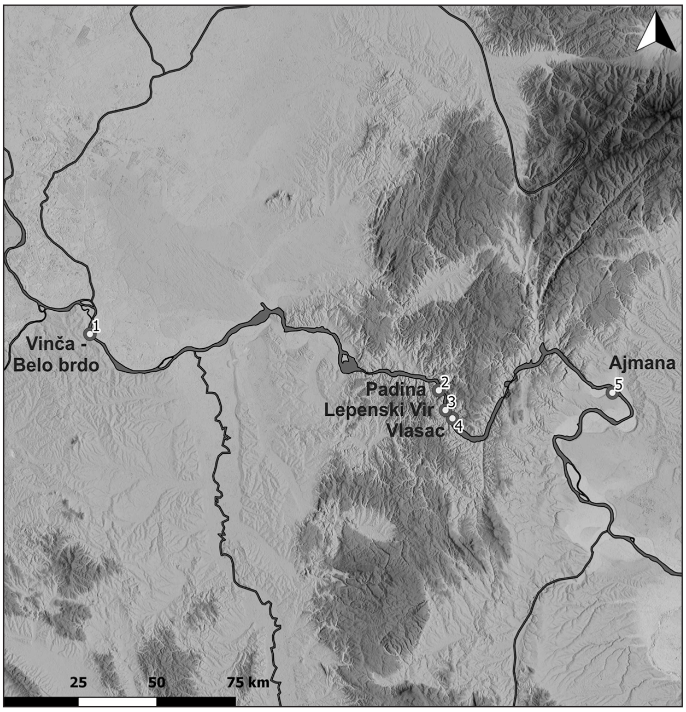

Fig. 1. Map of the research area with sites from which the samples in this study originate

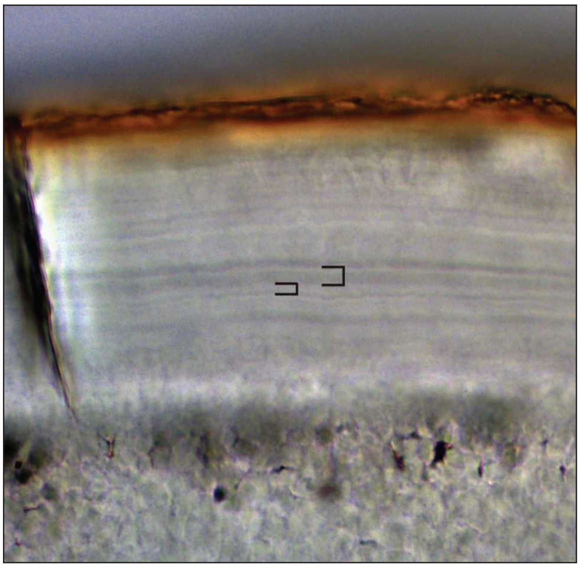

Fig. 2. The tooth cementum band under the microscope. The identification and measurement of a tooth cementum layer is illustrated.

Method 2 is based on calculating the average thickness of the incremental layers by measuring the thickness of the whole cement band at four different areas of a section. This procedure was done on three sections from the same tooth. The average value of the thickness of the band was divided by the number of layers counted from the specific section. Stress layers were determined visually by the observer, selecting layers that appeared wider and darker. Only these pre-determined layers were measured, and if their thickness was greater than the average incremental layer thickness, it was described as a stress layer. This method relies on the observer's pre-selection of stress layers.

In order to compare the results yielded by the different methods we compared the number of stress layers determined by each of the two approaches and counted the number of matches. Matching layers are those classified as stress layers by both methods applied to the same section and position in the cementum band. The percentage of matching stress layers for each section is presented in Table 2. As the match between the number and position of stress layers identified is very high (see the Results section) it was decided to use only the first method, as it is more objective (in that it does not involve the subjective preselection of layers).

Verifying and counting stress layers

After identification of the stress layers for each section and each tooth, the next step was the verification of the appearance of cementum stress layers on an individual level. A stress layer was considered as verified on an individual level if it appeared in the same position on at least two out of three sections of one tooth. Therefore, the total number of stress layers per individual (each individual is represented by a single tooth) is the total number of verified stress layers. In order to compare the positions of stress layers from different sections (which have different cementum band widths) the following procedure is applied (see Figure 3 for the illustration of the procedure):

❶ The sections are represented visually – each counted cementum layer is represented by a rectangle, stress layers marked in green.

❷ In order to make the sections of different widths comparable, they are stretched to same length.

❸ In order for a stress layer to be verified and counted, there have to be at least two layers at the same relative position (*i.e.* there is at least some overlap between the layers). The cementum band thickness profiles for each individual are shown in the Supplementary Material (SFig. 9–SFig. 16 at http://dx.doi.org/10.4312/dp.46.17). The evaluation of stress layers per individual was made according to this procedure, as shown in Figure 3.

Calculation of individual burden of stress

Cementum layer anomalies are indicators of stress burden, and the number of verified stress layers needs to be statistically corrected for the total number of TCA layers. This is done in order to account for the differences in age between individuals (as older individuals had more chance to experience stress). It is implemented by dividing the number of verified stress layers by the individual total number of TCA layers. Strictly speaking this is not an age correction, as the eruption time for different teeth may differ, therefore the differences in the total number of layers may not directly reflect differences in age, but for practical purposes it is equivalent to age correction given that differences in tooth eruption times are a few years at most. The resulting value can be interpreted as a number of verified stress layers per year of life covering the period after the specific tooth erupted.

Results

The stress layers are present in all individuals investigated in this study, with the number varying between two and 11 per person. The number of stress layers identified per person is consistent over all sections and between the methods. The results of the comparison of the two methods are presented in Table 2. The percentage of matching layers varies between 67 and 100 percent, with the mean value of 93.6 percent. In 64 percent of cases (sections) there is a full match between the layers identified as stress layers by both methods.

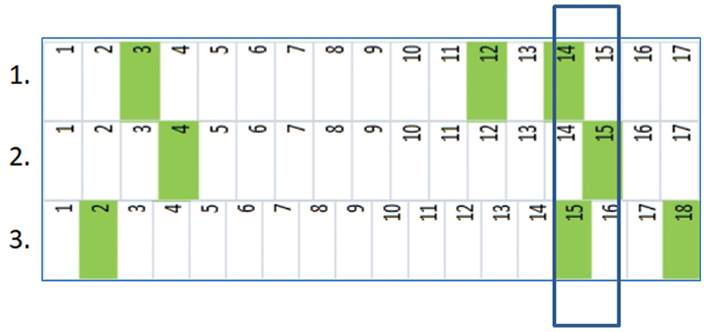

Fig. 3. Illustration of the stress layer verification: different rows represent different sections of the same tooth; stress layers (determined either by Method 1 or Method 2) in each section are marked in green; despite the fact that more than one stress layer is identified in each section individually (green rectangles), there is only one verified stress layer for this tooth, as only two layers from sections 2 and 3 overlap (the verified stress layer is marked).

The results show that both methods of stress layer identification yielded identical or very similar results in the majority of cases. However, it should be emphasized that this convergence refers only to the two specific protocols for classifying cementum layers as stress layers – it should not be interpreted as a measure of the absolute validity of any of the methods in terms of discriminating between the real stress layers and those not affected. The latter can only be achieved by a clinical study where the medical history of an individual is known.

The number of verified stress layers is correlated with the total number of TCA layers (r = 0.675, p = 0.033, see Fig. 4) which is not surprising given that, whatever the etiology of stress layers is, longer lifespan means more opportunity for stress layers to occur. The values of the number of verified stress layers per year of life (after tooth eruption) for each individual are presented in Table 3. The range of values is between 0.04 and 0.13 for the Mesolithic group, and between 0.08 and 0.15 for the Neolithic group. The average values of the number of verified stress layers corrected for the total number of verified stress layers (number of verified stress layers per year of life after tooth eruption) are 0.085 and 0.1 for the Mesolithic and the Neolithic groups, respectively. Therefore, the average number of verified stress layers per year of life after tooth eruption is higher in the Neolithic than in the Mesolithic, but there is a substantial degree of overlap (Fig. 5). No statistical tests are performed as the sample size and power of the test are too low for meaningful analysis, therefore we only report trends.

Individual	Section Nr.	Method 1, Nr. of stress layers	Method 2, Nr. of stress layers	Nr. of matching layers	Percent matching
Vlasac 38	1	7	8	7	87.5
Vlasac 38	2	6	7	6	85.71
Vlasac 38	3	9	9	9	100
Vlasac 24	1	4	4	4	100
Vlasac 24	2	4	4	4	100
Vlasac 24	3	6	6	6	100
Padina 18b	1	6	6	6	100
Padina 18b	2	3	3	3	100
Padina 18b	3	4	4	4	100
Vinča VII	1	3	3	3	100
Vinča VII	2	2	2	2	100
Vinča VII	3	2	2	2	100
Vinča VII	4	2	3	2	66.67
Lepenski Vir 66	1	6	7	6	85.71
Lepenski Vir 66	2	4	5	4	80
Lepenski Vir 66	3	4	5	4	80
Lepenski Vir 8	1	4	4	4	100
Lepenski Vir 8	2	5	5	5	100
Lepenski Vir 8	3	5	6	5	83.33
Ajmana 11	1	10	10	10	100
Ajmana 11	2	5	5	5	100
Ajmana 11	3	11	11	11	100
Lepenski Vir 9	1	9	9	9	100
Lepenski Vir 9	2	7	6	5	83.33
Lepenski Vir 9	3	7	8	7	87.5

Tab. 2. Comparison of the two methods for the identification of cementum band stress layers.

Discussion and conclusion

In this study, we explored the tooth cementum stress layers from the perspective of the differences in health and general stress between the Mesolithic and

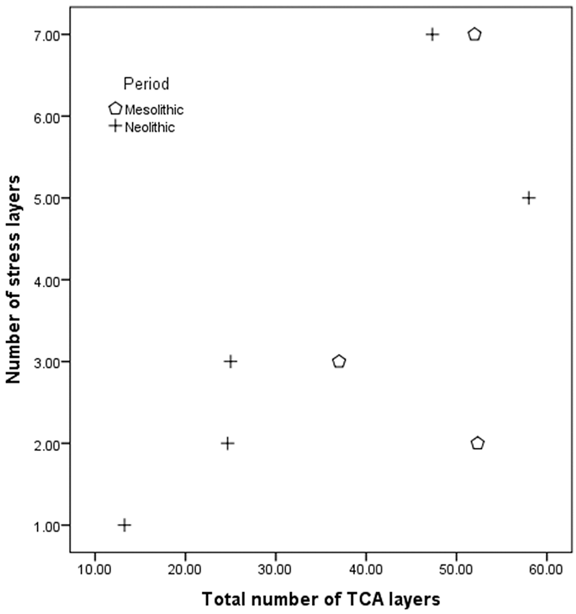

Fig. 4. Total number of TCA layers vs. *number of verified stress layers.*

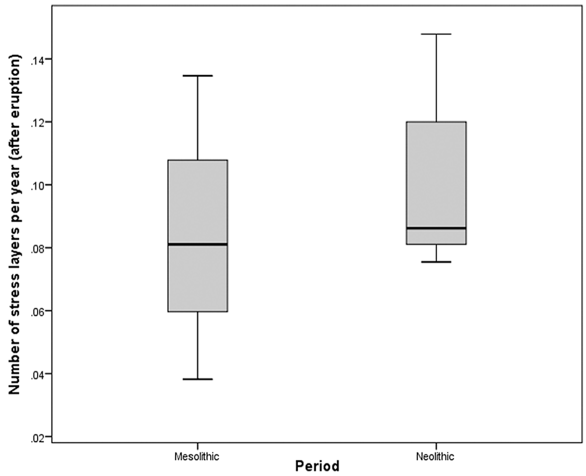

Fig. 5. Boxplot showing the distribution of the number of verified stress layers per year by chronological phases.

Neolithic populations. As expected, the number of stress layers when corrected for the total number of TCA layers is higher in the Neolithic group than in the Mesolithic group, but the statistical significance of this trend cannot be evaluated due to low sample size.

The results are also consistent with the picture of the Neolithic Demographic Transition formulated by Jean-Pierre Bocquet-Appel (*2008; 2011*), if some of the detected stress layers are induced by pregnancies. They might suggest both increased fertility, as the major driving force for Neolithic population growth, and increased burden of disease, as demonstrated by Ursula Wittwer-Backofen and Nicolas Tomo (*2008*). This would imply that TCA-based analysis of physiological stress can make a substantial contribution to the field of paleodemography. As teeth are among the most durable elements of the skeleton, in terms of resistance to decay and preservation, the analysis of TCA stress layers can be used in situations when the application of macroscopic methods of recording physiological stress is precluded due to missing bones. Moreover, some conditions detectable with macroscopic methods, such as hypoplasia, occur early in life, usually prior to permanent teeth eruption, whereas stress episodes that should theoretically be reflected in the cementum bands could occur later in life. To further support these first observations, a larger sample size will be evaluated in the next step in order to confirm or refute our preliminary results concerning the differences between the Mesolithic and the Neolithic populations with a statistically relevant sample.

Archaeological site	Grave	Sex	Period	Total number of verified stress layers	Number of stress layers per year
Vlasac	38	Female	Mesolithic	7	0.13
Vlasac	24	Male	Mesolithic	3	0.08
Padina	18b	Female	Mesolithic	2	0.04
Vinča–Belo brdo	VII	Female	Neolithic	1	0.08
Lepenski Vir	66	Male	Neolithic	2	0.08
Lepenski Vir	8	Female	Neolithic	3	0.12
Ajmana	11	Female	Neolithic	5	0.09
Lepenski Vir	9	Female	Neolithic	7	0.15

Tab. 3. Number of verified stress layers per year of life (after tooth eruption) for each individual.

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