

# Age and Morbidity at the beginning of Life

An evaluation of three ageing methods and assessment of infant mortality in a nineteenth century Dutch skeletal collection

*Sonja Jäger*



Cover picture:

Infant skeleton's weeping into handkerchiefs. featured in 'Alle de ontleed- genees- en heelkindige werken...van Fredrik Ruysch...vol. 3'. Frederik Ruysch (1638-1731), Dutch Anatomist. Etching with engraving, Amsterdam, 1744. National Library of Medicine. Retrieved from [http://www.nlm.nih.gov/exhibition/dreamanatomy/da\\_g\\_I-C-1-09.html](http://www.nlm.nih.gov/exhibition/dreamanatomy/da_g_I-C-1-09.html), accessed on 1 September 2014.

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# **Age and Morbidity at the beginning of Life**

An evaluation of three ageing methods and assessment of infant mortality in a nineteenth century Dutch skeletal collection

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## *Table of contents*

Preface	9
1. Introduction	11
1.1 Sources for the analysis of infant remains	12
1.2 Infant remains in archaeology	15
1.3 Absence of evidence is not evidence of absence	16
1.4 Infant osteological age estimation	17
1.4.1 Skeletal age estimation	17
1.4.2 Dental age estimation	19
1.5 The skeletal collection	22
1.6 Research questions	25
2. Infant survival in Middenbeemster during the Nineteenth Century	27
3. Skeletal and Dental Growth and Development	31
3.1 Skeletal growth and development	31
3.1.1 Growth sequence and velocity	32
3.1.2 Monitoring skeletal growth	33
3.1.3 Variation in skeletal growth	34
3.1.4 Skeletal growth as indicator of stress	35
3.2 Dental growth and development	37
3.2.1 Embryonic dental development	37
3.2.2 Dental tissues	39
3.2.2.1 Enamel	39
3.2.2.2 Dentine and pulp	40
3.2.2.3 Cementum	41
3.2.3 Dental growth and eruption pattern	41
3.2.4 Variation in dental development	42
3.3 Skeletal versus dental development	43
3.4 Summary	45
4. Skeletal and Dental age estimation	46
4.1 Skeletal age estimation	46

4.1.1	Potential drawbacks	47
4.2	Dental age estimation	48
4.2.1	The deciduous Demirjian stages by Liversidge and Molleson (2004)	49
4.2.1.1	Potential drawbacks	51
4.2.1.2	Accuracy	53
4.2.2	Dental height by Liversidge and colleagues (1993)	54
4.2.2.1	Potential drawbacks	54
4.2.2.2	Accuracy	55
4.3	Summary	55
5.	Materials and Methods	56
5.1	The Sample	56
5.2	Selection of individuals	58
5.3	Selection of dental ageing methods	59
5.4	Expectations and limitations	59
5.5	Skeletal age recording	60
5.5.1	Foetal age estimation: Fazekas and Kósa (1978)	62
5.5.2	Infant age estimation	62
5.5.2.1	The pars basilaris of the occipital: Scheuer and McLaughlin-Black (1994)	63
5.5.2.2	The Clavicle: Black and Scheuer (1996)	64
5.5.2.3	The ilium: Molleson and Cox (1993)	64
5.5.2.4	The scapula: Saunders and colleagues (1993)	65
5.5.2.5	Long bone length: Maresh (1955 and 1970)	65
5.5.3	Analysis	66
5.6	The Demirjian system for deciduous teeth by Liversidge and Molleson (2004)	66
5.6.1	Analysis	67
5.7	Dental height by Liversidge and colleagues (1993)	67
5.7.1	Analysis	68
5.8	Statistical analysis	68
5.9	Intra-Individual Error	70
6.	Results	71
6.1	Reproducibility	71
6.2	Accuracy	72
6.3	Results skeletal age estimation	75
6.3.1	Accuracy of the infant growth standards	79

6.3.2	Skeletal age estimation including all remains	80
6.3.2.1	Pars basilaris of the occipital	82
6.3.2.2	Clavicle	83
6.3.2.3	Scapula	83
6.3.2.4	Ilium	84
6.3.2.5	Sphenoid body	85
6.3.3	Conclusion skeletal age estimation	86
6.4	Results deciduous Demirjian stages	86
6.4.1	Results deciduous Demirjian stages including all individuals	89
6.4.2	Conclusion deciduous Demirjian stages	90
6.5	Results dental height by Liversidge and colleagues	91
6.5.1	Results Dental Height by Liversidge and colleagues including all Individuals	94
6.5.2	Conclusion dental height by Liversidge and colleagues	96
6.6	Comparison deciduous Demirjian stages with dental height	96
6.7	Infant mortality	97
6.7.1	Age distribution	97
6.7.2	Dental versus skeletal development	98
6.7.3	Conclusion infant mortality	99
7.	Discussion	100
7.1	Skeletal age estimation	101
7.1.1	The Standards	104
7.1.1.1	Foetal bone growth: The Fazekas and Kósa standard	104
7.1.1.2	Long bone length: The Maresh standard	104
7.1.2	Single bone infant standards	105
7.1.2.1	The pars basilaris: Scheuer and McLaughlin-Black	106
7.1.2.2	The Clavicle: Black and Scheuer	107
7.1.2.4	The scapula: Saunders and colleagues	108
7.1.2.3	The ilium: Molleson and Cox	109
7.1.3	The sphenoid body	110
7.2	The deciduous Demirjian stages by Liversidge and Molleson	112
7.3	Dental height by Liversidge and colleagues	115
7.4	Infant mortality	119
7.4.1	Dental versus Skeletal development	124
8.	Conclusion	129

Abstract	135
References	137
List of Figures	151
List of Tables	154
Appendix 1: Dataset for skeletal measurement recordings	156
Appendix 2: Dataset of deciduous Demirjian stage recordings	166
Appendix 3: Dataset of dental height recordings	172
Appendix 4: Comparison of the three ageing methods for each of the 39 remains	177
Appendix 5: Understanding the Neonatal line	182
Appendix 6: The dental nomenclature of the Fédération Dentaire Internationale	189



## *Preface*

This thesis took on a long journey at the beginning of the academic year in 2012. There were so many possibilities to investigate the infant category of the Middenbeemster collection, many of which I excavated myself during the summer of 2012 when the collection was retrieved from the ground. No studies had been conducted on these remains so any research would have to start with first, cleaning the bones, and a subsequent osteological analysis. But as there were no forms ready yet that listed the methods and procedures for the analysis, I had the opportunity to dive into the different methods that were available for age estimation of late foetal and infant remains. A general lack of studies providing accuracy levels for the various methods in existence struck me and this is where the idea emerged to try on a more fundamental methodological approach of evaluating several ageing methods. At that stage none of the individuals were identified, so it was anticipated to use histological age estimation based on deciduous dental enamel as standard against which I could test the other ageing methods. This is where the journey became much more complex as there was no histology lab established yet in the faculty and we would all have to learn while I became more acquainted with the methodology of thin section preparation and the materials that were needed for the procedure. Sometimes it would take months to get the right grinding paper, and not to mention the polishing machine which has caused us so much headache (and I will never use it again!). The subsequent microscopic analysis of the slides revealed the mistakes I made during preparation and some slides were lost while in others the microscopic features were obscured by taphonomic alterations. Unfortunately the technical problems were such that after two years of trial and error, interrupted by the the birth of my second daughter, I had to realise that the material would not provide the information I needed. Fortunately, meanwhile I had analysed in total 45 of the 49 infant remains and collected the data on skeletal and dental development that proved to contain more than enough information to write my thesis. Meanwhile, the identification

had proved successful in some cases, and I could now use the real age of ten individuals to evaluate the methods and, thus, being able to make a real contribution to the field of juvenile osteology.

This is where the journey ends and I am very thankful to my family who supported me during this long but rewarding process, taking care of the children when needed. I am thankful to Professor Maat and Job Aarents of the LUMC who provided me with some practical knowledge about thin section preparation, and to Professor Hillson of the UCL who shared some of his insights on dental histology, and who provided me with a more in depth knowledge on infant bone development. But most of all I would like to express my gratitude to my supervisor Dr. Andrea Waters-Rist who never gave up on me and who took the time to read my drafts and to provided me with tremendously valuable constructive criticism on my work. I also like to thank Professor Dr. Menno Hoogland who gave me with the opportunity to study human osteology at Leiden University.

## *1. Introduction*

Infant skeletal remains form a special category in osteology. The developing skeletal and dental elements are subject to rapid change in size and morphology during this time period. At birth the skeleton consists of 156 recognisable elements of which 30 constitute the cranium. In addition, the primary dentition, although not yet erupted, is forming rapidly within the jaws. Throughout development the number of skeletal and dental elements will change as new growth centres appear while other elements start to fuse and the permanent teeth start forming. Skeletal and dental development in subadults is a continuous process and this, in part, is why researchers lack consensus in their delineation of the boundaries of different age categories. Another reason is that different tissues (i.e. bone and teeth), and elements of the same tissue (i.e. different bones or teeth), have formation times that start and stop at different ages. Thus, researchers using different tissues or elements will utilise different age-related processes.

Definitions of age categories such as neonate, infant, or juvenile, are generally derived from disciplines such as medicine or behavioural biology, that are concerned with the living being and thus incorporate the development of the soft tissue as well as behavioural characteristics of the individual (see table 1 for definitions used in this thesis). In clinical literature the infant category is usually defined as being between birth and 12 months of age (Martin 2010, 55). Behavioural biologists, on the other hand, define infancy as the period when the individual is nursed, which can vary from birth to between one to three years of age (Scheuer, and Black 2000, 469). Human biologists usually define the infant category at from birth to three years (Bogin 1999; Steele and Bramblett 1988). In osteological research different definitions are used depending on the question asked (Baker *et al.* 2005; Lewis and Gowland 2007; Waters-Rist *et al.* 2011).

However, in osteological research the choice of an age category may also often be regulated by the availability of skeletal material. This study is primarily concerned with ageing methods based on aspects of crown development of the deciduous dentition. As deciduous crown development mainly covers the time from early intrauterine life until the end of the first year of life, it is most convenient to use the clinical definition whereby infancy is the period from birth to one year.

*Table 1. Definitions of time periods from fertilisation to the end of adolescence that are used in this thesis (Scheuer and Black 2000a, 468f).*

Embryo	The first 8 weeks of intra-uterine life
Foetus	From week 9 to birth
Preterm	From <37 weeks gestation
Fullterm	From 37-42 weeks gestation
Perinatal	Around the time of birth -from 24 weeks gestation to 7 postnatal days
neonatal	From birth to 28 days
infant	From birth to the age of 1 year
juvenile	Any age previous to adult

### *1.1 Sources for the analysis of infant remains*

Infant remains in archaeology are challenging in many ways. The tiny bones are easily overlooked during excavation and, being fragile, they have to be handled with care. In subsequent standard osteological analysis an assessment of the developmental state of the skeletal and dental remains has to be made to determine biological age. As stated above, growth during infancy proceeds very fast and the rapid changes in skeletal and dental dimensions, as well as morphology, can be used for age assessment, resulting in relatively high accuracy. However, age estimation methods that are available for the infant age category are

generally based on limited observations, owing to the scarcity of the source material.

The osteologist has to rely on different sources to compare his or her observations with. First, there are age estimation standards based on studies of modern-day infants, that are used to assess the growth and maturation of past subadults. These are generally based on roentgenographic measurements. Between 1930 and 1960 several longitudinal growth studies were conducted that followed individuals throughout their development (Greulich and Pyle 1959; Maresh 1955; Tanner and Whitehouse 1959). However, when health risks resulting from frequent exposure to radiation became known, they had to be halted (Scheuer and Black 2000, 8).

Several problems need to be addressed when comparing healthy modern juveniles with the non-survivors of archaeological samples. First, it may provide a distorted picture of past height, because individuals in many cases died due to disease or nutrition shortcomings which might have resulted in reduced height (Lewis 2007, 69; but see Saunders and Hoppa 1993). Second, it is likely that in the past growth generally followed a different and reduced path which, again, may result in individuals being aged younger. During the last 100 years a general increase in height has occurred in conjunction with individuals maturing earlier. This phenomenon is generally known as the secular trend (Ulijaszek 2001). It has altered growth curves and velocities of modern juveniles with the effect that archaeological specimens may be categorised as stunted, while being normal compared to their population of origin (Saunders and Barrans 1999).

A second type of standard is based upon analysis of relatively recent skeletal material where it has been possible to use historical documents to identify individuals and hence their age-at-death. Two such collections are the Granada subadult collection, with up to 230 individuals aged from birth to eight years of age, housed in the Laboratory of Anthropology of the University of Granada, Spain, and dating to the mid-twentieth century (Alemán *et al.* 2012), and the Lisbon collection housed at the Bocage Museum (National Museum of Natural History) in Lisbon, Portugal, with about 92 subadult individuals dating from the 19<sup>th</sup> and 20<sup>th</sup> centuries (Cardoso 2006). Both collections are still being researched

and studies based on these remains are limited (Cardoso 2007). Using growth standards that stem directly from skeletal material is generally preferred when assessing age in osteology, owing to potential problems of comparing dry bone to radiographic derived data (Beynon *et al.* 1998). However, some of the individuals in these collections may have already experienced the secular trend in growth, which makes them less useful for comparisons to older skeletal material.

Only a few archaeological historically documented skeletal collections exist that yield sufficient infant remains to facilitate the construction of reference standards. In order to assign an age to skeletal remains, the bones are compared to standards that list the length of a certain element together with an age range in which the element reaches a particular developmental state. Thus, in order to create a standard the collection needs to have historical records that list chronological age for the individuals. With this, maturation of the skeleton can be compared with real age. However, a growth standard requires a large sample size in order to capture at least part of the normal variability of the growth system. Archaeological documented collections are not only sparse but often only have limited numbers of individuals.

There are three well studied archaeological documented collections, that are contemporary with the skeletal material used in this thesis and dating to the 18<sup>th</sup> and 19<sup>th</sup> century. Two of them are from London: 1) the collection from the crypt of Christ Church Spitalfields (Adams and Reeve 1987), which is the best studied collection of juveniles at this moment including 63 infants and young children of known age, and 2) a small collection of about 14 subadults from the crypt of St. Bride's Church, Fleet Street, London (Gapert *et al.* 2009). The third partly documented collection comes from the St. Thomas' Church cemetery in Belleville Ontario, Canada and has yielded about 50 infant remains (Saunders *et al.* 1993).

This study will investigate several ageing methods based on dental and skeletal remains and apply these to a partly documented Dutch early modern skeletal collection. The purpose is to test the accuracy of two relatively recently developed dental ageing methods and skeletal age estimation for infant remains. Methods for this age category have not undergone systematic testing and thus more in depth knowledge on the reliability of the estimate they provide is needed.

## *1.2 Infant remains in archaeology*

Age estimation of infant remains stands at the beginning of an osteological inquiry into different aspects of the population under study. The resulting age distribution may give insights into periods that potentially increase stress and promote morbidity, such as the introduction of solid foods and weaning cessation (Lewis 2007, 97). Through cross-population comparison, differences in mortality patterns can be revealed that facilitate our understanding of social and environmental factors that influence infant survival.

Throughout the first year of life, different factors can be demarcated that shape infant mortality. Endogenous causes prevail during the perinatal and neonatal period, while environmental factors increase in influence postnatally (Lewis and Gowland 2007; Saunders and Barrans 1999). To determine the impact of the two factors on infant mortality requires that individuals are aged accurately. Today, neonatal mortality accounts for 40-60% of infant mortality in the developing world (Norton 2005, 2). Considering the hazards faced by woman from past populations during delivery, this percentage is considered standard. A mortality profile that reveals a greater number of infants that died during the post-neonatal period would, thus, indicate adverse environmental conditions and calls for further research.

The total number of infants that died within the first year of life (i.e. the mortality rate) has been conceived of as a measure of the adaptive success of a population to its environment (Lewis and Gowland 2007, 117). Infants are completely dependent on their environment for survival and their presence in the cemetery may indicate shortcomings in maternal and/or infant care, poor diet, lack of hygiene, disease outbreaks, or even infanticide (Lewis 2007; Mays and Evers 2011). However, a high number of infant remains should not automatically be interpreted to indicate a population under stress. It may also be due to increased fertility, where higher birth rates produce higher death rates without increasing the overall percentage of infants that die within the first year of life (Sattenspiel and

Harpending 1983, 489). Therefore, the observed number of infants in a cemetery sample does not necessarily indicate a population experiencing hardship.

### *1.3 Absence of evidence is not evidence of absence*

A number of limiting factors have to be addressed when conducting research on infant remains. In many cases few infants are recovered in archaeological excavations, with varying degrees of preservation. The small numbers are counter the expectations held for preindustrial societies, in which infant mortality is perceived to be high and to seldom drop below 25% (Guy *et al.* 1997; Saunders and Barrans 1999). An underrepresentation of infants potentially introduces a bias which renders inferences from the cemetery sample about the original population problematic (Paine and Harpending 1998; Lewis 2007). Several factors are said to be at the core of this problem. First, cultural practices may omit infant remains from common burial grounds (Baker *et al.* 2005). Second, their bones are found to disintegrate at a faster rate if soil conditions are very acidic (Guy *et al.* 1997). A third point is concerned with loss of material due to crude excavation methods and subsequent improper handling of the fragile bones (Milner *et al.* 2008). Fourth, without a trained human osteoarchaeologist analysing the material, small infant bones may be misidentified (i.e. as a small mammal or bird) (Scheuer and Black 2004). Moreover, in the past, it was common that infant remains were omitted from analysis because they were thought to be of little worth for scientific investigation (Baker *et al.* 2005).

Given the shortcomings, studies on subadults and especially infant remains are often based on few observations. But an increasing amount of literature has since demonstrated that infant remains are of great value to the study of past societies (Baker *et al.* 2005; Budnik and Liczbinska 2006; Guy *et al.* 1997; Halcrow and Tyles 2008; Halcrow and Tyles 2011; Lewis 2007; Saunders and Hoppa 1993).



## *1.4 Infant osteological age estimation*

From conception to one year, and to a lesser degree until three years, the subadult is growing at a high rate which will only happen once again, and at a lesser rate, during the adolescent growth spurt (Lewis 2007; Saunders and Barrans 1999). Growth in height is, therefore, happening very fast and, especially during the foetal and infant period, is highly correlated with age (Liversidge 1994, 39). Skeletal and dental development, however, more strongly reflect biological age, indicating the maturation of an individual. Chronological age starts with the birth event and it is this age that osteologists try to reconstruct (Liversidge 1994).

### *1.4.1 Skeletal age estimation*

Estimation of age from the skeleton is based on various measurements, most commonly of long bone length. Only a few reference standards exist that cover the foetal and infant period and most of them only cover the development of single bones (Black and Scheuer 1996; Fazekas and Kósa 1978; Maresh 1970; Molleson and Cox 1993; Saunders *et al.* 1993; Scheuer and McLaughlin-Black 1994). In many cases only few data points are provided for the used age intervals and ranges are generally very large. Whether the large ranges, that are only to increase after birth, are real, or a product of limited observations, remains to be established.

Variation in the timing and velocity of growth, however, is a known feature between populations resulting from genetic differences in height and body shape (Bogin 1999). This necessitates the standards used to predict age from skeletal development stem from populations of similar ancestry.

Skeletal development, as discussed above, does not function solely as an age indicator, but also as a monitor of the environment in which the individual is born. It has been shown that growth performance during the different stages of development (infancy, childhood and adolescence) is similar in well nourished, healthy children regardless their geographical origin (Bhandari *et al.* 2002, Bogin

1999, Onis *et al.* 2006). This shows that the trajectory of growth is universal which is why a marked divergence from this potential can be detected (Neumann and Harrison 1994; Shrimpton *et al.* 2001; Tanner *et al.* 2014). Adverse environmental conditions such as chronic malnutrition and increased risk of disease through prevalence of pathogens will eventually result in reduced weight gain and growth retardation, known as stunting (Frisancho *et al.* 1970; King and Ulijaszek 1999; Tanner *et al.* 2014). Such factors may already become apparent during foetal development if the mother is subjected to insufficient nutrition before and during pregnancy. Infants from chronically undernourished or diseased mothers tend to be small for gestational age (Barker 2001, Grantham-McGregor *et al.* 2007; Mahajan *et al.* 2004 ).

It is expected that insufficient nutrition or chronic disease affect skeletal growth more severely than dental development. A discrepancy between dental and skeletal age might indicate that the individual suffered a period of physiological stress which may have contributed to, or caused his or her death (King and Ulijaszek 1999, 16). As mentioned above, patterns of increased mortality during a particular developmental state may demarcate developmental periods of increased vulnerability or they may provide insights into the practice of child care (FitzGerald *et al.* 2006).

However, a discrepancy between the skeletal and dental developmental systems does not automatically imply reduced health, but may be due to normal inter-individual variability and/or sexual dimorphism (Liversidge *et al.* 1998). Therefore, differences between skeletal and dental age can only act as a preliminary indicator of stress in a sample.

### 1.4.2 Dental age estimation

Dental development is under tighter genetic control than skeletal development, creating increased stability in the sequence and chronology along which maturation is reached (Liversidge *et al.* 1998, 420). Dental age, therefore, better correlates with chronological age and produces a more reliable and accurate estimate (Huda and Bowman 1995). With dental development spanning the time from approximately six weeks post fertilisation until early adult life, it provides a very important tool for ageing subadults (Scheuer and Black 2000, 148). Many textbooks on dental material testify to this advantage (Hillson 1996; Hillson 2005; Hoppa and FitzGerald 1999; Alt *et al.* 1998). Crown mineralisation in deciduous teeth spans the time from around 15 weeks after fertilisation until about 18 months after birth with root formation continuing until about four years (Scheuer and Black 2000). This makes deciduous teeth especially relevant for the study of foetal and infants remains.

Teeth are better able to withstand the harsh conditions of the burial environment compared to bone (Hillson 2005). Teeth that are present in the jaw are covered by enamel, a highly mineralised tissue. Its density and structure make it almost impenetrable for food acids while being able to withstand the masticatory forces (Hillson 1996). The roots, that are made of dentine, are slightly less mineralised than enamel but remain protected by the surrounding alveolar bone in which they are anchored. Deciduous teeth are of comparable robusticity to permanent teeth, although their lower mineral content makes them more prone to diagenetic changes during burial (Shellis 1984). However, in young infants the developing deciduous tooth buds remain protected within their crypts surrounded by alveolar bone, increasing their chance of survival and of them being recovered from the ground during excavation. At age one, only a few teeth have usually started to erupt (Liversidge and Molleson 2004).

Three categories of methods to estimate the age of an individual from their deciduous teeth can be made. First, qualitative methods comprising assessment of maturation, eruption, and exfoliation of teeth (Demirjian *et al.* 1973; Moorrees *et al.* 1963a; Schour and Massler 1941; Ubelaker 1978, 1989). Second, quantitative

methods such as weight and height (Deutsch *et al.* 1981, 1984, 1985, Liversidge *et al.* 1993; Mörnsted *et al.* 1994; Stack 1964). The use of weight, however is not applicable for archaeological specimens, as the reconstruction of body mass from skeletal remains is rather imprecise and easily impacted by postmortem damage to the skeletal features that get measured (Scheuer and Black 2000, 155). And third, histological methods using microscopic incremental markers in dental tissues (Antoine 2000; FitzGerald 1998; FitzGerald and Saunders 2005; FitzGerald and Rose 2008; Huda and Bowman 1995; Mahoney 2011; Reid and Ferrell 2006; Smith 2006; 2008; Smith *et al.* 2006). Each method has strengths and weaknesses which is why research continues in order to produce more accurate results.

The choice of an ageing method is dependent on several factors: 1) the developmental stage of the individual, 2) the elements present for observation, and 3) the degree of accuracy that is desired. Another factor may encompass the number of investigators working on a project, which requires a method whose subjectivity is limited in order to limit inter-observer error (Hillson 2009, 145). When dealing with an age category that only comprises a single year, such as infancy, accuracy will and must be of utmost importance, otherwise it will be impossible to arrive at a well-differentiated age distribution. If accuracy is the aim, three dental ageing methods are considered most suited for infant remains. Two widely used qualitative systems exist that rely on maturation of single teeth, as developed by Moorrees and colleagues (1963a; 1963b) and Demirjian and colleagues (1973; Demirjian and Goldstein 1976). The former has been tested and discussed elsewhere (Saunders *et al.* 1993; Liversidge 1994) and will not be included here because it does not include the entire dentition.

Demirjian and colleagues have developed a system of eight qualitative stages ranging from initial mineralisation to the completion and closure of the root apex which can be applied to every tooth (Demirjian and Goldstein 1976; Demirjian *et al.* 1973). Originally developed for the permanent dentition, the method was recently adapted to the deciduous dentition by Liversidge and Molleson (2004). The Demirjian permanent system is widely applied but tooth stages have been reported to be delayed by almost one year (Liversidge *et al.*

2006, 460). Whether this delay is also present in the deciduous scoring system, still needs to be established.

Quantitative ageing methods rely on a correlation between tooth height and age. Liversidge and colleagues (1993) further developed a method originally introduced by Deutsch and colleagues (1981; 1984; 1985). Liversidge and colleagues provided regression equations to be used on single teeth (Liversidge *et al.* 1993, 308). The method has only been evaluated once by Cardoso (2007), who found a discrepancy between the maxillary and mandibular teeth and, as a consequence, critiqued the pooling of both jaws for the reconstruction of regression equations. A test of the quantitative method developed by Deutsch and colleagues (1985) which also relies on crown dimensions found a high correlation between dental height and chronological age for the first year of life with an accuracy of up to  $0.02 \pm 0.15$  years (Liversidge 1994, 39).

The most common histological ageing methods use regular incremental markings within the enamel of the crown, known as cross-striations and striae of Retzius (Fitzgerald and Rose 2008). Their formation is time dependent and can, therefore, be used in still forming teeth to establish the amount of time that passed from initial mineralisation to the moment of death (Antoine 2000; Antoine *et al.* 2009; Fitzgerald 1998; Smith 2006). Age is inferred from counting the number of cross striations, which represent the daily advance of the enamel secreting cells, or by measuring parts of the crown (Smith *et al.* 2006, 125). It is considered to be the most accurate method for ageing subadults with crowns still developing (Liversidge 1994, 41; Huda and Bowman 1995, 138). This holds true especially for deciduous teeth as they start mineralising during foetal development and, therefore, in most cases, possess the neonatal line, a hypo-mineralised band that forms at birth (Eli *et al.* 1989). By counting from the neonatal line to the last formed enamel, the exact number of days that the individual lived can be established. This method holds great potential but owing to the long preparation phase and the need for technical skills in thin section preparation and subsequent microscopic analysis, it should only be applied in collaboration with skilled personnel.

## 1.5 *The skeletal collection*

Increased attention to subadult skeletal remains in recent years has triggered research into the methods used to predict their chronological age (Antoine *et al.* 2009; Hillson 2009; FitzGerald and Saunders 2005; Phillips and Kotze 2009). In particular, the skeletal collection from Spitalfields, London (Adams and Reeve 1987), provides tremendously valuable source material which has resulted in the development of new ageing methods based on deciduous teeth such as the modified Demirjian stages (Liversidge and Molleson 2004) and crown height (Liversidge *et al.* 1993). However, the general scarcity of documented infant remains makes it difficult to conduct systematic testing of these new methods. Only very limited testing of ageing methods that use deciduous teeth has been conducted so far (Liversidge 1994; Saunders *et al.* 1993; Antoine *et al.* 2009).

This thesis will contribute to this need in that it will add new data from 49 infant remains of an unstudied, recently excavated 19<sup>th</sup> century cemetery collection from the Netherlands. The cemetery was excavated in Middenbeemster in the summer of 2011 by the Leiden University Faculty of Archaeology in collaboration with Hollandia archeologen (figure 1). Middenbeemster is a small Dutch village situated in the province of North-Holland (Noord-Holland). It belonged to a rural Protestant community, which colonised the area of a former lake, the 'Beemster', after its reclamation in the beginning of the 17<sup>th</sup> century (Danner 1986). The Beemster is the oldest reclaimed land in the Netherlands and its artificial landscape is of unique design (figure 2). It was classified as a UNESCO world heritage site in 1998 (de Jong 1998). At the centre of the Beemster polder a church was built (Alders 2006, 12). People from the entire Beemster polder were buried here, in what is now the oldest building of the district. The cemetery was in use from 1617 to 1866 AD (Lemmers *et al.* 2013).

The original clay bedding of the cemetery was cleared once during its use and filled with sand, possibly to ease the digging of graves. Only few burials from the earlier period survived and most interments come from after 1830, when the



Figure 1. Map of present day the Netherlands indicating the provinces and the position of the village of Middenbeemster (red dot) (Source: [http://d-maps.com/carte.php?num\\_car=4115&lang=en](http://d-maps.com/carte.php?num_car=4115&lang=en), accessed 24 July 2014).

land was bought by the municipality to be used as a public cemetery (Griffioen *et al.* 2012). Most of the burials were of wooden coffins, the silhouette of which was clearly visible in the soil during excavation. This helped in the recognition and recovery of the many subadult remains which constitute almost half of the collection. This creates a unique opportunity for the study of individuals under the age of eighteen years in a Dutch, rural, early modern setting. The preservation of the skeletal remains varies but in many cases can be considered good to excellent

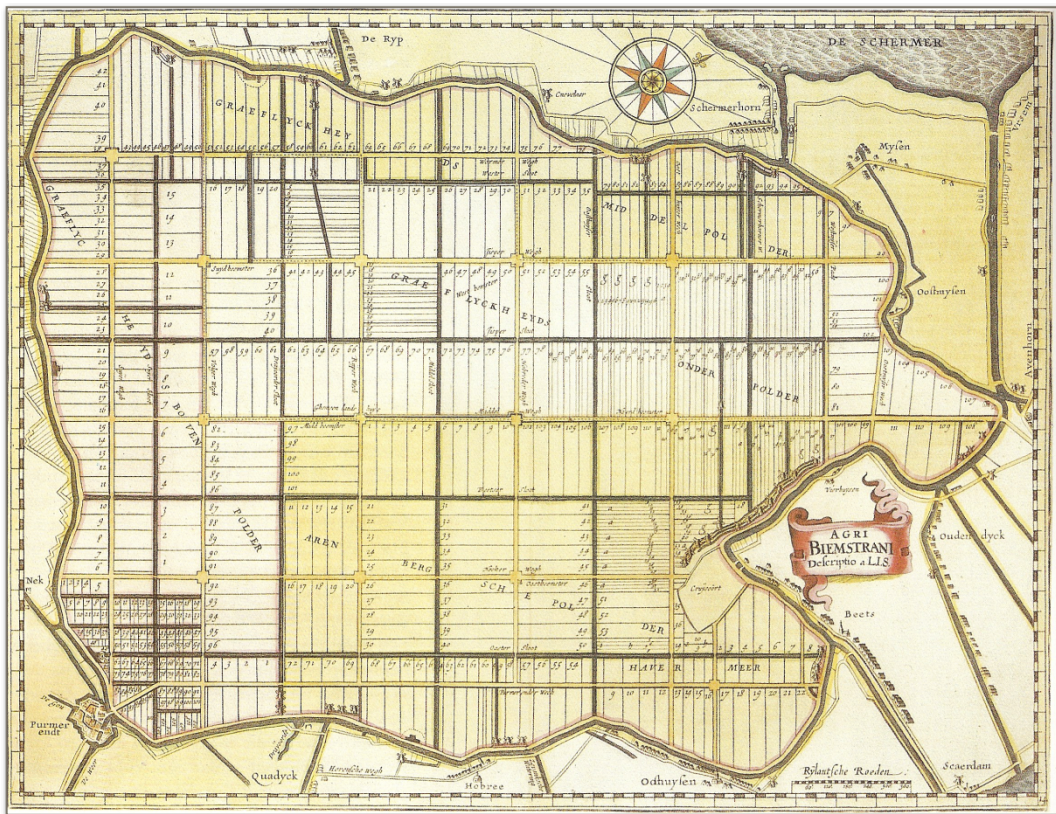


Figure 2. Map of the drained Beemster polder showing the subdivision of the land five years after its creation in 1612 (Danner 1986, 36).

(Lemmers *et al.* 2013). Regarding their fragile nature this is of special importance for the analysis of infant remains.

The archives of Middenbeemster provided information on the inhabitants of the Beemster, largely from the parish records. These records provide the names and ages for many of the individuals interred in the cemetery, together with a plan of the burials. From this it was possible to locate numerous interments and establish the name, age at death, and sex of the deceased. In total, 13 infants (from birth to age one year) have been identified so far of which ten provide an exact age at death.



## 1.6 Research questions

This study will compare the age-estimates of three methods, two of which are based on deciduous tooth development (the Demirjian system modified by Liversidge and Molleson 2004 and the dental height method of Liversidge and colleagues 1993), and the third which is based on skeletal maturation (Black and Scheuer 1996; Fazekas and Kósa 1978; Maresh 1970; Molleson and Cox 1993; Saunders *et al.* 1993; Scheuer and McLaughlin-Black 1994). Individuals with known chronological age will act as a means to statistically evaluate the accuracy of the three ageing methods.

Each method will be analysed separately in two steps: first, individuals of known age are analysed, and second, results are compared to the entire sample. The central research question is which age estimation method is the most accurate for the Middenbeemster infants.

Subsequently, it will be evaluated for all three methods whether the performance of the method is age dependent (i.e. more accurate during the neonatal period as opposed to the post-neonatal period). The rapid change in dental and skeletal development throughout the first year of life needs to be captured properly by the method in order to provide accurate results consistently.

The dental methods are subjected to two subsequent analyses. First, the performance of the individual tooth types (i.e. incisors, canines, and molars) is analysed. Possible patterns visible within the dentition may indicate differences in the timing of dental development between the Middenbeemster sample and the collection on which the methods were developed. Thus, the research question is whether there is a marked difference in the age estimates of the three tooth types.

The second question evaluates whether accuracy of the dental methods is dependent on the number of elements available. The methods make use of the entire dentition. However, archaeological specimens seldom have the entire dentition preserved. Thus, the question is if the accuracy of the dental ageing methods increases with an increasing number of observations.

A third question concerning only the method of Liversidge and colleagues evaluates the critique expressed by Cardoso (2007) on the pooling of the maxillary and mandibular teeth. The question is whether this critique can be substantiated (i.e. that there is a significant difference between the upper and lower jaw) and whether one of the two jaws is more accurate.

Skeletal age estimates are subjected to three additional analyses. First, the accuracy of each measurement of single bones or pairs of bones will be studied, to see whether there exist marked differences in their performance. This will aid in future application of the method to decide on whether or not a measurement is suited for this population. In a second step, it is evaluated whether cranial and post-cranial measurements differ in their accuracy. The third question is concerned with the accuracy of the different skeletal age standards that were employed in this thesis. Each skeletal age standard will be evaluated separately to see which one provides the most accurate results.

Subsequently, two sub-questions are concerned with the mortality of the Middenbeemster infants. In a first step, the age distribution of the infant sample will be studied to see if overall patterns can be discerned. The second step is concerned with the skeletal and dental growth systems to see whether there is a consistent lag between dental and skeletal age, and if so, at which age this begins to manifest itself. A discrepancy between dental and skeletal age will be discussed in light of possible stress periods suffered by the individuals prior to death and whether this can be tied to biological or cultural parameters.

The Middenbeemster skeletal collection is now being studied intensively and each year more biological and cultural aspects of the people living in the Beemster during the 19<sup>th</sup> century become known. The possibility to use historical data adds another dimension to the osteological analysis, providing a means to take on more fundamental methodological questions. This thesis provides much needed data on the applicability of ageing methods based on deciduous teeth. But it will also add to our understanding of infant growth and development from a preindustrial rural area in the Netherlands.

## *2. Infant survival in Middenbeemster during the Nineteenth Century*

The greater part of the skeletal population of Middenbeemster comes from the nineteenth century which was characterised by changing conditions in western Europe as a result of the upcoming industrial revolution (Komoso 1998). The Dutch economy grew steadily during this century even though the country was lagging behind in industrialisation. Together with economic improvements came a steady population growth. However, the agricultural sector could not keep up with the increasing population as well as the increasing demand in traded goods which created higher food prices that resulted in a rising amount of poor people and thus increasing socioeconomic inequalities (Bieleman 1996). Crop failures during the so called 'hungry forties' only added to the trend (Bergman 1967).

The land of the Beemster was mainly used for dairy farming, and the region is still known for its cheese today. Dairy farming was regarded as one of the most prosperous exporting sectors of the Dutch economy (Bieleman 1996), from which it can be assumed that the landowners of the district must have made a good living from their business. It could be argued that the dairy farmers would have a rather good nutritional status as opposed to large parts of the Dutch population who probably suffered from chronic undernutrition (Wintle 2000). The Dutch diet was generally very depleted in essential nutrients consisting of mainly potatoes with few vegetables and bread and sometimes meat (Wintle 2006,74). A more varied diet was only affordable for the middle and higher social classes. The dairy farmers of Middenbeemster would have had sufficient amount of milk and cheese at their disposal to counter the years of famine following the potato blight that struck Western Europe during the 1840's. But the fact that the export was particularly booming, it might have been more convenient for the farmers to trade

their products and to buy cheaper food instead, as did the Frisian farmers that were growing the much demanded wheat (de Vries 1974). Different social classes existed in Middenbeemster, from landowners, rich farmers, craftsmen, to labourers. The latter are considered to be among the poorest of the society. All different classes were buried in the cemetery of Middenbeemster.

While the Dutch population increased steadily, birth rates would remain relatively stable during the 19<sup>th</sup> century until the 1870's and on average 30 to 35 births would be registered yearly per thousand inhabitants of the population (Wintle 2006). A rise in population can therefore only be explained by an increase in the longevity of the dutch population (Wintle 2006). But in general, life expectancy until the 1870's was moderate and on average 36 years for males and 38 for females (Wintle 2000).

Before the 1870's the Dutch population experienced general high death rates that were particularly pronounced in the western (coastal) provinces. Total death rates were highest in the province of North Holland where the village of Middenbeemster is situated, averaging 32.4 per 1000 capita (average death rate for the Netherlands was 26.5/1000 capita) (Wintle 2000, 17). High infant mortality was the leading factor for these reported death rates, and one out of four individuals were likely to die during the first year of life (van Poppel *et al.* 2005). Thus, the prospects of infants born in Middenbeemster during in the 19<sup>th</sup> century were particularly dreadful. Only after the 1870's did a decline in mortality set in which in great part was the result of increased food supply, better hygiene, and improved water quality. The latter was the result of the introduction of the steam pump, which made possible the much more efficient drainage and pumping of the polders (Wintle 2006).

Drinking water in the coastal provinces was particularly bad. The area was almost devoid of fresh running water and especially regions of reclaimed land such as the Beemster, suffered from salination and open standing water where the windmills could not keep up with the rising sea level (Wintle 2000). In these brackish waters the *Anopheles maculipennis artoparvus* mosquito found an ideal place to breed resulting in malaria that was more or less endemic in the coastal parts of the Netherlands (Wintle 2000, 19). The danger of infectious disease was present throughout most of the year. It has been reported from Zeeland, another

coastal province situated in the south of the Netherlands, that from January until June respiratory infections were most common, and during the summer months gastrointestinal infections prevailed, while autumn saw intermittent fevers which were partly the result of malaria infection (Hogerhuis 2003, 46). The chronic gastrointestinal infections resulting from bad quality drinking water and the recurring fevers in autumn are the main factors held responsible for the high infant mortality in the western part of the Netherlands (Hogerhuis 2003; van Poppel and Mandenmakers 2002; Wintle 2000, 19).

Another problem that added to the awful circumstances of infants was the habit of woman to bottle feed their babies instead of providing breast milk (van Poppel *et al.* 2005). The replacement food was often of particular bad quality with very low nutritious content containing pap made of rusk thinned with water, some sugar and sometimes cow milk (Hogerhuis 2003, 47). Keeping in mind the condition of the drinking water, this mixture was potentially lethal to the infants. As reported by Lesthaeghe (1987, 3), the risk of dying was twice as high for bottle fed infants as opposed to breast fed infants. This has been supported by van Poppel and colleagues (2005) who showed that infant mortality differed greatly between provinces where breastfeeding was common practice and where it was not. Until the 1870's infant mortality in the coastal province of Zeeland counted about 250 per 1000 live births (mortality rates were similar to North-Holland), while in Friesland, where breast milk was commonly provided, on average 100 individuals died per 1000 live births. However, the stark difference between the provinces resulted from a combination of feeding practices and a more favourable environment (i.e. better sanitation levels). It was also found that, these two parameters were better able to explain the differences in infant mortality than did socioeconomic status (van Poppel *et al.* 2005). Thus in Friesland, where breastfeeding was common and drinking water was in a better condition than in the polders, families from the lower social classes were better able to provide protection from diseases than in the Province of Zeeland, where infants of the lower class had a much higher chance of dying during the first year of life.

It has been argued that the bottle feeding practices resulted from the workload of the mothers that lived in the country side. Woman would have to work the fields while leaving their newborns at home under the care of their

siblings and the elderly (Hogerhuis 2003). However, Saers (2012) researched the different activity levels of males and females interred in the cemetery of Middenbeemster using cross-sectional geometry of the major long-bones. Activity levels differed greatly among the females indicating that their tasks were more varied. Some woman would stay around the house to care for their children and perform all kinds of domestic tasks, while others who lived on the farms were expected to do the same work on the fields as the males. Thus not all woman would have to leave their infants which suggests that bottle feeding was (at least partly) culturally navigated rather than resulting from pure necessity.

In summarising, the dutch economy was improving during the nineteenth century resulting in overall population growth. However, the nutritious status and living conditions of the Dutch population would only start to improve after the 1860's. The province of North-Holland had a very high infant mortality during most of the 19<sup>th</sup> century resulting from unfavourable environmental conditions and inadequate feeding practices. Mortality can be considered most pronounced among the poorest, who would not be able to provide for clean water and a clean living environment to prevent gastrointestinal diseases. Infants born into the higher economic classes would have a better chance of survival but would equally be in danger of succumbing to the yearly occurring autumn sickness, which was adequately named the 'reclamation disease' (Wintle 2000, 40).

### *3. Skeletal and Dental Growth and Development*

An individual goes through several stages during his or her life cycle. The first two stages encompass the prenatal period and infancy, separated from each other by the birth event. Both stages are further divided into substages, the names and duration of which may differ between fields of research (Scheuer and Black 2000, 5). The prenatal period spans ten lunar months and is divided into three equal trimesters. The period from birth to the end of the first year of life is further divided into two stages, the neonatal period from birth to 28 days, and the post-neonatal period from 28 days to twelve months. See table 1 for definitions of various stages from conception to adulthood that are used in this thesis.

The following chapter is concerned with the general growth pattern of the skeletal and dental developmental systems during the foetal period and infancy, including their correlation. The information is presented to elucidate the limits inherent in the material when applying ageing methods based on skeletal and/or dental characteristics.

#### *3.1 Skeletal growth and development*

Growth is the combination of increase in size and maturity (Scheuer, and Black 2000, 4). The timing, magnitude, and velocity of growth is genetically regulated combining individual variability, sex differences, and ethnic variation (Hauspie and Susanne 1998, 127). However, environmental factors such as disease load, altitude, socioeconomic status, and climate determine whether the genetic potential is achieved at each moment during development (Lewis 2007, 61). Thus, growth needs to be described as an interaction of genetic and environmental

factors (Eveleth and Tanner 1990, 176). Genetic control is more apparent during foetal and early infant development and will slowly lessen with increasing age (Liversidge *et al.* 1998, 421).

### 3.1.1 Growth sequence and velocity

The skeleton starts developing during the embryonic period (Scheuer and Black 2000). By the time of birth the majority of the bones have started forming and are recognisable. Each bone follows its own growth and maturation pattern which is predictable and can be roughly correlated with chronological age (Norgan 1998, 195). The femur, for example, follows a very steady increase in size during the foetal period, creating one of the methods for foetal skeletal maturation assessment that is used to date (Deutsch *et al.* 1981, 236; Meire 1998, 21, but see Lampl and Jeanty 2003 for an opposing argument).

Foetal and infant growth are characterised by high velocity (figure 3). During the foetal period growth in length follows a linear increase that flattens

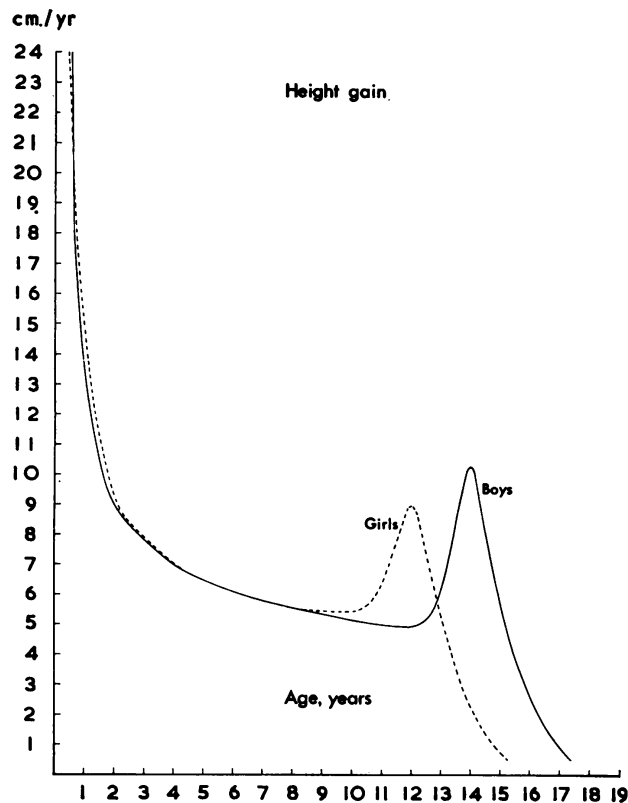


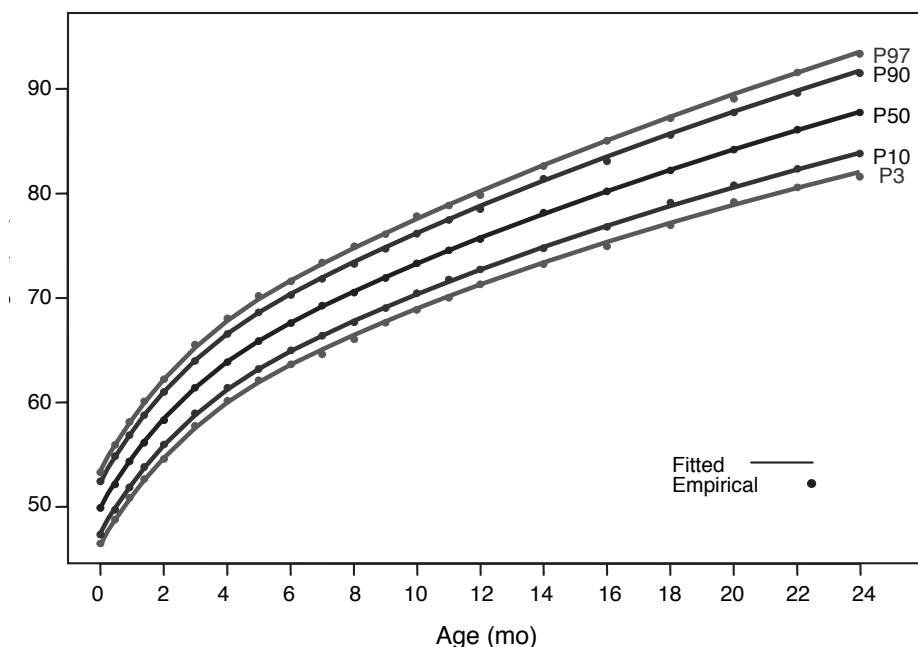
Figure 3. Average height velocity curves for boys and girls of normal growth from birth to cessation of growth (Tanner *et al.* 1966, 466).



slightly when approaching term. The flattening is associated with nutritional constraints of the foetus (Dunn 1985). Growth rate decelerates after birth but remains considerably high during the first three years (Bogin 2003, 16). Height gain will then remain at a low rate until the adolescent growth spurt when final height is reached (Karlberg 1998, 108).

### 3.1.2 Monitoring skeletal growth

Today growth in length is monitored for every child in most parts of the world and the data are used to record abnormal patterns and to take measures if needed. Length is compared to growth reference tables. These tables show the progressive increase in height as a smooth line broken up into several percentiles to account for variation in the speed and magnitude of growth (figure 4). A growth table is mathematically derived as the best fitting curve for distributions of size for age of individuals within a sample or population (Lampl and Thompson 2007, 643).



*Figure 4. Reference table for growth in height (in centimetres) for individuals aged from birth up to two years. The lines represent the 3rd, 10th, 50th, 90th and 97th smoothed percentile curves and the dots are empirical data (WHO Multicentre Growth Reference Study Group 2006, 81).*

There are two different kinds of reference data available that are used to create growth standards. First, longitudinal studies that follow individuals throughout their entire growth process recording gain in length at intervals (Lampl 1998). These longitudinal studies are important to reveal the individual trajectories of growth. Provided the sample is large enough, inferences can be made about the growth pattern and growth velocity of the population. Second, cross-sectional studies are aimed at recording the variation that exists in a population for a certain age category (Scheuer and Black 2004). From a large sample size, the mean height for that particular age group can be generated which will subsequently function to assess individual growth performance. Cross-sectional data represent a moment in time and give no information on the velocity of growth (Masci-Taylor 1998). Archaeological skeletal collections only provide cross sectional data, and consequently lack information on the individual trajectory of growth during that time period. In addition, archaeological reference collections often comprise only limited individuals which therefore are likely to fail to assess the entire variability of growth for each age category.

### 3.1.3 Variation in skeletal growth

Individuals can vary significantly in their timing and rate of growth and maturation (Lampl and Johnston 1996; Tanner 1998). Differences are apparent between males and females, with the latter being approximately ten percent advanced in maturation from early foetal development onwards until adolescence (Saunders 2008, 123). Genetic differences account for variation in growth among individuals and populations (King and Ulijaszek 1999). Environmental factors such as nutrition, disease, living conditions, and socioeconomic status determine if an individual reaches his or her potential height (Saunders and Barrans 1999, 184). Through studying the environment as well as living conditions, the magnitude of environmental interaction with growth can be explained.

While it was expected that normal individual growth progresses within one or two of the percentile lines of a growth chart it was found that this is often not the case. Maresh already observed in 1972 that the rate of growth of the individual

or of one or more of the long bones of the extremities would be more variable in terms of velocity. Growth of the individual would show a pattern that is sometimes faster or slower than the average of the population, and the same was observed for single bones. This pattern was substantiated by Lampl and Thompson (2003) who showed that individual patterns of growth are far more variable than are indicated by the reference tables generated by the World Health Organisation (the implications of this will be further discussed below in section 3.3). Thus, while the use of a standard reference is necessary to assess general growth and development, individual variability in the growth system has to be kept in mind.

#### 3.1.4 Skeletal growth as indicator of stress

Skeletal development is very sensitive to disturbances (Halcrow and Tayles 2011, 341). Retardation in growth and development, if not of congenital origin, is the consequence of adverse living conditions, which can result in a juvenile being short for age or stunted. If conditions are improved, catch up growth will occur (Lewis 2000, 67; Eveleth and Tanner 1990, 192). Growth retardation can already become apparent *in utero*. Apart from the genetic determinant which accounts for about 30 percent of foetal development other factors play an important role as well, such as the health, behaviour, nutritional, and emotional status of the mother (Barker 2001; Bogin 1999; Mahajan *et al.* 2004). Studies have shown that low birth weight infants have an increased risk of dying even if born full term (McIntire *et al.* 1999). In America during the 1980's it was found that 80 percent of late foetal and neonatal deaths were due to developmental retardation of the foetus, caused by a great variety of environmental and congenital conditions (Bogin 1999, 61). The effect of stunting, especially during infancy can have life-long consequences. It has been shown that growth faltering during the first months of life is the main cause for short adult stature in the developing world (Karlberg 1998, 112). Thus, while catch-up growth can occur it does not make up for all the deficit.

Susceptibility to growth disruption can differ throughout the life course and depending on the developmental stage, an individuals reaction to malnutrition and

disease may change (Halcrow and Tayles 2011, 337). From birth to approximately five years of age, the individual is most vulnerable to undernutrition and infection (Eveleth and Tanner 1976, 241).

Growth is very energy demanding and one of the main determining factors of normal juvenile growth is sufficient nutritional intake (Saunders and Barrans 1999, 184). Malnutrition, together with infectious disease (especially of the gastrointestinal and respiratory tracts), are listed as the leading cause for reduced height in juveniles (Humphrey 2000, 23; Ulijaszek 1997; Black *et al.* 2003). But general health, physical, and emotional stress are also of importance for normal growth and development (Eveleth and Tanner 1990, 1; Skuse 1998). There has been a noted difference in the growth outcome in children of similar origin but different socioeconomic status (Bogin 1999). Socioeconomic status shapes the entire environment the individual grows up in. It can determine the number of nutrients available for the individual. But it also defines the amount of education parents can get, which in turn determine the family income and the ability to provide for a safe and healthy environment (i.e. the amount of emotional and physical stress) for the growing child (Bradly and Corwyn 2002). Socioeconomic status is thus an important explanatory factor for a poor growth outcome.

In the past, a chronic shortage of certain nutrients (especially vitamins and minerals) was probably the norm rather than the exception (Bergman 1967). As has been shown by Wintle (2006) the Dutch diet during the nineteenth century was very monotonous and much depleted in essential nutrients such as vitamin D and iron. Thus, when assessing the age of late foetal and perinate archaeological remains it is of importance to be aware of these constrains because they might lead to an underestimation of age.

An ultimate aim of an osteologist would be to reconstruct the health of a population from the collection under investigation. However, such an inquiry is problematic as the sample reflects the health of non-survivors and will therefore be biased (Wood *et al.* 1992). What is being assessed instead is an individual frailty, or susceptibility to disease and death, of infants who found their way into the cemetery collection (Milner *et al.* 2008, 566). Patterns in the age distribution of a group will reveal factors that increased frailty for the specific age category.

Such patterns are a sensitive indicator for socio-economic and environmental conditions.

In this study skeletal growth will be assessed in conjunction with dental development. Non-specific stress markers such as Harris lines, cortical thinning of the long bones (Mays 1999), or non specific skeletal lesions such as cribra orbitalia and porotic hyperostosis (Lewis 2000; Halcrow and Tyles 2011; Magennis 1998; Wheeler 2012), are beyond the scope of this thesis, but will be studied in future research on the Middenbeemster collection.

### *3.2 Dental growth and development*

The newborn infant has ten deciduous tooth crowns developing in each jaw. Tooth development can be divided into several stages: initialisation of tooth formation, tissue secretion (crown and root formation), eruption, root resorption, and exfoliation. The last two stages only apply to the deciduous dentition as these teeth are shed from about six until eleven years of age to be replaced by the permanent dentition (Scheuer and Black 2000, 151). The deciduous dentition consists of four incisors, two canines and four molars for each jaw. From an evolutionary perspective, however, the molars should correctly be categorised as third and the fourth premolars (Hillson 2005, 44). The following description will focus on the deciduous dentition, however, the development of the permanent dentition follows the same principles. Deciduous teeth differ from permanent teeth in morphology, size, their developmental timing, a higher developmental rate, and a lesser degree of mineralisation.

#### *3.2.1 Embryonic dental development*

Tooth development starts six weeks after fertilisation (Nanci 2008, 89). An epithelial band forms over the mesenchyme, lining the oral cavity, at the location of the dental arcades of the future upper and lower jaws. From this band the dental

lamina differentiates which will form the teeth. The mesenchyme will eventually form the supporting tissues, such as muscles, cartilage, and bone of the jaw (Hillson 2005). During embryological development the tooth goes through three successive stages: the bud stage, the cap stage, and the bell stage (figure 5). The bud stage marks the thickening of the dental lamina at places where the deciduous teeth will be situated (Hillson 1996, 118). During the cap stage, the germ proliferates into the mesenchyme, forming the enamel organ, which is to form the enamel of the crown. Around the dental organ the mesenchyme condenses and becomes the dental papilla, which will eventually form the dentine and the cementum (Nanci 2008). The dental papilla is surrounded by another layer of condensed mesenchyme, known as the dental follicle.

The the bell stage includes 1) the establishment of the crown shape, called morpho-differentiation, 2) histo-differentiation, which involves differentiation of cells into ameloblasts (enamel secreting cells) and odontoblasts (dentine secreting cells), and 3) start of tissue secretion, known as initiation (Hillson *et al.* 2005,

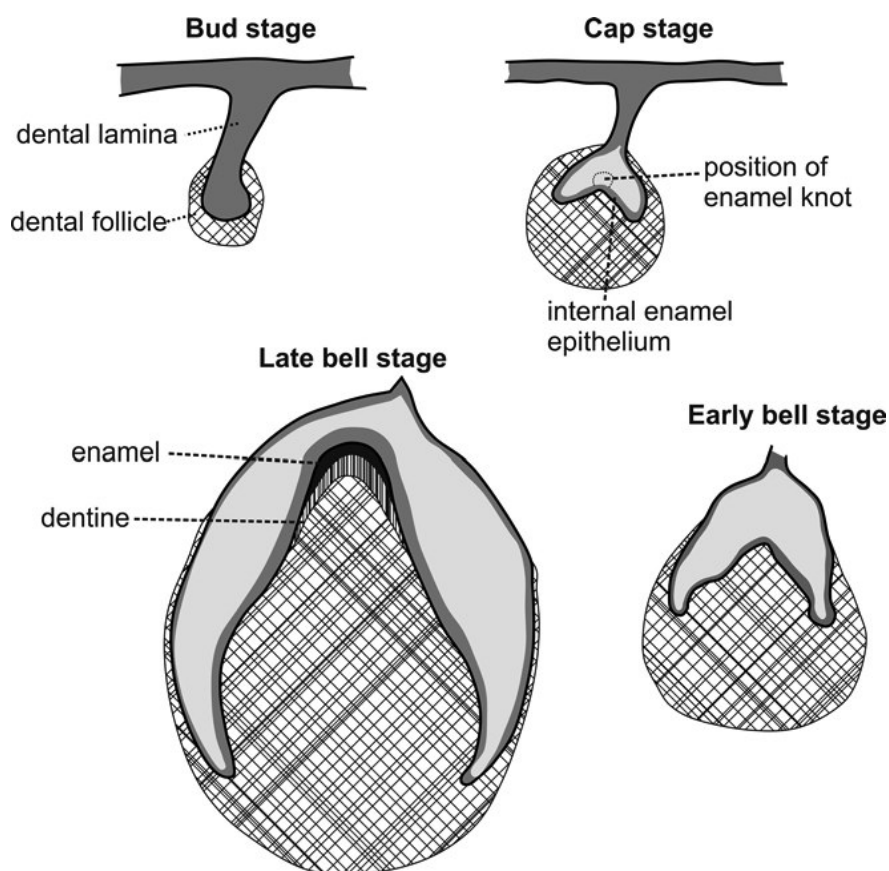


Figure 5. Successive stages of the developing tooth germ (Simon Hillson 2005, 209).

208). Differentiation of cells takes place along the border between the dental papilla and the dental organ. Odontoblasts start first to secrete the initial layers of dentine, triggering the ameloblasts to follow shortly to secrete the enamel in the opposite direction, producing the enamel dentine junction (EDJ). Ameloblasts move coronal towards the crown surface while odontoblasts are moving down apically towards the pulp chamber. As soon as secretion starts, the dental papilla is called the pulp (Nanci 2008, 198).

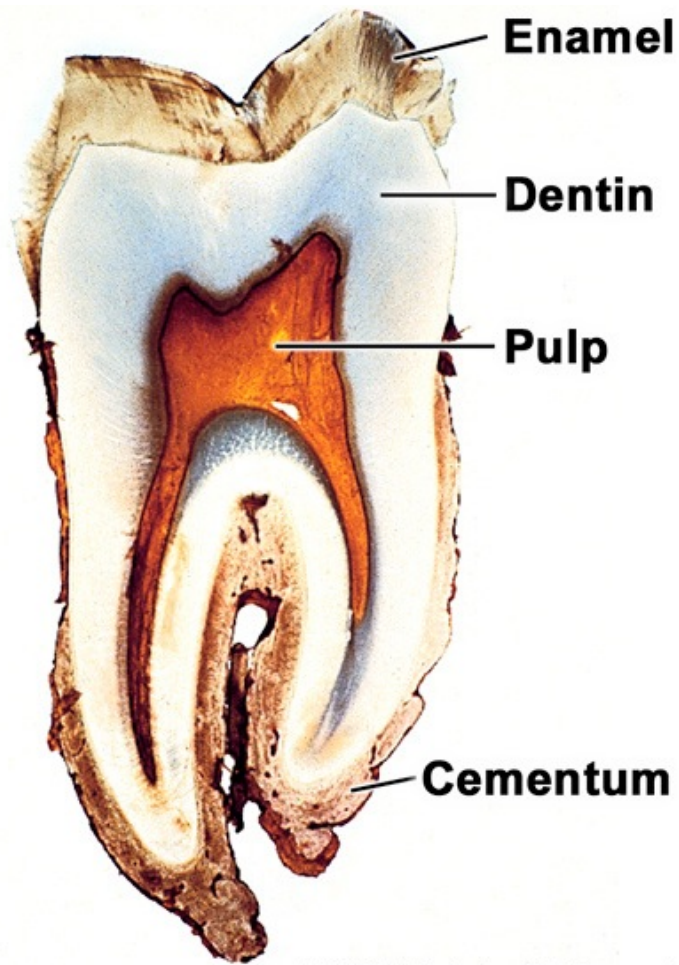
### 3.2.2 Dental tissues

Teeth consist of two parts: a crown and a root. The crown is the only part of the tooth visible *in vivo* and is covered by a hard, white substance called enamel. The root anchors the tooth in the bone and is covered by a layer of cementum forming the attachment for the periodontal ligament, which holds the tooth in place. The greater part of the tooth is formed by dentine which supports the enamel cap and makes up the root. The dentine encloses the pulp chamber and the root canal. The pulp chamber contains the soft tissue of the tooth while the root canal provides blood and nerve supply to the chamber (figure 6). The planes separating the different tissues are called: the enamel-dentine-junction (EDJ), the cemento-dentine-junction (CDJ), and the cemento-enamel-junction (CEJ). The outer junction between the crown and the root is called cervix.

#### *3.2.2.1 Enamel*

Enamel is the hardest tissue in the human body. It covers the softer parts of the tooth to protect it from the acidic environment of the mouth. Enamel is laid down in a rhythmic fashion giving it the appearance of layers that have been compared to the formation of tree rings (Massler *et al.* 1941, 33). Matrix secretion starts at the EDJ, where odontoblasts (dentine secreting cells) and ameloblasts start secreting dentine and enamel respectively, moving in opposite directions.

Ameloblasts leave behind bundles of rods/prisms as they travel from the EDJ toward the future surface of the crown (Nanci 2008). The undulating and intervening path of the ameloblasts cells create a very strong structure needed to



*Figure 6. A longitudinally sectioned tooth showing the different dental tissues (Liebgott 2001).*

withstand the masticatory forces applied to the teeth. When the ameloblasts reach the surface the prisms will undergo a maturation phase which reduces the organic content until the enamel consists of 96% inorganic material, less than one percent of organic matter and water (Hillson 2005, 155). After crown completion the ameloblasts remain inactive lining the surface of the crown and are subsequently shed during eruption of the tooth into the mouth. Enamel is a dead tissue and has no ability to remodel once it is formed.

#### *3.2.2.2 Dentine and pulp*

Dentine is less mineralised than enamel. It consists of 72% inorganic material, 18% collagen, and two percent other organic material (Hillson 2005, 184).



Dentine is formed by odontoblasts, which secrete the tissue in two steps: first, the pre-dentine is secreted, consisting of organic matrix in which during the second step crystallites are seeded, which grow until their expansion is hampered by one another. Dentine is a living tissue, although it does not remodel after it is formed. Secondary dentine, however is continuously laid down on the roof and the walls of the pulp chamber which contains the soft tissue of the tooth (Hillson 2005, 185). The odontoblasts do not die after matrix secretion but are lined around the margins of the pulp chamber. Their processes remain in so called *dentinal tubules* which run through the entire thickness of the dentine (Nanci 2008). Dentine forms the bulk of the tooth, but its higher organic content makes it more susceptible to diagenetic changes after burial than enamel. In archaeological material, dentine tends to become brittle and may be lost. However, specimens have been found with perfectly preserved dentine (Hillson 2005, 190).

#### 3.2.2.3 *Cementum*

Cementum contains 70% inorganic components and 22% organic material of which 21% is collagen (Hillson 2005, 193). It is formed by cementoblasts and covers the part of the tooth anchored in the socket of the bone. Cementum creates the attachment for the periodontal ligament. Small collagen fibres of the cementum are combined with large fibres of the periodontal ligament to create strong bondings between the two tissues (Hillson 1996, 199). Blood and nerve supply is provided only by the periodontal ligament, which also carries the cement forming cells. Cement, unlike enamel and dentine gets remodelled in case of injury or increased masticatory strain (Hillson 1996, 198). Cementum resembles bone very closely in its composition and in its ability to adapt to physical activities.

#### 3.2.3 *Dental growth and eruption pattern*

The sequence of tooth mineralisation generally commences with the anterior teeth between 16-18 weeks post-fertilisation, proceeding posteriorly until the second deciduous molar has started mineralising by about 35 weeks (Deutsch *et al.* 1981;

1984; 1985; Hillson 1996; Kraus 1959). Central incisors complete their crown approximately one month postnatally. Canines complete their crown between 0.7 and 1.4 years, crown completion for first molars ranges from 0.4 and 0.8 years, and second molars are more varied, ranging from 0.7 to 1.4 years (Liversidge *et al.* 1993, 309).

Eruption will commence about three month postnatally and end by about the age of 30 months, with root development and apex closure complete around four years of age (Scheuer and Black 2000; Schaefer *et al.* 2009). The order of eruption is the same as the order of crown completion in the deciduous dentition (Liversidge 2003, 84).

#### 3.2.4 Variation in dental development

Girls are advanced by three percent in development of their permanent dentition and differences of up to one year have been reported (Hillson 1996, 125). However, other studies found no significant difference between boys and girls under the age of five (Demirjian and Levesque 1980). In the deciduous dentition, the difference in timing of tooth development between girls and boys appears even less pronounced and has been reported to be of no significance (Demirjian and Levesque 1980). Especially the early stages are very similar between the sexes. A minor sexual dimorphism is present between tooth dimensions of the deciduous teeth, however not pronounced enough to be used to differentiate between the sexes (Black 1978; Hillson 2005).

Variation in tooth dimensions and/or morphology may also stem from population differences, inadequate nutrition, and poor health (Goodman and Song 1999, 219; Hillson *et al.* 2005). However, the amount of variation is less than is known for skeletal development (Hillson *et al.* 2005, 211). Dental developmental timing seems to be unaffected by adverse living conditions. A recent study by Elamin and Liversidge (2013) showed that malnutrition has no influence on the timing of human tooth formation.

Ethnic differences in the timing of dental development have been researched by Liversidge (2011). She tested the possible difference in permanent dental

maturation between Bangladeshi and white children living in London and found no significant variation. Sex differences in the combined groups were only apparent in root stages for the permanent canine and premolars using the dental maturity method by Moorrees and colleagues (1963b). As differences are generally less pronounced in the deciduous dentition it can be assumed that no significant variation exists between populations. However, when assessing the dental developmental state of an individual it is always recommended to use standards coming from populations of similar origin.

Other differences are of intrinsic nature. According to Stack (1967), deciduous teeth vary in their formation rate throughout their development. Differences also exist between tooth types as has been proven by Liversidge and colleagues (Liversidge *et al.* 1993, 309), who found that anterior teeth develop at a faster rate than molars.

### 3.3 *Skeletal versus dental development*

Several ways exist to assess poor growth in an archaeological skeletal sample. When using dental development as age indicator, the sample can be compared to other archaeological populations and to modern standards (Mays 1999). Comparison to a modern standard gives insights into the magnitude of stunting compared to modern children. But it does not account for variation that may exist in the timing of developmental stages between the compared populations. Therefore, growth in young individuals should also be assessed against dental development (Mays 1999, 291). A discrepancy between both developmental systems will indicate insufficient growth against individual development (Humphrey 2000, 29). However, this is not as straightforward as it appears. Dental and skeletal development follow different developmental tracks, which may not always coincide (Hillson 2005, 213).

As discussed above, a skeletal growth curve expresses development in a linear fashion, which may well be compared to dental development. However,

Michelle Lampl has proven the pulsatile nature of skeletal growth (1993; Lampl and Jeanty 2003). In her study, she followed individuals on a daily or weekly basis for a minimum of 40 days to a maximum of 21 months and observed that height increased in short bursts, lasting for approximately 24 hours, with periods of stasis in between, ranging from two days up to 51 days (Lampl 1993). These pulses proved to be non-periodic and their velocity, amplitude, and frequency varied between individuals (Lampl 1993, 648). This research has great implications for infant growth assessment in archaeological remains. The division of the youngest age category into foetal, perinate, neonate, and post-neonate operates with categories that may only encompass several weeks. If, for example, the neonatal period is examined (birth to 28 days), an individual may be characterised as short for age, while in fact it has died within two growth pulses. A discrepancy between dental and skeletal development could, therefore, equally mean, the individual experienced a period of normal growth stasis. However, as said, the saltations happened in a non-periodic manner and the magnitude and frequency of the bursts would differ between individuals. It should, therefore, be expected that delay in growth would be randomly distributed in the sample. Thus, clear patterns of delay in certain age groups should be taken as indicative for poor environmental conditions upsetting physical well-being. The occasional occurrence of a slight growth retardation, however, cannot be singled out from normal pulsatile growth.

Another difficulty may arise from the reported slowing down of skeletal growth when approaching term. Dunn (1985) reported the growth faltering to be minor, but with dental development advancing rhythmically every day (Antoine 2000), a slight discrepancy may develop, especially if the birth process proves to be difficult as well. Growth cessation after birth lasts about one week. If growth is normally constrained during the perinatal period, a pattern should be visible in which dental development is slightly advanced in the normal developing perinate. Such observations have not yet been reported, but this could well be due to lack of research on this matter.

An additional point that needs to be mentioned, is that different standards are used for skeletal and dental development. It is not known how the two systems correlated in the samples on which the standards were developed. This poses a major problem for using this kind of correlation on archaeological material as

there is no way this can be tested. A deviation from the standards can be indicative of genetic differences between the sample under study and the standard itself. Individuals in the past could well have had different genetic growth potentials (Humphrey 2000, 35).

The magnitude of the above posed problems has not been assessed at this point. Equally, it has not been defined when growth is considered retarded against dental development. This thesis will evaluate whether this stated discrepancy between both developmental systems is apparent in late foetal and young infant remains.

### *3.4 Summary*

Skeletal and dental development at first sight seem to encompass a straightforward process, but in fact are the result of a delicate interaction between the genotype of an individual and its environment. Both developmental systems follow different tracks and vary markedly in their susceptibility to adverse living conditions. Environmentally adverse conditions will potentially reveal themselves through growth retardation of the skeleton. In comparing the two developmental systems it is revealed that dental growth follows a steady path whereas skeletal growth has a pulsatile nature. A comparison of dental and skeletal development assesses the unique growth and maturation patterns of an individual. A greatly reduced height could be the outcome of stress suffered by the individual, while small differences are most likely the result of differences in the way the dental and skeletal systems grow.

## 4. *Skeletal and Dental age estimation*

The osteologist in general tries to assess the age of an individual using dental and skeletal indicators. Due to variability in skeletal growth outlined above, dental development is generally given more weight in the age estimation. But for very young age categories skeletal development should be considered an important age indicator too. This chapter briefly discusses osteological age estimation using skeletal and dental characteristics focussing on the methods chosen to be evaluated in this thesis.

### 4.1 *Skeletal age estimation*

Skeletal age in subadults is generally assessed through 1) appearance of primary and secondary ossification centres, 2) morphology and size of bones, and 3) fusion of ossification centres (Scheuer and Black 2000, 7). During infancy, size of the bones is a good indicator of age resulting from a strong genetic control on growth during foetal and early infant development (Liversidge 1994, 39). However, skeletal development in general reflects biological age, which describes the degree of adult maturity reached by the time death occurs (Scheuer and Black 2000, 6). As outlined in the introductory chapter, to be able to translate the maturity status of a person into chronological age, studies have focused on collecting growth data from living children or individuals of known age-at-death (Alemán *et al.* 2012; Black and Scheuer 1995; Cardoso 2006 Greulich and Pyle 1959; Maresh 1955; Rissech *et al.* 2013; Saunders *et al.* 1993; Tanner and Whitehouse 1975). Depending on whether the studies used modern or

archaeological individuals, these measurements are either based on dry bone or on radiographs.

#### 4.1.1 Potential drawbacks

As discussed above, genetic and environmental conditions create individual variation that is already apparent early in infancy. Such variation potentially creates wide ranges that only increase with increasing age. In addition, due to the inability to reliably estimate the sex of juveniles until after adolescence, larger age ranges have to be incorporated in order to account for differences in the timing of growth between boys and girls (Saunders 2008).

Apart from the variability inherent in the growth system itself, concerns surrounding its use as an age indicator in osteological research rests with the standards available. Several factors need to be discussed here. First, most growth standards have been developed using radiographs of modern healthy, living children (Saunders 2008). As already laid out in the introduction, the pattern of growth and maturation has changed substantially from the late nineteenth century until now. It is not known how much this will affect the method when applied to an archaeological sample that dates to before the onset of this secular trend.

Second, comparing radiographic images to dry bone can be problematic because they are in fact a two dimensional rendering of a three dimensional object which may lead to distortion of the original object (Hillson 2009, 142; Scheuer and Black 2004). In addition, the radiological image has limited sensitivity to detect newly mineralising tissue. By using reference data that are derived directly from skeletal remains problems related to the interpretation of radiographic images can be circumvented. But in general, the slight delay in detecting the advance of mineralisation in radiographic images is only of importance when assessing the initial mineralisation of new tissue such as the first appearance of primary and secondary ossification centres.

Using a combination of modern and archaeological standards will help in reducing the effects of the secular trend as well as radiographic differences, while

providing a large number of observations that is needed to capture the entire growth variation (modern standard).

A third aspect that needs to be kept in mind is that the population on which a standard is developed may differ in its sequence and timing of growth compared to the population under study (Black and Scheuer 1996). Thus, not all standards may be equally suited for the skeletal collection under study.

These apparent disadvantages have resulted in skeletal growth being described as the least reliable age indicator in juvenile osteology (Scheuer and Black 2000, 6). In fact, studies that try to estimate age based on skeletal remains generally confine their analysis to the dental record, and no study exists that actually tests the reliability of the growth standards on archaeological skeletal collections. It can be assumed that the strong genetic control during prenatal growth, and to a decreasing extent during infancy, should produce estimates with acceptable accuracy levels. This will be evaluated in this thesis.

## 4.2 *Dental age estimation*

Dental deciduous development has only limited interaction with the environment because a large part of it takes place in the protected uterine environment (Eveleth and Tanner 1976). This generally strong genetic control minimises environmental influences even after birth. Eveleth and Tanner (1976, 207) explain the adaptive significance of tight genetic control of development and eruption of deciduous teeth as due to an increased chance of survival of the infant. With delayed dental development the young individual will not be able to eat proper amounts of food and thus faces undernutrition. This makes dental ageing methods most reliable and although accuracy decreases with increasing age (or when crown formation is completed) it remains high for deciduous teeth (Liversidge 2003). Root formation has been found to be more variable than crown development in deciduous and permanent teeth (Liversidge *et al.* 1993). The following part will discuss the two macroscopic methods, chosen to be tested in this thesis.



#### 4.2.1 The deciduous Demirjian stages by Liversidge and Molleson (2004)

The Demirjian system comprises eight qualitative stages ranging from initial mineralisation to the completion and closure of the root apex named alphabetically from A to H (Demirjian and Goldstein 1976; Demirjian *et al.* 1973). Each stage is given an age-range in which it is expected to occur. The method scores single teeth separately which are combined and the resulting mean will act as the final age estimation. The method was first introduced in 1973 by Demirjian and colleagues and was developed on the left mandibular dentition which could be extrapolated to all mandibular teeth. The updated version by Demirjian and Goldstein (1976) included more individuals on the earlier and later stages to give a more complete picture of the development of single teeth. The original method was developed from panoramic radiographs from 1446 boys and 1482 girls of French Canadian origin (Demirjian and Goldstein 1976; Demirjian *et al.* 1973).

Liversidge and Molleson (2004) adapted the original system to the deciduous dentition. The authors developed the deciduous system on a sample of 121 skeletal remains from the crypt of Christ Church, Spitalfields, London, dating between AD 1729 and 1852. Individuals were aged between birth and 5.4 years of age. For 53 of the individuals chronological age was known. For the remaining individuals age was calculated using the dental height regression equations for deciduous and permanent teeth developed by Liversidge and colleagues (1993) and Liversidge and Molleson (1999), respectively. To remedy the lack of older individuals the sample was supplemented by rotational pantomographs of 61 modern living children. In addition, two Scottish archaeological skeletal collections of unknown age at death (n = 133) were studied. Their dental development was assessed in relation to mandibular molar development at stage D and F to see whether differences exist in the timing of development between the populations. This was found not to be the case and results of all samples were pooled.

Liversidge and Molleson used the eight stages defined by Demirjian and colleagues and included an additional root stage (table 2). Their description is adapted in such a way that the method can be used on skeletal material as well as

radiographs. As the stages have been adapted from the Demirjian system, in order to keep the terminology they will be called the deciduous Demirjian system/stages in this study. The general age range for this method is from one month postpartum to approximately four years of age (Liversidge and Molleson 2004, 176).

*Table 2. Descriptive criteria of the crown and root Demirjian developmental stages for deciduous teeth (Liversidge and Molleson 2004, 174).*

Stage A.	<b>Canine:</b> Beginning of mineralization is seen as a cusp tip, which has not yet reached maximum mesiodistal dimension of the crown.
Stage B.	<b>Incisors and canine:</b> Mineralized incisal edge/cusp tip has reached maximum mesiodistal width of tooth. <b>Molars:</b> Coalescence of cusp tips to form a regularly outlined occlusal surface.
Stage C.	<b>Incisors and canine:</b> a) Enamel of incisal surface is complete. Approximal edges of forming crown have reached future contact areas. b) Dentine is visible below incisal enamel. <b>Molars:</b> a) Enamel of occlusal surface is complete. Approximal edges of forming crown has reached future contact areas. b) Dentine is visible below occlusal enamel and beginning along sides (however, dentine is not full-thickness).
Stage D.	<b>Incisors and canine</b> a) Enamel is complete down to approximal enamel-cementum margins, with full-thickness occlusal dentine present, and roof of pulp chamber is mature. b) Beginning of root formation is seen as a dentine spicule approximally (both sides). <b>Molars:</b> a) Enamel is complete down to approximal enamel-cementum margins (not visible mesially if cusp of Zuckerkandl is present/pronounced), with full-thickness occlusal dentine present, and roof of the pulp chamber is mature. b) Beginning of root formation is seen as a dentine spicule approximally (both sides).
Stage E.	<b>Incisors and canine:</b> Root formation is more than a spicule, but root length is less than crown height (measured approximally). <b>Molars:</b> a) Initial formation of root bifurcation is seen in the form of a mineralized point or semilunar shape. b) Root length is less than crown height (measured approximally).
Stage F.	<b>Incisors and canine:</b> a) Root walls are very thin, and root length is equal to or greater than crown height (approximal). b) Root length is incomplete, with diverging apical edges. <b>Molars:</b> Midway down root, root wall is thinner than root canal.
Stage G.	<b>Incisors and canine:</b> Root length is almost complete, but apical edges are parallel or slightly converging. <b>Molars:</b> a) Mesial root length is almost complete, but apical edges are parallel or slightly converging. b) Midway down root, root wall is thicker than root canal.
Stage H1.	Root length complete, with apical walls converging, but apex is still open (width 1 mm). Mesial root of mandibular molars, mesiobuccal root of maxillary molars.
Stage H2.	Apical dentine edge is sharp. Apex is only just visible/closed (width 1 mm).

#### 4.2.1.1 *Potential drawbacks*

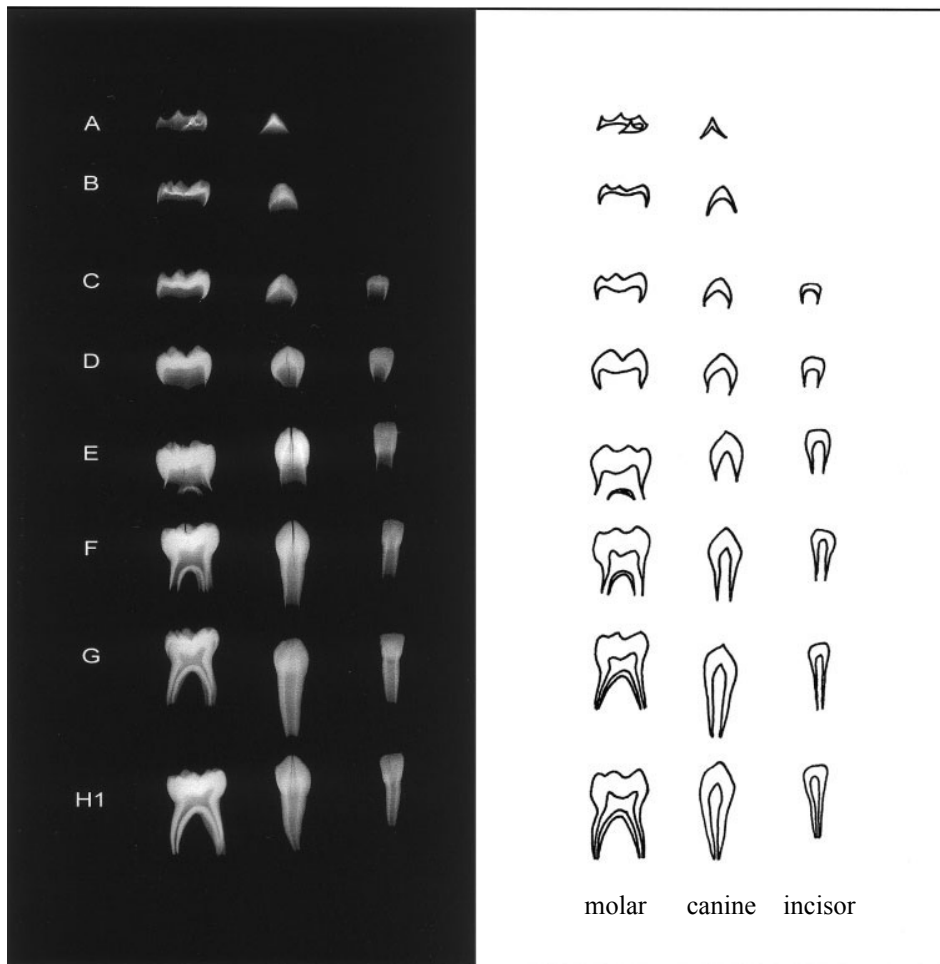
A major criticism towards qualitative ageing methods is that they divide dental growth and maturation into separate stages, and are thus failing to capture the continuous nature of the development (Liversidge 1993, 312). Larger ranges need to be applied in order to include all cases that have reached a particular stage within a certain time period. Additionally, teeth may be found in an intermediate stage which then need to be upgraded or downgraded according to the system (Hillson 2005, 130).

Another drawback concerning the Demirjian stages is that they originally stem from radiographs of living children. Radiographs only show fully mature enamel and may, therefore, be delayed by up to a month (Antoine *et al.* 2009). There is always the difficulty of translating a radiographic derived model image to the dry tooth assessment of archaeological specimens. See figure 7 for radiographic images of the stages and line drawings thereof.

In addition, developmental differences may be present between the archaeological population under investigation and the population on which the method was developed. Liversidge and Molleson tried to bridge such a potential gap by using archaeological material in combination with modern data. From this it may be assumed that the method is better suited for archaeological material than the original one. The fact that the material from Christ Church, Spitalfields, London is partially contemporary with the Middenbeemster collection can be considered a great advantage. Whether the London data are representative for a Dutch rural community, however, remains to be established.

A related problem to the use of arbitrarily defined stages rests in the subjectivity of stage assessment resulting in intra- and inter-observer error (Levesque and Demirjian 1980). Moreover, the method requires a certain amount of practice to produce consistent results.

Another possible drawback that might impair the accuracy of the method lies in the manner the method was developed. The data used for this method stemmed only partly from individuals of known age. In total 58 individuals could be used for the infant category, while the Spitalfields sample has 37 infants of known age at death. However, several of these individuals are aged younger than



*Figure 7. Radiographic images of the eight successive developmental stages of each tooth type (molars, incisors, and canines) including line drawings (Liversidge and Molleson, 2004, 175).*

the lower boundary of the method indicating that the proportion of individuals with known age at death is probably less. For half of the individuals of the Spitalfields collections age had to be estimated using the dental height regression equations by Liversidge and colleagues (1993). The same holds true for the additional data provided by the two Scottish collections. The 61 modern living children that added additional data were all aged two years and older, thus no contribution was made for the age category that is of concern in this thesis. The problem inherent in relying on an ageing method that is partly based on age estimations, is that it reflects biological age rather than chronological age and therefore represent a circular argument (Scheuer and black 2004, 3). In addition, the fact that two methods will be compared of which one (the deciduous Demirjian stages) is partly based on the other (dental height) is ironic and may

lead to false results. A general flaw in the comparison therefore arises, as agreement between the methods can either be interpreted as representing good accuracy or that the Demirjian system mimics dental height age estimates. Whether their similar origin leads to increased similarity between the age estimates of the two methods will be assessed through comparison with chronological age.

Another major drawback of the Demirjian stages for the deciduous teeth is a lack of sufficient data on the early stages of tooth development, and especially the anterior teeth. This creates a gap for data on perinates and neonates. Liversidge (1999) found that when applying the permanent stages to individuals that are aged around the lower boundary of the method, they tended to be underestimated. The same potential problem is dealt with in this thesis and will be discussed in more detail below (section 7.2). Whether the above mentioned drawbacks have major implications for accuracy and precision of age estimation will be assessed in this thesis.

#### 4.2.1.2 Accuracy

The permanent Demirjian stages are widely applied and studies have found varying degrees of accuracy, but with a general trend to overestimate age (Maber *et al.* 2006; Liversidge *et al.* 2006; Saunders *et al.* 1993; Willems *et al.* 2001). A test of several radiographic permanent dental ageing methods, including the permanent Demirjian stages, was conducted by Maber and colleagues (2006). The test sample included modern children of mixed ethnic origin, aged between three and 16.99 years. They found that the permanent Demirjian stages overestimated chronological age by 2.9 months ( $\pm 10.3$  month) (Maber *et al.* 2006, 68). Liversidge (1999), in her comparison of the Spitalfields collection with a modern sample, found that in both samples the permanent stages were underestimating younger individuals. This was interpreted as being the result of limited data on earlier permanent dental stages. The mean difference between chronological age and the maturity score was -5.5 months ( $\pm 12.7$  month). There has not been any testing on the deciduous scoring system.

#### 4.2.2 Dental height by Liversidge and colleagues (1993)

This quantitative ageing method relies on a strong correlation between tooth height and age during dental development. The method was originally developed by Deutsch and colleagues (1981; 1984; 1985), who found a good correlation between crown height and age during the foetal period (1984). Liversidge, Dean, and Molleson developed regression equations for deciduous and permanent teeth, that enable the easy use of dental measurements on single specimens (1993). The study was conducted on the same archaeological collection that provided data for the Demirjian stages for deciduous teeth, the Christ Church Spitalfields, London, sample (see above). In contrast to the Demirjian stages no additional collections were included. In total 304 single teeth were use form 63 individuals of known age. According to the authors quantitative assessment, dental height is less subjective in its assessment than the Demirjian developmental stages and has a higher accuracy than qualitative systems in general.

##### *4.2.2.1 Potential drawbacks*

Some disadvantages exist for the quantitative assessment of dental height. First, differences in initialisation of mineralisation can produce large ranges, as can individual variation in tooth size (Liversidge *et al.* 1993, 331). However, height differences are thought to be minor in foetal and early infant material (Liversidge *et al.* 2003).

Second, it has been questioned whether the pooling of the maxillary and mandibular teeth for the use of the regression equations is warranted, as there is a clear difference in size between the upper and lower anterior teeth. Cardoso (2007) evaluated the present method for permanent and deciduous teeth using the documented Lisbon skeletal collection of modern known age individuals. He found that maxillary deciduous teeth tend to overestimate age compared to mandibular teeth, but that both jaws gave relatively accurate results except the maxillary canine, which significantly overestimated chronological age. The study, however, suffered from a small sample size (n=52 teeth), and therefore could not provide meaningful results on the accuracy of the method. In addition, it was not

assessed whether a combination of both jaws would lead to a more accurate estimate. It will be evaluated in this thesis whether maxillary incisors tend to overestimate age, while mandibular incisors underestimate age.

#### *4.2.2.2 Accuracy*

Unfortunately the study by Cardoso was not concerned with the actual difference between estimated age and real age but rather with the difference between the jaws. Thus, no study exist to date that has put deciduous dental height to a test.

In a test of several quantitative ageing methods for permanent teeth, including dental height by Liversidge and colleagues (1993), Liversidge and colleagues (2003) found that the method significantly underaged individuals (mean difference = - 8.5 ± 11 month). However, no conclusions can be drawn from this regarding the accuracy of the regression equations for the deciduous dentition.

### *4.3 Summary*

Skeletal and dental development have been shown to be highly genetically regulated during foetal growth and early infancy which makes their development ideal for the assessment of age at death. Skeletal age in infants is determined through length of the developing bones. For assessment of dental age two methods were selected in this thesis: First, the deciduous developmental stages by Liversidge and Molleson (2004), and second, dental height regression regression equations developed by Liversidge, Dean, and Molleson (1993). The two dental methods as well as three infant reference standards have the advantage that they were developed on archaeological sample from England that is partly contemporary to the Middenbeemster collection which warrants their use on a northwest European cemetery sample. Accuracy of the dental and skeletal ageing methods have not been reported to date. This thesis will assess whether the three methods produce accurate results in the infant remains from Middenbeemster.

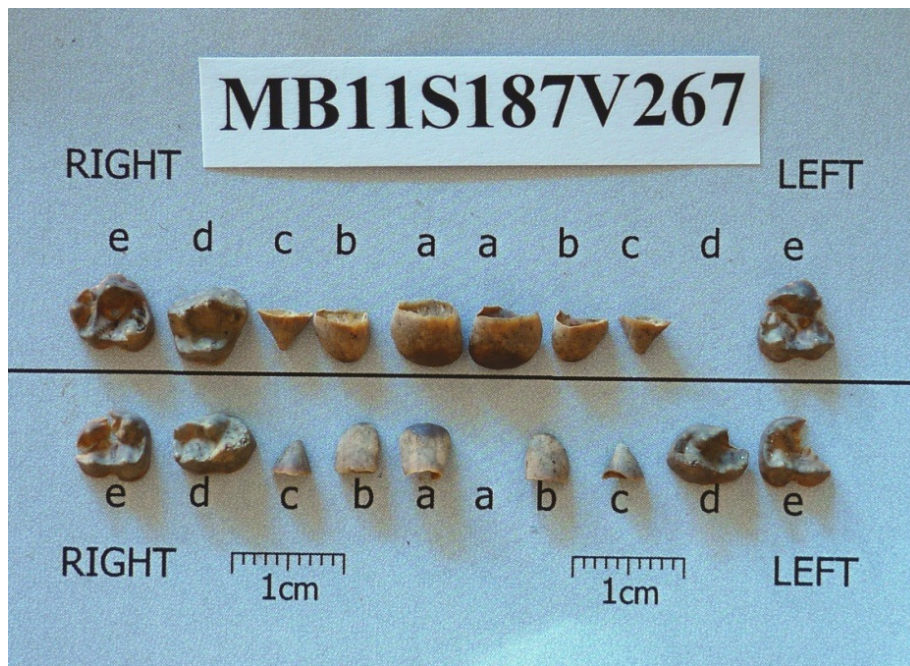
## 5. *Materials and Methods*

### 5.1 *The Sample*

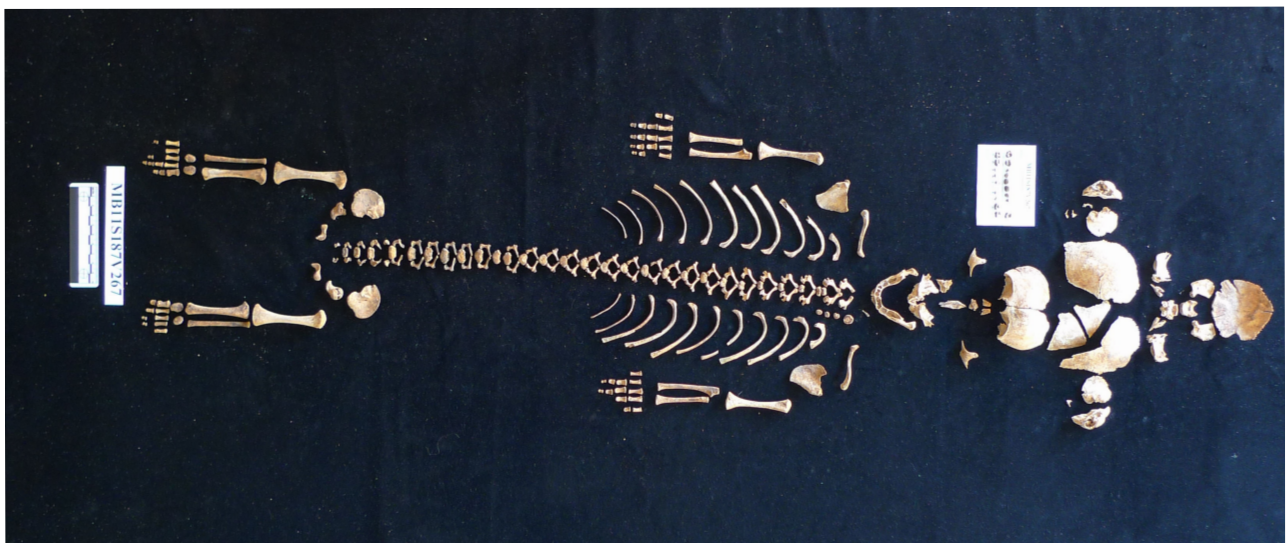
For this study 45 out of 49 foetal and infant remains from the Middenbeemster skeletal collection were analysed and photographed by the author herself (Fig. 8 a,b). The remaining four individuals were analysed by fellow students, however, their results were checked by the author prior to data collection. For the basic osteological analysis a standard recording form was used, provided by Dr. Andrea Waters-Rist of the osteoarchaeological laboratory of Leiden University Faculty of Archaeology. This analysis encompassed assessment of preservation, completeness, age, and the presence or absence of pathology. Age was assessed using dental and skeletal data. Skeletal elements were measured according to standards compiled by Schaefer and colleagues (2009). For a detailed description of the standards see section 4.3. Dental age was derived using a combination of several methods that evaluate the development and eruption of deciduous and permanent teeth (Liversidge and Molleson 2004; Moorrees *et al.* 1963a; 1963b; Ubelaker 1989) as well as dental height of deciduous and permanent teeth (Liversidge *et al.* 1993).

The Middenbeemster skeletal collection was chosen for this study because of the relatively high number of infant and foetal remains, which creates a great opportunity to study these very young age categories. The fact that additional historical data including age at death is known for some of the individuals provided the opportunity for an assessment of the accuracy of dental and skeletal ageing methods of infant remains.





a



b

*Figure 8a. Near complete dentition of one of the infants (MB11S187V0267) that was analysed by the author aged 4.2 weeks using dental height regression equations.*

*Figure 8b. Skeletal elements of the same individual. The very long spine results from the 24 vertebrae each consisting of three elements at that state of development which will fuse during childhood to form a single bone.*

## 5.2 Selection of individuals

Individuals were chosen for this study based on the presence of dental remains. In addition, as the aim was to compare two dental ageing methods and skeletal age, individuals were chosen that provided data on at least two out of the three methods. From the 49 individuals, ten skeletons yielded no dental remains, and these were excluded from further study. From the 39 remaining individuals, 25 provided data on the deciduous Demirjian stages, 37 on skeletal age, and all 39 could be used for dental height assessment (table 3). Ten individuals had archival data pertinent for this study. Their chronological age was given in days, weeks, or months. All ages were converted into weeks to facilitate comparison.

*Table 3. Inventory of the number of individuals and number of skeletal and dental elements used in this study.*

Total number of foetal, perinate, and infant (<1-year) individuals.	49
number of individuals excluded.*	10
number of individuals assessed for skeletal age estimation.	37
number of individuals assessed for the deciduous Demirjian stage assessment.	25
number of individuals assessed for dental height age estimation.	39
number of single teeth scored according to the deciduous Demirjian stages.	128
number of teeth measured.	300
number of individuals with known chronological age.	10

\*Individuals without dentition were excluded from the study because no comparison between methods would have been possible. In addition, these remains were very fragmentary.

### *5.3 Selection of dental ageing methods*

Several reasons added to the choice of the Demirjian deciduous system and dental height by Liversidge and colleagues methods for use in this study. First, both methods have an advantage over other methods, in that they use the entire dentition but can also be applied to single teeth. This makes these methods ideal for ageing individuals with differing degrees of completeness. Second, the methods used in this thesis needed to encompass, in as much as possible, foetal, neonate, and infant specimens. As already mentioned above, the deciduous Demirjian system has a disadvantage in this regard, as no data on foetal and neonatal dental development are provided. Third, both methods have been developed, at least partly, on archaeological populations. In an ideal situation a method would be created on the collection itself which could then incorporate special aspects of growth and development of that particular collection. However, in most cases this is not feasible, which is why using a method devised on a collection that dates from before the onset of the secular trend can be considered a great advantage. It is hypothesised that the accuracy of age estimation will be improved by this fact.

### *5.4 Expectations and limitations*

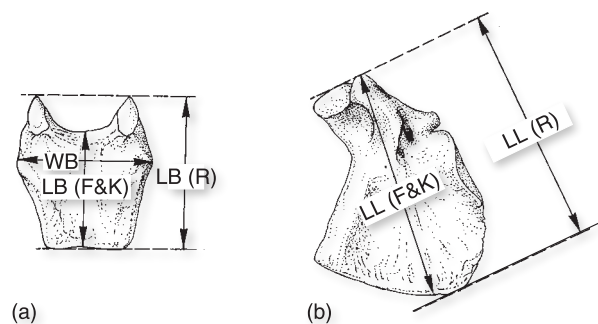
Several aspects need to be addressed prior to the analysis. First, it is expected that trends will become apparent that may help in future applications of ageing methods for Dutch populations. However, to be able to make a more general recommendation about the accuracy of the two dental ageing methods and skeletal age a larger sample size is needed with a somewhat even age distribution. Having a small sample size is a problem inherent in the material, because infant remains are generally sparse which is also reflected in the archaeological standards that are used to assess skeletal development (see below section 4.5).

Second, ethnic differences in the timing of dental and skeletal development between the standard and the test sample may lead to incorrect results. But in this thesis differences in the timing of dental, as well as skeletal development, as result of different ethnicity can be considered minor as the standards used are based on white/Caucasian groups.

Third, the accuracy of a method depends on the age distribution of the population on which the method was developed and the collection under study. Individuals whose age falls close to the lower boundary of an ageing method tend to become underestimated while at the higher boundary age will potentially be overestimated (Liversidge 1999). This effect will be discussed below.

### 5.5 Skeletal age recording

Skeletal development was assessed by measuring various bones of the cranium and the post-cranium using a sliding calliper, accurate to 0.1mm (figure 9). In total, 14 measurements were taken on eight cranial bones, six of which come in pairs. From the post-cranial skeleton all long bones were measured as well as the scapula and pelvis. See table 4a,b for a list of the bones and associated measurements



*Figure 9. Measurements taken for (a) the Pars Basilaris of the Occipital and (b) the Pars Lateralis of the Occipital (Scheuer and Black 2004, 75).*

WB = maximum Width of pars basilaris  
 LB (F&K) = Fazekas and Kósa's mid-sagittal length of pars basilaris  
 LB (R) = Redfield's maximum length of pars basilaris  
 LL (R) = Redfield's length of pars Lateralis  
 LL (F&K) = Fazekas and Kósa's length of pars Lateralis

Table 4a. Cranial measurements used in this study with associated standards.

Bone	Measurement(s)	Reference
Occipital	- Pars basilaris -maximum length, max width, saggital length	Fazekas and Kósa 1978; Scheuer and McLaughlin-Black 1994
	- Squama - height and width	Fazekas and Kósa 1978
	- Pars lateralis – height and width (left and right)	Fazekas and Kósa 1978
Parietal left and right	Chord height and chord width	Fazekas and Kósa 1978
Frontal left and right	Chord height and chord width	Fazekas and Kósa 1978
Temporal left and right	Pars petrosa length	Fazekas and Kósa 1978
Sphenoid	Body - length and width	Fazekas and Kósa 1978
Mandible left and right	Body length	Fazekas and Kósa 1978

Table 4b. Post-cranial measurements used in this study with associated standards.

Bone	Measurement(s)	Reference
Clavicle left and right	Maximum length	Fazekas and Kósa 1978; Black and Scheuer (1996)
Scapula left and right	Maximum length and height	Fazekas and Kósa 1978; Saunders <i>et al.</i> 1993
Humerus left and right	Maximum length	Fazekas and Kósa 1978; Mares 1955; 1970
Radius left and right	Maximum length	Fazekas and Kósa 1978; Mares 1955; 1970
Ulna left and right	Maximum length	Fazekas and Kósa 1978; Mares 1955; 1970
Ilium left and right	Maximum length and Maximum width	Fazekas and Kósa 1978; Molleson and Cox 1993
Ishium left and right	Maximum length and Maximum width	Fazekas and Kósa 1978; Molleson and Cox 1993
Pubis left and right	Maximum length	Fazekas and Kósa 1978; Molleson and Cox 1993
Femur left and right	Maximum length	Fazekas and Kósa 1978; Mares 1955; 1970
Tibia left and right	Maximum length	Fazekas and Kósa 1978; Mares 1955; 1970
Fibula left and right	Maximum length	Fazekas and Kósa 1978; Mares 1955; 1970

Skeletal measurements and associated standards that are commonly used in subadult osteology have been compiled into a manual by Schaefer and colleagues (2009), creating the most comprehensive field manual to-date. From this manual standards were chosen which included data on white Caucasian populations with sufficient amounts of perinatal and infant data. The standards are shortly discussed below.

#### 5.5.1 Foetal age estimation: Fazekas and Kósa (1978)

For foetal remains the standards developed by Fazekas and Kósa were used (1978). Fazekas and Kósa developed their reference standards using 138 foetal remains from a forensic context, aged between 12 and 40 prenatal weeks. The foetuses were from healthy Hungarian parents, and were either stillborn or died shortly after birth (Fazekas and Kósa 1967). Linear measurements of the dry bones were collected for all cranial and post-cranial bones that are recognisable during foetal development. Age of the individuals was based on crown-heel measurements, the common way to determine age in unborn foetuses. The fact that real age was not known for most of the remains and their association with forensic contexts raised some critic on the standards (Scheuer and Black 2004). However, this problem cannot be circumvented when dealing with unborn individuals as the exact day of conception is seldom known. The data were compiled into reference standards that record the associated length for a given age in two week intervals together with an age range.

On average nine measurements could be recorded per two week interval (range=5-15 measurements). While the standard is based on a very limited amount of observations, ethical considerations make it unlikely that larger standards will be available in the future.

#### 5.5.2 Infant age estimation

Reference standards for infant material aged from birth to twelve months are generally based on post-cranial remains. For cranial measurements, data exist only

for the pars basilaris of the occipital bone (Scheuer and McLaughlin-Black 1994) and the frontal and parietal bones (Young 1957). Because no infant frontal or parietal bones survived well enough in this collection these standards are not considered here.

No exhaustive standard exists that covers the entire post-cranial skeleton, which is why a collection of references had to be used. These include standards on the clavicle by Black and Scheuer (1996), the scapula by Saunders and colleagues (1993), the ilium by Molleson and Cox (1993), and measurements of the upper and lower limb bones collected by Maresh (1955; 1970). Except from the Maresh standards, all other references are at least partly based on archaeological material.

#### *5.5.2.1 The pars basilaris of the occipital: Scheuer and McLaughlin-Black (1994)*

The measurements of the pars basilaris that were originally defined by Fazekas and Kósa (1967, 50) and Redfield (1970, 214) (figure 9) were applied to a sample of 62 skeletal remains aged between about 26 weeks gestation and four years. Forty-six individuals were of known age at death from the St. Brides and Spitalfields archaeological collections (18<sup>th</sup> and 19<sup>th</sup> century). Provenance of the remaining 16 individuals is not stated by the authors. Age of these individuals was derived using the measurements of Fazekas and Kósa. However, six individuals are aged 40+ weeks to four months postnatally and therefore fall outside the range of the Fazekas and Kósa standard. Methods that were employed to estimate age in the other individuals are not stated by the authors. However, only individuals of known age at death are listed in the standard of the field manual by Schaefer and colleagues (2009). In total, 15 observations are available for the infant category from birth up to 12 months. There are no preset intervals for this time period as the measurements are mostly single point observations (7 out of 10) that are randomly spread throughout the year (at 2weeks, 3weeks, 4weeks, 7weeks, 3months, 5months, 8months, 9months, 11months, and 12months).

The variation between the different individuals shows that the standard does not capture the true variability present within a population for each age interval. For example, two individuals, one aged three months and the other aged eight

months both have the same mean saggital length, but differ for the other two measurements. In fact, this measurement hardly increases from three months until the end of the first year, rendering saggital length not very suited to age older infant remains. There seems to be great variability in the size of this bone between the individuals. One individual aged three weeks has a greater mean width as another individual aged three months. Such variability is seen all throughout the year and for each measurement. Thus, the use of this standard is not expected to yield very accurate results.

#### 5.5.2.2 *The Clavicle: Black and Scheuer (1996)*

Black and Scheuer (1996) developed a standard for age estimation based on the length of the clavicle. They compiled data from individuals aged from birth until adulthood using four documented white Caucasian skeletal collections: three archaeological collections from London, dating to the 18<sup>th</sup> and 19<sup>th</sup> century (Spitalfields, St. Bride's, and St. Barnabas) and the twentieth century Portuguese documented collection from Lisbon. Age of the examined individuals ranged from birth up to 30 years. Twenty individuals were aged between birth and 12 months. Unfortunately, the first year was only split into two intervals, from birth to six months and from seven months until the end of the first year. Therefore, no clear picture can be created about the development of this bone during the first year of life. In addition, the age distribution of the 20 individuals that contributed the measurements is not known. This is of importance because an uneven distribution might skew the mean age provided for the intervals as being either too high or too low. The use of archaeological material dating partly to the 19<sup>th</sup> century is considered an advantage in this study.

#### 5.5.2.3 *The ilium: Molleson and Cox (1993)*

Molleson and Cox (1993) created a standard for the age related changes in maximum length and maximum width of the ilium of the pelvis. The authors based their data on the London Spitalfields documented collection that provided data for 36 individuals aged from birth up to three years. Measurements, mean, and age-range are provided in three month intervals. For the age interval from



birth to three months, ten measurements are provided. However, the following three intervals from four up to twelve months are each based on only two measurements, which renders this standard less reliable for older infants.

#### *5.5.2.4 The scapula: Saunders and colleagues (1993)*

Reference data for the scapula were provided by Saunders and colleagues (1993). They developed standards for the long bones and scapula using a Canadian collection of partly documented skeletal material from the 19th century St. Thomas church, Belleville, Ontario. However, only references of the scapula are included in the manual by Schaefer and colleagues (2009). The inhabitants of the town who were buried in the cemetery were mostly settlers from north-western Europe, making comparison with a Dutch population from the same time period feasible. Age for the bones was based on dental development using the dental maturation standard of Moorrees and colleagues (1963a; 1963b) and substituted with chronological age in the few cases it was known. The collection provided data on 47 infants aged between birth and 12 months. Age and related skeletal measurements are recorded in six month intervals.

The fact that chronological age is substituted for dental age makes the use of this standard somewhat problematic, because dental development only approximates chronological age, reflecting biological age instead. In addition, it is not known for how many infant remains chronological age was provided (from 576 excavated adults and subadults, 80 individuals could be assigned a chronological age based on identification with the tombstones and coffin plates). However, in absence of other standards that provide data on the scapula, it is still used.

#### *5.5.2.5 Long bone length: Maresh (1955 and 1970)*

Marion Maresh (1955; 1970) has compiled roentgenographic measurements from healthy white American children living in Denver, Colorado as part of the Child Research Council study of physical growth. This longitudinal study collected data from about 180 boys and girls starting at the age of one-and-a-half months until adolescence. During the first half year three observations are made (at 1.5, 3.0,

and 6.0 months) and again at 12 months of age. Subsequent measurements were taken in half year intervals until age five and then every year until age 18. For each age interval, the mean length of the bone is given as well as the 10<sup>th</sup> and the 90<sup>th</sup> percentile. The study lasted for 45 years, starting in the year 1935 (Maresh 1972). The reference standards are widely applied and are thought to adequately represent the growth pattern of modern healthy white children (Schillaci *et al.* 2012).

### 5.5.3 Analysis

Skeletal age will be statistically compared to chronological age for individuals of known age (see section 4.8 for the statistics used in this thesis). The analysis includes 1) the entire skeleton, 2) single measurements, and 3) different age classes. Skeletal age estimates of single bones will be evaluated for consistent outliers to determine whether there exist bones with deviating growth patterns in the Middenbeemster collection. The results will aid future osteologists working on the present population and other Dutch archaeological skeletal collections to choose the best suited measurements. In addition, it will be looked at whether the standards differ markedly in their accuracy. The results will be compared to the remaining sample of unknown age at death infants.

## *5.6 The Demirjian system for deciduous teeth by Liversidge and Molleson (2004)*

The deciduous Demirjian stages are named in alphabetical order from A to H2, according to the description provided by Liversidge and Molleson (2004, 174) (table 2, p. 45). It was noted that the manual (Schaefer *et al.* 2009, 87) provided the original description of the stages described by Demirjian and colleagues (1973, 221f) instead of those that had been particularly adapted for this method by

Liversidge and Molleson (2004). As the adapted descriptions were found to be more comprehensive and more clear, they were used more often.

Only full dental stages were assigned. In cases where no exact match was found, the tooth was either upgraded or downgraded based on which stage provided the closest fit, taking into consideration the developmental stage of the remaining teeth. From the cumulative scores of single teeth the mean was calculated which was used as the final age estimate in each individual.

### 5.6.1 Analysis

The accuracy of the deciduous Demirjian stages is statistically evaluated for individuals of known age at death. The analysis includes 1) the entire dentition, 2) the different tooth classes, and 3) different age classes. The results are evaluated against the remaining sample.

## *5.7 Dental height by Liversidge and colleagues (1993)*

Maximum tooth length was established by measuring from the cervical margin to the highest developing cusp in molars, to the cusp tip in canines, and to the incisal edge in incisors. The teeth were measured with a sliding calliper, accurate to 0.1mm. Age was established by applying the regression equations (table 5) provided by Liversidge and colleagues (1998, 432). The cumulative age estimate was obtained for each individual by calculating the mean from estimates of the single teeth.

*Table 5. Regression equations for age estimation based on deciduous tooth length (Liversidge et al. 1998, 432).\**

<b>Tooth</b>	<b>regression equation for estimating age (yrs)</b>
first incisor	Age = -0.653 + 0.144 x length ± 0.19
second incisor	Age = -0.581 + 0.153 x length ± 0.17
canine	Age = -0.648 + 0.209 x length ± 0.22
first molar	Age = -0.814 + 0.222 x length ± 0.25
second molar	Age = -0.904 + 0.292 x length ± 0.26

\*Equations are applicable to the mandibular and maxillary teeth.

### 5.7.1 Analysis

The accuracy of dental height will be statistically evaluated using all individuals of known age at death. Analysis comprises 1) the entire dentition, 2) the upper and lower jaw, 3) the different tooth types, and 4) different age classes. Accuracy will also be determined for use of different amounts of teeth.

## 5.8 Statistical analysis

To evaluate the accuracy of the three ageing methods in relation to chronological age, two statistical analysis are performed for each subquestion. First, the means of estimated age and chronological age are compared. Second, the correlation between both variables is assessed. To statistically evaluate the means the analysis includes parametric tests as well as non parametric tests based on the nature of the data. If the sample size is greater than 10 the independent samples t-test can be used. In case of lower sample size, or when the Levene's test of equality of variance (F) is not passed, the nonparametric version is used instead, called the Mann-Whitney U (U) test.

The independent samples t-test compares the sample means of two unrelated groups. It establishes whether or not the means differ significantly from

each other. A statistically significant result indicates a significant difference. The Mann-Whitney U tests operates in a similar manner.

To evaluate the level of correlation between the estimated age and chronological age the Spearman rho correlation ( $r_s$ ) is used. The test is a statistical measure of the strength of a nonlinear (monotonic) relationship between two variables. The correlation is expressed as a value ranging from -1 to +1 which represent a negative strong and a positive strong correlation respectively. As the number approaches zero, the correlation is weaker. Table 6 shows the the Spearman rho values and associated strength of correlation.

*Table 6. Interpretation of the Spearman rho correlation coefficient (after Zou et al. 2003, 618).*

Correlation Coefficient Value		strength of correlation
1.0	-1.0	Perfect correlation
0.8	-0.8	strong correlation
0.5	-0.5	moderate correlation
0.2	-0.2	weak correlation
0.0		no correlation

Significance for all tests is set at  $p \leq 0.05$ . The statistical analyses were performed using SPSS 22 for mac. Accuracy of the methods is expressed as mean difference to chronological age. Results include the Standard Deviation (SD), which represents the amount of dispersion of the data from its mean and Standard Error (SE). The SE statistically measures the accuracy with which a sample mean represents a population (Medina and Zurakowski 2002).

### 5.9 *Intra-Individual Error*

To assess the possible effect of intra-individual error on the results, reassessment of the three methods was done for 10% of the individuals. These were picked at random in a separate session. Intra-observer agreement was calculated for skeletal measurements and dental height using the intraclass correlation coefficient (ICC). An ICC calculates the degree of agreement between the two measuring sessions as a number between 0 and 1, with the latter denoting a perfect agreement and zero no agreement. For the deciduous Demirjian stages the Cohen's Kappa coefficient was used. The Kappa statistic uses values that fall between -1 and +1. The strength of correlation is measured in the same way as with the Spearman correlation coefficient (see table 6).

## 6. Results

This chapter presents the results of the analysis of the three ageing methods. After establishment of the general accuracy of the methods for individuals of known age at death, each method will be evaluated separately in more depth. The first part of each analysis is concerned with the ten individuals of known chronological age, while in the second part the results from the first part will be discussed in light of the trends visible from the remaining individuals. Before this the intraobserver reproducibility results are presented.

### 6.1 Reproducibility

Reproducibility of skeletal and dental measurements show a very high level of agreement between the two recording sessions. For skeletal measurements the intraclass correlation coefficient (ICC) is 0.997 ( $p < 0.001$  and 95% CI 0.996, 0.998) and for the dental measurements the ICC is 0.990 ( $p < 0.001$  and 95% CI 0.975, 0.995).

The intrarater reproducibility for the two separate scoring sessions of the deciduous Demirjian stages was found to be Kappa = 0.312 ( $p < 0.001$ ). A kappa score of 0.21 to 0.40 is considered a fair agreement (Viera and Garrett 2005). While statistically significant, these results reveal that reproducibility is problematic for the Demirjian stages and that stages were not scored consistently. However, for the 32 observations (out of 65) that deviated it was never by more than one stage, thus not causing a major difference in age estimation. Whether the data are still adequately accurate will be further discussed in section 6.4 and in chapter seven.

## 6.2 Accuracy

Chronological age of the ten infants of known age is plotted against the deciduous Demirjian stages, dental height, and skeletal age in figure 10. Skeletal age and dental height estimates were available for all ten individuals, while the Demirjian stages could only be applied to seven individuals. What directly becomes apparent is that the deciduous Demirjian stages (blue label) are often far removed from chronological age (orange label). Skeletal age (green label) and dental height age (yellow label) are mostly grouped more closely around the known age but show some variation as well. Table 7 shows the difference of age estimation for the ten individuals for each method. Age in seven of the individuals is both overestimated as well as underestimated using different methods, indicating the discrepancies between the methods and chronological age are not caused by individual

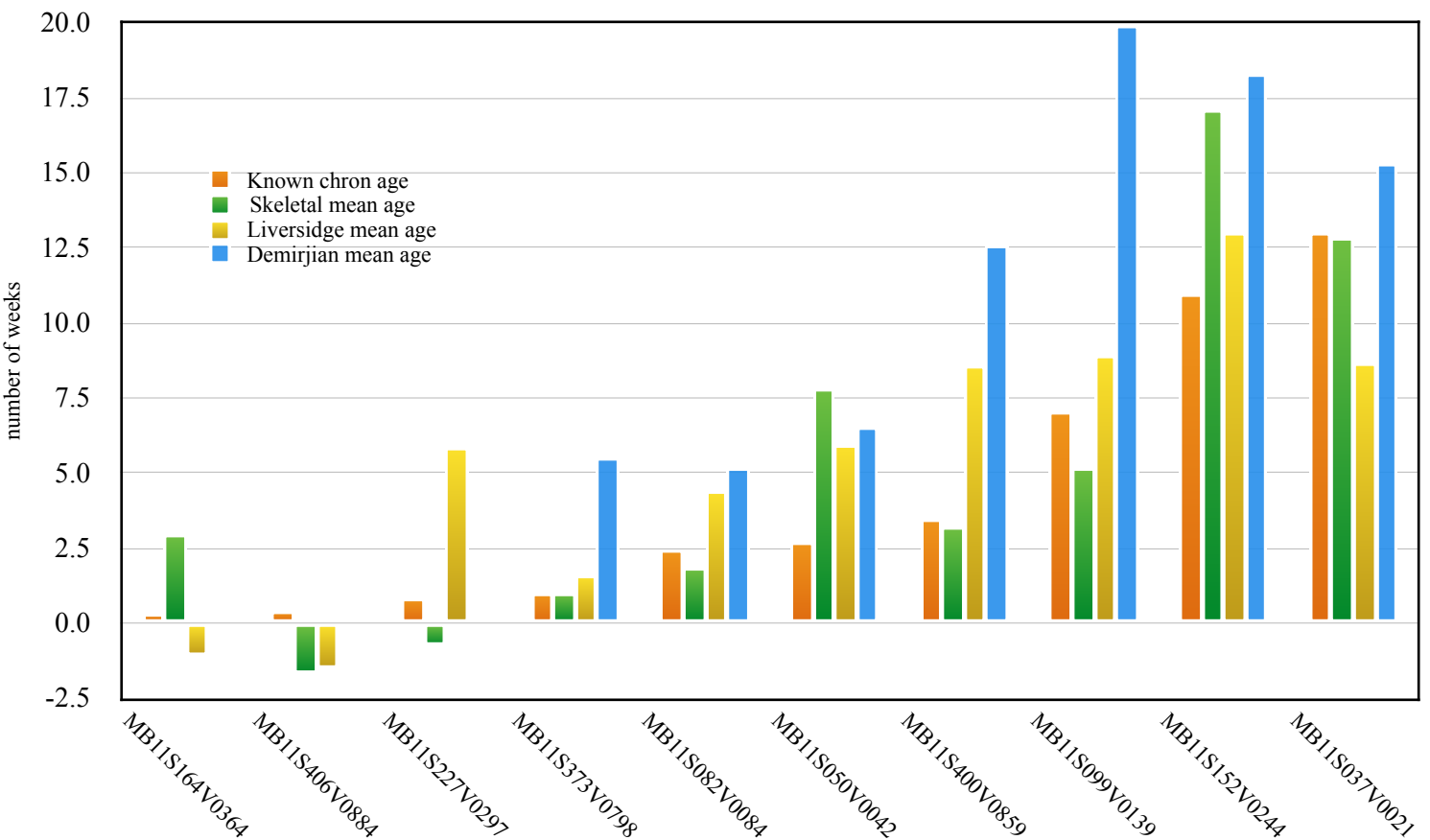


Figure 10. Chronological age plotted against estimated age of the three ageing methods in individuals of known age at death.



variability. If, for example, the individual was large for its age, and the methods were all working well, age would be consistently overestimated. As this is not the case, it can be assumed that differences are likely caused by the method itself and not by variability in the skeletal and dental development of the individual. However, it of course remains possible that the individual was advanced in one regard and behind in another, although this is less likely.

*Table 7. Mean difference to chronological age for all three ageing methods listed for each individual of known age at death.*

Individual	Method	n	mean difference in weeks	SD	SE
MB11S037V0021 Age=13.04 weeks	skeletal measurements	9	-0.21	8.14	2.71
	deciduous Demirjian stages	11	+2.28	4.77	1.44
	dental height regression equations	14	-4.39	3.39	0.91
MB11S050V0042 Age=2.71 weeks	skeletal measurements	2	+5.09	7.35	5.20
	deciduous Demirjian stages	4	+3.79	1.97	0.98
	dental height regression equations	5	+3.21	1.41	0.63
MB11S082V0084 Age=2.43 weeks	skeletal measurements	26	-0.70	5.99	1.17
	deciduous Demirjian stages	2	+2.77	1.59	0.79
	dental height regression equations	12	+1.95	2.27	0.66
MB11S099V0139 Age=7.0 weeks	skeletal measurements	1	-1.80	/	/
	deciduous Demirjian stages	3	+12.93	4.94	2.85
	dental height regression equations	2	+1.93	6.70	4.74
MB11S152V0244 Age=11.0 weeks	skeletal measurements	11	+6.08	17.78	5.36
	deciduous Demirjian stages	5	+7.72	3.98	1.78
	dental height regression equations	5	+2.00	5.53	2.47
MB11S164V0364 Age=0.28 weeks	skeletal measurements	10	+2.17	5.08	1.61
	deciduous Demirjian stages	-	-	-	-
	dental height regression equations	2	-1.37	2.42	1.71

Individual	Method	n	mean difference in weeks	SD	SE
MB11S227V0297 Age=0.86 weeks	skeletal measurements	27	-2.08	3.07	0.59
	deciduous Demirjian stages	-	-	-	-
	dental height regression equations	4	+4.96	2.91	1.45
MB11S373V0798 Age=1.0 week	skeletal measurements	25	-0.46	4.29	0.86
	deciduous Demirjian stages	3	+4.55	0.60	0.35
	dental height regression equations	17	+0.80	3.15	0.76
MB11S400V0859 Age=3.43 weeks	skeletal measurements	1	-0.18	/	/
	deciduous Demirjian stages	5	+9.15	6.33	2.83
	dental height regression equations	7	+5.11	3.69	1.39
MB11S406V0884 Age=0.43 weeks	skeletal measurements	30	-2.46	2.55	0.47
	deciduous Demirjian stages	-	-	-	-
	dental height regression equations	6	-1.03	2.62	1.07

n= number of observation per method. Whether the method overestimated age is indicated by a + or underestimated age is indicated by a - . SD= Standard Deviation, and SE=Standard Error. In some instances SD and SE could not be calculated (/).

An independent samples t-test was performed to evaluate how well the means of the methods matched with chronological age. In a subsequent step the Spearman rho correlation coefficient ( $r_s$ ) was calculated to assess if the methods showed a positive correlation. Table 8 shows whether the methods generally overestimates age (+) or underestimates (-) age.

Skeletal age estimation shows an underestimation of less than a week (-0.46 weeks), and the correlation is significantly moderate ( $r_s=0.638$   $p<0.001$ ). The sample did not pass the Levene's test of equality of variance ( $F=15.102$   $p<0.001$ ) so the nonparametric version of the t-test was used. The results of the Mann-Whitney U test indicate that there is a significant difference between the means of the two groups ( $U=4611.0$   $p<0.001$ ).

*Table 8. Mean difference in weeks to chronological age of all three ageing methods.*

method	n	mean difference to chronological age	SD	SE
Skeletal age	142	-0.46	8.51	0.71
Deciduous Demirjian stages	33	+5.53	6.46	1.41
Dental height	74	+0.57	5.01	0.58

Dental height deviates from chronological age by +0.57 weeks. In this case, an independent samples t-test found no significant difference between the means ( $t=0.70$   $df=146$   $p=0.45$ ) which is also expressed in a significant strong correlation ( $r_s=0.707$   $p<0.001$ ).

The deciduous Demirjian stages show a moderate significant correlation ( $r_s=0.597$   $p<0.001$ ). However, the method overestimates chronological age by an average of 5.53 weeks. The sample did not pass the Levene's test of equality of variance ( $F=4.257$   $p=0.043$ ) and the nonparametric version of the t-test was used. Results indicate that the overestimation leads to a significant difference between the means ( $U=244.000$   $p<0.001$ ). To better understand the differences in performance of the methods each of them is discussed separately below.

### *6.3 Results skeletal age estimation*

To better understand the performance of the skeletal age estimation method in the previous analysis, it was chosen to test each bone separately against chronological age as shown in table 9. For this analysis the left and right sides were pooled. In the case of multiple measurements taken from single bones, these were tested separately. For each measurement the  $r_s$  was calculated and an independent samples t-test was performed. In cases where less than ten observations were

Table 9. Mean difference to known age for single skeletal measurements and results of the rs and independent sample t-test /U-test.

Measurement	n	mean difference to known age	rs		t/U	
			rs	p	t/u	p
Occipital- pars basilaris width	6	-0.65	0.319	0.538	U=13.000	0.485
Occipital- pars basilaris length	6	+4.44	0.600	0.208	t=0.820	0.431
Occipital- pars basilaris saggital length	6	+10.66	0.580	0.228	U=16.000	0.818
Occipital- pars squama	1	-2.43	-	-	-	-
Occipital- pars lateralis height	8	-1.55	0.632	0.092	U=4.000	0.002
Occipital- pars lateralis width	2	-0.43	-	-	-	-
Sphenoid body max length	1	-0.43	-	-	-	-
Sphenoid body max width	3	-14.09	0.500	0.667	U=0.000	0.100
Frontal-Chord height	3	-1.79	-1.000	0.000	U=0.000	1.000
Frontal-Chord width	4	-1.46	-1.000	0.000	U=0.000	0.029
Temporal - pars petrosa	6	-2.36	0.953	0.003	t=-5.254	0.000
Mandibular length of body	6	-1.48	0.826	0.043	U=0.000	0.002
Clavicle max length	9	+0.67	0.535	0.138	U=24.000	0.161
Scapula max length	3	+8.25	-	-	U=3.000	0.700
Scapula max width	9	-0.35	0.859	0.003	U=32.000	0.489
Humerus max length	15	-2.24	0.894	0.000	t=-1.365	0.183
Radius max length	8	-1.76	0.778	0.023	t=-1.155	0.268
Ulna max length	9	-2.60	0.822	0.007	t=-1.561	0.138
Ilium max length	5	-2.14	0.818	0.047	U=0.000	0.008
Ilium max width	6	+7.25	0.667	0.219	U=18.00	1.000
Ishium max length	6	-4.03	0.985	0.000	U=2.500	0.009
Ishium max width	5	-1.94	0.745	0.148	U=0.000	0.008
Pubis max length	14	-1.61	0.310	0.282	t=-4.602	0.000
Femur max length	3	-1.24	1.000	0.000	U=0.000	0.100
Tibia max length	4	-0.55	-0.816	0.184	U=4.000	0.343
Fibula max length	3	+0.86	1.000	0.000	U=0.000	0.100

n=number of measurements. Mean difference to chronological age is indicated in weeks.

available, or the Levene's test for equality of variance was not passed, the Mann-Whitney U test was used instead of the t-test. From the analysis of the 26 measurements of single bones and pairs of bones it becomes apparent that performance varies considerably between them. Difference to chronological age ranges from -14.1 to +10.7 weeks. However, 77 percent of the measurements show a mean difference of less than three weeks (n=20), while 58 percent have a mean difference of less than two weeks (n=15). Variability is also reported in different measurements taken from single bones. The three measurements taken from the pars basilaris of the occipital bone (length, width and saggital length), for example, produce results that differ from each other by a maximum of 11.3 weeks.

In general, skeletal estimates underestimate chronological age in 76.9 percent of the cases (n=20). Eight measurements produce means that differ significantly from chronological age (Occipital- pars lateralis height; Frontal chord width; Temporal- pars petrosa; Mandibular length of body; Ilium max length; Ishium max width; Pubis max length), while three of them also fail to correlate in a meaningful way (Occipital- pars laterals height; Ishium max width; Pubis max length). Seven additional measurements do not correlate significantly with chronological age (Occipital- pars basilaris length, width and saggital length; Sphenoid body max width; Clavicle max length; Ilium max width; Ishium max width; Tibia max length). The height and width of the frontal bone had a very strong significant negative correlation. The result can probably be explained by the limited observations that are available (3 and 4 respectively). In general, it has to be kept in mind that sample sizes are very low and in most cases do not exceed ten observations. With such low numbers it cannot be ruled out that results are affected by chance. The variability among the different bones may explain why skeletal age differs significantly from the overall mean from chronological age. It also has to be kept in mind that the analysis used 26 different measurements from 18 different bones, each of them having their own level of accuracy and their own tendency to either overestimate or underestimate. The statistical analysis will be easily set off by such variety.

Clearly some bones are more closely correlated with chronological age than others. A significant difference to known age is confined to the flat bones and irregular bones, while all long bones perform very well. From the ten bones that

do not correlate significantly with chronological age, eight are again flat bones and irregular bones and two are long bones. As regards the mean difference to real age, the worst performing measurements are the pars basilaris length (occipital), the pars basilaris saggital length (occipital), the sphenoid body maximum width, the scapula maximum length, the ilium maximum width, and the ishium maximum length. These bones together have a mean difference of 8.1 weeks. The best performing measurements are the pars basilaris width (occipital), the pars lateralis width (occipital), the sphenoid body maximum length, the clavicle maximum length, the scapula maximum width, the tibia maximum length, and the fibula maximum length, with a mean difference of less than a week.

As the majority of the flat bones stem from the cranium it was decided to evaluate whether there is a significant difference in performance between the cranial measurements (n=52) and the post-cranial measurements (n=90). An independent samples t-test was conducted to compare their means with that of known chronological age. It was found that results differ only slightly between cranial and post-cranial measurements, with an average of 0.54 weeks. Mean difference to real age of the cranial bones is -0.11 weeks and for the post-cranial bones it is -0.66 weeks. In both cases the Levene's test was significant so the nonparametric version of the t-test was used (cranial measurements:  $F=7.774$   $p=0.006$ , post-cranial measurements:  $F=7.463$   $p=0.007$ ). Cranial measurements as well as post-cranial measurements are significantly different to chronological age at the 0.000 significance level (cranial measurements:  $U=557.500$ ; post-cranial measurements:  $U=1964.000$ ). While the means of the method seem to match very well with chronological age, the variation present within the method results in a significant difference between estimated and real age.

The variability seen above is also found within measurements of single individuals. Intra-individual variability increases with age from  $\pm 3.0$  in foetal remains (n=2), to  $\pm 8.5$  weeks in neonatal remains (n=5), to  $\pm 23.9$  weeks in post-neonatal remains (n=2). One of the individuals only provides one measurement and was not considered in this analysis. Variability for foetal and neonatal remains was calculated without the measurements of the pars basilaris of the occipital, the sphenoid body width, and the ilium, because these bones produce estimates that often differ greatly from the remaining bones and thus would skew the results.

### 6.3.1 Accuracy of the infant growth standards

It was then decided to test whether the different standards that had been employed during skeletal analysis differed in their performance. Testing the accuracy of the Fazekas and Kósa standard (1978) was not feasible as it only provides age estimates for foetal development up to the age of 40 weeks gestation. Thus, only the infant standards were analysed (Black and Scheuer 1996; Maresh 1955; 1970; Molleson and Cox 1993; Saunders *et al.* 1993; Scheuer and McLaughlin-Black 1994). Thirty-five out of 142 measurements could be used. Each step tested the agreement of the sample mean with the known chronological age by way of an independent samples t-test or Mann Whitney U test as well as their correlation using the Spearman rho correlation coefficient. Mean difference to known age of the standards as well as the standard deviation and standard error are presented in table 10.

*Table 10. Comparison of the infant bone standards with chronological age.*

Standard	n	mean difference in weeks	SD	SE
Long bone length (Maresh 1955; 1970)	13	+1.98	2.465	0.683
Occipital- Pars basilaris (Scheuer and McLaughlin-Black 1994)	10	+13.12	16.951	5.360
Clavicle (Black and Scheuer 1996)	3	+10.57	0.000	0.000
Scapula (Saunders <i>et al.</i> 1993)	5	+13.67	11.649	5.210
Ilium (Molleson and Cox 1993)	3	+19.32	0.115	0.006

The Maresh reference standards (1955; 1970) incorporate all the long bones, except the clavicle (n=13). Results overestimate chronological age by about two weeks and show a significant moderate correlation with known age ( $r_s=0.604$   $p=0.029$ ). The t-test results indicate no significant difference between the means ( $t=1.645$   $p=0.106$ ).

All the standards that only applied to one bone significantly overestimated chronological age. The pars basilaris of the occipital (Scheuer and McLaughlin-Black 1994) overestimates age by 13.1 weeks. The Levene's test for equality of

variance was not passed ( $F=6.837$   $p=0.015$ ) so the nonparametric version of the t-test was used. Results indicate no significant difference between the means ( $U=34.500$   $p=0.006$ ). But the estimates show no significant correlation between the method and real age ( $r_s=0.511$   $p=0.131$ ).

Only three measurements are available for the clavicle (Black and Scheuer 1996). The bone overestimates age by 10.6 weeks, but the difference between the means did not reach statistical significance ( $U=3.000$   $p=0.064$ ). Due to the low sample size the  $r_s$  could not be calculated.

The scapula (Saunders *et al.* 1993) shows an overestimation of 13.7 weeks. The means differ significantly ( $U=4.000$   $p=0.004$ ) and no significant correlation was found ( $r_s=0.000$   $p=1.000$ ).

The ilium provided two measurements that together overestimates real age by 19.3 weeks. The mean differs significantly from chronological age ( $U=0.000$   $p=0.005$ ) and a very strong negative correlation was found ( $r_s=-1.000$   $p=0.000$ ). This indicates that the bone is not able to predict real age. While sample size in most of the four single bone standards is very low, a combined analysis ( $n=21$ ) reveals that real age is still overestimated by 13.4 weeks. The independent samples t-test reveals that the means of the standards and chronological age differ significantly ( $t=5.988$   $p<0.001$ ).

The analysis above shows that the modern standard by Maresh (1955; 1970), performs much better than those standards that were at least partly based on archaeological reference collections and which are contemporaneous with the Middenbeemster skeletal collection (Black and Scheuer 1996; Molleson and Cox 1993; Saunders *et al.* 1993; Scheuer and McLaughlin-Black 1994). The assumption made in the previous chapter that the use of standards which are developed on individuals coming from the same time period should increase the accuracy of the age estimate, proves not to be valid. This will be considered further in the discussion section.

### 6.3.2 Skeletal age estimation including all remains

From the 49 infant individuals, 37 had skeletal remains available for observation. A total of 617 measurements were taken (varying from  $n=1$  to  $n=37$  within an



individual). As was shown in the analysis of individuals with known age, the variability in the age estimates of single bones increases with age. As done above, the analysis excluded measurements from the pars basilaris of the occipital, the sphenoid body width, and the ilium. During foetal development intra-individual variability is on average  $\pm 5.3$  (n=250) weeks, during the neonatal period variability increases to  $\pm 11.3$  weeks on average (n=320), and for the remaining infant period variability was found to be on average  $\pm 17.7$  weeks (n=47). These results are consistent with the general trend known in skeletal development of increasing variability with increasing age.

When comparing estimates from the cranial and post-cranial remains they show the strongest correlation during the foetal period, with a mean difference of 0.2 weeks. For the actual mean age of the different parts of the skeleton see table 11. The deviation increases with increasing age being 1.1 weeks in neonatal remains, and 4.9 weeks in infant remains. However, there is no pattern discernible indicating that one of them is generally delayed. When combined, cranial and post cranial estimates deviate by only 0.04 weeks. The results indicate no difference in the performance of cranial and post cranial measurements. In addition, increased variability with increasing age is noted, but this could equally point to variation in the performance of the standards.

*Table 11. Comparison of mean age from cranial and post-cranial estimates for three different age categories.*

	Age category	Mean age
Cranial estimates	combined	2.39
	foetal	-2.35
	neonatal	2.15
	post-neonatal	17.45
Post-cranial estimates	combined	2.35
	foetal	-2.54
	neonatal	3.26
	post-neonatal	12.54

To see whether the infant standards show similar variability in the entire infant sample, the Maresh standard was compared to the single bones standards of the pars basilaris of the occipital, the scapula, the clavicle, and the ilium. The Maresh standard could be used on 20 individuals while one or more of the other standards provided estimates for 19 individuals. Mean age for the Maresh standards is 7.87 weeks (range=1.0-32.53weeks) while the remaining standards produce a mean age of 16.95 weeks (range=1.0-46.23weeks), giving a mean difference of 10.92 weeks. These results confirm the observation that single bone standards provide much older estimates than the Maresh standard and it was decided to further evaluate the single bone standards. Other bones that consistently produced results that differed from the remaining estimates are analysed as well.

#### 6.3.2.1 *Pars basilaris of the occipital*

All three measurements (maximum length, maximum width and saggital length) of the pars basilaris of the occipital are available for 22 individuals. Results between the three measurements almost never agree except in two cases. In seven cases, two measurements provide the same estimate and in the remaining 13 instances all three estimates disagree. The divergence between measurements ranges from one week to up to a year with an average of 11.59 weeks. Table 12 shows the mean age of each measurement as well as the range.

*Table 12. Comparison of mean age and age range for the three measurements recorded from the Pars basilaris of the occipital.*

	n	mean age in weeks	range
Occipital- pars basilaris width	22	3.9	-4 to 17.4
Occipital- pars basilaris length	22	7.1	-10 to 28.2
Occipital- pars basilaris saggital length	22	9.3	-13 to 58.7

The table shows increasing mean age of the width, length and saggital length, respectively. The same trend is visible in individuals of known age, where the

youngest estimate also produces the least difference to chronological age (see table 9). The increase in mean age, also increases the range. The pars basilaris width shows a difference between the youngest and oldest estimate of 21.4 weeks, for the pars basilaris length the difference is 38.2 weeks, and the saggital length has a difference of 71.7 weeks. Thus, the increasing discrepancy with known age could be explained by an increasing variability in the maximum length and saggital length. The pars basilaris width performs very well (real age overestimation is less than a week) and results would improve if only the pars basilaris width was used in the age estimation.

#### *6.3.2.2 Clavicle*

Twenty-four individuals provided data on the length of the right and/or left clavicle (n=38). The average age for this bone is 2.15 weeks advanced above the mean age of all bones combined. The results for individuals with known age (n=9) were very accurate with a mean difference of +0.67 weeks. However, these results are misleading as the infant standard by Black and Scheuer (1996) overestimates age by about ten weeks while foetal estimates alone were underestimating age by about four weeks. In the entire sample the mean difference to mean skeletal age is +8.81 weeks for the infant standard and -1.5 weeks for the foetal standard. Thus, it can be concluded that the infant standard significantly overestimates chronological age.

#### *6.3.2.3 Scapula*

The length and width of the scapula were recorded for 21 individuals (n=35 measurements). The average difference to mean skeletal age is only +0.6 weeks and the two measurements do not differ in their results. However, when taking a closer look at the data it is shown that maximum width provides lower estimates in most of the cases with an average difference between length and width of 7.1 weeks. The reason why this did not show in the data is that scapula width is recorded more often in older individuals, thus increasing the average of that measurement. Splitting the sample into foetal and infant remains shows that foetal

estimates deviate from mean skeletal age by -0.57 weeks and infant estimates have an average difference of +5.7 weeks.

A relatively good agreement is found in the analysis of individuals of known age, which revealed a mean difference of +2.04 weeks and no significant difference between the estimates and real age. However, the difference between length and width did show in the analysis. The scapula maximum length overestimates age by +8.2 weeks and scapula maximum width underestimates age by -0.35 weeks, but no significant difference to chronological age is detected in either of the measurements. While the analysis confirms that maximum length overestimates age, it has to be kept in mind that for known age individuals only three measurements were available. For two of these measurements the foetal standard was used which underestimated chronological age by -1.6 weeks. The third measurement fell within the range of the infant standard and overestimated age by +28.05 weeks. When all estimates from the infant standard are considered it is shown that chronological age is significantly overestimated by more than 13 weeks on average.

To summarise, the scapula maximum length and maximum width differ in their relative accuracy to predict chronological age with the maximum width being more close to real age. Results are generally more accurate when foetal and infant estimates are combined indicating that the infant standard overestimates age substantially.

#### 6.3.2.4 *Ilium*

Two measurements were recorded from the left and right ilia, the maximum length and the maximum width. Twenty-four individuals provided data on at least one measurement. Results from the individuals of known age at death (n=11 measurements) showed good agreement with chronological age, with an average overestimation of 2.9 weeks. The infant standard by Molleson and Cox (1993), however, overestimated age by almost 20 weeks. It was found that there is a consistent discrepancy between the age estimate of the two measurements. Out of 19 cases that provide data on both measurements, 74 percent (n=14) do not agree with each other. From these cases, ilium width is always considerably advanced above ilium length except in two instances. The discrepancy is on

average 13.4 weeks but ranges from 2.0 up to 32 weeks. These inconsistencies between the measurements are apparent throughout all age classes and are, therefore, not interpreted to result from variation found in one of the standards. These findings will be further discussed in the discussion section.

### 6.3.2.5 Sphenoid body

Estimates for the sphenoid body could only be used in foetal remains, as no infant standard exists for this bone. The width and the length are recorded, and in total 21 measurements were taken (length=7, width=14). It was found that results from the body width consistently give very low age estimates. In the six cases that provide data on both measurements, the average divergence between them is 9.71 weeks (range=6.0-14.0w). Mean age for sphenoid body length deviates from the mean of all bones combined by -0.67 weeks. The body width on the other hand shows an average deviation of about -12.1 weeks (range=-3.05 to -21.63w) with a mean age of -10.43 weeks. The only case where the average came within three weeks of the mean age of the remaining bones, is in an individual of about 31 gestational weeks. This age falls close to the average delay the body width shows in most of the individuals, which suggests that the bone is growing only to a very limited extent during the late gestational period and throughout infancy. Table 13 shows the average measurement of the sphenoid body width and range for the different age categories. The deviation is present throughout all age classes and

*Table 13. Comparison of the average dimensions and range of the sphenoid body width in millimetres for three age categories.*

age category	n	body width mean	range
foetal	7	13.16	11.08-14.58
neonate	6	13.67	12.75-14.81
post-neonate	1	15.46	-

dimensions only increase slightly. It is rather surprising that the sphenoid body width was tested to have no significant difference with chronological age. At this point the author is not able to explain this issue but the small sample size might be contributing to this result.

### 6.3.3 Conclusion skeletal age estimation

The findings of this analysis show that while at a first glance skeletal age on average matches chronological age very closely, there is much variation between the single bones. In general, deviation from chronological age seldom exceeds a few weeks. Estimates that fall within four to five weeks of chronological age can be considered fairly accurate, given that birth may have occurred anywhere from 37/38 to 42 weeks gestation. Thus, a certain amount of size variation cannot be excluded.

An interesting finding is that mean accuracy is more close to chronological age when negative estimates are included, as they could be correcting for the tendency of the method to overestimate real age. However, the opposite is found, leaving room to further investigate this matter.

The statistical analysis suggests that there are two main areas of discrepancy: first, individuals that died during the late foetal stage and, second, the infant standards based on single bones. The Maresh standard alone produced results that consistently correlated with chronological age. However, whether the performance is a result of the standard alone or a result of a more stable growth track of the major long bones, needs to be established. Most of the single bone standards for infant remains performed poorly. In case of the length and width of the ilium and the sphenoid body, differences in the developmental pattern of the bone cannot be ruled out.

### *6.4 Results deciduous Demirjian stages*

The calculation of the intraobserver error revealed only fair agreement between the two scoring sessions which is potentially problematic, and could influence the results. A disagreement of one stage at first sight does not imply major differences in the age estimate but it should be noted that age intervals between the stages cover more than a month. For example stage D of the upper central incisors is

reached at age of 1.2 months while stage E is entered at 3.8 months giving a difference of 3.6 months. See table 14 for the dental developmental stages provided by Liversidge and Molleson and corresponding ages (2004, 179). Taking a closer look at the data it becomes apparent that individuals are consistently given younger stages during the second scoring session. In total, 20 canines and molars that had previously been assigned a stage C were assigned stage B in the second session, thus excluding them from the method. The same applied to the

*Table 14. Age of attainment of crown and root stages (mean + SD in years) (Liversidge and Molleson 2004, 179).*

	C	D	E	F	G	H1	H2
i <sup>1</sup>		0.12 ± 0.24	0.42 ± 0.31	0.98 ± 0.23	1.42 ± 0.35	2.38 ± 0.31	2.26 ± 0.15
i <sub>1</sub>		0.10 ± 0.20	0.32 ± 0.13	0.83 ± 0.27	1.20 ± 0.11	1.86 ± 0.34	1.98 ± 0.11
i <sup>2</sup>		0.28 ± 0.24	0.52 ± 0.19	0.96 ± 0.32	1.49 ± 0.04	2.42 ± 0.34	2.58 ± 0.49
i <sub>2</sub>		0.32 ± 0.07	0.47 ± 0.17	1.00 ± 0.28	1.60 ± 0.30	2.30 ± 0.30	2.39 ± 0.40
c <sup>1</sup>	0.34 ± 0.20	0.83 ± 0.26	1.07 ± 0.30	1.94 ± 0.18	2.47 ± 0.36	3.09 ± 0.25	3.33 ± 0.13
c <sub>1</sub>	0.38 ± 0.18	0.81 ± 0.12	1.02 ± 0.26	1.75 ± 0.13	2.38 ± 0.42	3.04 ± 0.27	3.51 ± 0.35
m <sup>1</sup>	0.18 ± 0.26	0.35 ± 0.11	0.70 ± 0.12	1.29 ± 0.12	2.30 ± 0.41	2.38 ± 0.35	2.87 ± 0.53
m <sub>1</sub>	0.13 ± 0.25	0.48 ± 0.18	0.78 ± 0.25	1.29 ± 0.12	2.49 ± 0.35	2.68 ± 0.28	2.91 ± 0.35
m <sup>2</sup>	0.29 ± 0.14	0.78 ± 0.26	1.23 ± 0.27	2.32 ± 0.47	3.05 ± 0.28	3.48 ± 0.69	3.92 ± 0.60
m <sub>2</sub>	0.39 ± 0.21	0.92 ± 0.26	1.34 ± 0.11	2.28 ± 0.51	2.78 ± 0.45	3.01 ± 0.61	3.54 ± 0.74

incisors, where in ten instances stage C was given instead of stage D, (one tooth was assigned stage D instead of stage C), which would equally exclude these teeth from being used for the final age estimate. The consequences of this finding will be discussed in the following chapter. While proceeding with the analysis, this potential error should be kept in mind.

The deciduous Demirjian system showed a significant difference with chronological age in the analysis above (section 5.2). To see whether the source of error could be found, two additional analyses were conducted. First, it was looked whether age played a role in the accuracy of the method, and second the different tooth classes were analysed. For both steps an independent samples t-test was done to see whether there was a difference between the sample mean and chronological age. In case of low sample size (n<10) the nonparametric version of the t-test was used instead. Subsequently, the Spearman rho correlation coefficient was calculated to test whether estimated and real age showed a positive correlation.

When looking at the age distribution of the individuals of known age it becomes apparent that only three of them fall within the age range of the method

(aged 7 weeks, 11 weeks, and 13.04 weeks). The remaining four individuals were aged younger but had some teeth that were in an advanced stage of development (aged 1 week, 2.4 weeks, 2.7 weeks, and 3.4 weeks). It is expected that the method would perform better in the older individuals, therefore, the derived estimates were arbitrarily divided into two age cohorts. One aged from 5.2 weeks to 6.9 weeks (n=12) to capture individuals that would only have some developing teeth available, and the other aged from 7.0 to 24.9 weeks (n=20). The sample mean of individuals younger than 7.0 weeks is found to differ significantly from chronological age ( $t=4.118$   $p<0.001$ ). However, the method overestimates known age by only +2.42 weeks and the correlation is significantly moderate ( $r_s=0.587$   $p=0.045$ ). The sample comprising individuals older than 7.0 weeks did not pass the Levene's test for equality of variance ( $F=10.790$   $p=0.002$ ) and the Mann-Witney U test was significant ( $U=16.000$   $p<0.001$ ) revealing that the means of chronological age and estimated age differ significantly from each other. The mean difference is +5.2 weeks and the Spearman test shows no correlation between the two groups ( $r_s=0.162$   $p=0.789$ ). The analysis shows that both age groups are unable to significantly predict chronological age but that in younger individuals estimated age is relatively close to known age which is the reverse of what had been expected. It was thought that age estimations around the lower cut-off age of a method would potentially be less accurate. It has to be noted that in the younger age cohort only incisors and molars are present, as canines only later reach a developmental stage that can be recorded by this method (at 4 months). To see whether the poor performance of the method is the result of the canines being variable, the different tooth classes were analysed separately.

The performance of the incisors, canines and molars is presented in table 15. Age estimates of all three tooth classes differ significantly from chronological age. Canines overestimate age on average by ten weeks and molars and incisors by about five weeks. Only age estimates from the incisors correlate positively with chronological age ( $r_s=0.779$   $p<0.001$ ), while the molars and canines fail in this regard (canine  $r_s=-0.544$   $p=0.456$ ; and molar  $r_s=-0.06$   $p=0.861$ ). The data shows that variability of the age estimates increases with age and is probably not the result of just one tooth being more variable than the others.



*Table 15. Mean difference of the Demirjian stages to chronological age for the different tooth classes, including results of the t/U test.*

Tooth class	n	mean difference in weeks	t/U	p
Incisors	18	4.67	t=2.54	0.016
Canines	4	10.11	U=0.000	0.029
Molars	11	5.27	t=2.213	0.039

#### 6.4.1 Results deciduous Demirjian stages including all individuals

Twenty-five individuals provided data for the developmental stages resulting in 128 single observations. On average five teeth were available per individual (range=1-15). A total of 208 teeth had to be excluded from analysis because their developmental stage was too young to be used for this method.

The sample was again split into two age categories, from 5.2 weeks to 6.9 weeks and from 7.0 weeks to 51 weeks. The resulting mean was compared to the mean of the different tooth classes to see whether one of them deviated strongly from it. Results are presented in table 16. The table shows that each tooth class

*Table 16. Mean difference of the tooth classes to the mean age of two age cohorts for the deciduous developmental stages.*

age cohort	tooth class	n	mean difference in weeks
5.2 - 6.99 weeks	incisor	8	-0.53
	canine	0	-
	molar	5	+1.46
7.0 - 51.0 weeks	incisor	13	-0.39
	canine	9	+2.04
	molar	14	-0.85

provides estimates that are very close to the mean of all teeth combined. Variation is limited to two weeks for the tooth classes regardless the age cohort. The estimates of the tooth classes reveal that, the method is consistent in itself. The

only tooth class that deviated slightly more than two weeks from the mean age were the canines. The overestimation was also apparent in individuals of known age at death where the canines overestimated age by more than ten weeks. However, as the deviation is only very limited in the entire sample and one should be careful to conclude that the canines are less suited for age prediction than the other two tooth classes. But that this represents a trend cannot be excluded either.

Regarding the accuracy of the method no conclusions can be drawn from the general consistency of the various tooth classes between the two age cohorts. Therefore, to better understand the results, the developmental stages are also compared to dental height below (see section 5.6).

#### 6.4.2 Conclusion deciduous Demirjian stages

It has been shown that the Demirjian tooth stages significantly overestimate chronological age in the infants from Middenbeemster by about five weeks. This difference can be considered moderate. Mean difference to chronological age was only 2.4 weeks in individuals younger than 7.0 weeks while variability in dental development seemed to increase with age. However, variability might be in part due to intra-observer error.

All three tooth classes are equally unable to predict chronological age, but the canines stand out as giving the greatest overestimation. It has to be kept in mind, however, that the entire analysis is based on a very low sample size, and that results should be interpreted with caution. In the analysis that combined all individuals, it was found that results were relatively consistent between the tooth classes.

## 6.5 Results dental height by Liversidge and colleagues

The analysis of known age infants revealed that dental height produces estimates that are in good agreement with chronological age. To be able to better evaluate the performance of the method, several additional analyses are conducted using the independent samples t-test.

First, the upper jaw (n=29) and lower jaw (n=44) are compared separately with chronological age to see whether results differ from each other. Estimates from both jaws are not significantly different to chronological age (upper jaw:  $t=1.679$   $p=0.099$ ; lower jaw:  $t=-0.524$ ;  $p=0.601$ ). The upper jaw overestimates chronological age by on average 2.31 weeks. The lower jaw, on the other hand, underestimates age by -0.52 weeks. When both jaws are combined estimated age deviates from real age by 0.57 weeks. Thus, the upper jaw is less preferable when estimating age from dental height but still provides good results.

Subsequently, the different tooth classes are analysed. Accuracy of each tooth class is presented in table 17. The mean age of all three tooth classes does not differ significantly from chronological age (incisors:  $t=1.631$   $p=0.107$ ; canines:  $t=0.490$   $p=0.63$ ; molars:  $t=-0.793$   $p=0.432$ ). The canines show the least deviation from real age of less than a week. Incisors overestimate age while molars underestimate age, but both stay below two weeks of deviation.

*Table 17. Mean difference of dental height to chronological age in weeks for the three tooth classes.*

tooth class	n	mean difference to chronological age	SD	SE
incisors	38	+1.68	4.10	0.67
canines	11	+0.95	4.54	1.37
molars	25	-1.28	6.27	1.25

Subsequently, estimates of incisors, canines and molars are evaluated separately for the upper and lower jaws to see whether differences between the

tooth classes stood out. Mean differences to chronological age are shown in table 18. Estimates from maxillary incisors differ from chronological age by +3.5 weeks which is indicated by the t-test to be of significance ( $t=2.079$   $p=0.046$ ), while having a moderate significant correlation ( $r_s=0.719$   $p=0.002$ ).

*Table 18. Mean difference to chronological age in weeks for the three tooth classes broken up into upper and lower jaw.*

Jaw	Tooth Class	n	mean difference to chronological age	SD	SE
upper jaw	Incisor	16	+3.5	4.73	1.18
	Canine	4	+0.16	4.93	2.47
	Molar	9	+1.13	7.54	2.51
lower jaw	Incisor	22	+0.35	3.46	0.74
	Canine	7	+1.4	4.62	1.74
	Molar	15	-2.68	5.45	1.41

However, significance for the t-test was only just reached, thus the result is interpreted as being still of relatively good agreement. The canines and molars provide very accurate results. The canines differ from known age by only +0.16 weeks ( $t=0.041$   $p=0.968$ ) and molars by +1.13 weeks ( $t=0.382$   $p=0.708$ ). The canines show a strong coloration that is not significant. The result may be explained by the small sample size ( $n=4$ ). The molars equally show a strong correlation with known age, which in this case is significant ( $r_s=0.896$   $p<0.001$ ). Thus, the analysis reveals that the slight overestimation seen in the comparison of maxillary and mandibular teeth results from the incisors alone. However, a difference to known age of 3.5 weeks in case of the maxillary incisors, is still considered a reasonably good match.

The mandibular incisors and canines overestimate chronological age only slightly by +0.35 weeks ( $t=0.272$   $p=0.787$ ) and +1.4 weeks ( $t=0.597$   $p=0.562$ ), respectively, and their correlation with real age is significantly moderate ( $r_s=0.659$   $p<0.001$ ) and significantly very strong ( $r_s=0.955$   $p<0.001$ ), respectively. Chronological age in the mandibular molars is underestimated by -2.68 weeks

( $t=-1.386$   $p=0.177$ ) with a strong significant correlation between estimated and real age ( $r_s=0.823$   $p<0.001$ ). The results show that the mandibular molars and the maxillary incisors almost equal each other out which leads to the general high accuracy for dental height estimates.

Subsequently, it is evaluated how many teeth were needed to reach accurate results. It was found that when less than seven teeth were used for the age estimate, the mean of dental height differs significantly from chronological age ( $t=2.013$   $p=0.049$ ). Dental height in this case overestimates chronological age by 2.47 weeks. However, the Spearman rho test showed overall strong correlation between the estimates and chronological age ( $r_s=0.795$   $p<0.001$ ). It should be noted that a discrepancy of two weeks is still a very good level of accuracy.

The final analysis evaluated whether the performance of the method was age dependent as was seen with the deciduous Demirjian stages. The sample was split into individuals aged from -8.7 weeks to +8.9 weeks ( $n=60$ ) and from 9.0 to 48.1 weeks ( $n=14$ ). The sample of individuals younger than nine weeks did not pass the Levene's test for equality of variance ( $F=47.942$   $p<0.001$ ) and the nonparametric version of the t-test was used which produced a significant result ( $U=1063.5$   $p<0.001$ ). Therefore, there is a significant difference between estimated and chronological age. However, the mean difference between real and estimated age is only 1.6 weeks and the Spearman rho correlation coefficient shows a moderate significant correlation between both groups ( $r_s=0.581$   $p<0.001$ ).

To test whether the reason for this result lies in the presence of negative estimates, the calculation was repeated including only positive estimates ( $n=55$ ). Mean difference increased to almost three weeks (2.82weeks) and again, the sample did not pass the Levene's test for equality of variance ( $F=9.625$   $p=0.002$ ) resulting in the use of the nonparametric version of the t-test. Results show a significant difference between the means of dental height and chronological age ( $U=458.5$   $p<0.001$ ).

The sample of individuals older than nine weeks only differ from chronological age by an average of 0.15 weeks. The Levene's test for equality of variance was not passed ( $F=8.987$   $p=0.005$ ). The older age cohort was able to significantly predict chronological age according to the Mann-Whitney U test ( $U=101.000$   $p=0.255$ ). However, the calculation of the Spearman rho correlation

coefficient revealed a weak negative correlation between real age and estimated age that was not significant ( $r_s=-0.365$   $p=0.199$ ).

The reason for the failing of the Mann-whitney U test for individuals younger than nine weeks was thought to be possibly the result of the great intra-individual variability. Variation within the dentition of single individuals ranges from between three to 17 weeks. It was decided to test whether the amount of individual variation had an influence on the performance of the method. Individuals were split into two groups of either low variation (<7 weeks) or high variation (>7weeks) and each group's mean was compared to chronological age. Results showed the reverse to what had been expected. For individuals with less variation within their dentition, the Mann-Whitney U test was significant ( $F=42.881$   $p<0.001$ ; and  $U=263.000$   $p=0.014$ ), indicating there was a difference between real and estimated age. Individuals with higher variability showed no significant difference between the means ( $t=-0.162$   $p=-0.186$ ) indicating that variation was not the cause of error. However, the mean age of individuals with less variability is 2.71 weeks, whereas the sample with increasing variability has a mean age of 8.14 weeks, which points out that the age of an individual is a determining factor for the accuracy of the method. The great variability found in individuals older than nine weeks also explains why the Spearmann test was not significant.

#### 6.5.1 Results Dental Height by Liversidge and colleagues including all Individuals

All 39 individuals provided information on one or more teeth, with a total of 300 observations. The number of teeth per individual varies between one and 17. The average amount of variation that exists within the teeth of single dentitions is 9.25 weeks, ranging from 2.14 up to 20.65 weeks. As with the sample of individuals of known age, all individuals were evaluated for a) differences between the the upper and lower jaw, b) the performance of the incisors, canines and molars, and c) differences in the three age cohorts. The results were compared with sample mean age.

The sample confirmed the observations made above that the upper jaw tends to provide older estimates. Mean difference between the jaws is 2.1 weeks (range=0.27-17.10weeks). The upper jaw has a mean age of 8.97 weeks (range=-12.5-45.04weeks) while the lower jaw gives an average age of 6.87 weeks. It was then looked whether these results could be caused by variation in the tooth classes. The incisors and molars show a similar extent of variation, but the former is overestimating age by +1.88 weeks while the latter is underestimating age by -2.12 weeks. The canine shows the least deviation of less than half a week (-0.43weeks). These results are almost identical to the ones derived from individuals of known age at death, the difference being that the canines are slightly underestimating age instead of overestimating it. The results from the tooth classes were split into the different age classes to see whether these showed consistency. Such was the case for the incisors and molars (table 19). The canines on the other hand were more variable, with a discrepancy between the age classes of six weeks. While on average the canines produced the best results, their variability throughout the age classes makes their estimates less well predictable and reliable.

*Table 19. Comparison of mean difference of each tooth class to the mean age of all teeth combined, separated into three age categories.*

tooth class	n	age class	mean difference in weeks	range
incisors	36	foetal	+2.51	-4.19 to +2.51
		neonatal	+1.62	
		post-neonatal	+1.86	
canines	27	foetal	-4.19	
		neonatal	-0.14	
		post-neonatal	+2.00	
molars	33	foetal	-0.57	
		neonatal	-2.80	
		post-neonatal	-2.11	

n= number of individuals

### 6.5.2 Conclusion dental height by Liversidge and colleagues

Dental height is able to predict chronological age very accurately with a discrepancy of less than a week. The upper jaw overestimates age by two weeks, but not significantly. It was found that the method decreases in accuracy when less than seven teeth were available for observation. In addition, dental height is less accurate in younger individuals, even if the method is corrected for the presence of negative estimates. The great variability in estimates, that is found in a single dentition, has no effect on the accuracy of the method. The analysis that included all individuals generally confirms the results made with individuals of known age. The more detailed analysis of the tooth classes reveals some variation within the canines, however, the overall performance of the method can be considered consistent.

### *6.6 Comparison deciduous Demirjian stages with dental height*

Twenty five individuals could be used for the comparison of the two dental ageing methods. The deciduous Demirjian stages give a higher age in 20 of the cases by an average of 3.77 weeks (range=0.12-13.10weeks). In the remaining five instances dental height gives a higher age by an average of 7.55 weeks (range=1.49-14.58weeks). Except in one case, all four individuals in which dental measurements produced an advanced age are from the oldest four individuals in the sample. The analysis with individuals of known age reveals that dental height is more accurate in older individuals. However, the analysis only incorporated individuals up to 13 weeks of age. Thus, the accuracy of the method for older infant remains has not been established. The developmental system shows a rather constant overestimation throughout the individuals which would support the findings above that the method is consistent in itself, but generally overestimates age.



## 6.7 Infant mortality

The last section analyses the age distribution of the Middenbeemster infants to see whether it exhibits abnormalities. Periods of increased stress can be shown through increased mortality but may also show in a delay of skeletal development. A comparison of skeletal and dental age follows the establishment of the age distribution.

### 6.7.1 Age distribution

Figure 11 shows the age distribution of the 39 foetal and infant remains from the Middenbeemster cemetery that were assessed in this thesis, broken into four week intervals. The distribution around the time of birth follows a gaussian curve which peaks during the neonatal period (0 - 4weeks). From 12 weeks onwards mortality remains stable until week 24, after which infant deaths become incidental. Late foetal mortality accounts for 20.5% of the total, neonatal mortality for

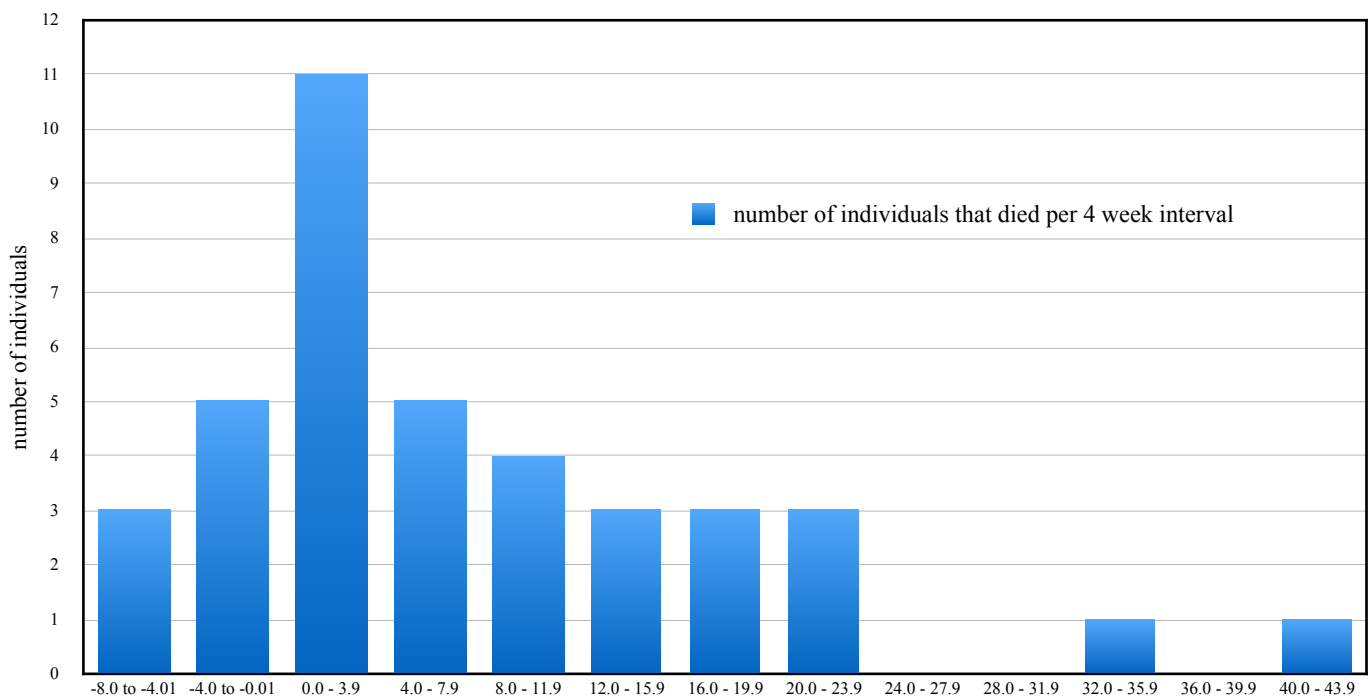


Figure 11. Age at death distribution\* of the infants from the Middenbeemster cemetery used in this thesis, broken down into four-week intervals.

\*For this figure the mean age of all three methods combined is used.

28.2%, while the post-neonate period until the age of 12 month accounts for 51.3% of the total. It has to be kept in mind that while the late foetal and neonatal period together encompass four months, the post-neonatal period represents 11 months. As a proportion of the amount of time, the number of individuals that died during the late foetal and neonatal period is thus much higher with 61.5% of the individuals dying within this period while only 38.5% died during post-neonatal period.

### 6.7.2 Dental versus skeletal development

Skeletal and dental age were compared to see whether there is a consistent difference between the two growth systems. As dental height showed a better correlation with chronological age, it was chosen to use this method for the comparison. Both methods were shown to have means that did not deviate strongly from chronological age, thus any deviation between the methods could be interpreted as an actual difference between the two growth systems. For this analysis all individuals were used and means of skeletal and dental age were compared using the independent samples t-test.

In 25 of the 37 individuals, dental age is advanced by an average of 4.6 weeks (range=0.12-12.30weeks). Skeletal age is advanced in 12 cases, on average by 2.9 weeks (range=0.19-6.73weeks). Results of an independent samples t-test were significant ( $t=7.076$   $p<0.001$ ) indicating a significant difference between the means of both groups.

It was looked whether the difference was more pronounced in one of the age groups. In the foetal remains 37.5% show skeletal delay while 82.4% of the neonates show delay of their skeletal system, and 58.3% of the infants have delayed skeletal development. Mean difference between skeletal and dental age is less than a week in foetal remains (0.98weeks). The sample did not pass the Levene's test for equality of variance ( $F=7.296$   $p=0.007$ ) so the nonparametric version was used. Results indicate no significant difference between the means of the methods ( $U=5949.500$   $p=0.80$ ).

Mean difference between skeletal and dental age in neonatal remains increased to 3.1 weeks. The Levene's test for equality of variance was not passed ( $F=19.209$   $p<0.001$ ) so the nonparametric version was used. Results indicate a significant difference between the means of the two methods for this age class ( $U=5785.500$   $p<0.001$ ). Post-neonates show a mean difference of 5.64 weeks and the results of a Mann-Whitney U test indicate a significant difference between the means of skeletal and dental age ( $F=7.336$   $p=0.007$ ;  $U=3153.500$   $p<0.001$ ).

From these observations it can be concluded that skeletal age tends to be delayed, and that comparatively more neonates are affected although the delay is more pronounced in the older infants. The fact that more individuals showed a delay during the neonatal period, and that this is the phase with the highest mortality, could indicate that increased stress during the neonatal period resulting from the hazards faced by the individuals adapting to the extrauterine environment led to a reduction of skeletal growth. However, it needs to be stated that because skeletal age tends to underestimate chronological age and dental age tends to overestimated real age, the real difference between skeletal and dental age could be less than indicated above.

### 6.7.3 Conclusion infant mortality

Infant mortality is most pronounced during the neonatal period. This age group also shows the greatest amount of individuals that have a delayed skeletal development. Skeletal age is delayed in all age groups but showed the greatest deviation in individuals older than 28 days.

## 7. Discussion

Before we proceed to the discussion of the results a word on accuracy seems necessary. Accuracy describes the degree of conformity of a measure to a standard or a true value ('accuracy' *Encyclopaedia Britannica Online Academic Edition*). In this thesis, accuracy measures how close the estimated age falls to chronological age. The degree of accuracy that is possible depends on the source material. Variability in timing of the developing dental and skeletal tissues needs to be incorporated as a degree of error one has to accept. The analyses pointed out that the degree of variability depends on the age category. Intra-individual variation is much more clustered in foetal remains, than in neonatal remains, while post-neonatal remains had the greatest amount of variation. Thus, in general, age estimation will be more accurate during the early developmental periods and will decrease with age.

Neonatal remains incorporate another error, which is the discrepancy between the moment of birth and the actual state of development of the individual. Chronological age represents a point in time, in this case defined as the birth event plus the days until death. Normal delivery takes place between 37 and 42 weeks post fertilisation. As the exact moment of birth during development cannot be known this range has to be used as the standard error when comparing estimated age to chronological age. While it should be expected that the early born and those born post term will equal each other out, it should be kept in mind the most prominent cause of neonatal death is inadequate prenatal growth and development (Bogin 1999, 61). According to Bogin four out of five neonatal deaths are due to the baby being small for gestational age (1999,59). Thus, neonatal remains will potentially show a delay to chronological age.

## 7.1 *Skeletal age estimation*

The susceptibility of skeletal development to adverse environmental conditions has led to that only very few studies exist who actually consider skeletal growth as a viable age indicator in subadult remains, and most of them are confined to foetal remains (Deutsch *et al.* 1981; Khan and Faruqi 2006; Rissech *et al.* 2013). In general, subadult remains are aged using the dentition, and skeletal development merely functions to show whether there has been any physiological impairment (Miles and Bulman 1994; Postel-Vinay and Sahn 2010; Saunders and Hoppa 1993). Despite these often stated shortcomings this study applied skeletal growth to estimate age to see whether the concerns are actually warranted in infant remains. Unfortunately this implies that very few studies exist to compare the present data with, and there have been no studies that test the accuracy of the standards that were consulted for this thesis.

Today skeletal growth and maturation in young children is generally determined by assessing the development of the hand and wrist bones. The developmental state of each bone is given a score and these are compared to an atlas providing an ideal representation of development at a given age (Greulich and Pyle 1959; Tanner *et al.* 1975; 1983; Todd 1937). Given the fragile nature of the small hand bones and carpal bones these are seldom fully retrieved from archaeological burial grounds which renders the method unsuited for osteological purposes. Thus, no comparison can be made between the measurements used in this study and modern data. A study by Hoffman (1979) compared diaphysial length of the radius and femur to dental eruption in individuals aged from two months until twelve years and found that variability was less pronounced in bone growth than in dental eruption. The variability in the emergence of the dentition is known which makes it a less preferable method for estimating age (Hillson 1996, 139; Scheuer and Black 2000, 152). The study by Hoffman, therefore, only indicates the upper limits of variability in bone growth and no degree of accuracy can be deduced from this. The degree of variability for skeletal growth can be

deduced from the standards developed by Maresh (1955). For example an individual with a diaphysial length of the humerus of about 86 cm can either be aged three month falling into the upper limits of its age cohort. But it could equally be a slowly growing infant of about six month of age (Schaefer *et al.* 2009, 174). To account for differences between males and females the ranges have to be increased in archaeological material. Maresh showed in her work that variation between males and females does not only pertain to the difference in absolute size between the sexes but that bones of males and females have different proportions to each other (1943).

Thus, the standard error used for skeletal age in infant remains needs to incorporate the discrepancy between moment of birth and actual gestational age as well as the normal variability of the growth system itself. Having an error of at least two months would seem appropriate. Therefore, the finding from the analysis of known age individuals that showed a general mean difference from chronological age of less than a week with a standard deviation of 8.5 weeks can be considered an excellent result. It suggests that skeletal age can be used as age estimator in perinatal and young infant remains.

The analysis revealed that the method contains a great deal of variability. But most of the bones showed a discrepancy to chronological age of less than one month which lies within the acceptable range. It is interesting that the greatest variability stems from flat and irregular bones which might suggest that the more complicated growth pattern of these bones is less well predictable than the linear increase in length of the long bones. In addition, the degree of error due to false interpretation of the description of the measurements, especially of the irregular bones could decrease the precision of the recordings. However, the high degree of intraobserver agreement shows that this was not a factor in this study.

The statistical analyses shows that in many cases there is a significant difference to known age, even though the mean difference to chronological age is minor, while other bones that have a larger difference to known age do not differ significantly. The pars basilaris saggital length, the sphenoid body maximum width, the scapula maximum length, and the ilium maximum width all overestimate chronological age substantially by about ten weeks (rang 7.25-14.09w), while the U-test did not indicate a significant difference to

chronological age. On the other hand, the pars lateralis height, frontal chord width, length of temporal pars petrosa, length of mandibular body, ilium maximum length, ishium maximum length, ishium maximum width, and pubis maximum length, together overestimate chronological age by only 1.8 weeks but turn out to be significantly different from real age. The differences are not the result of low sample size as both sets of measurements have on average the same amount of observations. What becomes apparent is that all measurements that tested significantly could only be applied to foetal bones thus producing only negative estimates. The same would, however, apply to the sphenoid body maximum width. But in this case, only three measurements were obtained and it is very likely that the result is due to chance. This finding is not well understood but it might be related to the statistical test that was used to look for a possible difference between the means of chronological age and estimated age. In all instances where no significant difference between the means could be detected even though the actual difference was more than two month, the Man Whitney U test was used. This test combines the two groups into one single sample and looks for differences in the distribution of the data. To do this, the test converts the continuous data into an ordinal ranked scheme, thereby removing the actual amount of difference in values. This could suggest, that the U-test is not a good choice of alternate test form small sample size for this type of data.

Another interesting finding is that the mean accuracy is more closely to chronological age when negative (foetal) estimates are included. This is odd as the method was shown to generally underestimate age. It was expected that the inclusion of negative estimates would increase this effect. However, the analysis of the infant standards revealed that all of them considerably overestimate age, which indicates that the apparent underestimation is probably the results of the great amount of individuals with a developmental state congruent with late gestational age. From the 17 neonatal remains aged based on dental height development, seven individuals have a skeletal age consistent with late foetal development. From the remaining 10 individuals, nine have at least one element that gives a negative age estimate or indicates 40 weeks of gestation. Thus, the apparent underestimation is not the result of a general tendency of one of the growth standards to underestimate age, rather it appears that individuals that died

around the time of birth were either small for gestational age or they were born earlier during development (between 37 and 40 weeks gestation).

### 7.1.1 The Standards

During data collection it was noted that there exists a gap for most of the neonatal period in which almost no observations exist. The foetal standards provide estimates in a weekly interval until the age of 40 weeks. The Maresh standard starts at 6.5 weeks, while the single bone standards only give ranges that incorporate the first three to six months. Only the clavicle provides some single observations from the second week onwards. Thus, in a sample that has a large amount of perinates the infant standards will automatically produce advanced estimates. Yet, the presence of the many negative estimates counteracts this shortcoming of the infant standards, thus, not causing major problems for the estimation of neonatal age in this sample. This potential bias should be kept in mind in future studies.

#### *7.1.1.1 Foetal bone growth: The Fazekas and Kósa standard*

Unfortunately the nature of foetal remains is that no exact age can be known. But from the estimates that are available for this age category it can be deduced that the genetic regulation of growth minimises variation in this age category. The intra-individual variability is one third of that of post-neonatal remains, and half of that from neonatal remains. This observation indicates that the foetal standards are predicting developmental age of the individual and variation therein very well.

#### *7.1.1.2 Long bone length: The Maresh standard*

The Maresh standard for the major long bones overestimated age by only two weeks with a standard deviation of 2.5 weeks. If compared to estimates of dental height which proved to be very accurate as well, the same good agreement is found and the two methods show a mean difference of 2.9 weeks. It was expected that infants from an archaeological sample would show a much reduced pattern of growth, and would thus be underestimated by the modern standard (Saunders



2008; Black and Scheuer 1996). These data suggest differently, and it can be assumed that foetal and infant growth in Middenbeemster followed a pattern that is comparable to growth today. However, it needs to be kept in mind that most of the data used for the standards was generated throughout the first part of the twentieth century which would suggest that the full potential of the secular trend was not yet reached. However, Maresh (1970) found no increase in length nor that individuals were maturing earlier throughout the course of the study, thus concluding that optimal growth and development was already established for individuals of this population. The Maresh standard is based on a large sample using longitudinal and cross-sectional data and therefore, may be adequately reflecting growth of children from a range of environmental conditions and genetic potentials, and thus may be actually quite suitable for age estimation of the Middenbeemster subadults.

Results of the statistical analysis are only based on a limited set of observations, but the overall sample suggests that the Maresh standard provided very consistent results, thus making the standard very reliable. It would be interesting to inquire whether the changes in the growth pattern caused by the secular trend are more restricted to childhood and the adolescent growth period. Future studies should incorporate the growth data of older subadults from Middenbeemster to see whether the standard produces comparable results in these age categories.

### 7.1.2 Single bone infant standards

It was expected that using standards that are developed on collections that are contemporaneous with the skeletal sample under study, would improve the accuracy of age estimates as the secular trend could be circumvented. The four standards each present reference data on a single bone using between one to three measurements, 1) the pars basilaris, 2) the clavicle, 3) the scapula, and 4) the ilium. Together these standards have a mean age that is twice as high as the mean age derived from the Maresh standards, which was able to predict age very well in the individuals of known age at death. There are several possible reasons why the single bone standards do not produce accurate results.

First, a technical problem is that age of single bone standards is generally expressed as a range. These ranges are very large, encompassing either 1.5, three or even six months. In order to be able to compare dental and skeletal data, as well as real age, the ranges were all converted into median ages. This partly explains why estimates are often not very precise. It should also be noted that ranges mostly derive from very limited observations which introduces the second problem inherent in the standards. In all four standards there are not enough cases to capture the normal variation for single bone development indicating that their use as standard may not be justified. In addition, it cannot be known whether there are extreme outliers in the sample that constitute the standard. The fact that the individuals probably died from disease or malnutrition should not cause a problem as the same can be implied for individuals from the Middenbeemster collection.

Third, the individuals in this sample could have had a different growth pattern for these bones. In case of the ilium, variation in the growth pattern could actually be correct (see below). Three out of the four standards are developed using the same skeletal collection (Spitalfields) with some substitution of other collections (clavicle, pars basilaris and ilium). This could indicate that differences in the developmental pattern are originating from the Spitalfields collection. However, the standard for the scapula which is developed using another collection, gives equally advanced age estimates.

Another aspect that has already been touched upon is the nature of the bones that constitute the Maresh standard as opposed to the single bone standards. While the former only applies to long-bones the latter use either flat or irregular bones (excluding the clavicle which is classified as a long-bone as well). Thus, two possibilities exist. First, the use of long bones is the best option when assessing the age of an individual, or second, the Maresh standard is generally more accurate which can be explained by the fact that it better incorporates the total variation that exists in a population. The single bone standards will be discussed in short below.

#### *7.1.2.1 The pars basilaris: Scheuer and McLaughlin-Black*

As discussed in the materials and methods section, the standard developed for the pars basilaris is based on a very limited number of observations (n=15). The

variation seen between the individuals is such that individuals of different ages show similar dimensions for selected measurements. This produced confusion when using the standard. In addition, there is also very little increase of the saggital length between three and 12 months. It was suggested that this measurement is not very well suited to estimate age of infant remains. This is confirmed in the analysis of individuals of known age where saggital length produced the least accurate estimates with an overestimation of more than 10 weeks (table 9). Maximum width on the other hand only showed a discrepancy of -0.69 weeks to real age. In the analysis of the entire sample, the saggital length was advanced to maximum width by 200%. Maximum length had an accuracy that lay in between the two other measurements.

For future use, the author would suggest to only use maximum width for age estimation. Another way to use the bone is to apply the rules of thumb that have been suggested by several authors (Fazekas and Kósa 1967, 58; Redfield 1970, 214; Scheuer and Maclaughlin-Black 1994, 380). Their studies showed that individuals aged less than 28 gestational weeks had a width that was smaller than the saggital length. Individuals that were older than five months had a width that was greater than mean length.

#### *7.1.2.2 The Clavicle: Black and Scheuer*

The clavicle overestimated age by about ten weeks in known age infants and differed from the mean skeletal age of the entire sample by almost five weeks. The unreliability of the standard is probably related to the age intervals provided by Black and Scheuer (1996, 427). The first year is divided into two intervals only. Thus, all individuals aged between birth up to six months and all individuals aged between six and 12 months would each get the same age assigned using this standard. Developmental differences between the standard population and the Middenbeemster collection cannot be deduced from using this standard as it is too imprecise to warrant such inquiry. To validate the use of this standard for age estimation, more cases need to be added in the future.

#### 7.1.2.4 *The scapula: Saunders and colleagues*

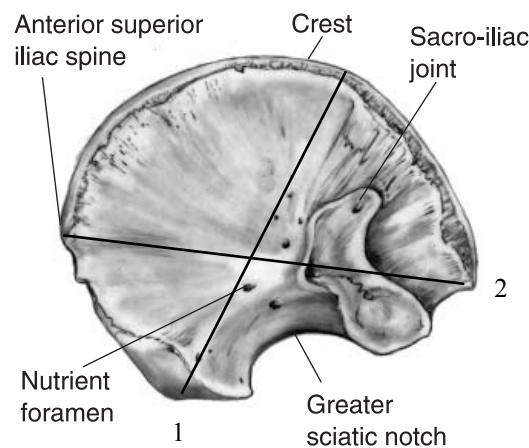
Results for the combined infant and foetal sample for age estimation using the scapula were within one week of chronological age, while the infant standard alone overestimated age by more than 13 weeks. In the material and methods section it was noticed that age was not known for all individuals that were used for generating this standard but were substituted using dental developmental stages by Moorrees and colleagues (1963a; 1963b). However, results do not differ from estimates of the other single bone standards. A slight impact on the results by using derived estimates cannot be ruled out but it seems likely that other aspects are overshadowing this condition. As only three measurements were available to test the infant standard against chronological age it is difficult to assess the extent of the overestimation but it seems substantial for this age category.

The results also showed a different level of accuracy between the two measurements. Maximum length significantly overestimated age in the entire sample and in individuals of known age, while maximum width showed a good agreement with known age. Whether the difference seen in the proportion of the two measurements to each other are the result of growth differences in the Middenbeemster collection needs to be addressed in future research. The present data will have to be compared to the adult sample to see whether size differences are still apparent in adulthood. Inter-population comparisons could reveal whether the exposed pattern is population specific.

Regarding the performance of the infant standard, the same caution that is raised for the clavicle, applies to the scapula standard, as it equally subdivides the first year into two intervals only. As has been noted already, the age distribution of the Middenbeemster sample shows a clustering of individuals around the time of normal delivery with the highest amount of individuals being aged as neonates. Standards that only use two or three intervals during the first year of life will inevitably overestimate all neonatal remains. In retrospect, using median ages to compare skeletal age with dental estimates and chronological age did only show that the standard used ranges by lack of sufficient individual cases to warrant the use of more closely defined age intervals.

### 7.1.2.3 *The ilium: Molleson and Cox*

Based on the ilium maximum width and maximum length the three infants of known age at death together overestimated chronological age by almost 20 weeks. It was found that the two measurements consistently provide estimates that differ from each other. The maximum length generally gives a younger estimate than ilium maximum width. The former estimated age with good accuracy with little more than two weeks difference to chronological age. This is confirmed in the entire sample where the maximum length is on average 2.3 weeks advanced to the mean of all bones combined. The maximum width on the other hand deviates from the mean of the entire sample by 9.8 weeks. This suggests that the ilium has proportions that differ from those of the individuals that were used to develop the standard (figure 12).



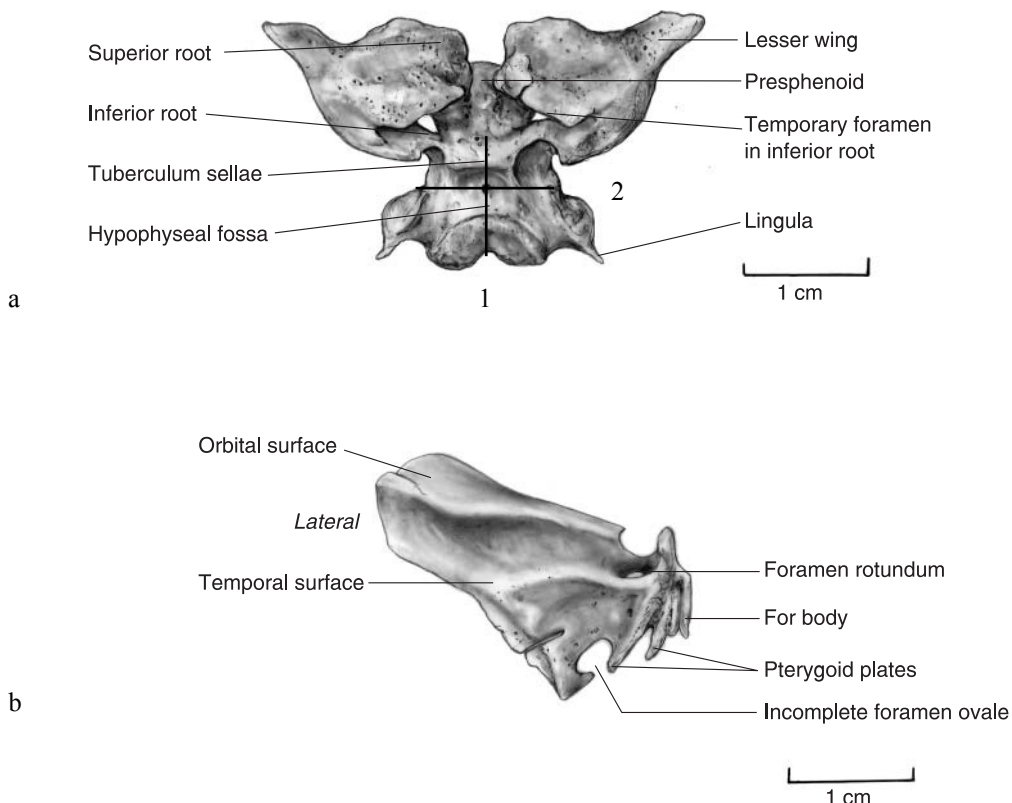
*Figure 12. Right perinatal ilium, pelvic view (after Scheuer and Black 2004, 319). The two measurements are indicated: 1=maximum length and 2=maximum width.*

A study by Saers on the activity patterns of the adult Middenbeemster sample based on limb bone geometry, found that the sample showed higher medio-lateral strengthening of the lower limb bones in both, males and females (2012, 66). This feature is correlated with wider pelvic breadth and is generally seen in woman, and in cold adapted populations. The relative differences in pelvic shape have not been investigated in the adult populations at this point. But variations in the relative dimensions of the foetal and infant ilium might suggest that the Middenbeemster collection has indeed a somehow different pelvic shape.

Further research will have to clarify this developmental characteristic of the Middenbeemster collection.

### 7.1.3 The sphenoid body

The sphenoid can legitimately be called the most complicated bone in the human skeleton. It lies in the centre of the cranium and articulates with eight different bones and five of them come in pairs (Scheuer and Black 2004, 94). The bone consists of a body, two lesser wings, two greater wings and the pterygoid plates (figure 13a,b). Development of the body includes two separate growth centres, the *pre-sphenoid* and the *post-sphenoid* that generally fuse before birth. At birth, the body is fused with the two lesser wings while the greater wings fuse during the first year. The body houses the sphenoidal sinus which is present at birth as two small separate cavities. Pneumatisation (i.e. the development of air cells or



*Figure 13a. Perinatal sphenoid body with fused lesser wings (after Scheuer and Black 2004, 96). The measurements are indicated: 1=Body maximum length; 2=Body maximum width.*

*Figure 13b. Perinatal right greater wing, inferior view (Scheuer and Black 2004, 97).*

cavities) will slowly progress throughout childhood while final shape of the sinus is achieved during puberty (Scheuer and Black 2004). Great variability in the size and shape of the sphenoidal sinus have been reported (Budu *et al.* 2013; Hewaidi and Omami 2008), which is directly related to variability in the shape of the body itself. The results from the analysis suggest differences in the developmental pattern between the individuals interred in the cemetery of Middenbeemster and the Hungarian sample used to develop the foetal standard (Fazekas and Kósa 1978). There is only a small increase in width dimensions from the foetal to the post-neonatal period and individuals that differ by more than 14 weeks in overall skeletal development have identical sphenoid body width dimensions. But a trend of increasing width can be discerned with the oldest individual of about 16 weeks of age (based on skeletal development) having the highest estimate of 35 weeks gestation. No older estimates were made.

If the differences in dimensions were coming from normal variability of the bone it should be expected to have more random results. Developmental differences can be deduced from the fact that the sphenoid body length gives estimates that are congruent with the remaining skeletal elements. The standard stops at 40 weeks gestation and the dimensions for the infant period are not known, but as the growth retardation already manifests itself during the foetal period, it can be concluded that the feature is real and does not result from the lack of information on older individuals.

It is at this point difficult to assess whether this feature comes from the present population or from the Hungarian foetal collection. Comparison with other populations would have to be made to determine whether this feature is more common or not. In addition, further research should aim at comparing the adult sphenoidal proportions in the Middenbeemster collection with other collections to determine whether the variation in dimensions persists into adulthood.

A study by Jeffery and Spoor (2004) investigated the shape variation of the cranial base during foetal development using morphometric features of MRI (Magnetic Resonance Imaging) scans of foetal remains. The changes in shape of the basicranium during foetal development of the cranium (resulting from retroflexion of the head) seemed to be centred around the sphenoid. The retroflexion results in disproportionate changes in the sphenoid height and length

which appears to be in direct relation to the development of the shape of the face and related internal tissues (Jeffery and Spoor 2004, 87). Whether variation in the size of the sphenoid, established in this study, are linked to a complex developmental pattern between different components of the cranium, and especially that of the facial components, should be addressed in future research.

## *7.2 The deciduous Demirjian stages by Liversidge and Molleson*

The deciduous Demirjian stages by Liversidge and Molleson produced inconsistent scores between the two recording sessions, which was shown to have a potential effect on the results. It was revealed that during the second scoring session the teeth were assigned younger stages which, in most cases excluded them from further analysis as they fell outside the age range of the method.

If, as suspected, the author was better acquainted with the method during the second session, this result suggests that the apparent overestimation may partly be the result of choosing the wrong stage as a result of inexperience. This fact highlights one of the pitfalls inherent in the method, as it requires a learning period. Thus, prior to data collection the rater will need to go through a period of training. The consistency with which stages were given during the second scoring session indicates that the author has reached the degree of confidence needed to apply the method. Unfortunately, the relatively long time lapse between two successive stages leads to an estimate that can differ significantly from chronological age if an error has been made in the stage assignment.

The author strongly urges to use the descriptive stages provided by Liversidge and Molleson (2004) instead of the original ones made by Demirjian and colleagues (1973). They were found to be missing in the manual by Schaefer and colleagues (2009) which is considered a shortcoming and should be added in future editions. The author only became aware of the difference between the descriptions after a certain amount of the skeletons were already analysed. It is



believed that switching from the original to the updated stage descriptions led to the difference seen in the two scoring sessions.

It should be noted that one of the reasons that added to the difficulty of using the method was due to the developmental stage of a great part of the dentition. Many teeth were just passing from stage C to stage D, which indicates the completion of the crown and the beginning of root formation. To judge when exactly the crown has completed its formation is very difficult especially when there has not yet been any root formation. In some teeth root formation will already commence although the crown has not yet been completed (Hillson 1996). This problem has also been acknowledged by Liversidge and Molleson (2004, 174).

The discussion above confirms the concerns that have been raised about the use of developmental stages to estimate age at death in subadults (Hillson 2005; Liversidge *et al.* 1993; Reid and Dean 2006). The degree of subjectivity is considerable especially when determining whether the tooth is still in a younger developmental stage or has already passed onto the next stage. Essentially, developmental stages should be consulted only when it is of importance to determine the biological age of a person.

Considering the difficulties the author faced during data collection it is of no surprise that the statistical analysis shows a rather poor performance of the deciduous Demirjian stages in individuals of known age. All statistical tests indicated a significant difference between estimated and known age. However, not in all cases does the difference to chronological age represent a problem. The age cohort of individuals aged between birth and seven weeks only overestimated chronological age by +2.4 weeks which is considered an accurate result. Given the fact that the method only applies to individuals older than 5.2 weeks, this result is surprising (see below). Individuals older than 7.0 weeks on the other hand overestimated age by +5.2 weeks on average and the overestimation was also confirmed in the entire sample where dental height was used as a comparison. The poor performance is probably also related to the age distribution of the known age sample, most of which are aged below the age range of the method.

The age range of the method clearly represented a confounding factor during data collection. Out of 39 individuals with teeth only 25 could be used, of which

only seven were of known age at death. Thus excluded 99 teeth from the analysis from individuals younger than 5.2 weeks of age. Fourteen of the individuals that could be used had on average eight teeth (range=1-16) that were outside the range of the method (i.e. at a younger developmental stage), excluding an additional 109 teeth from the analysis. This meant that age could only be estimated for the most advanced teeth in these individuals, and it was thought that this might potentially overestimate age. As mentioned above, it is, therefore, surprising that the method proved to be most reliable in younger individuals who were aged close to or even below the lower boundary of the method. However, Liversidge reported that when applying the permanent stages to individuals aged towards the lower boundary of the method, these individuals tend to be underestimated (Liversidge 1999). As discussed in section 4.2.1.2, Liversidge interpreted the underestimation to be the result of limited data on the earlier stages of the permanent dentition. If the same principle applies to the deciduous stages, this would support the general observation that the method overestimates age.

In the original article by Liversidge and Molleson (2004, 173) the age distribution of individuals younger than 12 months is reported in two intervals, one at three months (n=34) and the other at nine months (n=24). In what manner the age of the individuals is clustered around these intervals is not stated, but from this it can be deduced that there is a relatively large amount of data available for infants aged from 5.2 weeks to three months. This would provide a good picture of the variation in deciduous dental development present for the early post-neonatal period. Later stages are represented by far less individual cases. Whether the apparent difference in accuracy between the two age groups is directly related to the age distribution of the original sample used to develop the method, seems plausible, but more tests with other populations would have to be conducted to verify this hypothesis.

Differences in the accuracy of the method may also be related to differences in the growth pattern of the Spitalfields collection and the Middenbeemster sample. The statistical analysis shows that the canines are most advanced in this sample but that all teeth overestimate age considerably. This trend is confirmed in the entire sample, but the difference to mean age is much less. The canine is the last tooth class that starts developing and is thus the last to enter the the method. A

discrepancy in analysis might be related to the stage of development of the teeth, as most of the canines had just passed from stage B to stage C. Stage C alone covers a time span of about five months. Teeth that have just entered a stage will naturally be given a slightly older estimate for the individual than their developmental stage suggests, in order to cover the entire range of development that happens during that stage in a particular population.

In chapter four (section 4.2.1.1) concerns were raised about the use of dental height as substitute for known age at death in the Spitalfields collection in order to develop the deciduous Demirjian stages. First, the general problem of using derived age for the construction of a methods was highlighted (Bocquet-Appel and Masset 1985; Black and Scheuer 1996) and second, the ironic situation arose in which the author tries to compare two method of which one is partly developed using the data of the other. The comparison between the developmental stages and dental height showed that the methods produce results that have a different level of accuracy, which implies that the deciduous Demirjian stages do not mimic dental height, thus not introducing a circular argument. However, in fact it would not be feasible to test whether the two methods mimic each other as in the case of the deciduous stages providing similar results as dental height the method would be interpreted to be working well. To substitute real age with the derived estimates from crown length certainly introduced some degree of error, however, the level of accuracy reported for the method by Liversidge Dean and Molleson, shows that the error is kept at a minimum.

### *7.3 Dental height by Liversidge and colleagues*

The deciduous dentition has a fast developmental rate with a linear increase in height during crown formation and, therefore, a strong correlation between crown length and chronological age. This is shown in the results of the statistical analysis of the ten individuals of known age at death, where the mean age of dental height only deviated from chronological age by little more than half a week.

The results showed that dental height is very reliable in producing consistent results. Although, in three instances the statistical analysis revealed a significant difference between chronological and estimated age, 1) in maxillary incisors, 2) when less than seven teeth are available, and 3) in individuals of less than nine weeks of age. However, the difference would generally stay below two weeks and only in case of the maxillary incisors reached 3.5 weeks, which is still well within the acceptable range.

It is not well understood why in the case of individuals younger than nine weeks of age the mean differed significantly from chronological age. Large intra-individual variability, as well as a large amount of foetal estimates were ruled out to cause this result. Sample size can equally be rejected as more observations are available for the younger age cohort (n=60) than for individuals older than nine weeks (n=14). A possible explanation might be found in the age distribution of the Middenbeemster skeletal collection, which has a high amount of neonatal remains, while the Spitalfields documented collection has a relatively greater proportion of post-neonatal remains (Lewis and Gowland 2007). The difference in age distribution between the two samples might explain why the method is more accurate in post-neonatal remains. The effect however, is very small thus not causing major discrepancies in the age estimate.

The same conclusion can be drawn from analyses of the different tooth types, which shows that estimates are relatively constant throughout the neonatal and post-neonatal age classes. Foetal estimates of the incisors and canines deviate more strongly from the overall mean of the entire sample (incisors=+2.5; canines=-4.2; molars=-0.57), which can be explained by the lack of younger individuals in the Spitalfields sample. The results show that a lower boundary is reached for this method, and that estimates start to become more variable when applied to perinatal and foetal remains. From this it can be inferred that the method is best applicable to post-neonatal individuals.

Unfortunately, there is only limited data available on older infant remains aged toward the end of the first year. The oldest individual of known age at death is 13 weeks, thus, no accuracy is known for individuals aged between 13 and 48 weeks (n=10). The data suggest that variability increases with age while the method becomes less accurate in the oldest individuals. However, as the analysis

of older individuals has to rely on the comparison with the other two methods, which have proven to be generally less reliable than dental height, this conclusion should be treated with caution. See appendix 4 figure 16 for a graphic juxtaposition of the age estimates of all three methods for all individuals.

As expected, the analysis found that there was indeed a difference between the estimates of the upper and lower jaw, however, results did not differ significantly with chronological age except for the maxillary incisors. The upper jaw overestimated age by little more than two weeks while the lower jaw underestimated age by half a week. When the upper and lower jaw were each split into the different tooth classes a clearer picture emerged. It was revealed that the overestimation of the upper jaw resulted from the incisors while the underestimation of the lower jaw came from the molars. A combination of both jaws leads to an accurate age estimate of less than a week within chronological age (+0.57weeks) as the incisors and molars equalled each other out. This analysis was found missing in the considerations made by Cardoso who tested aspects of the present method on the documented Lisbon collection (2007).

Cardoso found the same difference between the jaws, in his study, but in his case, the maxillary canine differed significantly from chronological age. However, it must be kept in mind that the sample that was used by Cardoso is first, very small (n=62 deciduous teeth) and second, has a different age distribution compared to the Middenbeemster sample ranging from nine months to more than two years with an estimated mean age of about 14 months. It cannot be ruled out that the differences in accuracy seen in the two studies for the maxillary canines and incisors arise from differences in the rate of growth, which is reported to vary throughout development (Liversidge *et al.* 1993; Stack 1967). This argument might specially apply as many of the individuals used by Cardoso must have had considerable root development, which is known to be more variable (Liversidge *et al.* 1993). As both studies suffer from limited observations no general conclusions can be drawn. However, this thesis was able to add data on the earlier development of deciduous crown length to the discussion. Results suggest that there is indeed a difference between the two jaws but that they equal each other out in a fairly complete dentition.

Unfortunately, the study by Cardoso does not provide the data on mean difference to chronological age but only discusses the difference between the jaws. The present study and the work by Cardoso would greatly supplement each other to give a more substantial evaluation of the method for the entire infant period. Future research should aim at a more cross-population approach in order to increase sample sizes and to evaluate possible differences that are the effect of developmental differences between the collections. In light of the latter, differences in the accuracy seen in the incisors of the Middenbeemster collection could be the result of population differences in the general size of this tooth class or a difference in the timing of their development. Studying the final size of the dentition in the Middenbeemster subadults in comparison with other collections could reveal whether the apparent differences are due to generally greater size of the incisors. Differences in the timing of dental development should be studied in the entire subadult collection to inquire whether there are periods of greater increase in length of the teeth. However, the cross-sectional nature of this collection will not be able to fully differentiate between individual differences and population specific patterns. Unfortunately, population specific differences in the timing of dental development cannot be substantiated using the deciduous Demirjian stages. The method generally performed not well enough to warrant such an inquiry.

In conclusion, dental height proved to be a very easy to use method with minimised intraobserver error due to the simple application of one linear measurement per tooth. The method does not require a learning period and is therefore suited for the use on large projects that incorporate many researchers or less skilled students. The method showed that limited numbers of observations reduces the reliability but the mean would stay close to chronological age which makes this method very well suited for archaeological fragmented remains.

## 7.4 Infant mortality

The developmental stage of the Middenbeemster infant sample showed that mortality is clustered around the time of normal delivery with the relatively greatest percentage of deaths occurring during the late foetal and neonatal period (61%). Today, half of the individuals that die under five years of age, are accounted for by neonatal deaths and the mortality rate will slowly decrease throughout the first few years until it reaches a minimum rate that will remain low until risk of dying increases again when individuals become older (Klutke *et al.* 2003). This mortality curve is called a bathtub curve and is shown in figure 14. Today, in affluent western countries, infant mortality is minimised through advanced medical care. But in rural nineteenth century the Netherlands the hazard faced by mother and child during the birthing process and early extrauterine life would have been considerable. Thus, while the mortality curve is comparable to modern day distributions, the total amount of individuals dying would have been very different.

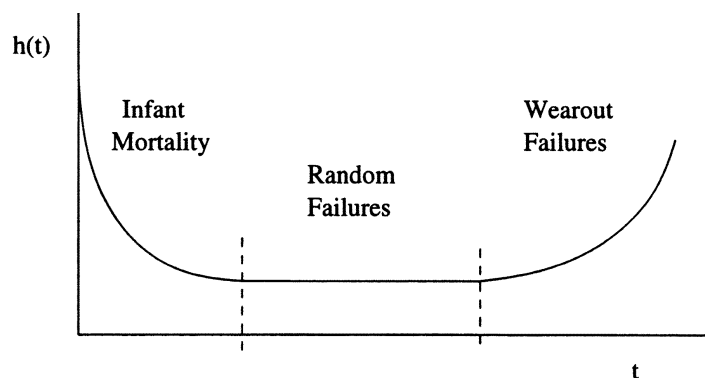


Figure 14. The classical bathtub curve showing the hazard ( $h$ ) of dying through time ( $t$ ). (Klutke *et al.* 2003, 126).

Lewis and Gowland (2007) studied the different mortality profiles from rural and urban sites of medieval England and showed that in the rural areas most individuals would die at birth or shortly after, whereas in the urban areas most

deaths occurred later during infancy. The different mortality profiles arise from different causes. First, exogenous causes are more apparent in urban areas where the population had access to care during and after birth with midwives and doctors close by. However, the pollution and disease load of the city posed a major threat to the health of the growing infant leading them to die later in infancy. Second, in the rural areas medical care was much more restricted causing more deaths to occur during parturition or shortly after death, while the environment was probably less stressful once the infant survived the neonatal period. Thus, endogenous factors such as difficulties to adapt to life in the extrauterine environment were more prevailing in the rural areas (Lewis and Gowland 2007). However, it cannot be ruled out that the decreased number of individuals dying during the post-neonatal period partly results from the most frail individuals already dying shortly after birth, while in the urban areas, these individuals would have survived only to succumb later to one of the prevailing infectious diseases.

The mortality pattern of the Middenbeemster infants falls in between the two extremes described for the medieval English sites. The great amount of perinatal and neonatal remains are congruent with the rural pattern of prevailing endogenous causes. But mortality was still relatively high during the first six months and would only become incidental during the second half of the first year. It should be noted that a pattern of high neonatal mortality does not imply that individuals were generally unfit for life. Rather, the environment was probably unable to provide for the proper care needed to help during complicated deliveries and to keep the more frail newborns alive. Thus, while the separation of endogenous versus exogenous causes is certainly warranted, exogenous causes are always at play, while endogenous factors will have their greatest impact at birth and during the first few days afterward, and to a lessening degree during the remaining neonatal period (Derosas 2003). This observation indicates that a strict separation of causes into endogenous and exogenous is probably not realistic when investigating mortality patterns in past populations.

A way to investigate the relative impact of the environment on infant mortality around the time of birth would be to look at the number of individuals that die very shortly after birth as opposed to individuals that survive for several weeks. From the ten individuals of known age, four died within the first week,



two died during the third week, and the remaining three at 7.0 weeks, 11.0 weeks and 13.0 weeks of age. In the entire sample, 10 individuals count as perinates (until seven days after birth). One should be careful, however, to imply that individuals with a derived age older than 40 weeks gestation actually lived. Many of these individuals could just have been large for gestational age or they were overdue. Today, five percent of deliveries take place post-term, after 42 week gestation (Shea *et al.* 1998). So, for most of the neonatal period it cannot be known for sure if the individuals lived and then for how long.

A possibility to determine whether the individuals survived birth would be to do an histological analysis of one of their teeth. Individuals that survive delivery generally show a hypo-mineralised band in the microstructure of the teeth that were developing at birth (i.e. deciduous teeth and mostly but not always the first permanent molar). This so called neonatal line can be seen as an abnormality of the otherwise very orderly structured enamel of the crown. It will develop as a result of stress suffered by the individual during the birthing process and subsequent adaptation to the new gaseous environment (Jakobsen 1975; Norén 1984). The neonatal line represents an impairment to the process of enamel secretion resulting in a temporary or complete cessation of secretion by the ameloblasts (enamel secreting cells). A more in-depth explanation of dental microstructure and the development of the neonatal line is provided in Appendix 5. Figure 15 shows the microscopic view of a longitudinal section of a molar from one of the Middenbeemster infants which shows the neonatal line as a band running through the enamel of the crown. Initially it was anticipated by the author to incorporate a histological analysis into this thesis but owing to the technical difficulties of preparing thin sections of the tiny teeth and subsequent time consuming analysis of the material, the data are not included here. Deciduous dental microstructure is very difficult to interpret owing to the reduced visibility of the features as compared to permanent teeth (Hillson pers. communication). From the preliminary analysis of the slides it was possible to determine the presence or absence of the neonatal line for the 16 individuals that had one tooth sectioned (age range of the individuals=1.9-48.1 weeks). Table 20 shows the derived age of those individuals and whether the neonatal line was present or not. In case it is known, chronological age has been added as well.



*Figure 15. Histo-montage of the longitudinally sectioned lower left second molar of individual S058V009275 seen under the light microscope through polarised light.*

*The individual is aged 48 weeks with dental height regression equations. The image shows the neonatal line (indicated by red arrows) in the enamel of the crown running roughly parallel to the crown surface (Magnification = X40). The tooth of this individual has already finished crown formation with root formation on its way.*

From the table it becomes apparent that the neonatal line is absent in many of the neonates and that the dental developmental state varies from two weeks to six weeks for those that do not possess a neonatal line. It has to be kept in mind however, that the neonatal line only becomes visible under the light microscope when the individual survives birth for about two weeks (Antoine 2000). In this way enough enamel forms afterwards to show the structure clearly under the

*Table 20. Presence or absence of the neonatal line (NNL) in the sectioned tooth of 16 Middenbeemster infants.*

Individual	Estimated age in weeks*	Known age in weeks	NNL visible
MB11S315V0656	1.96		No
MB11S156V0046	2.40		No
MB11S187V0267	4.19		Yes
MB11S082V0084	4.38	2.43	No
MB11S130V0173	5.10		possible
MB11S227V0297	5.82	0.86	No
MB11S050V0042	5.92	2.71	Yes
MB11S287V0450	6.11		Yes
MB11S320V0662	6.17	stillborn	No
MB11S493V1069	7.81		Yes
MB11S400V0859	8.54	3.43	Yes
MB11S037V0021	8.65	13.04	Yes
MB11S335V0711	13.49		Yes
MB11S314V0655	14.36		Yes
MB11S352V0747	16.62		Yes
MB11S058V0092	48.11		Yes

\*The age estimates of dental height are used for this table, as these most adequately approach chronological age.

microscope. It is, therefore interesting to note that in two individuals of known age at death that died at age of 2.4 and 2.7 weeks the younger individual had no neonatal line while the older individual did. The observation confirmed the concerns raised above about assigning real ages to individuals that show a developmental stage congruent with a neonate. It could well be that many of the individuals characterised as neonates in this sample died during birth or shortly afterwards, thus, succumbing to endogenous factors. Any research to clarify this issue is beyond the scope of this thesis but the potential of histological analysis to aid in the study of neonatal and infant remains has been indicated.

#### 7.4.1 Dental versus Skeletal development

Skeletal growth is delayed to dental height age by 4.6 weeks in 67 % of the sample (range=0.6-8.9 weeks). While also taking into account the slight growth restriction toward the end of pregnancy and a general growth cessation during the first week after birth, this observation confirms the above posted expectation that skeletal growth will lag behind dental development in neonatal remains (see section 3.3). However, in the background chapter the research on the saltatory nature of skeletal growth by Lampl (1993; Lampl and Jeanty 2003) was introduced, which is expected to manifest itself in a random pattern of slight advances and delays as the short bursts of growth would be non-periodic in manner. Results indicate that this pattern does not show in the sample. Whether the saltatory growth pattern is obscured by a more pronounced stress related growth faltering, or whether the model of saltatory growth cannot be substantiated at this point and remains an open question thus far (for a critique on the saltatory nature of growth see Heinrichs *et al.* 1995).

To determine whether the delay can be considered pathological poses a problem as is has not been defined how much of a delay needs to be manifested to make the distinction. In light of the research done by Lampl, where intervals of growth stasis between bursts would last for a maximum of 51 days (=1.6 months), it could be suggested that a discrepancy of about one month should be accepted as normal variation between the two growth systems. However, the sample shows a strong pattern in which the greater proportion of individuals show delayed skeletal growth and this delay increases with increasing age (foetal=-1week; neonates=-3weeks; post-neonates=-5.6weeks). In addition, among the age groups most of the individuals with delayed skeletal growth are neonates which correlates with the general mortality pattern. In many instances the delay of individual cases is not pronounced enough and would, therefore, be considered a normal variation between the growth systems. It is only through the general pattern seen in the entire sample that growth faltering emerges as an explanatory factor.

The fact that the severity of the delay increases with age is congruent with the increased environmental susceptibility of skeletal growth to environmentally adverse conditions, resulting from decreasing genetic control on the development.

A study by Lampl and Johnston (1996) compared skeletal and dental age in children from present day Mexico who live in conditions comparable to past populations that had restricted access to medical care and who suffered from mild to severe undernutrition, while subjected to relative high infectious disease load. In their study 60% of the individuals aged 4.0-4.5 years showed skeletal development that was delayed by 1.0-2.0 years while 30% would be underaged by 2.0-3.0 years. They showed that stunting increased with increasing age, and so did the variability. This confirms the observations made in this thesis.

Stunting early in life can have adverse effects for the development of a child and potentially leads to reduced adult height which is supported by clinical research (Youxue *et al.* 2000). However, life in the nineteenth century rural Netherlands must have been much less predictable and adverse conditions such as starvation or disease outbreaks could strike a child at any given age. Thus, to limit the reasonable time of insult to one age group is unrealistic. The fact that the average adult male in this skeletal collection would only reach a height of about 171 cm and the average female about 160 cm (Lemmers *et al.* 2013) indicates the general environmental constraints on the growth potential of children living in Middenbeemster at that time. On a population level, however, conditions of people living in Middenbeemster were not as dreadful, giving that the average height of Dutch males for most of the century lay below 165 cm (Jacobs and Tassenaar 2004).

In the second chapter it was outlined that infant mortality was possibly in great part the result of inadequate feeding practices. Chances of survival would have declined rapidly for infants that were deprived of the colostrum, the first milk produced by a new mother, which contains a great amount of antibodies and nutrients essential to the survival of the new born (Molleson and Cox 1993, 44). In combination with inadequate substitute foods the prospects of the individuals would have been dreadful. Future research will gain insights into the diet of the Middenbeemster infant remains through the study of stable isotopes and in particular the stable  $\delta^{15}\text{N}$  (nitrogen) isotopes to reconstruct infant feeding. This isotope is found in bone collagen and different so called trophic levels of  $\delta^{15}\text{N}$  are observed for plants, herbivores and carnivores. Breastfed infants generally show a

trophic level increase compared to adult humans which will decrease when other food is introduced (Katzenberg 2008). Studies using this isotope could successfully indicate the period of weaning in skeletal populations (Eerkens and Bartelink 2013; Herring *et al.* 1998; Katzenberg *et al.* 1996; Pearson *et al.* 2010; Richards *et al.* 2002; Waters-Rist *et al.* 2011). In the Middenbeemster collection an isotopic study could clarify whether the breastfeeding hypothesis (Lesthaeghe, 1987; van Poppel *et al.* 2005; Wintle 2000) can be substantiated for the infant sample.

Apart from factors related to infant care, neonatal mortality is in great part the result of insufficient foetal development which manifests itself *in utero* mainly through reduced weight gain (McIntire *et al.* 1999). A study by Shrimpton and colleagues (2001) on the worldwide pattern of growth faltering in developing countries showed that foetal growth was generally unaffected by environmental conditions that shaped the life of the mother. Even though conditions were adverse, growth during pregnancy followed the normal pattern, while stunting started directly after birth. Thus, during intrauterine life, weight gain is the proper measure of inadequate development while after birth, growth in length becomes the leading symptom. The reason for early stunting is thought to be related to nutritional constraints during foetal development (Shrimpton *et al.* 2001).

The described pattern explains why the foetal remains generally show only very little delay, while already during the neonatal period growth some individuals show a potential skeletal developmental retardation. As the developing foetus depends on its mother for the provision of nutrients, neonatal stunting, apart from congenital abnormalities, will indicate an impairment in this regard. The discussion also indicates that the mother must have suffered from chronic undernutrition to cause such an effect. The picture that emerges is that of a general lack of adequate nutrition for many of the individuals that were interred in the cemetery of Middenbeemster, which already started during pregnancy and would continue afterwards if the mother would not provide the much needed milk. Unfortunately the skeletal sample has not yet been fully identified. If the remains were to be linked to the years in which the individuals died, it could be investigated whether their death is related to the years of famine during the 1840's. However, inadequate nutrition was a problem for a great part of the

population for most of the nineteenth century (Bergman 2008; Wintle 2006) and pregnancy, especially during the winter months, would have been difficult for woman of the lower socioeconomic classes. Scalone (2013) in his study on maternal mortality in several German villages during the 18<sup>th</sup> and 19<sup>th</sup> century showed that maternal mortality was directly related to nutritional stress around and during pregnancy. Nutritional stress does not need to imply the general lack of food but can be related to shortage of some essential nutrients such as iron or vitamin D. Today, 35-75% of pregnant woman in the developing world have iron-deficiency anaemia, which manifests itself by a low number of red blood cells (Allen 2000, 1280). Apart from distressing symptoms for the mother, iron depletion can reduce general health of the foetus and leads to reduced birth weight and risk of premature deliveries, thus increasing the risk of neonatal mortality. In addition the risk for the infant of becoming anaemic itself is much higher when born from an anaemic mother, which will subsequently lead to reduced mental and motor development (Allen 2000, 1282). Research into skeletal manifestations that have been linked to anaemia such as cribra orbitalia or portic hyperostosis (Walker *et al.* 2009) and rickets (Mays *et al.* 2006) are beyond the scope of this thesis but will be and are being studied in this skeletal collection (Veselka 2013). The fact that the Dutch diet was very depleted in essential nutrients (consisting mainly of potatoes, bread, some vegetables, and occasionally meat) makes it very likely that anaemia was among the causes of infant mortality.

To conclude, historical studies suggest that life in the western part of the Netherlands during the nineteenth century was hazardous in many ways. Infants who survived birth faced an uncertain future, as regardless of their social class infants may have been mostly spoon or bottle fed, thus increasing the risk of diarrhoea through contaminated water and spoiled ingredients (van Poppel *et al.* 2005). The yearly recurring late summer and autumn fevers (Wintle 2000) would have been disastrous for the new born and their mother, while chronic undernutrition of many mothers must have played an important role in causing some delay in skeletal development. Only after 1870 did the general living conditions improve, which did not only make the drinking water less lethal but also meant a better nutritional status for the population and thus of the pregnant

woman. Infants born to healthy and well fed woman would have a much better start into their life, thus increasing their chance of survival.

At this point, the data on skeletal development are not unequivocally pointing towards a strong stress induced delay in skeletal development of the Middenbeemster infants. Results from the Maresh reference standard revealed that the Middenbeemster infants could be accurately aged using modern reference data, indicating that individuals might have been reaching their growth potential. However, the pattern of increasing amount of delay with age in skeletal growth compared to dental development supports the assumption that conditions in the Beemster polder, during most of the nineteenth century, were hazardous for new borns and infants. The problem is also inherit in the age class as the amount of delay will generally be limited in individuals that only lived for a few days or weeks. Future research should address this issue in the entire subadult sample to see whether the observed trends in skeletal delay continue in older individuals or whether they are in fact growing according to modern standards, thus leading a relatively healthy life despite what has been suggested by historical sources.



## 8. Conclusion

Estimating age at death stands at the beginning of an osteological analysis. In subsequent inquiries into other aspects of the skeletal collection under study, such as age specific death rates, the osteologist needs to be aware of the limits inherent in the methods that are used to assess age at death. This thesis aimed at providing much needed data on some age estimation methods. The starting point for this research was 49 infant skeletons aged between around birth and twelve months, that were recently excavated from a cemetery in Middenbeemster, The Netherlands, dating mainly to the nineteenth century. The objective was to evaluate the accuracy of three age estimation methods: 1) a collection of standards of skeletal measurements of various bones (Black and Scheuer 1996; Fazekas and Kósa 1978; Maresh 1955; Molleson and Cox 1993; Saunders *et al.* 1993; Scheuer and McLaughlin-Black 1994), 2) dental height regression equations by Liversidge, Dean, and Molleson (1993), and 3) the deciduous Demirjian system by Liversidge and Molleson (2004) that uses dental developmental stages to estimate age at death. In addition, the skeletal and dental growth systems were compared. It was anticipated to evaluate whether differences in the developmental pattern between the two systems were apparent and what could be deduced from this in terms of the amount of stress suffered by the individuals during their short lives. The use of dental ageing methods excluded ten individuals from the analysis leaving the remains of 39 infants to be investigated. Historical records provided the chronological 'known' age at death of 10 individuals that functioned as a means to best evaluate the accuracy of the ageing methods.

The initial result of the analysis of individuals of known age showed that skeletal measurements and dental height provided age estimates that differed from chronological age by about half a week, while the deciduous Demirjian stages overestimated age by 5.5 weeks. While skeletal age suffered from some inconsistencies in the estimate of several bones and some standards, the general accuracy of the method was well within the acceptable range of one month. Dental

height proved to be very reliable for the entire sample providing consistent results in most of the cases. The deciduous Demirjian system was also very consistent in itself but produced age estimates that were much older compared to chronological age or dental height age estimates.

A subquestion was concerned with age specific accuracy of the methods and it was revealed that the three methods showed different degrees of accuracy throughout the first year. Skeletal age and the dental developmental stages both gave better results in neonate and young post-neonate remains. This result is thought to be related to a general trend of the methods to overestimate age. The many neonate remains with developmental characteristics congruent with late foetal development provided very young estimates in many cases which compensated for the general overestimation trend. Only in older individuals did the overestimation start to show, thus decreasing the accuracy of age estimation in this group. Dental height was generally very accurate throughout most of the year, but proved most accurate in post-neonatal remains, where the mean difference to chronological age was only 0.15 weeks.

Method specific questions were evaluated as well. For the dental methods, differences in accuracy of the tooth types were investigated. The deciduous Demirjian stages showed a constant overestimation of about five weeks for the molars and incisors throughout the age classes. The canines stood out with an overestimation of ten weeks on average. For dental height, the tooth classes were analysed for each jaw separately and the maxillary incisors were found to significantly overestimate known age by 3.5 weeks. However, this was compensated for by an underestimation of age from the mandibular molars (-2.7 weeks), hence cancelling each other out and leading to the high level of accuracy of the method. The remaining teeth proved to provide ages that lay within two weeks of chronological age. The observations only partly confirmed the results obtained by Cardoso (2007), who showed that the entire maxillary dentition gave older estimates compared to the mandibular dentition. The fact that the present study and the one by Cardoso used samples with different age distributions could explain the differences seen between studies. From the results of the present study, it is suggested that in a complete dentition it is warranted to use all the teeth, but in fragmented remains the maxillary incisors and mandibular molars should be

avoided. The combined mandibular teeth, however, give the same high level of accuracy as the entire dentition with a mean difference to chronological age of half a week.

For the dental height method another subquestion inquired whether accuracy would be affected by the number of teeth that could be used per individual. The reliability of age prediction decreases in individuals with less than seven teeth, but the overall difference to chronological age still remains below 2.5 weeks, thus the method potentially provides very reliable results in fragmented remains.

The evaluation of skeletal measurements proved to be a more complicated process, owing to the great number of bones that were used and an even greater amount of measurements. In addition, in total six different standards had to be consulted: one foetal standard (Fazekas and Kósa 1978) and the remaining five for infant remains. (Black and Scheuer 1996; Maresh 1955; Molleson and Cox 1993; Saunders *et al.* 1993; Scheuer and McLaughlin-Black 1994). One subquestion inquired whether there was a differences in accuracy of age estimation between the 26 measurements that were recorded. The results showed a great variability in the mean difference to known age ranging from -14.1 to +10.7 weeks. However, variability was confined to only a few bones, while 77% of the bones produced results of acceptable accuracy. Variability was found to mainly come from infant standards that provided reference data for single bones, namely the pars basilaris (of the occipital), clavicle, scapula, and ilium, which gave the worst performance with a combined mean difference to chronological age of +14 weeks.

Several observations were made regarding the single bone infant standards: 1) the standards are based on a limited amount of observations which necessitated the use of very large ranges, thus failing to produce accurate results, and 2) a gap exists for the first weeks after birth where no estimates are provided by the standards, therefore, proving to be inadequate for neonatal remains. A third observation is concerned with standards that used multiple measurement per bone, the pars basilaris of the occipital (3 measurements), the scapula (2 measurements), and the ilium (2 measurements). The three bones showed different levels of accuracy for each measurement which seem to result from differences in the shape of these bones. One measurement generally provided relatively accurate results (i.e. it fell within the mean age of the entire sample) such as the pars basilaris

width, the scapula maximum width, and the ilium maximum length, while the other measurement(s) was/were considerably off (pars basilaris length; pars basilaris saggital length; scapula maximum length; ilium maximum length).

A similar observation was made for the sphenoid body, the measurements of which could only be recorded in the foetal standard. In this bone, maximum width underestimated age by an average of -14 weeks, while maximum length gave results that were in concordance with the remaining estimates of the individual. A closer look at the results of the sphenoid and the single bone reference standards revealed that these discrepancies are not the product of one standard and that they manifest themselves throughout the foetal, neonatal, and post-neonatal age classes. Therefore, it can be suggested that developmental variation may exist for these bones. Whether such variation is present in the Middenbeemster sample or results from differences between the reference and analysed samples, needs to be addressed in future research.

The only infant standard that provided reliable results was based on length of the major long bones and was developed on healthy American children during the first part of the 20<sup>th</sup> century (Maresh 1955). It was hypothesised that 19<sup>th</sup> century Middenbeemster individuals lived with a comparatively high level of environmentally induced physiological stress that would have affected their growth. However, the data suggests that the Middenbeemster infants were reaching their growth potential, thus, indicating that skeletal development might have been less impaired by the environment. However, the amount of stunting will always be limited in a sample of primarily neonatal and young post-neonatal remains as their life was generally too short to manifest an extensive amount of growth faltering. Future study should address developmental differences in the growth of long bones compared to flat and irregular bones to see whether the different levels of accuracy found between the Maresh standards and the remaining single bone standards can partly be found there.

This thesis also introduces the reader to the mortality pattern seen in the infant sample. The age distribution revealed a high percentage of individuals dying during the neonatal period (birth - 28 days), and to a lesser degree during the late foetal and early post-neonatal periods, while only a few cases are recorded during the second part of the first year. This mortality pattern can be considered

normal in an environment that does not have the benefits of medical care of modern affluent countries.

The mortality pattern was studied in conjunction with a comparison of the dental and skeletal growth systems, to gain some preliminary insights into the reasons for infant morbidity and mortality in this population. It was shown that skeletal age was delayed to dental height age by about one month in almost 70% of the individuals, and the amount of delay increased with age, while the greatest percentage of individuals with delayed skeletal development, were among the neonates. In addition, all neonatal remains had many skeletal elements that provided a foetal age estimate and it was concluded that individuals were relatively small for their age, which is also reflected in the general trend of the skeletal ageing method to underestimate age.

To investigate whether individuals died due to endogenous or exogenous causes a preliminary study of the dental microstructure was conducted in a small sample. It was shown that neonates oftentimes did not survive long enough to show the neonatal line in the enamel of the crown, which needs about two weeks to become visible. Thus, many neonates probably died due to endogenous reasons.

It is at this point difficult to determine whether there exists a stress induced delay of skeletal growth in the infant remains. On the one hand the good accuracy provided by the modern Maresh standard suggests a growth potential comparable to modern conditions, while on the other hand the sample shows some delay in skeletal development which increases in severity with age, starting directly after birth, which is in accordance with the general pattern of growth faltering seen in the developing world today. Future study should try to establish whether this trend of increasing delay continuous in older individuals to assess whether it is indeed a real phenomenon.

In summary, from the three infant ageing methods that were evaluated in this thesis, dental height by Liversidge and colleagues (1993) stood out as being most reliable, producing relatively consistent results throughout the first year of life. Skeletal age was relatively accurate in neonatal remains but during the post-neonatal period only the standard by Maresh (1955) provided good results that differed little from known age. The single bone standards of the pars basilaris (Scheuer and McLaughlin-Black 1994), clavicle (Black and Scheuer 1996),

scapula (Saunders *et al.* 1993), and ilium (Molleson and Cox 1993), all overestimated age to an extent that the author does not recommend their use for age estimation. The deciduous dental development stages by Liversidge and Molleson (2004) proved the least reliable method for this age category of infants under one year. The method consistently overestimated chronological age and all statistical analyses indicated that the method was not able to predict known age. As well, the deciduous Demirjian method is less accurate because it produced rather high intra-observer error, which was not the case for the other two methods that relied upon simple measurements. The dental height method of Liversidge, Dean, and Molleson (1993) was the easiest to use of all methods and produced the most comprehensively accurate results, making it the method that this thesis recommends should be used in future analyses of archaeological Dutch infant material.

This thesis introduces the infant sample of a newly excavated Dutch skeletal collection. It provides much needed data on the early stages of skeletal and dental development, while the presence of historical records for 10 individuals opened a window into a more rigorous evaluation of ageing methods. The fact that this thesis provides the actual differences to chronological age for each age estimation method will be very helpful to other researchers aiming at using these methods on other north-west European skeletal samples. Hopefully in the future, more individuals can be added to the known age at death sample to make the observations more robust.

The extensive amount of information that was collected for this research will aid in future studies into the developmental pattern of the skeletal and dental growth systems, and will shed light on developmental variation that may be unique to the present skeletal collection. A more fundamental inquiry into morbidity and mortality should include the entire subadult sample to see whether the observed patterns in the infant age class continue throughout childhood and adolescence. Among the age classes, infants are the most frail and will be the first to become victim to their environment, if not provided with the proper care. Thus, infant remains are providing us with a proxy for the general conditions that shaped the life of the people living in the Beemster polder, the Netherlands, during the preindustrial era of the nineteenth century.

## *Abstract*

Skeletal and dental growth and development is investigated in 39 perinate and infant skeletons aged between 32 weeks gestation to 42 weeks after birth in order to determine the accuracy of three ageing methods and to assess possible periods of increased stress in the sample. The skeletal remains belong to a recently excavated, partly documented cemetery, from Middenbeemster, a rural village in the Netherlands, dating mainly to the nineteenth century. Three ageing methods were chosen to be evaluated, the accuracy of which had not been systematically investigated: 1) the deciduous Demirjian stages by Liversidge and Molleson (2004); 2) the dental height regression equations by Liversidge and colleagues (1993), and; 3) skeletal age estimation using 26 measurements from 18 different bones utilising six different standards (Black and Scheuer 1996; Fazekas and Kósa 1978; Maresch 1955; Molleson and Cox 1993; Saunders *et al.* 1993; Scheuer and McLaughlin-Black 1994). Accuracy of the methods is tested on a subsample of ten individuals for whom age at death is known from the Beemster district archives, and the results are further evaluated using the entire sample. Results from individuals of known age indicate high levels of accuracy for skeletal age and dental height with mean difference to chronological age of only -0.4 and +0.6 weeks, respectively. The deciduous developmental stages significantly overestimate chronological age by +5.5 weeks. These observed trends are confirmed in the entire sample where age was compared to the sample mean. In neonatal remains, skeletal age is most accurate regardless the standards used, but for post-neonates only the Maresch (1955) standard provides accurate results (+1.9 weeks). Dental developmental stages are more accurate in individuals less than two months (+2.4 weeks), increasing in older individuals to +5.2 weeks. Dental height gave an outstanding performance with consistent high levels of accuracy in neonatal (+1.6 weeks) and post-neonatal remains (+0.15 weeks), making it the preferred method for age estimation in the infant category. The mortality pattern followed a normal declining curve with the greatest percentage of individuals

dying during the neonatal period. Skeletal development lagged behind dental development by about one month in almost 70% of the individuals, showing a trend of an increasing amount of delay with age. But differences in age between skeletal and dental development were not unambiguously pointing to a stress induced delay and more research is needed to clarify the observed trends. This thesis provides new information on the accuracy of dental and skeletal ageing methods of infant remains and should guide our application of these methods in future research of north-west European skeletal samples.



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## *List of Figures*

Figure 1:	A map of present day the Netherlands indicating the provinces and the position of the village of Middenbeemster (red dot) (Source: <a href="http://d-maps.com/carte.php?num_car=4115&amp;lang=en">http://d-maps.com/carte.php?num_car=4115&amp;lang=en</a> , accessed 24 july 2014).	23
Figure 2:	Map of the drained Beemster Polder showing the subdivision of the land five years after its creation in 1612 (Danner 1986, 36).	24
Figure 3:	Average height velocity curves for boys and girls of normal growth from birth to cessation of growth (Tanner <i>et al.</i> 1966, 466).	32
Figure 4:	Reference table for growth in height (in centimetres) for individuals aged from birth up to two years. The lines represent the 3rd, 10th, 50th, 90th and 97th smoothed percentile curves and the dots are empirical data (WHO Multicentre Growth Reference Study Group 2006, 81).	33
Figure 5:	Successive stages of the developing tooth germ (Simon Hillson 2005, 209).	38
Figure 6:	A longitudinally sectioned tooth showing the different dental tissues (Liebgott 2001).	40
Figure 7:	Radiographic images of the eight successive developmental stages of each tooth type (molars, incisors, and canines) including line drawings (Liversidge and Molleson, 2004, 175).	52
Figure 8a:	Near complete dentition of one of the infants that was analysed (MB11S187V0267) aged 4.2 weeks using dental height regression equations.	57
Figure 8b:	Skeletal elements of the same individual. The very long spine results from the 24 vertebrae each consisting of three elements at that state of development which will fuse during childhood to form a single bone.	57
Figure 9:	Measurements taken for (a) the Pars Basilaris and (b) the Pars Lateralis (Scheuer and Black 2004, 75).	60

Figure 10:	Chronological age plotted against estimated age of the three ageing methods in individuals of known age at death.	72
Figure 11:	Age at death distribution of the infants from the Middenbeemster cemetery used in this thesis, broken down into four-week intervals.	97
Figure 12:	Right perinatal ilium, pelvic view (after Scheuer and Black 2004, 319). The two measurements are indicated: 1=maximum length and 2=maximum width.	109
Figure 13a:	Perinatal sphenoid body with fused lesser wings (after Scheuer and Black 2004, 96). The measurements are indicated: 1=Body maximum length; 2=Body maximum width.	110
Figure 13b:	Perinatal right greater wing, inferior view (Scheuer and Black 2004, 97).	110
Figure 14:	The classical bathtub curve showing the hazard (h) of dying through time (t). (Klutke <i>et al.</i> 2003,126).	119
Figure 15:	Histo-montage of the longitudinally sectioned lower left second molar of individual S058V009275 seen under the light microscope through polarised light. The individual is aged 48 weeks with dental height regression equations. The image shows the neonatal line (indicated by red arrows) in the enamel of the crown running roughly parallel to the crown surface (Magnification = X40). The tooth of this individual has already finished crown formation with root formation on its way.	122
Figure 16:	Comparison of age estimates in weeks from skeletal measurements, dental height, and the deciduous Demirjian stages for each of the 39 individuals that are discussed in this thesis.	179 ff
Figure 17:	Schematic drawing of a sectioned crown showing different incremental markers (FitzGerald and Rose 2008, 253).	183
Figure 18:	Microscopic image of a section of the upper right first molar of individual MB11S287V045. The image is taken at 400X magnification. It shows the prisms running almost vertically from the bottom of the image to the top (dotted black line). Other structures are visible as well. Cross-striations are clearly visible in some areas (three closely spaced arrows). A dark band running obliquely to the long axis of the prisms, indicated by three larger arrows, represents either a Brown striae of Retzius or a Wilson Band.	184



- Figure 19: Microscopic image showing a section of the imbricational enamel of the lower left second molar of individual S058V009275. Three clearly visible BSR are marked with black arrows. 185
- Figure 20: The mesiobuccal cusp of a longitudinally sectioned upper right first molar (FDI=54) from individual MB11S287V045, showing the neonatal line. The two dental tissues (Enamel and Dentine) are marked, as well as the the boundary between them known as the Dentine Enamel Junction. (DEJ). To ease orientation the cervical margin and the cusp tip are marked as well. 187

## *List of Tables*

Table 1:	Definitions of time periods from fertilisation to the end of adolescence that are used in this thesis (Scheuer and Black 2000, 468f)	12
Table 2:	Descriptive criteria of the crown and root Demirjian developmental stages for deciduous teeth (Liversidge and Molleson 2004, 174).	50
Table 3:	Inventory of the number of individuals and number of skeletal and dental elements used in this study.	58
Table 4a:	Cranial measurements used in this study with associated standards.	61
Table 4b:	Post-cranial measurements used in this study with associated standards.	61
Table 5:	Regression equations for age estimation based on deciduous tooth length (Liversidge <i>et al.</i> 1998, 432).	68
Table 6:	Interpretation of the Spearman rho correlation coefficient (after Zou <i>et al.</i> 2003, 618).	69
Table 7:	Mean difference to chronological age for all three ageing methods listed for each individual of known age at death.	73f
Table 8:	Mean difference in weeks to chronological age of all three ageing methods.	75
Table 9:	Mean difference to known age for single skeletal measurements and results of the r t-test /U-test.	76
Table 10:	Comparison of the infant bone standards with chronological age.	79
Table 11:	Comparison of mean age from cranial and post-cranial estimates for three different age categories.	81
Table 12:	Comparison of mean age and age range for the three measurements recorded from the Pars basilaris of the occipital.	82
Table 13:	Comparison of the average dimensions and range of the sphenoid body width in millimetres for three age categories.	85

Table 14:	Age of attainment of crown and root stages (mean + SD in years) (Liversidge and Molleson 2004, 179).	87
Table 15:	Mean difference of the Demirjian stages to chronological age for the different tooth classes, including results of the t/U test.	89
Table 16:	Mean difference of the tooth classes to the mean age of two age cohorts for the deciduous developmental stages.	89
Table 17:	Mean difference of dental height to chronological age in weeks for the three tooth classes.	91
Table 18:	Mean difference to chronological age in weeks for the three tooth classes broken up into upper and lower jaw.	92
Table 19:	Comparison of mean difference of each tooth class to the mean age of all teeth combined, separated into three age categories.	95
Table 20:	Presence or absence of the neonatal line in the sectioned tooth of 16 Middenbeemster infants.	123
Table 21:	Skeletal measurement recordings and corresponding age for 37 individuals of the Middenbeemster infant sample.	157 ff
Table 22:	Deciduous Demirjian stage recordings and corresponding age estimates for 39 foetal and infant remains of the Middenbeemster sample.	167 ff
Table 23:	Dental height recordings and calculated age estimates using the regression equations provided by Liversidge and colleagues (1993) for 39 individuals of the Middenbeemster infant sample.	173 ff

## *Appendix 1: Dataset for skeletal measurement recordings*

Table 21 shows the recordings of all measurements collected from the 37 Middenbeemster infants that possessed teeth and skeletal remains. The amount of data that was recorded made it necessary to split the table into nine segments each presented on a different page.

For each individual (indicated by the rows) the table provides information on whether the bone is present (P) or absent (A) and, when present, if the measurement could be taken/is observable (O) or if the bone/part of the bone was unobservable/damaged (U). Subsequently, the measurement is provided and the corresponding age estimate. Foetal age is provided as a single estimate (mean age), while infant age is given as 1) mean age, 2) minimum age, and 3) maximum age.<sup>1</sup> At the end of the table, summary ages are listed for each individual: the mean age of all measurements combined, together with the minimum age, and maximum age, and the intra-individual variability (calculated as the difference between minimum age and maximum age). In case it is known chronological age is listed and the mean difference to it. Other mean ages that are provided are from 1) the marsh standard, 2) the single bone standards, 3) cranial measurements, and 4) post-cranial measurements. Finally, for each individual it is listed a) the number of elements/measurements that were present and observable, b) the number of elements/measurements that were absent c) the number of elements/measurements that were present and unobservable, and d) the number of measurements that could not be used because no standard existed to provide a corresponding age estimate. The table provides all ages in weeks.

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<sup>1</sup> The mean age in estimates from individuals older than 40 weeks gestation was calculated by the author from the range provided by the infant standards. This, to make skeletal age comparable to the other methods and chronological age.

Table 21. Skeletal measurement recordings and corresponding age for 37 individuals of the Middenbeemster infant sample.

specimen	Pars basilaris of occipital - length				Pars basilaris of occipital - width				Pars basilaris of occipital - saggittal length				Sphenoid body length			Sphenoid body width			Occipital - squama height			Occipital - squama width					
	P/A/O/U	measurment	mean Age	min age	max age	P/A/O/U	measurment	mean Age	min age	max age	P/A/O/U	measurment	mean Age	min age	max age	P/A/O/U	measurment	mean Age	P/A/O/U	measurment	mean Age	P/A/O/U	measurment	mean Age	P/A/O/U	measurment	mean Age
MB11S037V0021	P/O	17.09	17.35	13.00	21.70	P/O	16.94	13.00			P/O	13.55	26.05	13.00	39.10	A			P/U			P/U					
MB11S050V0042	A					A					A					A			A			P/U					
MB11S058V0092	A					A					A					P/U			A			A					
MB11S080V0049	A					A					A					A			A			A					
MB11S082V0084	A					A					A					A			A			A					
MB11S090V0107	P/O	12.61	-10.00			P/O	12.55	-2.00			P/O	9.30	-13.00			P/O	9.22	-6.00	P/O	12.86	-12.00	A	54.23	0.00			
MB11S099V0139	A					A					A					A			A			A					
MB11S102V0151	A					A					A					A			A			A					
MB11S122V0161	A					A					A					A			A			A					
MB11S130V0173	A					A					A					A			A			A					
MB11S133V0299	P/O	17.03	17.35	13.00	21.70	P/O	15.56	0.00			P/O	12.24	-2.00			P/O	12.24	-2.00	P/O	13.94	-11.00	A					
MB11S139V0215	P/O	14.98	-2.00			P/O	13.01	-4.00			P/O	11.61	-4.00			P/U			P/U			A	9.51	-4.00			
MB11S152V0244	P/O	19.13	28.20	21.70	34.70	P/O	16.51	3.00			P/O	15.00	58.69	56.50	60.87	A			A			P/O					
MB11S156V0046	P/O	16.78	0.00			P/O	15.77	0.00			P/O	13.05	0.00			P/O	10.16	-2.00	P/O	13.56	-10.00	P/U					
MB11S164V0364	P/O	17.10	8.70	0.00	17.39	P/O	15.83	8.70	0.00	17.39	P/O	13.47	10.85	0.00	21.70	A			A			A					
MB11S187V0267	P/O	18.34	21.70			P/O	17.25	17.35	13.00	21.70	P/O	13.81	23.89	13.00	34.78	P/O			P/O	14.81	-8.00	P/O	59.77	1.00			65.60
MB11S189V0332	A					A					A					P/O			P/O	15.46	-5.00	P/U					
MB11S191V0374	P/O	15.17	-2.00			P/O	12.80	-4.00			P/O	12.71	0.00			P/O			P/U			P/O					58.60
MB11S227V0297	P/O	15.36	0.00			P/O	12.71	0.00			P/O	11.59	0.00			P/O			P/O	11.08	-16.00	P/U					
MB11S245V0390	A					A					A					A			A			A					
MB11S287V0450	A					A					A					A			A			A					
MB11S295V0485	P/O	16.80	1.00			P/O	15.01	0.00			P/O	13.12	0.00			P/O			A			A					
MB11S296V0486	P/O	14.66	0.00			P/O	13.51	-2.00			P/O	11.61	-4.00			P/O			A			A					
MB11S314V0655	P	18.79	21.70			P/O	17.47	17.35	13.00	21.70	P/O	13.97	39.10			A			A			A					
MB11S315V0656	P/O	16.22	10.85	0.00	21.70	P/O	15.26	13.00			P/O	13.47	10.85	0.00	21.70	P/O			P/O	13.89	-10.00	P/O	57.83	0.50			59.75
MB11S320V0662	P/O	16.64	-8.00			P/O	15.56	0.00			P/O	13.24	0.00			P/O			P/O	12.75	-12.00	P/O	50.71	-2.00			0.00
MB11S323V0650	P/O	17.34	17.35	13.00	21.70	P/O	15.17	0.00			P/O	13.95	39.10			P/O			P/O	13.99	-10.00	P/O	54.79	0.00			
MB11S335V0711	P/O	17.96	17.35	13.00	21.70	P/O	17.96	17.35	13.00	21.70	P/O	13.88	23.89	13.00	34.78	P/O			P/O	12.91	-12.00	A					
MB11S352V0747	A					A					A					A			A			A					
MB11S373V0798	P/O	16.21	1.00			P/O	14.91	-1.00			P/O	13.08	0.00			P/O			P/O	13.04	-10.00	A					
MB11S376V0900	A					A					A					A			P/O			P/O	55.64	0.00			58.35
MB11S400V0859	A					A					A					A			A			A					
MB11S404V0867	P/O	16.71	0.00			P/O	16.08	0.00			P/O	13.12	0.00			P/O			P/O	14.58	-8.00	P/O	52.31	-2.00			56.74
MB11S406V0884	P/O	15.45	-2.00			P/O	13.80	-1.00			P/O	11.50	-5.00			P/O			P/O	12.21	-14.00	A					-2.00
MB11S418V0906	P/O	15.18	0.00			P/O	12.93	-3.00			P/O	10.89	-7.00			P/O			A			A					
MB11S421V0940	A					A					A					A			A			A					
MB11S493V1069	P/O	16.82	17.35	13.00	21.70	P/O	15.15	13.00			P/O	12.62	6.50	0.00	13.00	P/O			P/O	14.49	-8.00	P/O	56.05	0.00			

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 21 continued.

specimen	Occipital - pars lateralis height left			Occipital - pars lateralis height right			Occipital - pars lateralis width left			Occipital - pars lateralis width right			Frontal - Chord height left				Frontal - Chord height right			
	P/A/O/U/ N	measu rement	mean Age	P/A/O/U/ N	measu rement	mean Age	P/A/O/U/ N	measu rement	mean Age	P/A/O/U/ N	measu rement	mean Age	P/A/O/U/ N	measu rement	mean Age	P/A/O/U/ N	measu rement	mean Age	min age	max age
MB11S037V0021	P/U			P/U			P/U			P/U			A			A				
MB11S050V0042	A			A			A			A			A			A				
MB11S058V0092	P/O/N			P/O/N			P/O/N			P/O/N			P/O/N			P/U				
MB11S080V0049	A			A			A			A			A			A				
MB11S082V0084	P/O	26.08	0.00	P/O	26.78	0.00	P/O/N			P/O/N			P/O	54.98	0.00	P/O	55.88	0.00		
MB11S090V0107	A			P/O	19.58	-6.00	A			P/O	11.11	-4.00	P/U			P/U				
MB11S099V0139	A			A			A			A			A			A				
MB11S102V0151	A			A			A			A			P/O	50.13	-4.00	P/O				
MB11S122V0161	A			A			A			A			P/O/N			P/O/N				
MB11S130V0173	P/U			P/U			P/U			P/U			A			A				
MB11S133V0299	P/U			P/U			P/U			P/U			P/U			P/U	49.29	-5.00		
MB11S139V0215	P/O	22.65	-2.00	P/O	23.49	-2.00	P/O	15.28	0.00	P/O	16.44	0.00	P/O			P/U				
MB11S152V0244	A			A			A			A			A			A				
MB11S156V0046	A			A			A			A			A	43.59	-5.00	P/O				
MB11S164V0364	A			A			A			A			A			A				
MB11S167V0267	P/O	27.54	1.00	P/O	28.13	1.00	P/O/N			P/O/N			P/U			P/U				
MB11S169V0332	A			A			A			A			A			P/U				
MB11S191V0374	P/U			P/O	22.25	-4.00	P/U			P/U			P/U			P/U				
MB11S227V0297	P/O	24.99	0.00	P/O	25.72	0.00	P/U			P/O/N			P/U			P/U				
MB11S245V0390	A			A			A			A			A			A				
MB11S287V0450	A			A			A			A			A			A				
MB11S295V0485	A			A			A			A			A			A				
MB11S296V0486	A			A			A			A			A	45.00	0.00	P/O				
MB11S314V0655	P/U			P/U			P/O/N			P/U			A			A				
MB11S315V0656	P/O	26.88	0.00	P/O	26.68	0.00	P/O/N			P/U			P/U			P/O	56.11	1.00		
MB11S320V0662	P/O	26.77	0.00	P/O	27.30	0.00	P/O/N			P/O/N			P/O	52.73	0.00	P/O	55.64	0.00		
MB11S323V0650	P/O	26.65	0.00	P/O	27.10	0.00	P/O/N			P/O/N			P/O	59.03	1.00	P/U				
MB11S335V0711	P/U			P/U			P/U			P/U			P/U			A				
MB11S352V0747	A			A			A			A			P/U			P/O	62.82	2.15	0.00	4.30
MB11S373V0798	P/O	27.92	0.50	P/O	27.62	0.50	P/U			P/U			P/O	55.60	0.50	P/O				
MB11S376V0900	A			A			A			A			P/O	56.88	2.15	P/O	58.28	2.15	0.00	4.30
MB11S400V0859	A			A			A			A			A			A				
MB11S404V0867	P/O	26.32	0.00	P/O	24.74	0.00	P/O/N			P/O/N			P/U			P/U				
MB11S406V0884	P/O	24.07	-2.00	P/O	23.75	-2.00	P/O	14.28	0.00	P/O	15.54	0.00	A			A				
MB11S418V0906	P/O	21.67	-4.00	A			A			P/O	14.86	0.00	A			A				
MB11S421V0940	P/U			P/U			P/U			P/U			P/U			P/U				
MB11S493V1069	P/O/N			P/O/N			P/U			P/U			P/O	54.92	0.00	P/O	53.31	0.00		

P=Present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 21 continued.

specimen	Frontal – Chord width left		Frontal – Chord width right		Temporal - pars petrosa length left		Temporal - pars petrosa length right		Mandibular length of body left		Mandibular length of body right		Clavicle maximum length left				
	P/A/O/U/N	measurment Age	P/A/O/U/N	measurment Age	P/A/O/U/N	measurment Age	P/A/O/U/N	measurment Age	P/A/O/U/N	measurment Age	P/A/O/U/N	measurment Age	P/A/O/U/N	measurment	mean Age	min age	max age
MB11S037V0021	A		A		P/O/N		P/U		P/O/N		P/O/N		P/O	48.19	13.00	0.00	26.00
MB11S050V0042	A		A		A		A		A		A		A				
MB11S058V0092	P/U		P/U		P/O/N		P/O/N		P/U		P/U		P/U				
MB11S080V0049	A		A		A		A		A		A		P/O	54.96	41.02	30.04	52.10
MB11S082V0084	P/O	44.96	P/O	44.08	A	0.00	P/O	39.84	P/O/N	25.19	-10.00	P/O/N	P/O	41.27	-2.00		
MB11S090V0107	P/U		P/U		P/O	29.11	P/O	29.63	P/O	35.58	0.00	P/O	P/O	30.39	-12.00		
MB11S099V0139	A		A		A		A		A		A		A				
MB11S102V0151	P/O	40.41	P/O	32.24	P/O	-4.00	P/O	32.33	P/O	36.18	0.00	P/O	P/O	38.71	-4.00		
MB11S122V0161	P/O/N		P/O/N		A		A		A		A		A				
MB11S130V0173	A		A		A		P/O	37.28	P/O	36.99	0.00	A	P/O	42.67	-2.00		
MB11S133V0299	P/U		P/U		P/U		P/O	38.49	P/O/N			P/O/N	P/O	40.09	-3.00		
MB11S139V0215	P/U		P/U		P/O	33.23	P/O	32.75	P/U	35.06	-2.00	P/O	P/O				
MB11S152V0244	A		A		P/O/N		A		P/O/N			A	P/U				
MB11S156V0046	P/O	48.59	P/O	35.96	P	-2.00	A		P/O	34.56	-2.00	P/O	P/O	42.98	-2.00		
MB11S164V0364	A		A		A		A		P/O	33.47	-2.00	P/O	P/O	48.31	39.05	26.00	52.10
MB11S187V0267	P/O	50.56	P/O	50.18	P/O/N	1.00	P/O/N		P/O/N			P/O/N	P/O				
MB11S189V0332	P/U		P/U		A		P/O/N		A			A	A				
MB11S191V0374	P	46.58	P/O	47.26	P/O	0.00	P/O	31.86	P/O	37.78	0.00	P/O	P/O	37.73	0.00		
MB11S227V0297	P/U		P/U		P/U		P/U	35.98	P/O	35.36	-1.00	A	P/O				
MB11S245V0390	P/O	47.90	P/U		P/O	-2.00	P/U	39.16	P/O	38.40	1.00	P/U	P/O				
MB11S287V0450	A		A		P/O	-2.00	P/O		P/O/N			P/O/N	A				
MB11S295V0485	A		A		P/O	-2.00	A		A			A	A				
MB11S296V0486	P/O	46.13	P/O	46.13	A		A		A			A	P/O	42.82	-2.00		
MB11S314V0655	A		A		P/U		P/U		P/U			P/U	P/O	49.18	26.00	0.00	52.10
MB11S315V0656	P/O	49.48	P/O	48.73	P/O	1.00	P	38.28	P/O	37.95	0.50	P/O	P/O	45.69	1.00		
MB11S320V0662	P/O	49.81	P/U		P/O/N		P/O/N		P/O/N			P/O/N	P/O	44.75	0.00		
MB11S323V0650	P/O	47.01	P/U		P/O	0.00	P/O	38.93	P/O/N			P/O/N	P/O	43.57	-0.50		
MB11S335V0711	P/O/N		A		P/U		P/U		P/O/N			P/O/N	P/O	44.94	13.00	0.00	26.00
MB11S352V0747	P/U		P/O/N		A		A		A			A	P/U				
MB11S373V0798	P/O	48.49	P/O	48.97	P/O	-1.00	P/O	35.98	P/O	35.52	0.00	P/O	P/O	46.78	13.00	0.00	26.00
MB11S376V0900	P/O	47.71	P/O	48.45	P/O/N	0.00	P/O/N		A			P/O	P/O	37.59	0.50		
MB11S400V0859	A		A		A		A		A			A	A				
MB11S404V0867	P/U		P/U		P/U		P/O	37.49	P/O/N			P/O/N	P/O	45.59	13.00	0.00	26.00
MB11S406V0884	A		A		P/O	-2.00	P/O	35.64	P/O	38.33	0.00	A	P/O	41.90	-2.50		
MB11S418V0906	A		P/O	40.55	P/O	-5.00	P/O	32.25	P/O	37.04	0.00	P/O	P/O				
MB11S421V0940	P/U		P/U		P/U		P/U		P/O/N			P/O/N	A				
MB11S483V1069	P/O/N		P/O/N		P/O/N		P/O/N		P/O/N			P/O/N	P/O	45.25	13.00	0.00	26.00

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 21 continued.

specimen	Clavicle maximum length right				Scapula length (height) left				Scapula length (height) right				Scapula width left				Scapula width right			
	P/A/O/U	measu rement	mean Age	min age	P/A/O/U	measu rement	mean Age	min age	max age	P/A/O/U	measu rement	mean Age	min age	max age	P/A/O/U	measu rement	mean Age	min age	max age	
MB11S037V0021	P/U				P/U					P/U					P/U					
MB11S060V0042	A				A					P/U					A	32.31	13.00	0.00	26.00	
MB11S068V0092	P/O	52.01	41.20	30.40	52.10	P/U				P/U					P/O	33.50	39.05	26.00	52.10	
MB11S080V0049	A				A					A					A					
MB11S082V0084	P/O	41.60	-2.00		A					P/O	27.52	-1.00			A					
MB11S090V0107	A				A					A					A					
MB11S099V0139	A				A					A					A					
MB11S102V0151	P/O	38.62	-4.00		P/U					P/U					P/O	26.11	-2.00			
MB11S122V0161	A				A					A					A					
MB11S130V0173	P/O	42.17	-2.00		P/U	33.25	-2.00			P/O					P/U					
MB11S133V0289	P/O	41.48	-3.00		P/U	33.11	-2.00			P/O					P/U					
MB11S139V0215	P/U				P/U					P/U					P/U					
MB11S162V0244	A				P/O	40.77	39.05	26.00	52.10	P/U					P/O	32.20	13.00	0.00	26.00	
MB11S166V0046	P/O	42.47	-2.00		P/O	33.97	0.00			P/U					P/U					
MB11S164V0364	P/O	42.36	-2.00		A					A					P/O	28.53	-1.00			
MB11S187V0267	P/O	48.52	39.05	26.00	52.10	P/U				P/U					P/U					
MB11S189V0332	P/U				A					A					A					
MB11S191V0374	P/U				P/U					P/O					P/U					
MB11S227V0297	P/O	43.89	0.00		P/O	34.45	0.00			P/O	28.48	-4.00			P/U					
MB11S245V0390	P/O	41.83	-2.00		P/U					P/U					P/U					
MB11S287V0450	P/U				A					P/O	36.72	1.00			P/O					
MB11S295V0485	A				A					A					A					
MB11S296V0486	A				P/U					P/U					P/U					
MB11S314V0655	P/O	50.70	26.00	0.00	52.10	P/U				P/U					P/U					
MB11S315V0656	P/U				P/U					P/U					P/O	28.66	1.00			
MB11S320V0662	P/O	45.60	0.00		P/U					P/U					P/O	28.56	0.00			
MB11S323V0650	P/O	42.61	-0.50		P/O	30.86	-3.00			P/U					P/O	28.33	-1.00			
MB11S335V0711	P/U				P/U					P/U					P/O	28.82	0.00			
MB11S352V0747	A				P/U					P/U					P/U					
MB11S373V0798	P/O	46.57	13.00	0.00	26.00	P/U				P/U					P/O	28.93	0.00			
MB11S376V0900	A				A					A					A					
MB11S400V0859	A				A					A					A					
MB11S404V0867	P/O	46.59	13.00	0.00	26.00	P/U				P/U					P/O	31.13	0.00			
MB11S406V0884	P/O	42.90	-2.50		A					P/O	31.80	-2.00			P/O	25.90	-4.00			
MB11S418V0906	A				P/U					P/U					P/U					
MB11S421V0940	A				P/U					P/U					P/U					
MB11S493V1069	P/O	46.00	13.00	0.00	26.00	P/O	38.28	13.00	0.00	26.00	38.46	13.00	0.00	26.00	P/O	29.17	0.00			

P=Present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.



Table 21 continued.

specimen	Humerus maximum length left							Humerus maximum length right							Radius maximum length left							Radius maximum length right							Ulna maximum length left									
	P/A/O/U	measu- re- ment	mean Age	min age	max age	P/A/O/U	measu- re- ment	mean Age	min age	max age	P/A/O/U	measu- re- ment	mean Age	min age	max age	P/A/O/U	measu- re- ment	mean Age	min age	max age	P/A/O/U	measu- re- ment	mean Age	min age	max age	P/A/O/U	measu- re- ment	mean Age	min age	max age	P/A/O/U	measu- re- ment	mean Age	min age	max age			
MB11S037V0021	P/O	73.74	5.20			P/O	72.74	5.20			P/U				P/U						P/U					P/U												
MB11S050V0042	P/O	70.70	2.60	0.00	5.20	A					A				A						A					A												
MB11S058V0092	A					P/O	91.77	39.05	26.00	52.10	A				P/O	72.47	39.05	26.00	52.10		P/O					P/O												
MB11S080V0049	A					A					A				A						P/O					P/O												
MB11S082V0084	P/O	67.89	0.00			P/U					P/O	54.13	0.00		P/O	55.02	0.00				P/O					P/O	61.27	3.25	0.00	6.50								
MB11S090V0107	A					P/O	49.18	-9.00			P/O	35.95	-12.00		P/O	35.97	-12.00				P/O					P/O	38.23	-13.00										
MB11S099V0139	P/O	67.83	5.20			A					A				P/O	49.07	-2.00				A				A													
MB11S102V0151	P/O	61.01	-2.00			P/O	60.20	-2.00			P/O				P/O	48.62	-2.00				P/O					P/U												
MB11S122V0161	A					A					A				A						A				A													
MB11S130V0173	P/O	62.11	-2.00			P/O	61.39	-2.00			P/O	51.61	0.00		P/O	51.09	0.00				P/O				P/O	58.09	-0.50											
MB11S133V0299	P/O	62.41	-1.00			A					A				A						A				P/U													
MB11S139V0215	P/U					P/U					P/O	46.59	-3.00		P/O	46.83	-3.00				P/O				P/U													
MB11S152V0244	P/O	73.52	9.75	6.50	13.00	P/O	73.40	9.75	6.50	13.00	A				P/O	57.89	6.90	4.30	9.50		P/O				P/O	64.32	3.25	0.00	6.50									
MB11S156V0046	P/O	66.12	0.50			P/O	66.18	0.50			P/O	67.14	0.00		P/O	52.87	0.00				P/O				P/O	60.33	0.00											
MB11S164V0364	A					P/O					A				A						A				A													
MB11S187V0267	P/O	70.31	3.25	0.00	6.50	P/O	70.50	3.25	0.00	6.50	P/O	56.64	3.25	0.00	6.50	P/O	57.31	6.50			P/O				P/O	66.13	6.50											
MB11S189V0332	A					A					A				A						A				A													
MB11S191V0374	P/O	68.59	0.00			P/U					P/O	56.00	0.00		P/O	57.32	6.50				P/O				P/O	49.24	-6.00											
MB11S227V0297	P/O	62.67	-1.00			P/O	63.29	-1.00			P/O	50.11	0.00		P/O	48.85	0.00				P/O				P/O													
MB11S245V0390	P/O	62.76	0.00			P/O	62.49	0.00			P/U				P/O						P/O				P/U													
MB11S287V0450	A					P/O	70.60	3.25	0.00	6.50	A				P/O	57.08	3.25	0.00	6.50		P/O				P/O	63.00	3.25	0.00	6.50									
MB11S295V0485	P/O	67.80	3.25	0.00	6.50	P/O	67.90	3.25	0.00	6.50	P/O	56.20	3.25	0.00	6.50	P/O	56.60	3.25	0.00	6.50		P/O			P/O													
MB11S296V0486	P/O	61.81	-2.00			P/O	61.12	-2.00			P/U				P/O	52.19	0.00				P/O				P/O													
MB11S314V0655	P/O	73.55	9.75	6.50	13.00	P/O	73.10	9.75	6.50	13.00	A				P/O	59.80	15.10	4.20	26.00		P/O				P/O	66.98	6.50											
MB11S315V0656	P/O	64.66	0.00			P/O	63.99	0.00			P/O	51.26	0.00		P/O	51.34	0.00				P/O				P/O	58.73	0.00											
MB11S320V0662	P/O	66.94	0.00			P/O	66.99	0.00			P/O	53.09	0.00		P/O	53.02	0.00				P/O				P/O	62.15	3.25	0.00	6.50									
MB11S323V0650	P/O	68.30	3.25	0.00	6.50	P/O	68.30	3.25	0.00	6.50	P/O	53.20	1.00		P/O	52.89	1.00				P/O				P/O	60.52	0.50											
MB11S335V0711	P/O	66.58	3.25	0.00	6.50	P/O	66.59	3.25	0.00	6.50	P/O	52.64	3.25	0.00	6.50	P/O	53.44	3.25	0.00	6.50		P/O			P/O	62.17	3.25	0.00	6.50									
MB11S352V0747	P/U					P/U					P/U				P/U						P/U				P/O	60.61	0.50											
MB11S373V0798	P/O	65.44	0.00			P/O	65.14	0.00			P/O	52.51	0.00		P/O	51.98	0.00				P/O				P/O	59.93	0.00											
MB11S376V0900	P/O	65.74	3.25	0.00	6.50	A					P/O	54.94	3.25	0.00	6.50	A					A			A														
MB11S400V0859	P/O	68.12	3.25	0.00	6.50	A					A				A						A			A														
MB11S404V0887	A					P/O	69.13	3.25	0.00	6.50	A				P/O	57.53	3.25	0.00	6.50		P/O				P/O	58.60	-1.00											
MB11S406V0884	P/O	61.70	-2.00			P/O	62.10	-2.00			A				P/O	50.10	-1.00				P/O				P/O													
MB11S418V0906	P/O	57.80	-4.00			P/O	57.90	-4.00			P/O	48.40	-2.00		P/O	47.95	-2.00				P/O				P/U													
MB11S421V0940	P/U					P/U					P/U				P/U						P/U				P/O	73.48	19.50	13.00	26.00									
MB11S493V1069	P/O	65.47	3.25	0.00	6.50	P/O	65.31	3.25	0.00	6.50	P/O	53.12	3.25	0.00	6.50	P/O	53.55	3.25	0.00	6.50		P/O			P/O	62.37	3.25	0.00	6.50									

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 21 continued.

specimen	Ulna maximum length right						Ilium maximum length left						Ilium maximum length right						Ilium maximum width left						Ilium maximum width right					
	P/A/O/U	measu rement	mean Age	min age	max age		P/A/O/U	measu rement	mean Age	min age	max age		P/A/O/U	measu rement	mean Age	min age	max age		P/A/O/U	measu rement	mean Age	min age	max age		P/A/O/U	measu rement	mean Age	min age	max age	
MB11S037V0021	P/U						P/U						P/U						P/U						P/O	40.20	21.70	17.39	26.00	
MB11S050V0042	A						A						A						A						A					
MB11S058V0092	A						P/O	49.50	67.39	56.52	78.26		P/O	49.50	67.39	56.52	78.26		P/O	46.00	34.78	30.43	39.13		P/O	45.50	34.78	30.43	39.13	
MB11S060V0049	A						A						A						A						A					
MB11S082V0084	P/O	61.95	3.25	0.00	6.50		P/O	33.23	0.00				P/O	32.78	0.00				P/O	36.70	21.70	13.00	30.43		P/O	37.10	21.70	13.00	30.43	
MB11S090V0107	A						P/U						P/O	22.55	-10.00				P/U						P/O	21.46	-10.00			
MB11S099V0139	A						A						A						A						A					
MB11S102V0151	P/U						P/O	27.32	-4.00				P/O	27.43	-4.00				P/O	31.71	0.00				P/O	31.99	0.00			
MB11S122V0161	A						A						A						A						A					
MB11S130V0173	P/O	57.48	-0.50				P/O	28.76	-4.00				P/O	34.73	1.50	0.00	3.00		P/O	38.44	21.70	17.39	26.00		P/O	37.16	21.70	17.39	26.00	
MB11S133V0299	A						P/U						P/U						P/U						P/U					
MB11S139V0215	P/O	53.65	-3.00				P/O	32.55	-2.00				P/O	32.10	-2.00				P/O	29.05	-2.00				P/O	28.48	-2.00			
MB11S162V0244	P/O	64.64	3.25	0.00	6.50		A					A						A						A						
MB11S166V0046	P/O	60.72	0.50				P/U						P/O	31.96	-2.00				P/O	36.14	0.50				P/O	35.65	0.50			
MB11S164V0364	A						A						A						A						A					
MB11S187V0267	P/O	66.96	6.50				P/O	34.64	1.50	0.00	3.00		P/O	34.73	1.50	0.00	3.00		P/O	38.10	21.70	17.39	26.00		P/O	37.16	21.70	17.39	26.00	
MB11S189V0332	A						P/O	35.80	21.70	17.39	26.00		A						P/O	38.10	21.70	17.39	26.00		A					
MB11S191V0374	P/U						P/U						P/U						P/U						P/O	29.88	-4.00			
MB11S227V0297	P/O	56.59	-2.00				P/O	30.77	-2.00				P/O	30.77	-2.00				P/O	32.29	0.00				P/O	32.29	0.00			
MB11S245V0390	A						P	30.64	-2.00				P/O	30.18	-2.00				P/O	33.06	0.00				P/O	34.31	0.00			
MB11S287V0450	P/O	65.85	3.25	0.00	6.50		A					A						P/O	33.79	0.00				P/O	38.97	34.78	30.43	39.13		
MB11S295V0485	P/O	64.40	3.25	0.00	6.50		P/O	35.15	0.00				P/O	35.10	0.00				P/O	31.37	0.00				P/O	31.79	0.00			
MB11S296V0486	P/U						P/U						P/U						P/U						P/U					
MB11S314V0655	P/O	66.75	6.50				P/U						P/U						P/U						A					
MB11S315V0656	P/O	59.56	0.00				P/O	31.06	-2.00				P/O	31.11	-2.00				P/O	34.06	19.50	13.00	26.00		P/O	34.07	19.50	13.00	26.00	
MB11S320V0662	P/O	62.10	3.25	0.00	6.50		P/O	34.34	21.70	17.39	26.00		P/O	32.35	6.50	0.00	13.00		P/O	32.35	6.50	0.00	13.00		P/O	34.13	1.00			
MB11S323V0650	P/O	60.47	1.00				P/U						P/O	32.39	-2.00				P/O	34.78	8.00	0.00	16.00		P/O	34.77	8.00	0.00	16.00	
MB11S335V0711	P/O	62.31	3.25	0.00	6.50		P/U						P/O	32.39	-2.00				P/O	34.78	8.00	0.00	16.00		P/O	34.77	8.00	0.00	16.00	
MB11S352V0747	P/U						P/U						P/U						P/U						P/U					
MB11S373V0798	P/U						P/U						P/O	32.10	-2.00				P/U						P/U					
MB11S376V0900	A						A						A						A					A						
MB11S400V0859	A						A						A						A					A						
MB11S404V0867	P/O	64.47	0.00				P/O	34.60	0.00				P/O	35.09	0.00				P/O	36.99	6.50	0.00	13.00		P/O	38.17	6.50	0.00	13.00	
MB11S406V0884	P/O	58.30	-1.00				P/O	32.50	-1.00				P/O	32.80	-1.00				P/O	28.90	-1.00				P/O	28.90	-1.00			
MB11S418V0906	P/O	55.04	-2.00				A					A						P/O	28.43	-4.00				P/O	28.40	-4.00				
MB11S421V0940	P/U						P/U						P/U						P/U						P/U					
MB11S493V1069	P/O	62.40	3.25	0.00	6.50		P/O	31.60	-2.00				P/O	31.50	-2.00				P/O	36.23	21.70	13.00	30.43		P/O	36.04	21.70	13.00	30.43	

P=Present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 21 continued.

specimen	Ishium maximum length left			Ishium maximum length right			Ishium maximum width left			Ishium maximum width right			Pubis maximum length left			Pubis maximum length right			Femur maximum length left					
	P/A/O/U	measu- rement	mean Age	P/A/O/U N	measu- rement	mean Age	P/A/O/U	measu- rement	mean Age	P/A/O/U	measu- rement	mean Age	P/A/O/U	measu- rement	mean Age	P/A/O/U	measu- rement	mean Age	P/A/O/U	measu- rement	mean Age	min age	max age	
MB11S037V0021	P/U			P/O	20.16	1.00	P/U			P/O/N			A			P/U								
MB11S050V0042	A			A			A			A			A			A								
MB11S058V0092	A			A			A			A			A			A								
MB11S080V0049	A			A			A			A			A			A								
MB11S082V0084	P/O	20.00	1.00	P/O	19.70	1.00	P/O	12.82	0.00	P/O	12.44	0.00	P/O	16.00	0.00	P/O	16.18	0.00	A					
MB11S090V0107	A			A			A			A			A			A								
MB11S099V0139	A			A			A			A			A			A								
MB11S102V0151	P/O	14.92	-5.00	P/O	14.99	-5.00	P/O	10.14	-4.00	P/O	10.85	-4.00	P/O	13.55	-4.00	P/O	13.26	-4.00	P/O	68.78	-2.00			
MB11S122V0161	A			A			A			A			A			A								
MB11S130V0173	P/O	17.13	-2.00	A			P/O	11.10	-2.00	A			A			A								
MB11S133V0299	P/O	18.33	0.00	P/O	18.12	0.00	P/O	11.37	-2.00	P/U			A			A								
MB11S139V0215	P/O	16.39	-4.00	P/O	15.56	-4.00	P/O	10.94	-3.00	P/O	10.92	-3.00	P/O	14.85	-2.00	P/O	14.66	-2.00	P/U	71.86	-0.50			
MB11S152V0244	A			A			A			A			A			A								
MB11S156V0046	P/U			P/U			P/U			P/U			P/U	16.56	0.00	A								
MB11S164V0364	A			A			A			A			P/O			A								
MB11S187V0267	P/U			P/U			P/O/N			P/U			P/U			P/U								
MB11S189V0332	A			A			A			A			A			A								
MB11S191V0374	P/O	14.83	-4.00	P/U			P/O	9.72	-6.00	P/U			A			A								
MB11S227V0297	P/O	17.15	-2.00	A			P/O	11.27	-2.00	A			P/O	14.82	-2.00	A								
MB11S245V0390	P/O	17.85	0.00	P/O	17.67	0.00	P/O	11.33	-2.00	P/O	10.94	-2.00	P/U			P/U								
MB11S287V0450	P/U			P/O	19.90	1.00	P	12.40	0.00	P/O	12.75	0.00	A			A								
MB11S295V0485	A			P/O	18.33	0.00	A			P/O	12.47	0.00	P/O	15.07	-2.00	P/O	15.12	-2.00	P/O	76.40	0.00			
MB11S296V0486	P/U			P/U			P/U			P/U			A			A								
MB11S314V0655	A			A			A			A			A			A								
MB11S315V0656	P/O	17.65	0.00	P/O	17.87	0.00	P/O	12.41	0.00	P/O	12.40	0.00	P/U			P/U								
MB11S320V0662	P/O	18.70	0.00	P/O	18.75	0.00	P/O	12.88	0.00	P/U			P/U			P/U								
MB11S323V0650	P/U			P/U			P/U			P/U			P/U			P/U								
MB11S335V0711	P/O	18.70	0.00	A			P/O	12.06	0.00	A			P/U			P/U								
MB11S352V0747	A			P/U			A			P/U			A			A								
MB11S373V0798	P/O	17.72	-1.00	P/U			P/O	15.05	0.00	P/O	12.59	0.00	A			P/U								
MB11S376V0900	A			A			A			A			A			A								
MB11S400V0859	A			A			A			A			A			A								
MB11S404V0867	A			A			A			A			A			A								
MB11S406V0884	A			P/O	17.30	-2.00	A			A			A			A								
MB11S418V0906	P/U			P/O	13.85	-6.00	P/U			P/O	9.90	-6.00	A			P/U								
MB11S421V0940	P/U			P/U			P/U			P/U			P/U			P/U								
MB11S493V1069	P/O	18.97	0.00	P/O	19.24	0.50	P/O/N			P/O/N			P/U			A								

P=Present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 21 continued.

specimen	Femur maximum length right						Tibia maximum length left						Tibia maximum length right						Fibula maximum length left						Fibula maximum length right							
	P/A/O/U	measu- rement	mean Age	min age	max age		P/A/O/U	measu- rement	mean Age	min age	max age		P/A/O/U	measu- rement	mean Age	min age	max age		P/A/O/U	measu- rement	mean Age	min age	max age		P/A/O/U	measu- rement	mean Age	min age	max age			
MB11S037V0021	P/U						P/U						P/U						P/U						P/U							
MB11S050V0042	A						A						A						A						A							
MB11S058V0092	P/U						P/U						P/U						P/U						A							
MB11S080V0049	A						A						A						A						A							
MB11S082V0084	A						A						A						A						A							
MB11S090V0107	P/U						P/O	48.42	-8.00				P/U						P/U						A							
MB11S098V0139	A						A						A						A						A							
MB11S102V0151	P/O	68.84	-2.00				A					P/O	59.82	-2.00				A						P/O	56.37	-2.00						
MB11S122V0161	P/O	93.35	9.75	6.50	13.00		P/O	82.35	13.00			P/O	82.22	13.00				A						A								
MB11S130V0173	A						A						A						A						A							
MB11S133V0299	P/O	72.62	-0.50				P/U					P/U							P/U						P/U							
MB11S139V0215	P/O	66.77	-3.00				P/U					P/U							P/O	57.66	-3.00				P/O	55.38	-3.00					
MB11S152V0244	A						A						A						A						A							
MB11S156V0046	P/O	74.14	0.00				A					P/O	65.75	0.00				A						A								
MB11S164V0364	A						A					P/O	70.29	3.25	0.00	6.50		A						A								
MB11S187V0267	P/O	79.53	3.25	0.00	6.50		P/O	68.11	3.25	0.00	6.50	P/O	69.97	3.25	0.00	6.50		P/O	67.04	6.50				P/O	66.99	6.50						
MB11S189V0332	P/U						P/U						P/U						P/U						A							
MB11S191V0374	P/O	67.21	-3.00				P/O	60.15	-2.00			P/O	60.30	-2.00				A						A								
MB11S227V0297	P/O	72.89	0.00				P/O	62.06	-1.00			P/O	62.28	-1.00				P/O	59.39	0.00				P/O	59.27	0.00						
MB11S245V0390	P/U						P/U						P/U						P/U						A							
MB11S287V0450	P/U						P/O	70.24	6.50			P/O	69.22	3.25	0.00	6.50		P/O	69.38	9.75	6.50	13.00		P/O	68.75	9.75	6.50	13.00				
MB11S295V0485	P/O	75.80	0.00				P/O	68.40	3.25	0.00	6.50	P/O	68.10	3.25	0.00	6.50		P/O	68.10	6.50				A								
MB11S296V0486	P/O	73.34	0.00				A					A						A						A								
MB11S314V0655	P/O	82.30	3.25	0.00	6.50		P/O	74.58	9.75	6.50	13.00	P/O	74.39	9.75	6.50	13.00		P/O	74.39	9.75	6.50	13.00		P/U								
MB11S315V0656	P/O	74.75	0.00				P/O	64.50	0.00			P/O	64.59	0.00				P/O	62.52	0.00				P/O	62.28	0.00						
MB11S320V0662	P/O	77.94	0.00				P/O	66.01	0.00			P/O	66.41	0.00				P/O	66.41	0.00				P/O	62.69	0.00						
MB11S323V0650	P/O	78.27	1.00				P/O	67.39	1.00			P/O	67.04	1.00				P/O	67.04	1.00				P/O	61.97	1.00						
MB11S335V0711	P/O	80.00	3.25	0.00	6.50		P/O	68.24	2.15	0.00	4.30	P/O	69.40	2.15	0.00	4.30		P/O	69.40	2.15	0.00	4.30		P/U								
MB11S352V0747	P/O	79.20	3.25	0.00	6.50		P/O	71.08	6.50			P/O	70.91	6.50				P/O	70.91	6.50				A								
MB11S373V0798	A						A					A						A						A								
MB11S376V0900	A						A					P/O	82.00	0.00				P/O	82.00	0.00				A								
MB11S400V0859	A						A					A						A						A								
MB11S404V0867	A						A					A						A						A								
MB11S406V0864	P/O	72.90	-1.00				P/O	63.10	-1.00			A						A						P/O	68.60	3.25	0.00	6.50				
MB11S418V0906	A						A					A						A						A								
MB11S421V0940	P/U						P/U					P/O	95.27	26.00				P/O	95.27	26.00				P/U								
MB11S493V1069	P/O	76.34	0.50				P/U					P/U						P/U						P/U								

P=Present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 21 continued.

specimen	mean age all bones combined	min age	max age	inter-individual variability	known age	mean difference to known age	mean age Marsh standard	mean single bone infant standards	mean cranial measurements	mean post-cranial measurements	number of elements observable (P/O)	Number of elements Absent (A)	Number of elements unobservable (PIU)	Number of elements with no standard (N)
MB11S037V0021	14.31	5.20	26.05	20.85	13.04	1.27	1.00	17.35	18.80	9.85	9	12	26	0
MB11S050V0042	7.80	2.60	13.00	10.40	2.71	5.09	13.00	2.71	7.80	7.80	2	48	1	0
MB11S058V0092	35.81	19.50	67.39	47.89			32.53	46.23	42.12	42.12	10	18	18	5
MB11S080V0049	41.02	30.04	52.10	22.06			41.02	41.02	41.02	41.02	1	50	0	0
MB11S082V0084	1.81	-2.00	21.70	24.70	2.43	-0.62	3.25	21.70	0.00	2.61	26	15	6	4
MB11S090V0107	-8.95	-13.00	-2.00	11.00			too young	too young	-7.73	-10.57	19	22	10	
MB11S099V0139	5.20				7.00	-1.80	too young	too young	7.00	7.00	1	50	0	0
MB11S102V0151	-2.68	-5.00	2.00	7.00			too young	too young	-2.83	-2.64	28	16	7	0
MB11S122V0161	11.38	9.75	13.00	3.25			11.38	11.38	11.38	11.38	4	43	0	4
MB11S130V0173	-1.04	-4.00	0.50	4.50			too young	too young	0.00	-1.42	15	27	9	0
MB11S133V0299	-1.50	-11.00	17.35	28.35			too young	too young	-0.11	-1.33	14	12	21	4
MB11S139V0215	-2.67	-4.00	0.00	4.00			too young	too young	-2.55	-2.75	26	2	23	0
MB11S152V0244	17.08	3.00	58.69	55.69	11.00	6.08	6.58	25.82	29.96	12.24	7	34	2	8
MB11S156V0046	-1.05	-10.00	0.50	10.50			too young	too young	-2.30	-0.23	25	10	16	0
MB11S164V0364	2.94	-2.00	10.85	12.85	0.28	2.66	3.25	9.42	4.85	0.05	10	41	0	0
MB11S187V0267	7.30	-8.00	34.78	42.78			4.60	20.83	5.99	9.99	29	0	14	8
MB11S189V0332	16.63	-5.00	21.70	26.70			6.5	21.70	-5.00	16.63	4	32	13	2
MB11S191V0374	-2.36	-6.00	0.00	6.00			too young	too young	-1.64	-3.09	22	4	25	0
MB11S227V0297	-1.27	-16.00	0.00	16.00	0.86	-2.13	too young	too young	-2.38	-0.84	26	7	16	2
MB11S245V0390	-0.73	-2.00	1.00	3.00			too young	too young	-0.33	-0.83	15	20	16	0
MB11S287V0450	3.00	0.00	34.78	34.78			17.89	5.57	1.00	5.41	15	30	5	1
MB11S295V0485	1.20	-2.00	6.50	8.50	stillborn		3.61	1.00	-0.25	1.50	23	28	0	0
MB11S296V0486	-2.00	-8.00	0.00	8.00	stillborn		too young	too young	-2.80	-1.20	10	27	14	0
MB11S314V0655	14.55	3.25	39.10	35.85			8.18	26.03	26.05	11.42	10	20	16	5
MB11S315V0656	0.08	-2.00	19.50	21.50			too young	10.81	1.95	1.68	37	13	0	1
MB11S320V0662	0.40	-12.00	21.70	33.70	stillborn		3.25	14.10	-1.83	1.74	34	0	13	4
MB11S323V0650	1.00	-10.00	39.10	49.10			1.50	14.61	4.40	0.61	29	1	16	5
MB11S335V0711	5.38	-12.00	34.78	46.78			3.03	12.51	8.92	3.37	22	11	15	3
MB11S352V0747	5.70	4.30	6.50	2.20			5.42		2.15	4.19	5	26	19	1
MB11S373V0798	0.54	-10.00	13.00	23.00	1.00	-0.46		9.00	-0.73	2.27	26	13	12	0
MB11S376V0900	0.33	0.00	3.00	3.00			3.25		0.76	2.17	10	39	0	11
MB11S400V0859	3.25	0.00	6.50	6.50	3.43	-0.18	too young	too young			1	49	1	0
MB11S404V0867	0.15	-8.00	13.00	21.00			3.25	9.75	-1.20	4.43	23	13	13	4
MB11S406V0884	-2.03	-14.00	0.00	14.00	0.43	-2.46	too young	too young	-2.50	-1.72	30	21	0	0
MB11S418V0906	-3.40	-7.00	0.00	7.00			too young	too young	-3.00	-3.80	20	22	9	0
MB11S421V0940	22.75	19.50	26.00	6.50			22.75			22.75	2	13	34	2
MB11S493V1069	4.99	-8.00	30.43	38.43			3.25	12.02	4.12	5.62	27	1	12	11

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

## *Appendix 2: Dataset of deciduous Demirjian stage recordings*

Table 22 shows the recordings of the dental deciduous developmental stages for all Middenbeemster infants who possessed teeth (n=39). The teeth are named using the code of the Federation Dentaire Internationale (FDI). (see appendix 6 for an explanation of the FDI system). The table is split into five segments each presented on a different page.

For each individual (indicated by the rows) the table provides information on whether the tooth is present (P) or absent (A) and when present if the tooth is observable (O) or unobservable/damaged (U). Moreover the stage of development is recorded with the corresponding age estimate (if provided by the method), and the Standard Deviation (SD). At the end of the table, summary ages are listed for each individual: the mean age of all teeth combined, the minimum age, and maximum age, as well as mean age for incisors, canines and molars each listed separately. Known age is recorded in case it is known, and the mean difference to chronological age. Finally, for each individual is listed a) how many teeth are scored, b) the number of teeth that were too young to be used for this method, c) the number of teeth that were unobservable, and d) the number of teeth that were missing. The table provides all ages in weeks.

Table 22. Deciduous Demirjian stage recordings according to Liversidge and Molleson (2004) and corresponding age estimates for 39 foetal and infant remains of the Middenbeemster sample.

Specimen	51				52				53				54				55					
	P/A	O/U	stage	age w	SD	P/A	O/U	stage	age w	SD	P/A	O/U	stage	age w	SD	P/A	O/U	stage	age w	SD		
MB11S037V0021	A					P	O	D	14.56	0.24	P	O	C	17.68	0.20	A						
MB11S050V0042	P	O	D	6.24	0.24	A					A					P	O	C	9.36	0.26	A	
MB11S058V0092	P	O	G	50.96	0.35	A					P	U				A						
MB11S080V0049	P	O	E	21.84	0.31	A					A					A						
MB11S082V0084	A					A					A					P	O	B				
MB11S090V0107	A					A					A					A						
MB11S091V0110	P	U				P	U				A					P	U					
MB11S099V0139	A					A					A					A						
MB11S102V0151	P	O	C			A					A					P	O	B				
MB11S122V0161	P	O	E	21.84	0.31	A					A					A						
MB11S130V0173	A					A					A					A						
MB11S133V0299	A					P	O	C			P	O	B			A						
MB11S139V0215	A					A					A					A						
MB11S142V0289	P	U				P	U				P	U				P	O	D	18.20	0.11	P	U
MB11S152V0244	A					A					A					A						
MB11S156V0046	A					A					A					P	O	B				
MB11S164V0364	A					A					A					A						
MB11S187V0267	P	O	D	6.24	0.24	P	O	C			P	O	B			P	O	C	9.36	0.26	P	O
MB11S189V0332	A					A					A					A						
MB11S191V0374	A					A					A					P	O	D	18.20	0.11	A	
MB11S227V0297	P	O	C			P	O	C			P	O	B			P	O	B				
MB11S245V0390	A					P	O	C			A					P	O	B				
MB11S287V0450	P	O	D	6.24	0.24	P	U				P	O	B			P	O	C	9.36	0.26	P	O
MB11S295V0485	A					A					A					A						
MB11S296V0486	A					A					A					A						
MB11S314V0655	P	O	E	21.84	0.31	P	O	D	14.56	0.24	P	O	C	17.68	0.20	P	O	D	18.20	0.11	P	O
MB11S315V0656	P	O	D	6.24	0.24	P	O	C			A					A						
MB11S320V0662	P	O	C			A					P	O	B			P	O	C	9.36	0.26	P	O
MB11S323V0650	P	O	C			P	O	C			P	O	B			P	O	B				
MB11S335V0711	A					P	O	C			A					P	O	C	9.36	0.26	P	O
MB11S352V0747	P	O	E	21.84	0.31	P	O	E	27.04	0.19	P	O	C	17.69	0.20	P	O	D	18.20	0.11	A	
MB11S373V0798	A					P	O	C			P	O	B			P	O	B				
MB11S376V0900	A					A					A					A						
MB11S400V0859	A					A					A					A						
MB11S404V0867	P	O	C			A					P	O	B			P	O	B				
MB11S406V0884	P	O	C			P	O	C			A					P	O	B				
MB11S418V0906	P	O	C			A					A					A						
MB11S421V0940	A					P	U				A					P	O	D	18.20	0.26	A	
MB11S493V1069	P	O	D	6.24	0.24	P	O	C			P	O	C	17.68	0.20	P	O	D	18.20	0.26	P	O
SUM				10					3					4					11			5

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 22 continued.

Specimen	61				62				63				64				65			
	P/A	O/U	stage	age w	SD	P/A	O/U	stage	age w	SD	P/A	O/U	stage	age w	SD	P/A	O/U	stage	age w	SD
MB11S037V0021	P	O	E	21.84	0.31	P	O	D	14.56	0.24	P	U				P	O	C	15.08	0.14
MB11S050V0042	A	U				P	U				A					A				
MB11S058V0092	P	U				A					A					P	O	E	36.40	0.12
MB11S080V0049	A					A					A					A				
MB11S082V0084	P	O	C			P	O	C			P	O	B			A				
MB11S090V0107	P	O	B			A					A					A				
MB11S091V0110	P	U				P	U				A					P	U		15.08	0.14
MB11S099V0139	A					A					A					A				
MB11S102V0151	P	O	C			A					P	O	B			A				
MB11S122V0161	A					A					A					A				
MB11S130V0173	A					P	O	C			A					A				
MB11S133V0299	A					P	O	C			A					A				
MB11S139V0215	A					P	O	B			P	O	B			A				
MB11S142V0289	A					P	O	E	27.04	0.19	A					P	U			
MB11S152V0244	A					A					A					A				
MB11S156V0046	P	O	C			P	O	C			P	O	B			A				
MB11S164V0364	P	O	C			A					A					A				
MB11S187V0267	P	O	D	6.24	0.24	P	O	C			P	O	B			A				
MB11S189V0332	A					A					A					A				
MB11S191V0374	P	O	C			P	O	C			A					A				
MB11S227V0297	A					P	O	B			P	O	B			A				
MB11S245V0390	A					P	O	B			P	O	B			A				
MB11S287V0450	P	O	D	6.24	0.24	P	U				P	O	B			A				
MB11S295V0485	P	O	C			P	O	C			P	O	B			A				
MB11S296V0486	A					A					P	O	A			A				
MB11S314V0655	A					A					P	O	C	17.68	0.20	A				
MB11S315V0656	P	O	D	6.24	0.24	A					A					P	O	D	18.20	0.11
MB11S320V0662	P	O	C			A					A					P	O	C	9.36	0.26
MB11S323V0650	P	O	C			P	O	C			P	O	B			P	O	C	9.36	0.26
MB11S335V0711	P	O	D	6.24	0.24	P	O	D	14.56	0.24	A					P	O	C	9.36	0.26
MB11S352V0747	A					A					A					P	O	D	18.20	0.11
MB11S373V0798	P	O	D	6.24	0.24	P	O	B			A					P	O	B		
MB11S376V0900	A					A					A					A				
MB11S400V0859	A					P	O	D	14.56	0.24	A					A				
MB11S404V0867	A					P	O	C			P	O	B			A				
MB11S406V0884	A					A					A					A				
MB11S418V0906	A					A					A					A				
MB11S421V0940	P	O	E	21.84	0.31	A					A					A				
MB11S493V1069	A					P	O	C			P	O	C	17.68	0.20	P	O	D	18.20	0.11
SUM				7				4					2					8		3

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.



Table 22 continued.

Specimen	71			72			73			74			75			
	P/A	O/U	stage	age w	SD	P/A	O/U	stage	age w	SD	P/A	O/U	stage	age w	SD	
MB11S037V0021	P	O	E	16.64	0.27	P	O	C			P	O	C	6.76	0.25	A
MB11S050V0042	P	O	D	5.20	0.20	P	O	C			A					A
MB11S058V0092	P	U				P	U				P	U				P
MB11S080V0049	A					A					A					A
MB11S082V0084	P	O	D	5.20	0.20	P	O	C			P	O	B			A
MB11S090V0107	A					A					A					A
MB11S091V0110	P	U				A					A					A
MB11S099V0139	A					A					P	O	D	24.96	0.18	A
MB11S102V0151	P	O	C			A					P	O	B			P
MB11S122V0161	A					A					A					A
MB11S130V0173	A					P	O	D	16.64	0.07	A					A
MB11S133V0299	P	O	D	5.20	0.20	A					A					A
MB11S139V0215	P	O	C			A					A					A
MB11S142V0289	P	O	E	16.64	0.13	P	O	E	24.44	0.17	P	O	C	19.76	0.18	P
MB11S152V0244	P	O	E	16.64	0.13	A					A					A
MB11S156V0046	A					A					A					A
MB11S164V0364	A					A					A					A
MB11S187V0267	A					P	O	C			P	O	B			P
MB11S189V0332	A					A					A					A
MB11S191V0374	A					A					A					A
MB11S227V0297	A					A					A					A
MB11S245V0390	A					P	O	B			A					A
MB11S287V0450	A					P	O	C			P	O	B			P
MB11S295V0485	A					P	O	B			P	O	B			P
MB11S296V0486	A					A					A					A
MB11S314V0655	A					P	O	D	16.64	0.07	P	O	C	19.76	0.18	P
MB11S315V0656	P	O	D	5.20	0.20	P	O	C			A					A
MB11S320V0662	P	O	C			A					P	O	B			P
MB11S323V0650	P	O	D	5.20	0.20	P	O	C			A					A
MB11S335V0711	P	O	E	16.64	0.13	P	O	C			P	O	C	19.76	0.18	P
MB11S352V0747	A					A					A					A
MB11S373V0798	P	O	D	5.20	0.20	P	O	C			A					A
MB11S376V0900	A					A					A					A
MB11S400V0859	P	O	D	5.20	0.20	P	O	D	16.64	0.07	P	O	C	19.76	0.18	P
MB11S404V0867	P	O	C			P	O	C			P	O	B			P
MB11S406V0884	P	O	C			P	O	C			A					A
MB11S418V0906	A					P	O	C			A					A
MB11S421V0940	P	O	E	16.64	0.13	A					P	O	D	24.96	0.18	P
MB11S493V1069	A					P	O	C			P	O	C	6.76	0.25	P
SUM				12				4		5			11		4	

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 22 continued.

Specimen	81			82			83			84			85				
	P/A	O/U	stage age w SD	P/A	O/U	stage age w SD	P/A	O/U	stage age w SD	P/A	O/U	stage age w SD	P/A	O/U	stage age w SD		
MB11S037V0021	P	O	16.64 0.18	P	O	C	P	O	C	19.76 0.18	P	O	C	6.76 0.25	P	O	B
MB11S050V0042	P	O	5.20 0.20	A			P	U							A		
MB11S058V0092	P	U		P	U		P	U							P	O	D
MB11S080V0049	A			A			A								A		
MB11S082V0084	P	O	5.20 0.20	P	O	C	P	O	B						A		
MB11S090V0107	A			P	O	B	P	O	A						A		
MB11S091V0110	A			A			A								P	U	
MB11S099V0139	A			A			P	O	C	19.76 0.18	A				A		
MB11S102V0151	A			P	O	B	P	O	B						P	O	B
MB11S122V0161	A			A			A								P	O	B
MB11S130V0173	A			A			P	O	C	19.76 0.18	A				A		
MB11S133V0299	A			A			P	O	B						A		
MB11S139V0215	P	O		P	O	C	P	O	B						P	O	B
MB11S142V0289	A			P	O	E	24.44 0.17	U							P	U	
MB11S152V0244	P	O	16.64 0.18	A			A								P	O	C
MB11S156V0046	P	O		P	O	C	P	O	B						P	O	B
MB11S164V0364	A			A			A								A		
MB11S187V0267	P	O	5.20 0.20	P	O	C	P	O	B						A		
MB11S189V0332	A			A			P	O	B						A		
MB11S191V0374	A			A			A								A		
MB11S227V0297	A			A			A								A		
MB11S245V0390	P	O		P	O	B	A								A		
MB11S287V0450	P	O	5.20 0.20	P	O	U	P	O	B						P	O	B
MB11S295V0485	A			A			A								A		
MB11S296V0486	P	O		P	O	C	A								A		
MB11S314V0655	P	O	16.64 0.18	P	O	D	16.64 0.07	P	O	C	19.76 0.18	A			P	O	C
MB11S315V0656	P	O	5.20 0.20	A			P	O	B						P	O	C
MB11S320V0662	P	O		P	O	C	A								P	O	C
MB11S323V0650	P	O	5.20 0.20	P	O	C	P	O	B						P	O	B
MB11S335V0711	A			A			P	O	C	19.76 0.18	P	O	C	6.76 0.25	P	O	C
MB11S352V0747	A			A			A								A		
MB11S373V0798	P	O	5.20 0.20	P	O	C	P	O	B						P	O	B
MB11S376V0900	A			P	O	B	P	O	B						P	O	B
MB11S400V0859	A			A			A								A		
MB11S404V0867	A			P	O	C	P	O	B						P	U	
MB11S406V0884	P	O		A			A								A		
MB11S418V0906	A			A			A								A		
MB11S421V0940	P	O	16.64 0.18	A			A								A		
MB11S493V1069	P	O	5.20 0.20	P	O	C	P	O	C	19.76 0.18	P	O	C	6.76 0.25	P	O	B
SUM			12			2			6				10				5

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 22 continued.

Specimen	mean age all teeth combined	minimum age	maximum age	SD	mean age incisors	mean age canines	mean age molars	known chronological age	average difference to known age	number of teeth Observable P/O	number of teeth unobservable P/U	number of teeth absent A	number of teeth too young
MB11S037V0021	15.32	6.76	21.84	11.5	16.85	18.72	11.70	13.04	2.28	11	2	4	3
MB11S050V0042	6.50	5.20	9.36	12.0	5.55		9.36	2.71	3.79	4	4	10	2
MB11S058V0092	44.72	36.40	50.96	13.0	50.96		43.16			5	10	5	0
MB11S080V0049	21.84			16.2	21.84					1	0	19	0
MB11S082V0084	5.20	5.20	5.20	10.4	5.20			2.43	2.77	2	0	8	10
MB11S090V0107	under 5.2									0	0	15	5
MB11S091V0110	16.81	15.08	20.28	8.3			16.81			3	9	8	0
MB11S099V0139	19.93	15.08	24.96	8.8		19.76	20.02	7.00	12.93	3	0	17	0
MB11S102V0151	under 5.2									0	0	8	12
MB11S122V0161	23.40	21.84	24.96	15.1	21.84		24.96			2	0	18	0
MB11S130V0173	18.20	16.64	19.76	6.8	16.64	19.76				2	0	15	3
MB11S133V0299	5.98	5.20	6.76	12.0	5.20		6.76			2	0	7	11
MB11S139V0215	under 5.2									0	0	12	8
MB11S142V0289	22.21	16.64	27.04	8.3	23.14	19.76	21.58			7	10	3	0
MB11S152V0244	18.27	15.08	24.96	9.4	16.64		20.11	11.00	7.27	5	0	15	0
MB11S156V0046	under 5.2									0	0	9	11
MB11S164V0364	under 5.2							0.28		0	0	18	2
MB11S187V0267	6.76	5.20	9.36	12.5	5.89		7.63			6	0	2	12
MB11S189V0332	18.20			5.7			18.20			1	0	19	0
MB11S191V0374	under 5.2									0	0	14	6
MB11S227V0297	under 5.2									0	0	15	5
MB11S245V0390	under 5.2							0.86		0	0	8	12
MB11S287V0450	6.76	5.20	9.36	12.5	5.89		9.36			4	3	5	8
MB11S295V0485	under 5.2							stillborn		0	0	14	6
MB11S296V0486	under 5.2							stillborn		0	0	14	6
MB11S314V0655	18.20	14.56	24.96	8.8	17.26	18.72	18.63			15	0	5	0
MB11S315V0656	6.53	5.20	9.36	12.0	5.72		7.63			7	0	7	6
MB11S320V0662	9.36	6.76	9.36	13.0			8.06	stillborn		4	0	4	12
MB11S323V0650	5.20	5.20	5.20	10.4	5.20					2	0	2	16
MB11S335V0711	13.61	6.76	20.28	11.5	12.48	19.76	12.13			11	0	5	4
MB11S352V0747	20.59	17.69	27.04	9.4	24.44	17.69	18.20			5	0	15	0
MB11S373V0798	5.54	5.20	6.24	11.0	5.55			1.00	4.54	3	0	3	14
MB11S376V0900	under 5.2									0	0	16	4
MB11S400V0859	12.58	5.20	19.76	10.0	12.13	19.76	6.76	3.43	9.15	5	1	13	1
MB11S404V0867	under 5.2									0	3	4	13
MB11S406V0884	under 5.2							0.43		7	0	13	0
MB11S418V0906	under 5.2									0	1	17	2
MB11S421V0940	20.84	16.64	24.96	11.0	18.37		21.74			8	1	11	0
MB11S493V1069	13.62	5.20	19.76	11.0	5.72	18.72	12.48			10	0	3	7
SUM										133	45	399	203

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

### *Appendix 3: Dataset of dental height recordings*

Table 23 shows the recordings of dental height for all Middenbeemster infants who possessed teeth (n=39). The teeth are named using the code of the Federation Dentaire Internationale (FDI). (see appendix 6 for an explanation of the FDI system). The table is split into five segments each presented on a different page.

For each individual (indicated by the rows) the table provides information on whether the tooth is present (P) or absent (A) and when present if the tooth is observable (O) or unobservable/damaged (U). Moreover the measurement is recorded with the corresponding age estimate which was calculated using the regression equations provided by Liversidge and colleagues (table 5 p.68), and the Standard Deviation (SD). At the end of the table, summary ages are listed for each individual: the mean age of all teeth combined, minimum age, and maximum age, as well as intra-individual variability (calculated as the difference between minimum age and maximum age). Real age and the mean difference to chronological age are given if known for the individual. In addition the mean of the upper and lower jaw are included as well as the difference between the two (calculated as the difference between minimum age and maximum age). Furthermore, mean age for incisors, canines and molars are each listed separately. Finally, for each individual is listed a) how many teeth are present and observable, b) the number of teeth that were present but unobservable, and c) the number of teeth that were absent. All ages are provided in weeks.

Table 23. Dental height recordings and calculated age estimates using the regression equations provided by Liversidge and colleagues (1993) for 39 individuals of the Middenbeemster infant sample.

Specimen	51				52				53				54				55				
	P/A	O/U	Measure ment	Age w SD	P/A	O/U	Measure ment	Age w SD	P/A	O/U	Measure ment	Age w SD	P/A	O/U	Measure ment	Age w SD	P/A	O/U	Measure ment	Age w SD	
MB11S037V0021	A				P	O	5.27	11.44	0.17	P	O	4.17	11.44	0.22	A						
MB11S050V0042	P	O	5.66	8.42	0.19	A				A					P	U					
MB11S058V0092	P	O	12.31	58.29	0.19	A				P	U				A					5.68	39.20
MB11S080V0049	P	O	9.34	35.98	0.19	A				A					A						
MB11S082V0084	A					A				A					P	O	3.89	2.50	0.25	A	
MB11S090V0107	A					A				A					A						
MB11S091V0110	P	U				P	U			A					P	U				4.90	27.39
MB11S099V0139	A					A				A					A						
MB11S102V0151	P	O	4.44	-0.70	0.19	A				A					P	O	3.22	-5.15	0.25	A	
MB11S122V0161	P	O	8.63	30.62	0.19	A				A					A						
MB11S130V0173	A					A				A					A						
MB11S133V0299	A					P	O	4.83	8.20	0.17	P	O	3.97	9.44	0.22	P	O	4.24	6.61	0.25	A
MB11S139V0215	A					A				A					A						
MB11S142V0259	P	U				P	U			P	U				P	U					
MB11S152V0244	A					A				A					A						
MB11S156V0046	A					A				A					P	O	3.97	3.49	0.25	A	
MB11S164V0364	A					A				A					A						
MB11S187V0267	P	O	5.67	8.50	0.19	P	O	4.57	6.10	0.17	P	O	3.62	5.60	0.22	P	O	3.84	2.00	0.25	P
MB11S189V0332	A					A				A					P	O	4.97	15.02	0.25	A	
MB11S191V0374	A					P	O	3.88	0.65	0.17	P	O	2.57	-5.76	0.22	P	U				
MB11S227V0297	P	O	5.44	6.76	0.19	P	O	4.77	7.69	0.17	A				P	U					
MB11S245V0390	A					P	O	4.27	3.74	0.17	A				P	O	3.80	1.53	0.25	A	
MB11S287V0450	P	O	5.99	10.89	0.19	P	U			P	O	3.44	3.68	0.22	P	O	3.69	0.26	0.25	P	U
MB11S295V0485	A					A				A					A						
MB11S296V0486	A					A				A					A						
MB11S314V0655	P	O	6.98	18.20	0.19	P	O	5.20	10.92	0.17	P	U			P	O	3.10	-6.41	0.25	A	
MB11S315V0656	P	O	5.44	6.77	0.19	P	O	4.74	7.99	0.17	A				P	O	4.99	15.08	0.25	P	O
MB11S320V0662	P	O	5.43	6.65	0.19	A				P	O	3.96	9.30	0.22	P	O	3.81	1.61	0.25	P	O
MB11S323V0650	P	O	5.56	7.67	0.19	P	O	4.68	7.02	0.17	P	O	3.47	4.01	0.22	P	O	4.06	4.50	0.25	P
MB11S335V0711	A					P	O	5.30	11.90	0.17	A				P	O	4.54	10.04	0.25	P	O
MB11S352V0747	P	O	6.98	18.20	0.19	P	O	6.28	19.24	0.17	P	O	5.1	21.32	0.22	P	O	4.79	12.48	0.25	A
MB11S373V0798	A					P	O	4.47	5.30	0.17	P	O	3.15	0.52	0.22	P	O	3.98	3.58	0.25	P
MB11S376V0900	A					A				A					A						
MB11S400V0859	A					A				A					A						
MB11S404V0867	P	O	5.62	8.11	0.19	A				P	O	3.46	3.90	0.22	P	O	3.68	0.15	0.25	P	U
MB11S406V0884	P	O	5.11	4.32	0.19	P	O	3.77	-0.21	0.17	A				P	O	3.37	-3.43	0.25	P	U
MB11S418V0906	P	O	4.77	1.76	0.19	A				A					A						
MB11S421V0940	A					P	U			A					A						
MB11S493V1069	P	O	5.98	10.82	0.19	P	O	4.86	8.45	0.17	P	O	3.98	9.55	0.22	P	O	4.22	6.38	0.25	P
Sum				17				13				11					19				12

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 23 continued.

Specimen	61					62					63					64					65				
	P/A	O/U	Measurement	Age w	SD	P/A	O/U	Measurement	Age w	SD	P/A	O/U	Measurement	Age w	SD	P/A	O/U	Measurement	Age w	SD	P/A	O/U	Measurement	Age w	SD
MB11S037V0021	P	O	6.62	15.60	0.19	P	O	5.35	11.96	0.17	P	U			P	O	4.52	9.36	0.25	P	O	3.84	10.92	0.26	
MB11S050V0042	A					P	U				A				P	U					A				
MB11S059V0092	P	U				A					A				P	O	6.93	37.64	0.25	A					
MB11S080V0049	A					A					A				A						A				
MB11S082V0084	P	O	5.34	5.80	0.19	P	O	4.57	6.00	0.17	P	O	3.53	4.50	0.22	A				A					
MB11S090V0107	P	O	4.30	-1.73	0.19	A					A				A					A					
MB11S091V0110	P	U				P	U				A				P	U				P	O	5.10	30.43	0.26	
MB11S099V0139	A					A					A				A					A					
MB11S102V0151	P	O	4.34	-1.45	0.19	A					P	O	2.43	-7.28	0.22	P	O	3.20	-5.38	0.25	A				
MB11S122V0161	A					A					A				A					A					
MB11S130V0173	A					P	O	4.81	7.80	0.17	A				A				A						
MB11S133V0299	A					P	O	4.96	9.24	0.17	P	O	4.11	10.97	0.22	P	O	4.20	6.15	0.25	A				
MB11S139V0215	P	O	3.75	-5.87	0.19	A					P	O	2.36	-8.00	0.22	A				A					
MB11S142V0259	A					P	O	7.41	28.74	0.17	A				P	U				P	U				
MB11S152V0244	A					A					A				A					A					
MB11S156V0046	P	O	5.67	8.47	0.19	P	O	4.62	6.50	0.17	P	O	3.21	1.14	0.22	P	O	3.97	3.48	0.25	A				
MB11S164V0364	P	O	4.62	0.62	0.19	A					A				A					A					
MB11S187V0267	P	O	5.57	7.75	0.19	P	U				P	O	3.53	4.66	0.22	A				P	U				
MB11S189V0332	A					A					A				A					A					
MB11S191V0374	P	O	4.50	-0.26	0.19	P	O	3.82	0.15	0.17	A				A				A						
MB11S222V0297	A					P	O	4.72	7.33	0.17	P	O	3.24	1.50	0.22	A				A					
MB11S245V0390	A					P	O	4.27	3.74	0.17	P	O	3.48	4.10	0.22	P	O	3.80	1.53	0.25	A				
MB11S287V0450	P	O	6.07	11.49	0.19	P	U				P	O	3.44	3.68	0.22	A				A					
MB11S295V0485	P	O	5.28	5.58	0.19	P	O	4.59	6.29	0.17	P	O	3.37	2.91	0.22	P	O	3.30	-4.21	0.25	A				
MB11S296V0486	A					A					P	O	2.15	-18.59	0.22	A				A					
MB11S314V0655	A					A					P	U			P	O	5.02	15.60	0.25	P	O	4.16	16.12	0.26	
MB11S315V0656	P	O	5.61	8.05	0.19	A					A				P	O	3.87	2.34	0.25	P	O	3.15	0.82	0.26	
MB11S320V0662	P	O	5.69	8.63	0.19	A					P	O	3.97	9.41	0.22	P	O	3.88	2.44	0.25	P	O	3.55	-2.18	0.26
MB11S323V0650	P	O	5.68	8.57	0.19	P	O	4.75	7.50	0.17	A				P	O	4.50	4.42	0.25	P	O	3.30	3.09	0.26	
MB11S335V0711	P	O	6.47	14.46	0.19	P	O	5.58	14.14	0.17	A				P	O	4.61	10.87	0.25	P	O	4.04	14.30	0.26	
MB11S352V0747	A					A					A				P	O	4.65	10.92	0.25	A					
MB11S373V0798	P	O	5.72	4.83	0.19	P	O	4.41	1.40	0.17	A				P	O	3.79	1.40	0.25	P	O	3.14	0.62	0.26	
MB11S376V0900	A					A					A				A					A					
MB11S400V0859	A					P	O	5.79	15.60	0.17	A				A					A					
MB11S404V0867	A					P	O	4.49	5.46	0.17	P	O	3.35	2.70	0.22	P	O	3.70	0.38	0.25	A				
MB11S406V0884	A					A					A				A					A					
MB11S418V0906	A					A					A				A					A					
MB11S421V0940	P	O	7.78	24.28	0.19	A					A				A					A					
MB11S493V1069	A					P	O	4.87	8.53	0.17	P	O	3.84	8.03	0.22	P	O	4.24	6.27	0.25	A				
Sum																									8

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 23 continued.

Specimen	71				72				73				74				75								
	P/A	O/U	Measure- ment	Age w SD	P/A	O/U	Measure- ment	Age w SD	P/A	O/U	Measure- ment	Age w SD	P/A	O/U	Measure- ment	Age w SD	P/A	O/U	Measure- ment	Age w SD					
MB11S037V0021	P	O	5.28	5.20 0.19	P	O	4.77	7.28 0.17	A				P	O	4.20	5.72	0.25	A							
MB11S050V0042	P	O	5.29	5.65 0.19	P	O	4.43	5.03 0.17	P	O	3.59	5.32	0.22	A				A							
MB11S058V0092	P	U			P	U									P	U			P	O	6.83	56.68	0.26		
MB11S080V0049	A				A										A				A						
MB11S082V0084	P	O	4.97	3.10 0.19	P	O	4.72	7.10 0.17	P	O	3.57	4.90	0.22	P	O	3.67	0.03	0.25	A						
MB11S090V0107	A				A										A				A						
MB11S091V0110	P	U			P	U									A				P	O	5.10	30.43	0.26		
MB11S099V0139	A				A										A				P	O	4.03	4.19	0.25	A	
MB11S102V0151	P	O	3.86	-5.05 0.19	A										P	O	3.08	-6.77	0.25	P	U				
MB11S122V0161	A				A										A				A						
MB11S130V0173	A				P	O	4.59	6.24 0.17	A						A				P	O	3.28	2.60	0.26		
MB11S133V0299	P	O	5.24	5.28 0.19	A						3.84	7.80	0.22	A					P	U					
MB11S139V0215	P	O	3.62	-6.78 0.19	A										A				P	O	4.95	14.81	0.25	P	U
MB11S142V0259	P	U			P	O	6.90	21.50 0.17	P	U					A				P	O	3.56	-1.19	0.25	P	U
MB11S152V0244	P	O	5.91	10.29 0.19	A										A				P	O	3.55	-1.30	0.25	P	U
MB11S156V0046	A				A										A				P	O					
MB11S164V0364	A				A										A				P	O	2.91	-2.80	0.26		
MB11S187V0267	A				P	O	4.40	4.79 0.17	P	U					A				P	O	3.40	-3.06	0.25	P	U
MB11S189V0332	A				A										A				A						
MB11S191V0374	A				A										A				A						
MB11S227V0297	A				A										P	U			A						
MB11S245V0390	A				P	O	3.93	1.05 0.17	A						A				P	O	4.46	8.84	0.25	A	
MB11S287V0450	A				P	O	4.88	8.61 0.17	P	O	3.63	5.75	0.22	P	U				P	O	3.67	0.03	0.25	P	O
MB11S295V0485	A				P	O	3.27	-4.21 0.17	P	O	2.96	-5.08	0.22	A					P	O	3.62	-0.52	0.25	P	O
MB11S296V0486	A				P	O	5.34	11.96 0.17	P	O	4.61	16.12	0.22	P	O				P	U					
MB11S314V0655	A				P	O	4.03	1.85 0.17	A						A				P	O	4.46	8.84	0.25	A	
MB11S315V0656	P	O	4.63	0.71 0.19	P	O	4.40	4.78 0.17	A						P	O	3.41	-2.91	0.25	P	O	2.94	-2.34	0.26	
MB11S320V0662	P	O	5.53	7.43 0.19	A										A				P	O	3.41	-2.91	0.25	P	O
MB11S323V0650	P	O	5.05	3.85 0.19	P	O	4.77	7.73 0.17	A						A				P	O	4.13	5.20	0.25	P	O
MB11S335V0711	P	O	6.17	12.22 0.19	P	O	5.46	10.66 0.17	P	O	4.44	14.51	0.22	P	O				P	O	3.94	3.15	0.25	P	O
MB11S352V0747	A				A										A				P	O	4.66	20.07	0.25	P	O
MB11S373V0798	P	O	5.18	4.78 0.19	P	O	4.40	4.78 0.17	A						A				P	O	3.41	-2.91	0.25	P	O
MB11S376V0900	A				A										A				P	O	3.41	-2.91	0.25	P	O
MB11S400V0859	P	O	5.58	7.80 0.19	P	O	5.27	11.44 0.17	P	O	3.75	6.76	0.22	P	O				P	O	4.13	5.20	0.25	P	O
MB11S404V0867	P	O	4.80	1.97 0.19	P	O	4.19	3.12 0.17	P	O	3.25	1.61	0.22	P	O				P	O	3.39	-3.17	0.25	P	O
MB11S406V0884	P	O	4.35	-1.38 0.19	A										A				P	O	3.39	-3.17	0.25	P	O
MB11S418V0906	A				P	O	3.69	-0.85 0.17	A						A				P	U					
MB11S421V0940	P	U			A										P	U			P	O	5.91	25.90	0.25	P	O
MB11S493V1069	A				P	O	4.84	8.29 0.17	P	O	3.92	8.90	0.22	P	O				P	O	4.12	5.23	0.25	P	O
Sum				15				18				11					18						12		

P=Present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

Table 23 continued.

Specimen	81					82					83					84					85				
	P/A	O/U	Measurement	Age w	SD	P/A	O/U	Measurement	Age w	SD	P/A	O/U	Measurement	Age w	SD	P/A	O/U	Measurement	Age w	SD	P/A	O/U	Measurement	Age w	SD
MB11S037V0021	P	O	5.40	6.24	0.19	P	O	4.45	4.68	0.17	P	O	3.90	8.32	0.22	P	O	4.00	3.64	0.25	P	O	3.72	9.36	0.26
MB11S050V0042	P	O	5.23	5.20	0.19	A					P	U				P	U				A				
MB11S058V0092	P	U				P	U				P	U				P	U				P	O	6.31	48.77	0.26
MB11S080V0049	A					A					A					A					A				
MB11S082V0084	P	O	4.91	6.40	0.19	P	O	4.66	6.60	0.17	P	O	3.54	4.60	0.22	P	O	3.73	1.00	0.25	A				
MB11S090V0107	A					P	O	3.31	-3.87	0.17	P	U				P	U				A				
MB11S091V0110	A					A					A					P	U				P	U			
MB11S099V0139	A					A					P	O	4.36	13.67	0.22	A					A				
MB11S102V0151	A					P	O	3.38	-3.32	0.17	P	O	2.42	-7.39	0.22	P	O	3.03	-7.34	0.25	P	U			
MB11S122V0161	A					A					A					P	O	4.84	13.52	0.25	A				
MB11S130V0173	A					A					P	O	3.83	7.80	0.22	A					A				
MB11S133V0299	A					P	O	4.76	7.65	0.17	P	O	3.85	8.14	0.22	P	O	3.89	2.57	0.25	P	U			
MB11S139V0215	P	O	4.08	-3.38	0.19	P	O	3.65	-1.14	0.17	P	O	2.34	-8.21	0.22	P	O	3.10	-6.50	0.25	P	O	2.86	-3.53	0.26
MB11S142V0259	A					P	U				P	U				P	U				P	U			
MB11S152V0244	P	O	5.70	8.68	0.19	A					P	O	2.97	-1.40	0.22	P	O	4.59	10.60	0.25	P	O	3.45	12.94	0.26
MB11S156V0046	P	O	4.86	2.39	0.19	P	O	4.14	2.70	0.17	P	O				P	O	3.61	-0.62	0.25	A				
MB11S164V0364	A					A					A					A					A				
MB11S187V0267	P	O	5.00	3.48	0.19	P	O	4.51	5.66	0.17	P	O	3.25	1.62	0.22	P	O	3.80	1.53	0.25	P	U			
MB11S189V0332	A					A					A					A					A				
MB11S191V0374	A					A					A					P	U				A				
MB11S227V0297	A					A					A					A					A				
MB11S245V0390	P	O	4.46	-0.52	0.19	P	O	4.46	5.25	0.17	A					P	O	3.54	-1.56	0.25	P	U			
MB11S287V0450	P	O	5.27	5.50	0.19	P	U				P	O	3.58	5.21	0.22	A					A				
MB11S295V0485	A					A					A					A					A				
MB11S296V0486	P	O	4.37	-1.20	0.19	P	U				A					A					A				
MB11S314V0655	P	O	6.25	12.48	0.19	P	O	5.52	13.52	0.17	P	O	4.69	17.16	0.22	A					P	O	3.99	13.52	0.26
MB11S315V0656	P	O	4.44	-0.70	0.19	A					P	O	3.24	1.51	0.22	P	O	3.61	-0.65	0.25	A				
MB11S320V0662	P	O	5.75	9.10	0.19	P	O	4.83	8.16	0.17	A					P	O	3.69	0.26	0.25	P	O	3.51	6.24	0.26
MB11S323V0650	P	O	5.09	4.15	0.19	P	O	4.80	7.97	0.17	P	O	3.35	2.71	0.22	P	O	3.98	3.61	0.25	P	U			
MB11S335V0711	A					A					P	O	4.41	14.20	0.22	P	O	4.52	9.83	0.25	P	O	4.24	17.37	0.26
MB11S352V0747	A					A					A					A					A				
MB11S373V0798	P	O	5.19	4.88	0.19	P	O	3.92	0.93	0.17	P	O	2.94	-1.71	0.22	P	O	3.42	-2.80	0.25	P	O	2.82	-4.16	0.26
MB11S376V0900	A					P	O	4.14	2.72	0.17	P	O	2.80	-3.26	0.22	P	O	3.29	-4.34	0.25	P	O	2.96	-2.06	0.26
MB11S400V0859	A					A					A					A					A				
MB11S404V0867	A					P	O	4.35	4.36	0.17	P	O	2.79	-3.32	0.22	P	O	3.18	-5.61	0.25	P	U			
MB11S406V0884	P	O	4.35	-1.38	0.19	P	O	3.61	-1.49	0.17	A					A					A				
MB11S418V0906	A					A					A					A					A				
MB11S421V0940	P	O	7.87	24.96	0.19	A					A					P	O	5.91	25.90	0.25	P	O	4.96	28.29	0.26
MB11S493V1069	P	O	5.19	4.90	0.19	P	O	4.78	7.81	0.17	P	O	3.84	8.03	0.22	P	O	4.13	5.34	0.25	P	U			
Sum																									10
																									19

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.



Table 23 continued.

Specimen	Mean age all teeth combined	minimum age	maximum age	intra-individual variability in weeks	Known chronologic al age	mean difference to real age	mean upper jaw	mean lower jaw	mean difference upper/lower jaw	mean incisors	mean canines	mean molars	number of teeth observable P/O	number of teeth unobservable P/U	number of teeth absent A
MB11S037V0021	8.65	3.64	15.60	-11.96	13.04	-4.39	11.79	6.31	5.48	8.91	9.88	7.80	14	2	4
MB11S050V0042	5.92	5.03	8.42	-3.39	2.71	3.21	8.42	5.30	3.12	6.08	5.32		5	5	10
MB11S058V0092	48.11	37.64	58.29	-20.65			45.04	52.73	7.68	58.29		45.57	5	10	5
MB11S080V0049	35.98	-					35.98			35.98			1	0	19
MB11S082V0084	4.38	0.03	7.10	-7.07	2.43	1.95	4.70	4.22	0.48	5.83	4.67	1.18	12	0	8
MB11S090V0107	-2.81	-3.87	-1.73	-2.14			-1.73	-3.87	2.14	-2.80			2	3	15
MB11S091V0110	29.42	27.39	30.43	-3.04			28.91	30.43				29.42	3	9	8
MB11S099V0139	8.93	4.19	13.67	-9.48	7.00	1.93		8.93		13.67		4.19	2	1	17
MB11S102V0151	-4.90	-7.39	-0.70	-6.69			-3.99	-5.78	1.78	-2.63	-7.34	-5.96	10	2	8
MB11S122V0161	22.07	13.52	30.62	-17.10			30.62	13.52	17.10	30.62		13.52	2	0	18
MB11S130V0173	5.10	1.04	7.80	-6.76			4.42	5.55	1.13	7.02	7.80	1.82	5	0	15
MB11S133V0299	7.47	2.57	10.97	-8.40			8.44	6.29	2.15	7.59	9.09	5.11	11	2	7
MB11S139V0215	-5.43	-8.21	-1.14	-7.07			-6.94	-4.92	2.01	-4.29	-8.11	-5.02	8	0	12
MB11S142V0259	21.68	14.81	28.74	-13.93			28.74	18.16	10.59	25.12		14.81	3	14	3
MB11S152V0244	13.00	8.68	22.51	-13.83	11.00	2.00	22.51	10.63	11.88	9.49		15.35	5	0	15
MB11S156V0046	2.40	-1.40	8.47	-9.87			4.62	0.38	4.24	5.02	-0.13	1.29	10	2	8
MB11S164V0364	-1.09	-2.80	0.62	-3.42	0.28	-1.37	0.62	-2.80	3.42	0.62		-2.80	2	0	18
MB11S187V0267	4.19	-1.30	8.50	-9.80			5.77	2.63	3.14	6.05	3.96	0.74	12	6	2
MB11S189V0332	15.02	-					15.02					15.02	1	0	19
MB11S191V0374	-1.30	-5.76	0.65	-6.41			-1.31			0.18	-5.76		4	2	14
MB11S227V0297	5.82	1.50	7.69	-6.19	0.86	4.96	5.82			7.26	1.50		4	3	13
MB11S245V0390	1.58	-3.06	5.25	-8.31			2.93	0.23	2.70	2.65	4.10	-0.39	10	2	8
MB11S287V0450	6.11	0.26	11.49	-11.23			6.00	6.27	0.27	9.12	4.58	0.26	9	6	5
MB11S295V0485	0.21	-5.08	6.29	-11.37	stillborn		2.64	-4.65	7.29	2.55	-1.09	-4.21	6	0	14
MB11S296V0486	-8.73	-18.59	-1.20	-17.39	stillborn		-12.50	-1.20	11.30	-1.20	-18.59	-6.41	3	3	14
MB11S314V0655	14.36	8.84	18.20	-9.36			15.51	13.37	2.14	13.42	16.64	14.39	13	2	5
MB11S315V0656	1.96	-2.36	8.05	-10.41			3.94	0.27	3.66	4.11	1.51	-0.16	13	0	7
MB11S320V0662	6.17	-2.18	14.24	-16.42	stillborn		5.69	6.66	0.98	7.99	10.98	3.23	16	0	4
MB11S323V0650	5.08	2.03	8.57	-6.54			5.69	4.40	1.29	6.81	3.36	3.60	17	1	2
MB11S335V0711	13.49	9.83	20.07	-10.24			12.75	14.14	1.39	12.68	14.35	13.79	15	0	5
MB11S352V0747	16.62	10.92	21.32	-10.40			16.43			18.72	21.32	11.70	5	0	15
MB11S373V0798	1.60	-4.16	5.30	-9.46	1.00	0.78	2.36	0.16	2.20	3.84	-0.60	-0.94	17	0	3
MB11S376V0900	-1.73	-4.34	2.72	-7.06				-1.74		2.72	-3.26	-3.20	4	0	16
MB11S400V0859	8.54	5.20	15.60	-10.40	3.43	5.11	11.18	7.49	3.69	11.61	6.76	6.07	7	0	13
MB11S404V0867	1.47	-5.61	8.11	-13.72			3.45	-0.15	3.60	4.60	1.22	-2.06	13	3	4
MB11S406V0884	-1.50	-3.43	4.32	-7.75	0.43	-1.31	0.23	-1.42	1.64	-0.03		-3.43	6	1	13
MB11S418V0906	0.45	-0.85	1.76	-2.61			1.76	-0.85	2.61	0.46			2	1	17
MB11S421V0940	26.51	24.28	30.73	-6.45			24.89	27.15	2.26	24.62		27.26	7	2	11
MB11S493V1069	7.81	4.90	10.82	-5.92			8.50	7.13	1.36	8.13	8.63	6.95	16	1	3
Sum													300	83	397

P=present, A=Absent, O=Observable, U=Unobservable, SD=Standard Deviation, all ages are provided in weeks.

## *Appendix 4: Comparison of the three ageing methods for each of the 39 remains*

In the following figure 16, skeletal age, dental height and the dental developmental stages are graphically displayed for each of the 39 remains. The graph is split into three segments showing individuals with increasing age. The first shows remains that could only be aged using dental height and skeletal age estimations (range 31 weeks gestation and 3 weeks after birth). The second sections gives the age estimates for individuals aged between 39 weeks gestation and 18 weeks after birth, and the third section covers individuals aged between four weeks and 48 weeks after birth.

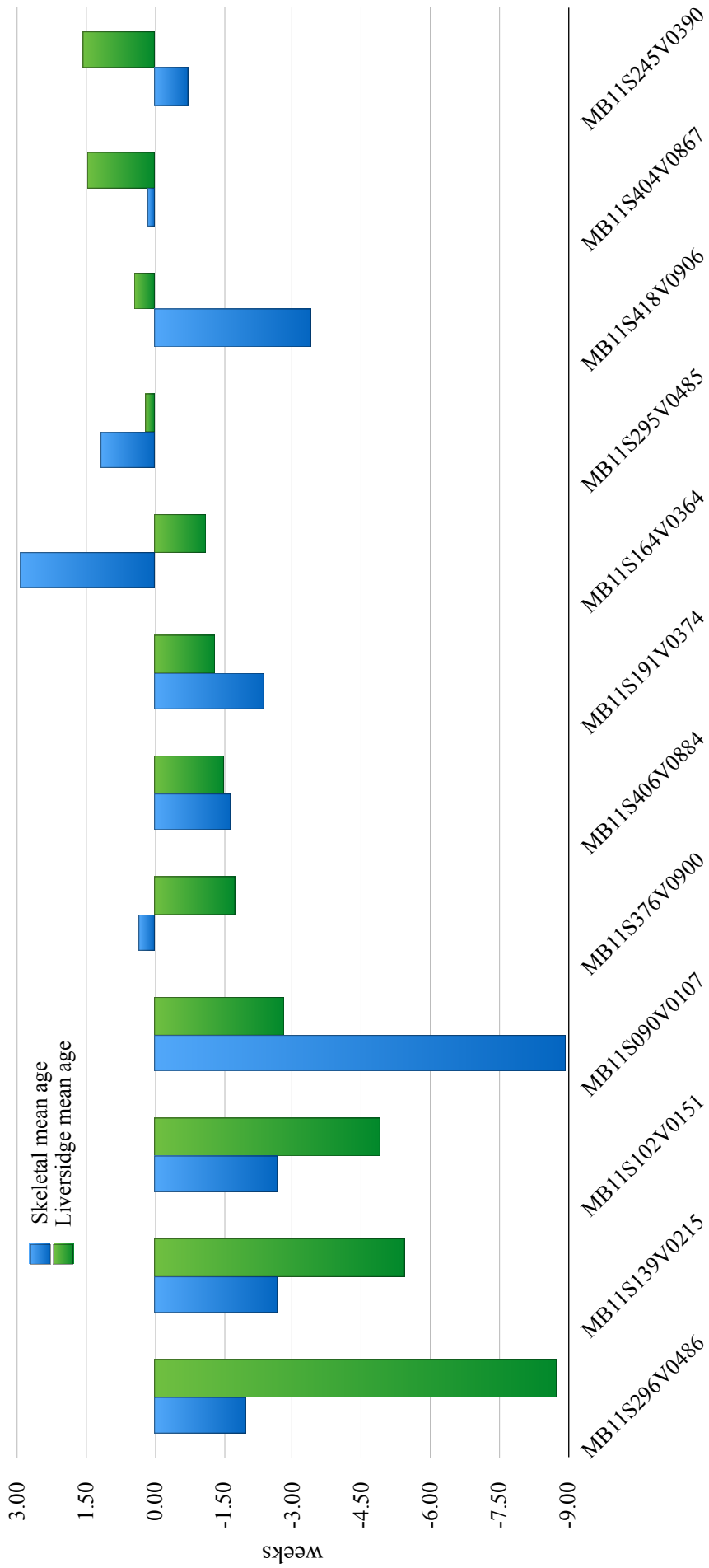


Figure 16. Comparison of age estimates in weeks from skeletal measurements, dental height, and the deciduous Demirjian stages for each of the 39 individuals that are discussed in this thesis.

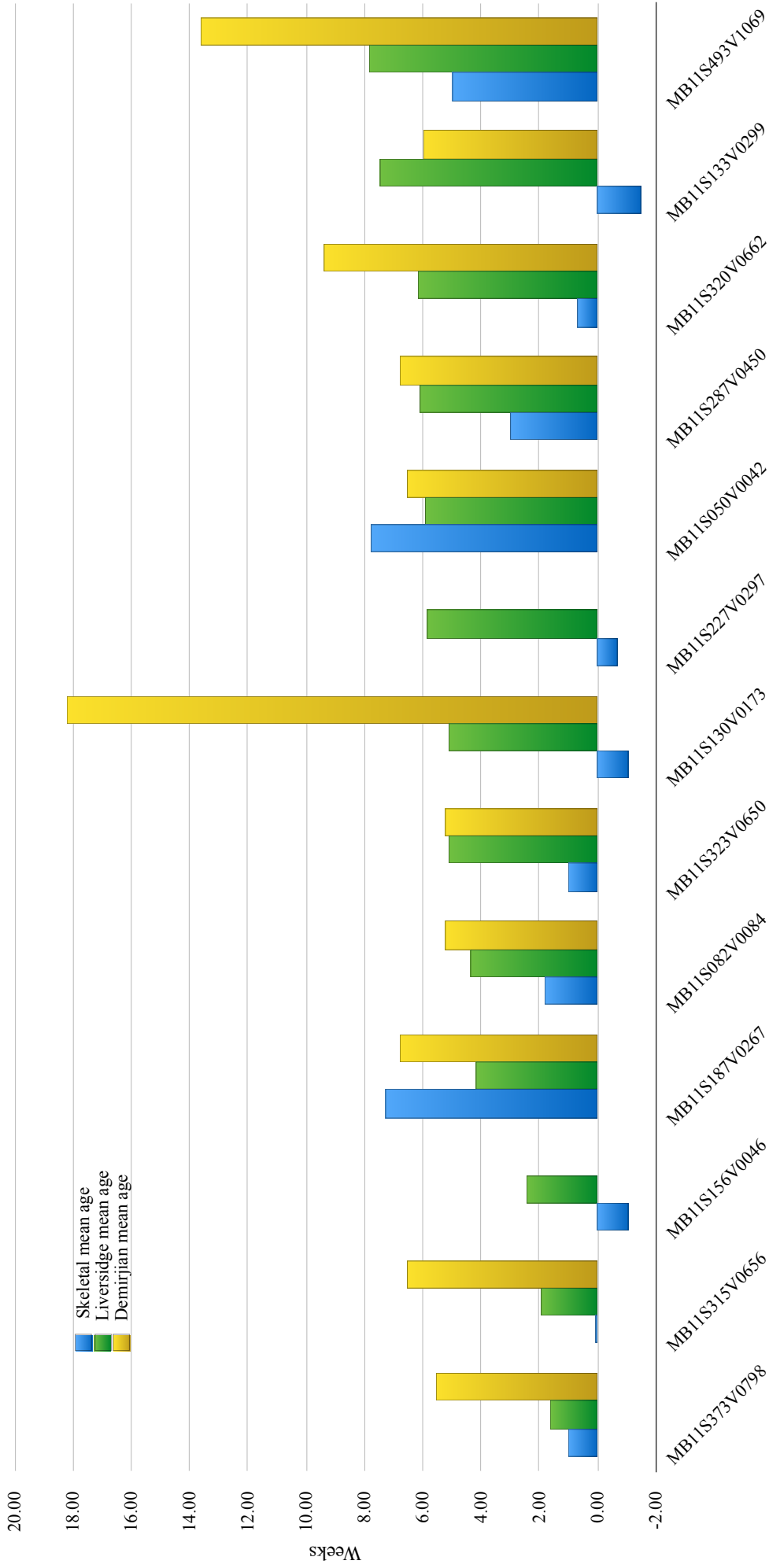


Figure 16 continued.

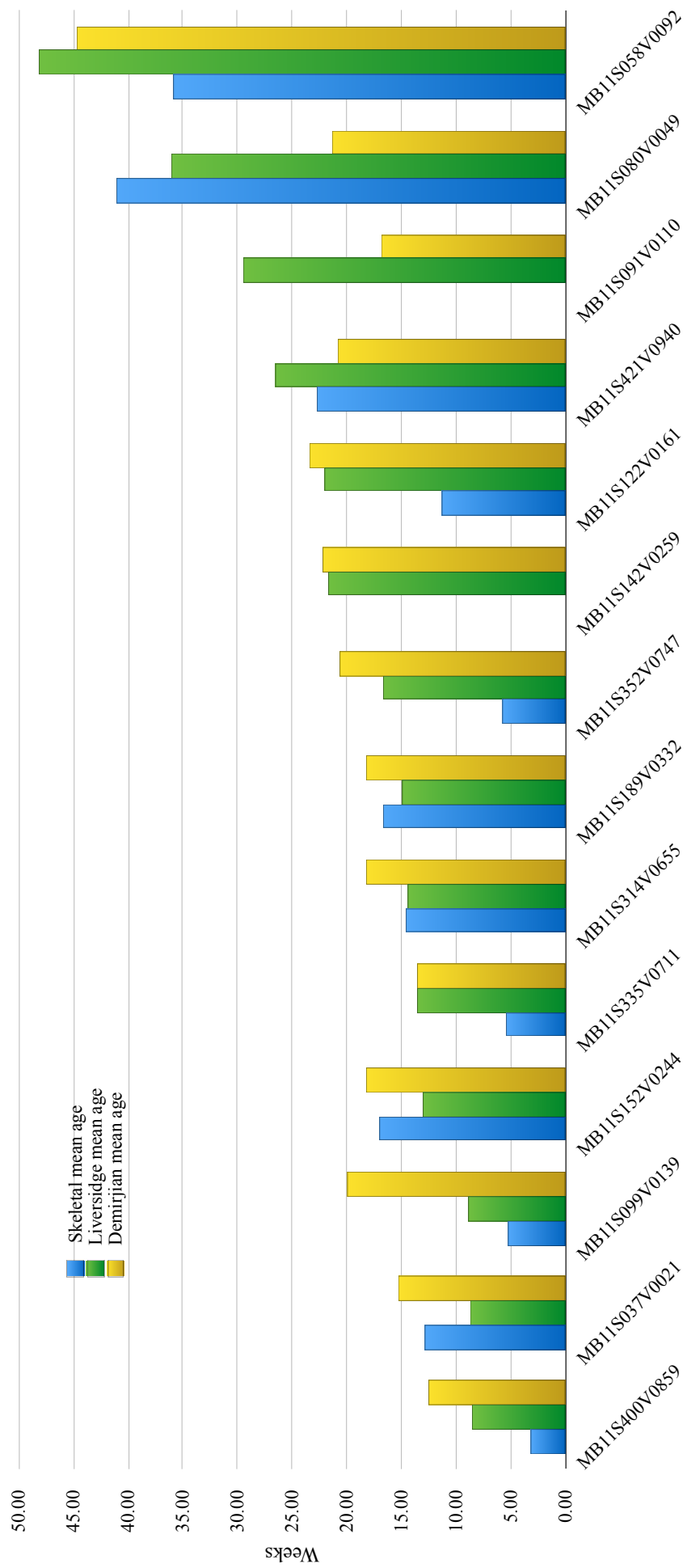


Figure 16 continued.

## *Appendix 5: Understanding the Neonatal line*

To understand the development of the neonatal line (NNL) and its placement among the other histological features of enamel, the internal dental structure will be discussed shortly. It should be added that the material properties of enamel are outstanding for archaeological purposes. It resists degradation for thousands of years and, as it does not remodel, may preserve a record of disruptions during crown formation, visible as macroscopic and microscopic defects. Dental histology is applied to match teeth of the same dentition and to estimate age at death very accurately using incremental markers (Hillson 2005).

### *Enamel histology*

Enamel is laid down in a rhythmic fashion giving it the appearance of layers that have been compared to the formation of tree rings (Massler *et al.* 1941, 33). Enamel is first laid down in dome like layers that grow wider and higher to form the cusps of the teeth (Hillson *et al.* 2005, 209). This part of the crown is called cuspal or appositional enamel. After the ameloblasts reach the final height of the cusp, deposition continues in sleeve-like layers down the sides of the cusps towards the cervical margin (see figure 17 for a schematic drawing of a section of a crown with the various incremental markers). The lateral part of the crown is called incremental enamel.

When the enamel secreting cells (ameloblasts) travel from the enamel-dentine-junction (EDJ) to the crown surface they leave behind bundles of crystallites, called prisms. Prisms are visible with normal light microscopy as thin lines extending from the EDJ to the surface of the crown (figure 18). Dental microscopy, however, reveals several other structures that can be grouped in 1)

regular incremental markers, reflecting periodic differences in ameloblast activity, and 2) irregular incremental markers giving insights into external systemic disruptions during amelogenesis (enamel formation).

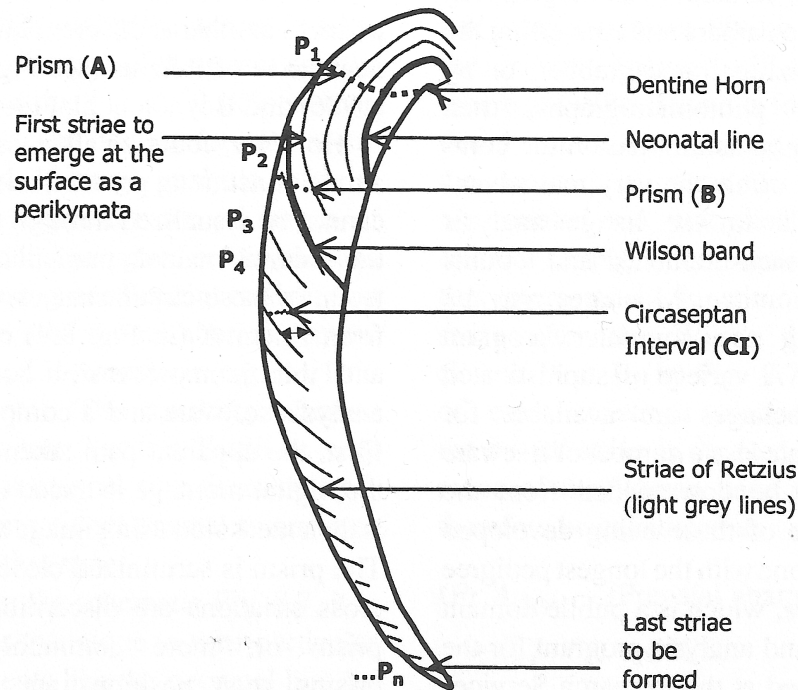


Figure 17. Schematic drawing of a sectioned crown showing different incremental markers (FitzGerald and Rose 2008, 253).

### Regular Incremental Markers

#### *Cross-striations*

With polarised light microscopy, cross-striations (CS) are visible as alternating dark and light bands that run perpendicular to the long axis of the prism (Antoine et al. 2009, 45) (figure 18). These bands correspond to alternating constrictions and enlargements of the enamel prism when viewed under scanning electron microscope (SEM) (FitzGerald and Rose 2008, 242). Cross-striations have been proven to represent a circadian cycle of the ameloblasts metabolic rhythm with one bright and a dark band combined marking 24 hours of secretion (Antoine 2000; Antoine et al. 2009; FitzGerald 1998; Smith 2006). The circadian cycle has been explained through the rhythmic secretion of melatonin. This hormone



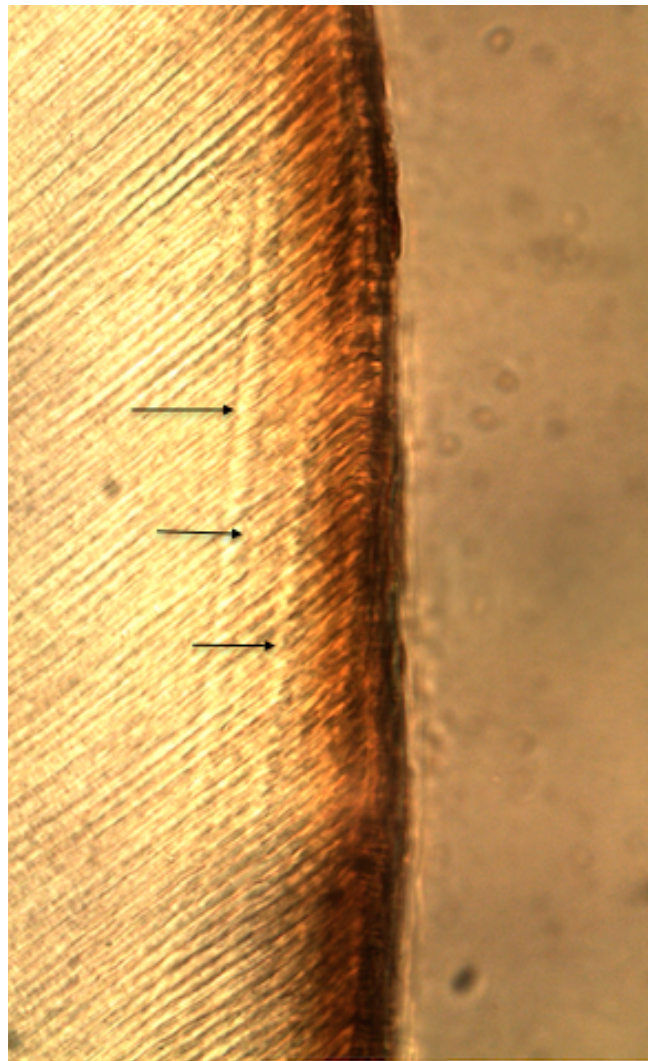
*Figure 18. Microscopic image of a section of the upper right first molar of individual MB11S287V045. The image is taken at 400X magnification. It shows the prisms running almost vertically from the bottom of the image to the top (dotted black line). Other structures are visible as well. Cross-striations are clearly visible in some areas (three closely spaced arrows). A dark band running obliquely to the long axis of the prisms, indicated by three larger arrows, represents either a Brown striae of Retzius or a Wilson Band (see below).*

regulates cell metabolism, creating cyclic changes in mineral content and secretion rate of ameloblasts (Smith 2006). If it were the aim to age the individual using these histological structures one were to count the number of cross striations that were formed from the neonatal line (which represents the moment of birth, and, thus, day zero) until the last formed enamel just prior to death and would arrive at the exact age at death. However, as is seen in figure 18 the features are not always visible and instead of counting, parts of the crown will need to be measured using the average thickness of cross striations (FitzGerald and Rose 2008).



### *Brown striae of Retzius*

Brown striae of Retzius represent a disturbance affecting the entire ameloblast front, that follows a nearly weekly or circaseptan rhythm (FitzGerald 1998; FitzGerald and Rose 2008, 244). Brown striae of Retzius (BSR) appear as dark lines in longitudinally sectioned teeth (Nanci 2008, 181). In appositional enamel they form dome like structures, while in imbricational enamel they are visible as lines running from the EDJ to the crown surface as shown in figure 17 (FitzGerald and Rose 2008, 243). Imbricational lines run obliquely to the EDJ and cut prisms at an acute angle (figure 19). Li and Risnes (2004) found in their study on SEM images of incremental structures, that BSR were associated with several abnormalities in the prism course: 1) the dark band was less mineralised, 2) a



*Figure 19. Microscopic image showing a section of the imbricational enamel of the lower left second molar of individual S058V009275. Three clearly visible BSR are marked with black arrows.*

change in prism direction was occasionally observed, 3) in some cases prisms became narrower, increasing the amount of inter-prismatic matrix, and 4) prism boundaries were found to become blurred. As with CS, varying ratios in mineral content have been proposed for the formation of BSR (Smith 2006) .

The rhythm by which BSR are formed varies between individuals but is the same for the whole dentition of a single individual. The number of daily increments between two adjacent BSR varies from six to eleven (Hillson 2005, 164). The origin of this circaseptan interval is not well understood and several explanations have so far been produced: 1) BSR result from the interference of two or more rhythms that create a new one, 2) BSR have a similar aetiology as cross-striations, based on melatonin secretion which is thought to be responsible for the maintenance of other cycles as well, and 3) BSR are explained as being of chaotic origin (FitzGerald and Rose 2008, 244).

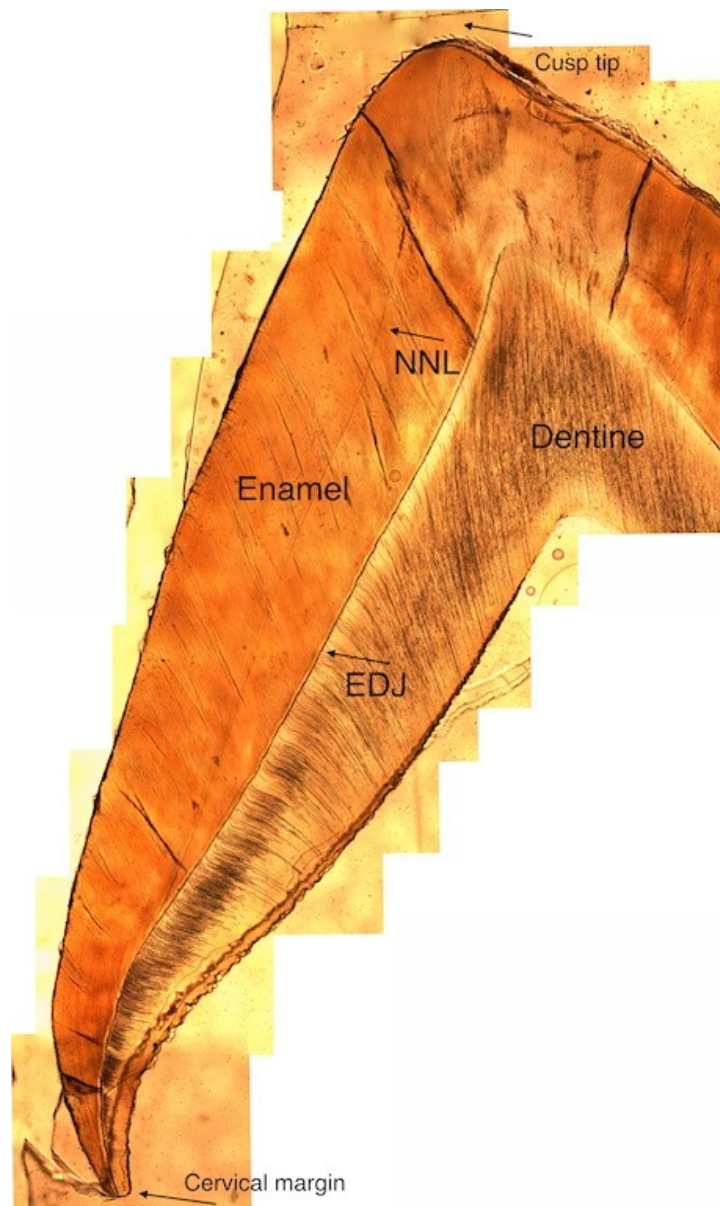
### *Irregular Incremental Markers*

#### *Accentuated striae of Retzius*

Accentuated striae of Retzius or Wilson bands (WB's) are incremental lines that represent a disturbance of normal enamel secretion by an external stressor (Nanci 2008, 182; Witzel et al. 2008, 401). Because the stress signal affects all ameloblasts at the same time, WB's exhibit the same orientation as BSR, but are frequently more broad and accentuated than the latter (Witzel et al. 2008, 401). To facilitate proper separation of both incremental features, WB's have been defined as being visible for at least 75 per cent of their length from the EDJ to the crown surface (FitzGerald and Saunders 2005, 287; Goodman and Rose 1990, 93). WB's may coincide with BSR but may well be found at any point within a circaseptan cycle, providing another means of separating between both features. WB's are produced by a vast variety of stress factors such as disease or chronic undernutrition and are believed to be a measure of individual morbidity during crown development (FitzGerald et al. 2006, 180; Goodman and Rose 1990, 102; Teivens et al. 1996, 176).

### *The neonatal line*

The neonatal line (NNL), which marks the moment of birth, has been characterised as being similar to an accentuated striate of Retzius (Antoine 2000, 43; Antoine et al. 2009, 49; Eli et al. 1989, 220). The neonatal line, therefore, is the only Wilson band, the cause of which is known (figure 20). The NNL represents the physiological and metabolic stress experienced by an individual during parturition (Jakobsen 1975). According to Norén, the metabolic stress is



*Figure 20. The mesiobuccal cusp of a longitudinally sectioned upper right first molar (FDI=54) from individual MB11S287V045, showing the neonatal line. The two dental tissues (Enamel and Dentine) are marked, as well as the the boundary between them known as the Dentine Enamel Junction. (DEJ). To ease orientation the cervical margin and the cusp tip are marked as well.*

caused by hypocalcaemia, a decrease in plasma calcium, occurring within the first 48 hours after birth (1984, 155). Physiological stress, represented by the mode of delivery, however, may influence the thickness of the NNL (Eli *et al.* 1989). A difference in accentuation has been shown for a) normal deliveries, exhibiting a NNL of 11-12  $\mu\text{m}$ , b) complicated/operative deliveries, which show a NNL of up to 17  $\mu\text{m}$  and c) elective/caesarean deliveries, with NNL thickness of only 6-7  $\mu\text{m}$  (Eli *et al.* 1989, 221). The NNL can be distinguished from other striae of Retzius as it divides the prenatal enamel from the postnatal enamel, both having a different structure when viewed under light microscope. The Prenatal enamel is very regular showing CS but no BSR. The postnatal enamel is characterised by good visibility of BSR and cross-striations (Antoine *et al.* 2009, 49).

## *Appendix 6: The dental nomenclature of the Fédération Dentaire Internationale*

The description of the FDI system for designating single teeth of the primary and secondary dentition is taken from Alt and Türp (1998). For a more thorough discussion of this system, the reader is kindly directed to the chapter by these authors.

The two digit system of the Fédération Dentaire Internationale has been proposed in 1970 and is widely used in the field of dentistry and related scientific research. The system uses two digits, the first digit denotes the quadrant. To be able to correctly designate a single tooth in the mouth, the mouth is split into four quadrants, upper right, upper left lower left and lower right. The quadrants are numbered successively from one to four in the permanent dentition and from five to eight in the deciduous dentition. Numbering starts in the upper right quadrant moving counter clockwise to the upper left, then lower left and finally to the lower right quadrant. The second digit specifies the tooth within the quadrant. Each quadrant consist of either eight teeth in the permanent dentition or five teeth for the deciduous dentition. The teeth are numbered successively starting with the central incisors, proceeding posteriorly:

Permanent central incisor.....1	Deciduous central incisor.....1
Permanent lateral incisor.....2	Deciduous lateral incisor.....2
Permanent canine.....3	Deciduous canine.....3
Permanent first premolar.....4	Deciduous first molar.....4
Permanent second premolar.....5	Deciduous second molar.....5
Permanent first molar.....6	
Permanent second molar.....7	
Permanent third molar.....8	

The following squares show the correct numbering of the two dentitions. The first digit denotes the jaw, while the second digit is the number of the particular tooth. Together they form unique code for each tooth of both dentitions.

This thesis is only concerned with the primary dentition but to understand the deciduous numbering the permanent dentition needed to be included as well.

*FDI codes for the permanent dentition*

Upper jaw right	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	Upper jaw left
Lower jaw right	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	Lower jaw left

*FDI codes for the deciduous dentition*

Upper jaw right	55	54	53	52	51	61	62	63	64	65	Upper jaw left
Lower jaw right	85	84	83	82	81	71	72	73	74	75	Lower jaw left