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Linear and appositional growth in infants and children from the prehistoric settlement of Ban Non Wat, Northeast Thailand: Evaluating biological responses to agricultural intensification in Southeast Asia

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ABSTRACT

The bioarchaeological model of health change predicts a deterioration in population health with the adoption and intensification of agriculture. However, research in mainland Southeast Asia challenges this model, showing no clear pattern of health deterioration associated with the intensification of rice agriculture. Childhood growth, a sensitive indicator of general population stress, is used in this paper to test the applicability of the bioarchaeological model at the prehistoric site of Ban Non Wat in Northeast Thailand. Agricultural intensification at Ban Non Wat is most apparent in the Iron Age rather than the earlier periods. Linear and appositional growth patterns of infants and children (n = 95) at Ban Non Wat were compared among the Neolithic, Bronze and Iron Age periods (1750 BCE–430 CE) to assess differences in growth patterns associated with agricultural intensification over time. Comparative analysis of linear growth found no evidence for differences among the chronological phases at the site. A detailed assessment of appositional growth from the larger Bronze Age sample showed no evidence for extreme nutritional stress. These findings are consistent with other bioarchaeology health research in prehistoric Southeast Asia. A gradual transition to intensified agriculture over time and retention of a broad-spectrum based diet at Ban Non Wat may have provided a buffer from the biological stress exhibited in other parts of the world during agricultural intensification.

1. Introduction

The adoption and intensification of agriculture has been traditionally viewed as an important landmark in the history of human civilization, resulting in social, economic, human biological and cultural transformations (Childe, 1936; Pinhasi and Stock, 2011). Bioarchaeologists reconstructing health among prehistoric agricultural populations in America and Europe found that with an increased reliance on agriculture, there was a corresponding deterioration in health. These studies, collectively interpreted in the form of a generalized model, posit that changes in subsistence strategies with an increased focus on agricultural activities led to significant alterations in the physical and cultural environments that negatively affected the health of farming populations (Cohen and Armelagos, 1984a; Cohen and Crane-Kramer, 2007). According to this model, although agricultural intensification facilitated producing and controlling food surpluses, it caused a shift in the dietary focus of populations from a broad range of resources exploited by earlier hunting foraging populations, towards a more restricted diet of less nutritious food crops (Cohen and Armelagos, 1984b). Studies have shown that the subsistence shift to intensified agriculture in some parts of the Old and New Worlds led to an increase in physiological stress (Larsen, 2006). The adoption of agriculture in some regions of the world also resulted in changes in social structure as food surplus allowed increasing social complexity, the expansion of trade networks and technological advancement (Pinhasi and Stock, 2011). Agrarian populations also had to adapt to an increase in pathogen loads with sedentism, increasing population density and proximity to domestic animals (Armelagos et al., 1991; Cohen and Armelagos, 1984b; Pearce-Duvet, 2006). These changes resulted in health deterioration as communities adapted to the changing biosocial environment.

Although many bioarchaeological studies have reported this change in health deterioration associated with increasing reliance on agriculture, some studies have showed no clear changes in health associated with the adoption and intensification of agriculture, questioning its universal applicability (Bridges, 1989; Hodges, 1987; Pietrusewsky and Douglas, 2001; Tayles and Oxenham, 2006). In particular, samples from Mainland Southeast Asia (MSEA) do not show evidence for deterioration in health with an increasing reliance on agriculture (Domett and Tayles, 2007; Douglas and Pietrusewsky, 2007; Halcrow et al., 2013;

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Tayles et al., 2000). There is recent research reporting some evidence for a rise in infectious diseases in the Iron Age, consequent with the time of increased population density, trade networks and contacts with neighboring regions (Halcrow et al., 2016; Oxenham et al., 2005; Tayles and Buckley, 2004; Tayles et al., 2007). What is apparent from earlier research in MSEA is that the intensification of agriculture and its consequent health effects is different from other regions. This difference may be partly explained by the particular gradual transition in subsistence strategies in the context of high tropical biodiversity of MSEA, which allowed for the retention of a broad subsistence spectrum of resources naturally available in the region (Domett and Tayles, 2006; Douglas and Pietrusewsky, 2007; Pietrusewsky and Douglas, 2001).

The understanding of health change in Southeast Asian prehistoric populations has thus far been largely based on a diachronic assessment using multiple sites that range temporally from the Neolithic to the Iron Age from different geographic locations (Domett and Tayles, 2007; Douglas, 1996; Douglas and Pietrusewsky, 2007; Halcrow, 2006; Halcrow et al., 2013; Halcrow and Tayles, 2008b; Halcrow et al., 2016; Oxenham et al., 2006; Oxenham et al., 2005). Until recently, there has not been a site that spans the period of agricultural intensification in the region, preventing scholars from making temporal comparisons of population health with a continuous population history over time and at one location. Ban Non Wat situated in the Upper Mun River Valley (UMRV) catchment in Northeast Thailand (Fig. 1), provides a unique opportunity to examine prehistoric health during the period when agriculture intensified. Ban Non Wat, with a large skeletal sample of around 690 burials (Newton, 2014; Tayles and Halcrow, 2016) spanning from ca. 1750 BCE to 430 CE, from incipient rice farming to a developed wet-rice agricultural economy and a technologically advanced society (Higham, 2014; Higham, 2015; O'Reilly, 2014), is an ideal sample for investigating any temporal changes in physiological stress to increasing reliance on agriculture. The isotopic evidence from this site indicates there was little long distance immigration (Cox et al., 2011; King et al., 2015), suggesting the genetic composition of the sample is homogenous, which provides a particularly strong platform for addressing the question of health change over time.

Childhood growth is a sensitive indicator of the overall health of a population and is helpful in assessing health in modern and past populations (Eveleth and Tanner, 1990; Goodman and Armelagos, 1989; Halcrow and Tayles, 2008a). As such, this paper assesses the growth of infants (individuals between 0 and 0.9 years of age) and children (1 to younger than 12 years of age) as a proxy to evaluate physiological stress at Ban Non Wat. The analysis aims to test the applicability of the bioarchaeological model of health change by examining whether there is evidence for growth retardation in the infants and children as agriculture intensified over time at the site. In most of the previous bioarchaeological studies assessing prehistoric health in Southeast Asia, the direct assessment of childhood growth was not possible owing to limited skeletal sample size of infants and children at the sites (Halcrow, 2006). However, with the recovery of a large skeletal

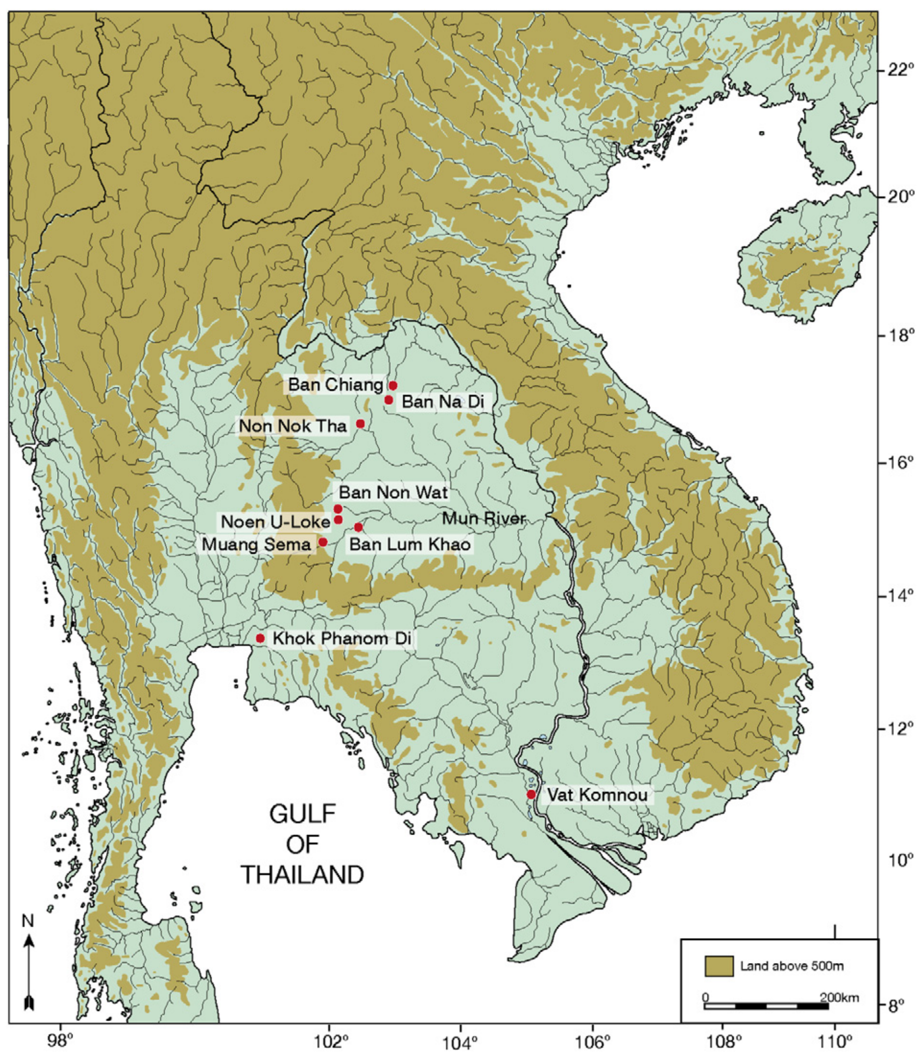

Fig. 1. Location of Ban Non Wat, situated in the upper Mun River valley, northeast Thailand (and other sites mentioned in this paper).

sample of infants and children from Ban Non Wat (n = 216), it is now possible to specifically assess childhood growth from a single site spanning the period of changes in the environment associated with changing subsistence strategies.

In light of previous bioarchaeological research from Southeast Asia (Domett and Tayles, 2007; Douglas and Pietrusewsky, 2007; Oxenham and Tayles, 2006a; Tayles et al., 2009), it is hypothesized that there will be no significant variation in the linear and appositional growth of infants and children indicative of growth retardation from the Neolithic Age to the Iron Age.

1.1. Environmental and socio-cultural changes over time in prehistoric Northeast Thailand

The environment in the UMRV was relatively wet during the Early Holocene period compared with today (Boyd and Chang, 2010; Kealhofer and Penny, 1998). Palaeoenvironmental research has shown that the region was a lacustrine environment with high biodiversity during this period. Thereafter, the region experienced increasing dryness from around 1500 BCE, which gradually led to the present day environmental conditions in the region (Boyd and Chang, 2010). Palaeohydrological studies also show a corresponding increase in moats and water management activities around the settlements in the early Iron Age, which probably facilitated water retention for the rice fields during dry periods (Boyd, 2008; Boyd and Chang, 2010; O'Reilly, 2014; Scott and O'Reilly, 2015).

Although rice was present from the early Neolithic levels at Ban Non Wat, the evidence for wet-rice agricultural intensification is not found until the Iron Age in the region (Higham, 2014). This intensification is supported by the increasing number of iron implements such as ploughshares and other implements found at other Iron Age sites in the upper Mun river catchment such as Noen-U-Loke and Non Ban Jak (Higham, 2014). It has also been argued that the ritual importance of rice grew in the Iron Age as expressed in the burials filled with rice at Noen-U-Loke (Boyd and Chang, 2010; Higham, 2014).

Archaeological evidence suggests increasing social complexity over time at Ban Non Wat (Higham, 2011). During the Bronze Age 2 phase (~1000–850 BCE) there is possible evidence for a change in social structure as indicated by the presence of elaborate burials and mortuary wealth for a short period of time. This was interpreted as the rise of social aggrandizers, who may have exercised certain control over the society during this period (Higham, 2011; O'Reilly, 2014). This expression of social hierarchy was absent in the subsequent Bronze Age phases and at other Bronze sites in Southeast Asia, where a more hetararchical or flexible ranking system social order is evident (Boyd and Chang, 2010; O'Reilly, 2000; White, 1995). Fundamental social changes occurred in the Iron Age, during which time a more complex system of social hierarchy developed in the region (Higham, 2014). During later phases of the Iron Age, there is a dramatic increase in metal weaponry such as spearheads, arrowheads and projectile points which may be a response to interpersonal violence with neighboring populations (Higham, 2014). Technological advancement can be seen through the skillfully cast bronze and iron tools which were, as noted, useful in tilling the land during this period of agricultural intensification (Higham, 2014).

1.2. Assessing physiological stress and childhood growth during subsistence transitions in archaeological contexts

Human growth is known for its plasticity and is influenced by environmental and genetic factors (Bogin, 1999). In particular, nutrition and disease play an important role, with poor nutrition and/or infection having major negative impacts on growth (Goodman and Armelagos, 1989; Larsen, 1997; Scrimshaw et al., 1968). Studies of modern populations demonstrate growth retardation, stunting and low BMI in response to poor nutrition and disease (Bogin, 1999; Bogin et al., 1989; Eveleth and Tanner, 1990; Frisancho, 1978; Martorell, 1995).

Research on the growth of infants and children in the past has been instrumental in testing the bioarchaeological theory of health change associated with subsistence transition (Larsen, 2015). For example, studies in prehistoric American populations found evidence for growth faltering in the form of smaller-for-age diaphyseal lengths in the maize agriculturalists compared with the earlier populations with foraging and mixed subsistence economies (Cook, 1979; Cook and Buikstra, 1979; Goodman et al., 1984; Lallo, 1973). In addition to linear growth, appositional bone growth (contributing to increasing width of bones) is a complementary source for assessing stress (Huss-Ashmore et al., 1982; Keith, 1981; Larsen, 2015) as cortical bone growth has been shown to display a greater sensitivity to changes in the environment compared with diaphyseal length (Himes et al., 1975; Hummert, 1983; Huss-Ashmore et al., 1982; Mays, 1999; Mays et al., 2009; Ruff et al., 2013). Research has shown that cortical bone tissue is under both physiological and mechanical influences (Carter et al., 1996; Ruff and Hayes, 1983; Ruff et al., 2006). However, the exact extent of the relationship between physiological and mechanical influences on the bone is still not clear (Gosman et al., 2013; Eleazer and Jankauskas, 2016). Nevertheless, it is accepted that the assessment of cortical bone parameters are useful for assessing physiological stress response (Mays et al., 2009; Newman and Gowland, in press; Temple, 2014).

1.3. Previous childhood growth studies in prehistoric Southeast Asia

Although there has been a significant increase in the amount of bioarchaeological research focusing on infants and children in Southeast Asia (Clark et al., 2014; Domett, 2001; Douglas, 1996; Halcrow, 2006; Halcrow et al., 2014; Halcrow et al., 2013; Halcrow and Tayles, 2008b; Halcrow et al., 2007; Halcrow et al., 2016; Halcrow et al., 2008; Ikehara-Quebral, 2010; Oxenham et al., 2008), growth research has been limited by small sample sizes (Halcrow, 2006). Therefore, the majority of these studies include growth as a minor component of the broader research evaluating overall population stress.

Douglas (1996) included subadult growth analysis as a part of the health assessment at Ban Chiang and Non Nok Tha, spanning from the pre-metal to the Bronze and Iron Age periods in Northeast Thailand. The long bone length comparisons between pre-metal and subsequent periods at both sites indicated no significant changes in stress experience over time. Ikehara-Quebral (2010) assessed the overall health in the early historic sample from Vat Komnou (Angkor Borei, Cambodia) including linear growth based on a small sample of femora (n = 4). Statistical analyses was not possible for such a small sample but the femoral lengths of the Vat Komnou individuals were similar in lengths by age to those from Northeast metal age sites of Ban Chiang and Non Nok Tha (Ikehara-Quebral, 2010). Halcrow (2006) analyzed the growth patterns of children as part of an overall health and disease evaluation of infants and children from late prehistoric Thailand. With a larger number of skeletal samples pooled from seven Thai archaeological sites spanning the Neolithic and the Iron Age (4000 BCE–1500 CE) (N = 325), the comparative analysis found no variation in growth over time between these sites. Inter-population comparison of growth patterns between the combined sample from the seven Thai sites and the medieval British Wharram Percy sample revealed a lag in the linear growth of the Thai sample suggesting slower linear growth (Halcrow, 2006). On the other hand, Halcrow (2006) found that a comparison of appositional growth showed higher cortical indices in the Thai children compared with Wharram Percy. This overall comparison indicated that the Thai individuals were potentially healthier compared to Wharram Percy based on the cortical indices values.

2. Materials

The site of Ban Non Wat was excavated over successive field seasons from 2002 to 2011 (Higham and Kijngam, 2012). Bayesian analysis of radiocarbon dates shows the earliest occupation at the site in the

Table 1
Number of infants and children at Ban Non Wat.

Period	Dates	Number of infants and children	Number of children with complete long bone diaphyses included in the study
Neolithic	~1750–1050 BCE	31	8
Bronze Age	~1050–420 BCE	123	71
Iron Age	~420 BCE–430 CE	62	16
Total		216	95

Neolithic period (ca. 1750 to 1050 BCE) (Higham and Higham, 2009). The subsequent Bronze Age spanned ca. 1050 BCE to 420 BCE, followed by the Iron Age, dating from ca. 420 BCE to 430 CE (Higham and Higham, 2009; Higham and Kijngam, 2013).

Of the 216 individuals identified as infants and children, skeletal growth assessment was carried out on 95 individuals with complete long bone diaphyses. The distribution of infants and children among the three archaeological periods is listed in Table 1. The number of individuals with complete diaphyses used for growth comparison is shown in Table 2. For assessing appositional growth, all 47 complete femoral diaphyses were radiographed (Table 2). The assessment of appositional growth (cortical index and residual analysis) was restricted to the Bronze Age sample as only a small sample of femora were available for radiography in the Neolithic ($n = 5$) and Iron Age samples ($n = 4$).

3. Methods

Age estimation of individuals was based on the eruption and development of the deciduous and permanent dentitions following three methods: absolute tooth lengths (Liversidge et al., 1993; Liversidge and Molleson, 1999); dental formation (Moorrees et al., 1963a; Moorrees et al., 1963b), as modified by Smith (1991) and Lewis (2002); and dental eruption (Ubelaker, 1989). Age estimation using tooth lengths was preferred, as it is not based on the subjective categorization of formation stages for each tooth (Cardoso, 2007). Tooth length was measured as the distance from the cusp-tip to the developing edge of the crown or root margin, following the recommendations of Liversidge and Molleson (1999). Dental formation and eruption methods of ageing were applied only when information on tooth lengths was not available. To maximize the sample available for aging the individuals, digital radiographs of teeth in the alveoli were also taken. The Genoray PORT X II portable radiography machine was used for dental radiography. DIGORA software was used for processing the images and measuring tooth lengths. Digital radiographs of the femora were taken using Kellex radiography equipment and Carestream dental imaging system.

3.1. Linear and appositional growth assessment

Diaphyseal lengths and femoral cortical measurements are plotted against dental age to examine any alterations in growth patterns of the infants and children among the time periods. All complete diaphyses were measured, with preference given to the left side. Maximum diaphyseal lengths were measured using Mitutoyo calipers (to the accuracy of 0.01 mm). For bones longer than 150 mm an osteometric board was used. The measurements used followed standard methods (Buikstra and Ubelaker, 1994).

Cortical bone growth comparative analyses were carried out for the Neolithic, Bronze and Iron Age samples at the femoral midshafts. Femoral cortical measurements were chosen as the cylindrical shape of femoral midshaft allows appropriate measurement of the subperiosteal and medullary area (Garn, 1970). The total subperiosteal (T) and medullary width (M) of the bones were measured at the midshaft from radiographs (Fig. 2). *Image J* software was used for measuring the dimensions from the digital radiographs.

As mentioned earlier, cortical bone growth analyses was restricted to the Bronze Age sample in the absence of sufficient data in the Neolithic and Iron Age sample. This allowed the assessment of the interaction between linear and appositional growth trends in infants and children (0–12 years) from the Bronze Age sample only. Cortical growth was calculated measuring the cortical thickness (C) and cortical indices (CI). Cortical thickness (C) is calculated as $(T - M)$. Cortical indices standardize the cortical volume for bone size to evaluate changes in the appositional growth with increasing age. CI was calculated as $(T - M / T) * 100$.

3.2. Statistical analysis

All statistical analyses were carried out using Stata 13 (StataCorp, 2013) for Mac.

3.2.1. Intra-observer error

To test the reliability of metric observations, a random sample of bones ($n = 15$) and teeth ($n = 15$) were re-measured by ND. Agreement between the original and repeated measurements was tested using Intra-class Correlation Coefficients (ICCs). Values of 0.92 or higher were considered to represent sufficient intra-rater reliability following Knapp (1992); Ulijaszek and Kerr (1999). These analyses revealed ICCs of 0.99 between the original and repeated measurements of both the long bones and teeth (see Supporting information, Table 1).

Finally, to examine the validity of radiographic measurements, paired *t*-tests were used to investigate evidence for potential biases (the lack of which would not necessarily demonstrate an appropriate level agreement at the sample level) between actual and radiographic measurements of tooth lengths and femoral lengths. The results of the paired *t*-test are described in the Supporting information, Table 2.

Table 2
The number of individuals with complete long bones available for growth comparison between the three archaeological periods at Ban Non Wat.

Period	No. of individuals with complete long bones included in linear growth assessment						No. of individuals included in appositional growth assessment
	Humerus	Radius	Ulna	Femur	Tibia	Fibula	Femur
Neolithic	0	4	4	6	5	4	5
Bronze Age	41	28	29	47	31	36	38
Iron Age	7	5	6	7	7	9	4
Total	48	37	39	60	43	49	47

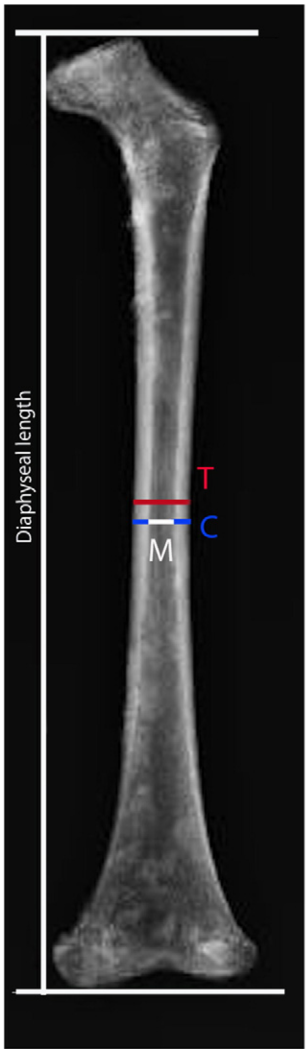

Fig. 2. Femur radiograph showing 'T' = total subperiosteal width of the midshaft and 'M' = medullary width and 'C' cortical thickness.

As these differences were not statistically significant, suggesting that there was no bias between the two approaches, radiographic measurements were included in cases where actual measurements of teeth were unavailable.

3.2.2. Linear growth comparison

Linear regression models were used to describe the growth pattern by age for each period. Where sufficient numbers of samples were available, the addition of a quadratic age term was formally investigated, this would allow modeling plausible non-linearity in growth patterns. Where sufficient data was not available to evaluate adding a quadratic term, visual inspection of scatter plots showing the data overlaid with lines of best fit were used to determine if a linear regression model was able to adequately fit data from each of the three archaeological periods. Two-sided $p < 0.05$ was considered statistically significant in all cases. The formal evaluation of adding a quadratic term to understand the relation between diaphyseal lengths and age was possible only for the Bronze Age sample, where it was statistically significant (femora $p = 0.004$; humeri $p = 0.001$; radii $p = 0.012$; fibulae $p = 0.002$). Over the reduced age ranges in the Neolithic and Iron Age, possibly due to smaller sample sizes, a quadratic term did not appear to be needed and so the linear term was accepted as sufficient. Growth comparisons between periods were based on interactions between the period

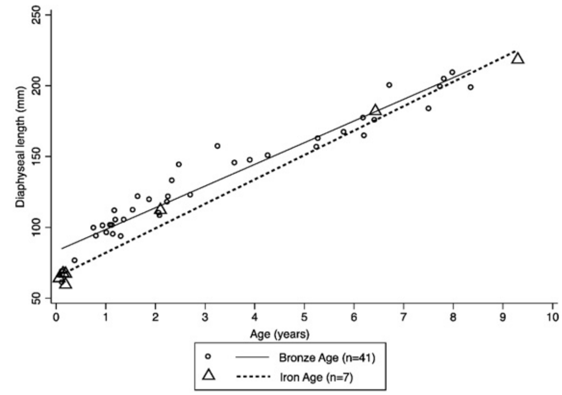

Fig. 3.1. Humeral linear growth comparisons between Bronze and Iron Age samples. No humeral lengths were available to study growth comparison between Neolithic and Bronze Age samples.

and the linear growth slope, which addressed the question of whether the slope for age differed between two periods. A statistically significant interaction ($p < 0.05$) would indicate that there was evidence that linear growth differed between the periods. Although diaphyseal linear growth is not linearly associated with increasing age (Bogin, 1999; Cameron, 2012; Pinhasi, 2008; Ulijaszek et al., 1998), a linear regression model may be adequate over some age ranges (0–10 years), and such a comparison would not be biased as long as the periods covered the same age range.

3.2.3. Appositional growth comparison

Femoral cortical bone growth for all three archaeological groups was also assessed using a linear regression model for the age group of 0–8 years (the age comparison was restricted due to the comparable age range among the periods). In addition to cortical thickness, total bone width and medullary bone width have also been compared among the three archaeological periods for the age group of 0–8 years. Cortical indices were compared for all three archaeological groups using a scatterplot and fitting a LOWESS (locally weighted scatterplot smoothing) line.

Further assessment of the Bronze Age sample was carried out to explore the relationship between femoral cortical indices and diaphyseal linear growth. A cubic regression model provided good fit to explain the relationships between age and femoral length ($R^2 = 0.97$), and a reasonable fit between age and cortical index ($R^2 = 0.46$). The standardized residuals of femoral lengths and cortical indices, which measured the departures of actual measurements from expected measurements, were compared with each other to examine the association between diaphyseal linear growth and appositional growth of individuals from the Bronze Age.

4. Results

4.1. Linear growth comparison

The unequal age distribution in the three periods made it necessary to conduct pairwise comparisons of growth between the Neolithic and Bronze Age samples and between the Bronze and Iron Age samples, rather than a single model comparing growth over all three periods (see Supporting information, Fig. 1.a–f). The Neolithic and Iron Age samples have not been directly compared, as further age restrictions for each bone would be necessary due to the uneven distribution of samples. The age group for comparative analysis of the Neolithic and Bronze Age linear growth differed for each long bone (radius, ulna and tibia (0–4 years), femur (0–8 years) and fibula (0–10 years)). The

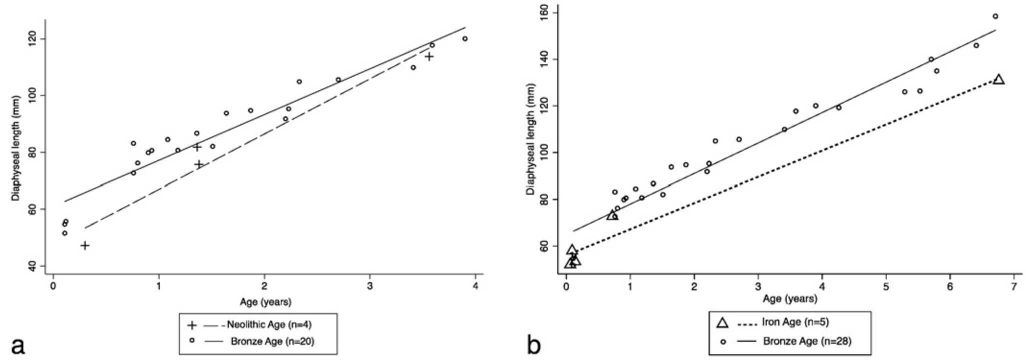

Fig. 3.2. a, b. Radial linear growth comparisons of Neolithic, Bronze Age and Iron Age samples.

humeral growth was not included in comparing the Neolithic and Bronze Age samples, as no humeral diaphyses were available in the Neolithic sample. The age group for growth comparison between the Bronze Age and Iron Age samples also differed for each long bone (humerus (0–10 years), radius, ulna, tibia and fibula (0–7 years) and femur (0–8 years)) (Figs. 3.1–3.6). Limited sample sizes in the Neolithic and Iron Age groups made it difficult to understand the nature of growth patterns older than 8 years of age in the case of most long bones.

The results show that there was no statistically significant evidence for variation in linear growth profiles from the Neolithic and Bronze Age, and from the Bronze Age to Iron Age periods (Table 3, all $p \geq 0.135$). Although not statistically significant for any particular long bone, a visual comparison of slopes does show a consistent pattern of the Bronze Age slopes indicating increased growth rate compared with the Neolithic and Iron Age slopes. Slopes and 95% confidence intervals for the Neolithic, Bronze Age and Iron Age samples are shown in Table 3. The possibility of children in the Bronze Age having meaningfully longer diaphyses compared to those in the other two time periods cannot be ruled out based on these results. However, the small sample sizes in the Neolithic and Iron Age groups make it difficult to precisely estimate any differences.

4.2. Appositional growth

As mentioned, appositional growth has been shown to have greater sensitivity to environmental stressors, compared with linear growth. The cortical thickness of individuals from all chronological periods was assessed over time. A linear regression line best explained the relationship of cortical growth with age (Fig. 4). Pairwise comparisons showed no significant differences in the cortical thickness (Neolithic to Bronze Age, $p = 0.327$; Bronze to Iron Age, $p = 0.138$).

In addition to cortical thickness, it is necessary to look at changes in the total bone width (T) and medullary width (M), to assess total bone width and medullary width individually (Garn, 1970; Ruff et al., 2013). Fig. 5 shows the total (T) and medullary (M) widths in the Neolithic, Bronze and Iron Age samples. Due to small samples sizes in the Neolithic and Iron Age, a linear regression model was used to compare T and M among the three periods. The total width (T) shows a normal growth process, which is more or less linearly associated with increasing age. Pairwise comparisons showed no statistically significant differences in the T over time (Neolithic to Bronze Age, $p = 0.255$; Bronze to Iron Age, $p = 0.232$). M shows a decrease in the resorption at the endosteal surface with increasing age. Pairwise comparisons show no statistically significant differences in M growth over time (Neolithic to Bronze Age, $p = 0.326$; Bronze to Iron Age, $p = 0.294$). This pattern of appositional bone growth is expected in normal growing children (Garn, 1970).

The data for cortical indices (CI) in the samples was plotted fitting a LOWESS regression line (bandwidth = 0.95) to explore the association with age (Fig. 6). Initially, there is a clear decline in the CI from 0 to around 1.5 years of age. There is considerable variation in the cortical index values around the age of 2 years. Some individuals show a lower CI below 45%, while two individuals show a CI as high as 60%. Beyond the age of 4 years, cortical index values show a gradual increase.

Given that earlier studies have recorded greater sensitivity of cortical bone growth compared with linear growth to environmental stress (Himes, 1978; Himes et al., 1975; Himes et al., 1976; Huss-Ashmore et al., 1982; Mays, 1999), the relationship between linear and appositional growth of infants and children in the Bronze Age sample was explored

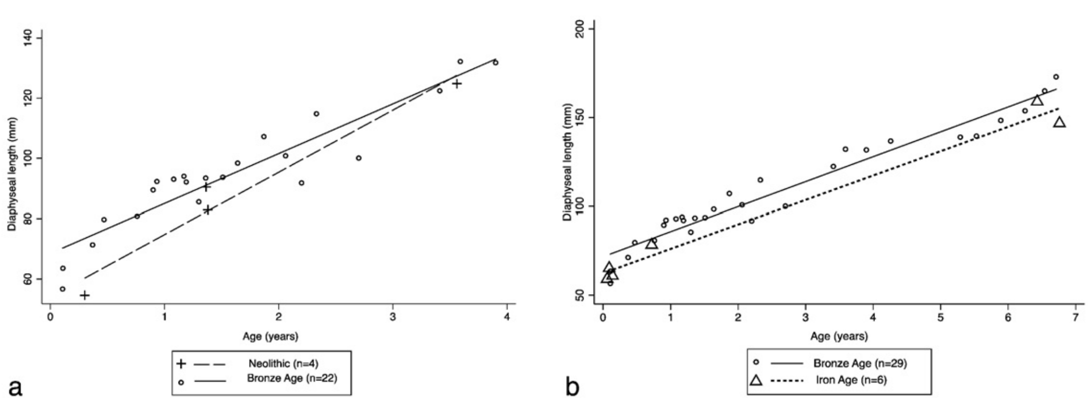

Fig. 3.3. a, b. Ulnar linear growth comparisons of Neolithic, Bronze Age and Iron Age samples.

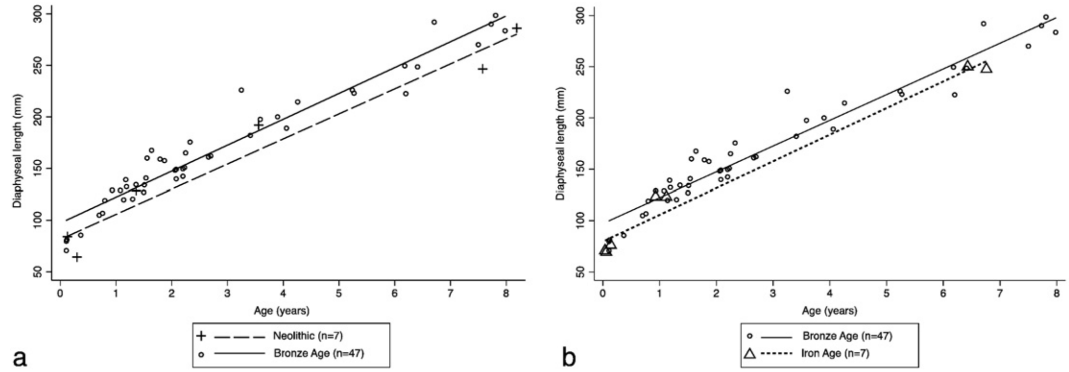

Fig. 3.4. a, b. Femoral linear growth comparisons of Neolithic, Bronze Age and Iron Age samples.

by comparing their femoral cortical indices (CI) to corresponding linear growth (Fig. 7).

The quadrant display of residuals of CI and diaphyseal lengths shows an age-wise distribution of individuals from the Bronze Age sample. The residual plot shows that the distribution of standardized residuals of femoral lengths and cortical index is concentrated mostly within ± 2 standard deviation (SD), which is expected to be the case for approximately 95% of observations. However, a few outliers beyond ± 2 SD, circled in the figure, are discussed in detail. Those individuals with comparatively shorter femoral lengths than the CI fall in quadrant A. None of the individuals in this quadrant had significantly shorter diaphyseal lengths in relation to their CI than the normal mean except for B49, which is the only outlier in this group with significantly shorter diaphyseal length below -2 SD. Quadrant B shows those individuals having relatively higher CI and lengths compared to other individuals. Of these, B168 shows relatively longer length compared to the CI. Quadrant C represents those individuals whose linear growth was normal whereas the appositional growth seemed to be lower. However, most of the individuals lie within the CI of -1 SD, except for B465, which has lower CI (close to -2 SD) compared with the length. This implies that most individuals had a “normal” CI for their age. Those individuals with negative residuals for both length and cortical index suggest linear and cortical bone growth below the normal mean, and are shown in Quadrant D. A single individual, B84 lies below -1 SD in length and below -2 SD in CI, suggestive of overall deficient growth.

5. Discussion

The hypothesis predicted that there would not be any significant variation in the linear and appositional growth patterns of infants and children from the Neolithic compared with those from the Bronze and Iron Age at Ban Non Wat. When linear growth in children from the different cultural phases was compared, there was no statistically significant variation in growth over time. Visual observations of the graphs suggested the possibility that individuals in the Bronze Age may have experienced less physiological stress compared with those in the Neolithic and Iron Age samples. However, the regression slopes failed to statistically confirm this. Given the small sample sizes from the Neolithic and Iron Age, only substantial differences would have been reliably detected and smaller but still meaningful differences cannot be ruled out here.

Appositional growth comparison using cortical thickness also showed no statistically significant changes over time. The total bone width and medullary width were assessed to check for surface-specific changes in appositional growth. Both total width and medullary width indicate normal growth, displaying no evidence for a sudden increase in bone apposition or excessive resorption that could result out increased mechanical load or nutritional stress. The residual plot analysis explored the relationship between linear and appositional growth of children belonging to the Bronze Age. This method of analyzing the interaction between different aspects of growth may be useful for determining deviations from expected growth patterns in children. However, there is little evidence for the continuation of linear growth at the expense of appositional growth in the Ban Non Wat sample. Only one individual (B84) seemed to be markedly deficient in both linear and appositional bone growth.

5.1. Inter-population linear and appositional growth comparison

In the absence of comparable growth data from Southeast Asia, these results are compared to a few published studies in other parts of the world. The samples from the late medieval rural site of Wharram

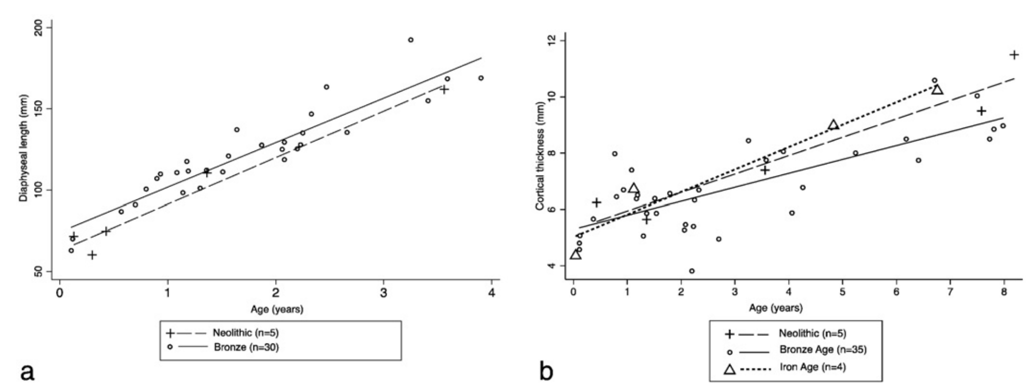

Fig. 3.5. a, b. Tibial linear growth comparisons of Neolithic, Bronze Age and Iron Age samples.

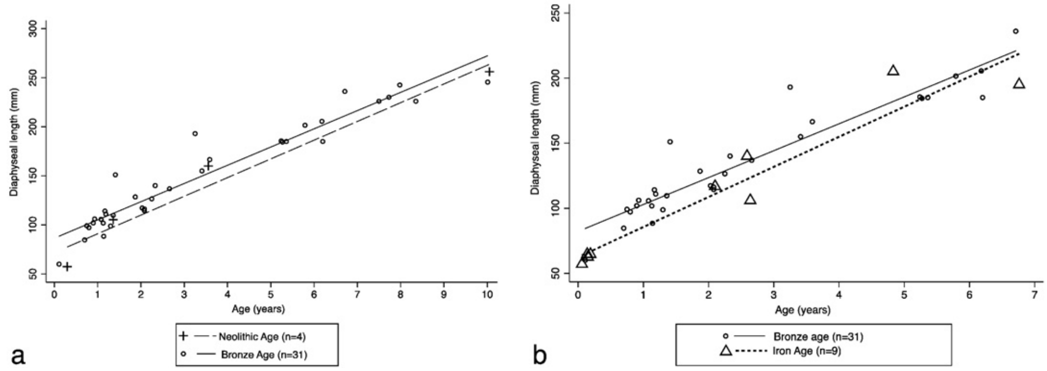

Fig. 3.6. a, b. Fibular linear growth comparison of Neolithic, Bronze Age and Iron Age samples.

Table 3
Growth comparisons between the Neolithic, Bronze and Iron Age growth patterns tested using linear regression analysis:

Long bone growth	Slope (per year) (95% confidence interval)			Pairwise comparison (p-values)[a]	
	Neolithic	Bronze Age	Iron Age	Neolithic vs. Bronze Age	Bronze vs. Iron Age
Humerus	–	15.29 (14.0, 16.5)	17.22 (15.0, 19.4)	–	0.135
Radius	19.45 (13.7, 25.2)	16.15 (13.7, 18.5)	11.20 (8.9 13.5)	0.241	0.157
Ulna	20.64 (14.3, 26.9)	16.54 (13.5, 19.4)	13.72 (11.6, 15.7)	0.225	0.793
Femur	24.29 (20.4, 28.1)	25.09 (23.0, 27.1)	25.98 (21.9, 30.0)	0.712	0.694
Tibia	28.62 (20.9, 36.2)	27.42 (23.2, 31.5)	23.98 (19.8, 28.2)	0.780	0.162
Fibula	19.07 (14.8, 23.2)	18.55 (16.5, 20.5)	23.10 (18.4, 27.7)	0.821	0.373

[a] Pairwise comparisons between a) Neolithic vs. Bronze Age slopes of radius, ulna, tibia (0–4 years), femur (0–8 years) and fibula (0–10 years). b) Bronze Age vs. Iron Age slopes of humerus (0–10 years), radius, ulna, tibia and fibula (0–7 years) and femur (0–8 years).

Percy, England (10th–16th century CE) (Mays, 1999) are used here for comparative analysis. In the comparison of Wharram Percy sample and Ban Non Wat, perinates have been excluded as Mays (1999) used long bone length to estimate age of the perinates from Wharram Percy, potentially creating a circular bias of growth analyses.

Tables 4.1–4.2 describe the comparative mean diaphyseal lengths and cortical index values by age in Ban Non Wat children from 1 to 10 years. Wharram Percy consistently has smaller mean lengths and cortical indices by age compared with Ban Non Wat (Tables 4.1 and 4.2; Figs. 8.1 and 8.2). Visual assessment shows that the Ban Non Wat sample has wider mean CIs throughout the growth period of 1 to 10 years of age.

The current findings are consistent with earlier bioarchaeological analyses of Thai infant and childhood growth and health (Halcrow, 2006) where no significant differences were found in linear growth with the intensification of agriculture. When compared with Wharram Percy, the children from Ban Non Wat showed higher cortical indices, which may suggest that they experienced relatively low physiological stress through better nutrition and/or lower pathogen load. Although genetic differences between the two populations probably influenced these findings, other studies have reported nutritional stress and elevated childhood mortality in the Wharram Percy sample as a reflection of poorer living conditions compared to modern UK populations (Mays, 1985, 1999).

Additional information on the ontogenetic patterns of cross-sectional geometric properties of cortical bone in children can contribute to

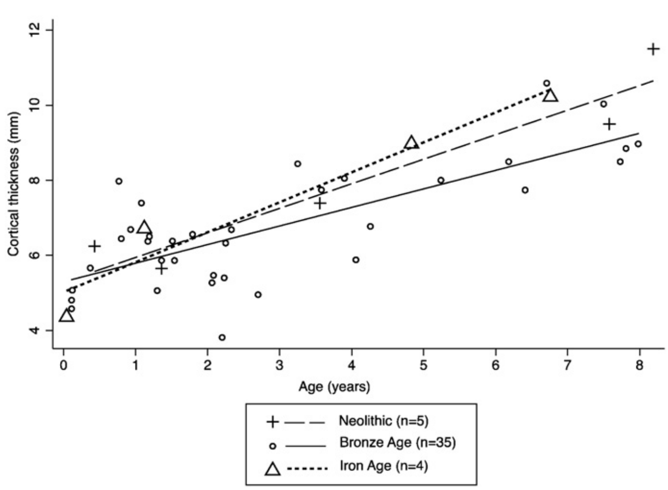

Fig. 4. Cortical thickness (C) comparisons of the Neolithic, Bronze and Iron Age samples.

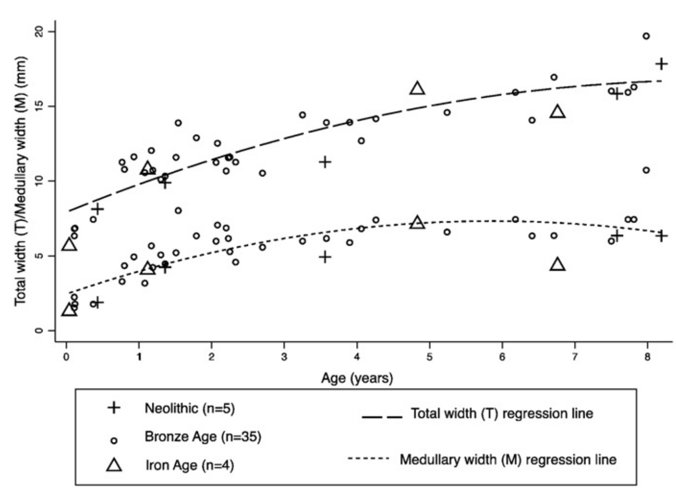

Fig. 5. Total bone width (T) and medullary width (M) in infants and children (0–10 years) from the Neolithic, Bronze and Iron Ages. A quadratic regression line fit for T ($R^2 = 0.85$) and M ($R^2 = 0.70$), but statistical comparison could not be carried out using a quadratic model due to the small sample sizes in the Neolithic and Iron Age.

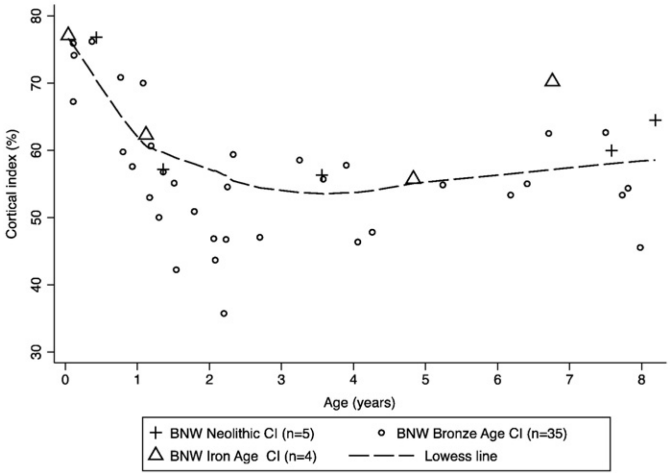

Fig. 6. Growth in cortical indices in children (0–8 years) from the Neolithic, Bronze and Iron Age samples at Ban Non Wat.

understanding the disturbances in cortical bone accrual. Evaluating body mass using cross-sectional properties of bone in past populations has been applied in the recent times to evaluate possible physiological stress in children (Robbins Schug and Goldman, 2014; Pinhasi et al., 2014). Such analyses may help in tracing further evidence for stress in infants and children.

5.2. Childhood stress within the Southeast Asian environmental context

Returning to the main question of variability in growth patterns with increasing agricultural intensification, it is interesting that no significant changes occur in childhood linear growth over time at Ban Non Wat. These findings fit with the general model of health change in the region where there are no obvious patterns of increased levels of stress in the form of non-specific health indicators and infectious disease over time (Domett and Tayles, 2007; Douglas and Pietrusewsky, 2007; Oxenham and Tayles, 2006b). However, visual assessment does suggest better growth attainment in the Bronze Age compared with the Neolithic and Iron Age, although this trend could not be statistically tested owing to smaller sample sizes.

Halcrow et al. (2016) have recently assessed if there is any evidence for health deterioration in infants and children with the intensification of agriculture in MSEA by assessing several non-specific indicators of stress (cribra orbitalia, porotic hyperostosis, periostitis and endocranial new bone formation) from seven sites spanning the pre-metal to the Iron Age periods. They found little evidence for a change in stress experience from the pre-metal to the Bronze Age sites. There was evidence for very poor health in the early pre-Metal sample of Khok Phanom Di in Southeast Thailand, but this is probably due to the localized coastal environmental conditions and a genetic form of anemia in the sample, which has not been found in the inland Northeast Thailand samples. This research further suggests that there is little evidence that agricultural intensification had a significant impact on health of populations due to the retention of a broad subsistence base of non-agricultural food crops to supplement domesticated rice (Halcrow et al., 2016). Although agrarian populations in other regions, such as the Americas and Europe, relied heavily on crops such as maize and wheat, the tropical environment in mainland Southeast Asia provided a variety of supplementary resources that the population exploited (Domett and Tayles, 2007; Kealhofer and Penny, 1998; Oxenham and Tayles, 2006b). In addition, the type of staple crop and the tempo with which it was introduced is the key difference between agricultural adoption in Thailand compared with the temperate environments. Although rice agriculture intensified during the Iron Age, MSEA experienced a gradual transition towards intensified rice agriculture, with continued heavy reliance on foraging, hunting and fishing of a multitude of other naturally available resources in the region throughout the entire sequence (Oxenham and Tayles, 2006b). Further, this retention of a broad spectrum subsistence base is echoed in the oral pathology research in the region that has found an absence of temporal patterns of increasing caries, also partly due to the relatively low cariogenic potential of rice compared with the carbohydrate staples depended on in

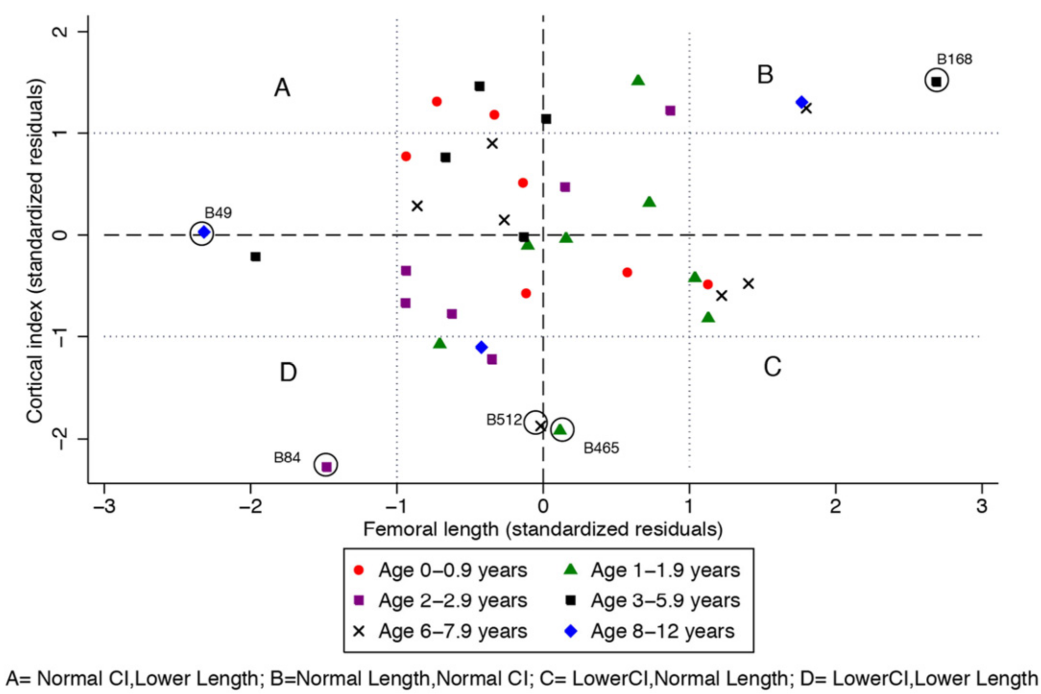

Fig. 7. Residual graph illustrating the interaction between the femoral CI (appositional) and diaphyseal (linear) growth in the Bronze Age infants and children. The circled and numbered individuals represent statistical outliers defined as either standardized residual having an absolute value ≥ 2 (i.e. not being expected to occur by chance > 5% of the time).

Table 4.1
Comparative study of femoral diaphyseal lengths between Wharram Percy (WP) and Ban Non Wat (BNW):

Age (years)[a]	Wharram Percy (Medieval)	n	WP (SD)	BNW (Neolithic, Bronze and Iron Age samples combined)	n	BNW (SD)
1	125	10.9	10	123.79	13	2.7
2	151.9	10.3	10	153.07	16	3.7
3	173	12.8	11	182.75	4	15.2
4	186	12.1	11	198.60	5	4.4
5	195	9.9	6	224.50	2	1.5
6	214	12.8	21	242.62	4	6.7
7	241	23.9	8	269.75	2	22.2

[a] Data presented employing age categories following Mays (1999), such that Age category 1 includes children aged 0.5–1.49 years.

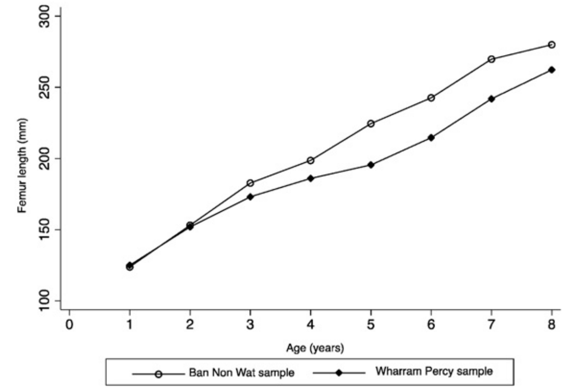

Fig. 8.1. Linear growth comparison of Ban Non Wat sample with Wharram Percy sample (0–8 years).

other parts of the world (Halcrow et al., 2016; Halcrow et al., 2013; cf. Newton et al., 2013; Tayles et al., 2000, 2007; cf. Willis and Oxenham, 2013). Bioarchaeological research assessing systemic stress in the Jomon foragers and Yayoi agriculturists of East Asia using linear enamel hypoplasia has also shown a reduction in stress levels from foragers to agriculturists. The author interpreted this as evidence that supplementary nutritionally "fall-back" foods such as marine resources and terrestrial mammals were maintained as a significant part of the Yayoi agriculturalist diet (Temple, 2010).

There was, however, some evidence for a significant increase in mortality and morbidity in the late northeast Thai Iron Age period sites of at Noen-U-Loke and Muang Sema (Halcrow et al., 2016). This high infant mortality seen in the Noen U-Loke indicative of high levels of stress is also seen in the Iron Age period of Ban Non Wat (Tayles and Halcrow, 2016). The changes in stress response in the infants and children occurring during the Iron Age were attributed to the major socio-economic and environmental changes occurring in the region during this time (Halcrow et al., 2016). During this period there is evidence for the adoption of intensified wet-rice agriculture, increased settlement density and population contact through trade, modification of water-ways, and the clearing of forests (Boyd and Chang, 2010; Higham, 2014; King et al., 2013; Welch and McNeill, 1991). The population and environmental changes consequent of wet-rice production probably led to increased exposure to infectious pathogens and a higher rate of pathogen transmission (Halcrow et al., 2016). In particular the partial destruction of forest areas and intentional flooding of areas for rice cultivation would have produced standing bodies of water suitable for the *Anopheles* genus mosquito, the vector for malaria, which likely had a detrimental effect on health of these past populations (Halcrow et al., 2016).

The assessment of retrospective childhood stress by Clark et al. (2014) from Ban Non Wat through the analysis of linear enamel hypoplasia and stature in adults (survivors) revealed an improvement in health from the Neolithic to the middle Bronze Age, and a subsequent deterioration in health in late Bronze Age. This suggested an increase in childhood stress in the later Bronze Age that may relate to subsistence change and climate change during this time (Boyd and Chang, 2010; Clark et al., 2014). While this may appear on face value contradictive to our evidence for Bronze Age, the findings of childhood stress in the adults may be indicative of the change in environmental conditions right at the end of the Bronze Age that was not able to be assessed in our relatively small sample. Poor preservation of the Iron Age sample of adults from this site inhibited the assessment of Iron Age individuals in Clark et al.'s (2014) analyses.

In the current research we also experienced problems with assessing growth of infants and children during the Iron Age due to relative poor bone preservation. Future assessment of stress and growth may be possible using the recently excavated, well-preserved Iron Age samples from Non Ban Jak, a matter of kilometers from Ban Non Wat (Higham et al., 2014). Current investigations of childhood health at Ban Non Wat assessed through non-specific stress indicators and pathology, in conjunction with the current growth results will also aid in a broader understanding of the biological consequences of the adoption and intensification of agriculture in this region.

6. Conclusion

This study aimed to investigate growth variability over time among infants and children as a proxy for evaluating physiological stress in the skeletal sample of Ban Non Wat in response to agricultural intensification. The linear growth assessment results did not show any significant evidence for growth retardation with agricultural intensification.

Table 4.2
Summary of the mean cortical index values between Wharram Percy (WP) and Ban Non Wat (BNW) Bronze Age sample:

Age(years)[a]	Mean CI BNW	n	BNW (SD)	Mean CI WP	n	WP (SD)
1	58.24	7	2.4	37.5	9	7.0
2	47.57	8	2.6	38.1	9	7.1
3	53.37	3	3.3	40.4	11	8.3
4	51.90	4	2.8	38.6	11	3.4
5	54.85	1	–	35.3	5	1.7
6	54.16	2	0.8	41.1	21	7.4
7	62.50	1	–	46.0	8	6.9

[a] Data presented employing age categories following Mays (1999), such that Age category 1 includes children aged 0.5–1.49 years.

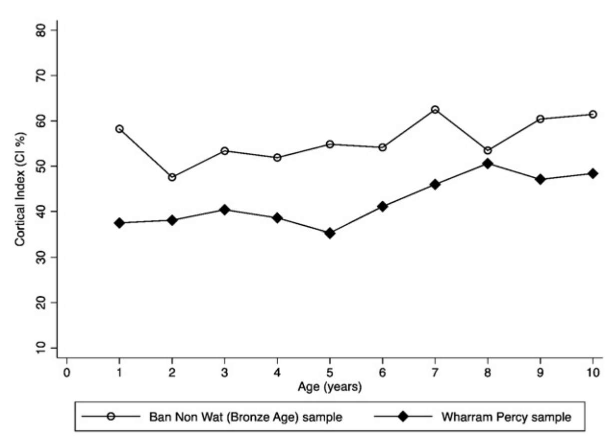

Fig. 8.2. Comparisons of mean cortical indices (CI) of Ban Non Wat (Bronze Age) sample with the Wharram Percy (WP) sample (0–10 years).

Appositional growth comparison, measured by cortical width also showed no significant differences among the three archaeological periods. Both linear and appositional growth analyses support the hypothesis that there would not be significant temporal differences in growth patterns associated with agricultural intensification. A detailed assessment of cortical bone growth in the Bronze Age individuals suggests an absence of stress severe enough to affect growth. Cortical bone growth is more or less maintained throughout the growth period, with evidence for variation around the age of 2 years. However, the overall cortical index values do not show any consistent trend of growth change. The overall results of growth assessment in children from Ban Non Wat are consistent with the earlier bioarchaeological research in this part of the world showing no clear evidence for deterioration in health with the intensification of agriculture.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jasrep.2016.12.019.

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