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Paleopathological characteristics of Neolithic early rice farmers in the lower reaches of the Yangtze river

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Paleopathological investigations of human remains from the Neolithic Hemudu culture in the lower reaches of the Yangtze River in southern East Asia were conducted to clarify the health status of people in early rice-farming societies. Our results show that the occurrence ratios of cribra orbitalia and enamel hypoplasia did not differ significantly between early rice farmers and hunter-gatherers. By contrast, the occurrence ratios of periosteal reactions, dental caries, and antemortem tooth loss in adults were higher among the early rice farmers. Based on these findings and the results of archaeological research on the Hemudu culture, it was suggested that: 1) the Hemudu culture adopted a diversified livelihood strategy that was not overly dependent on rice as a food resource, which did not lead to an extreme decline in health status, 2) the work in the rice fields or the working environment caused stress to the workers, and 3) the rice-farming society's dietary habits led to a decline in oral health. Our results provide new paleopathological insights into the health status of early rice farmers in East Asia. However, the sample size of early rice farmers used in this study was small, and more data are needed to verify the validity of the views presented here.

KEYWORDS

palaeopathology, stress marker, oral health, early rice farmers, Hemudu culture, East Asia

1 Introduction

The beginning of agriculture was a significant event in human history as it brought about a means of food production under human control. Among the various agricultural plants used as food, rice is undoubtedly one of the most important, along with wheat. The lower reaches of the Yangtze River in southern East Asia is considered one of the origins of rice agriculture (Fuller et al., 2009; Fuller, 2011; Larson et al., 2014). Rice cultivation and utilization were initiated during the early Holocene period (Shangshan culture, 11,000-8,600 years ago). Subsequently, during the Hemudu culture around 7,000-5,500 years ago, systematic rice cultivation began with the establishment of rice paddies (Zhejiang, 2003; Nakamura S. 2010; Zhao, 2011; Liu and Chen, 2012; Zuo et al., 2017; Crawford, 2022). Large quantities of carbonized rice, farming tools, animal remains, and stilt-house ruins were found at the Hemudu and Tianluoshan sites of the Hemudu culture period, revealing the specific aspects of life and society of early rice-farming people.

TABLE 1 Human skeletal materials (number of individuals).

	Adult female	Adult male	Adult of unknown sex	Juvenile	Total
Early rice farmers (Hemudu site)	1	2		5	8
Early rice farmers (Tianluoshan site)	4	5		5	14
Hunter-gatherers (Huiyaotian and Liyupo sites)	38	36	8	9	91

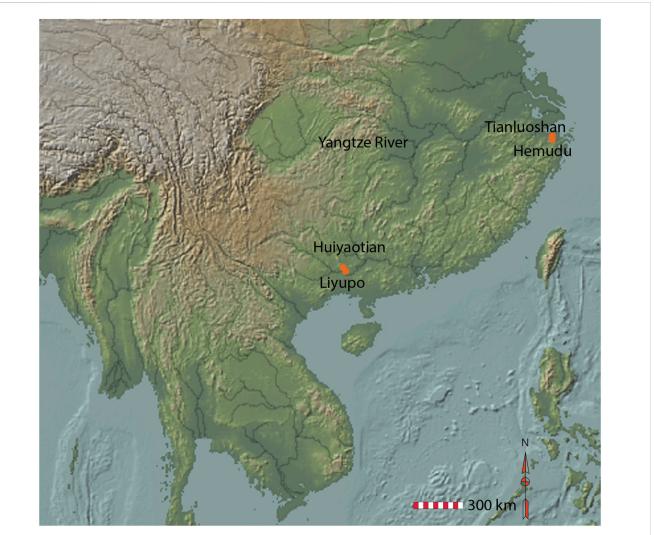


FIGURE 1 Map showing the location of the sites discussed. Map by C.F.W. Higham courtesy GeoMapApp (www.geomapapp.org), CC by Ryan et al. (2009).

Paleopathological studies examining changes in human health status during the adoption of agriculture as a new means of livelihood can suggest important implications for exploring the development of human societies. For instance, when Native American populations began cultivating corn in North America before the arrival of Europeans, linear enamel hypoplasia (LEH), a stress marker, became more frequent than that during the preceding hunter-gatherer period. This might have resulted from the fact that the early agricultural food production system was immature and reliance on a single crop may have led to nutritional deficiencies, or changes in population density and habitat at the beginning of agriculture made the spread of infectious diseases more likely (Goodman et al., 1980; Larsen, 1995; Larsen, 1999; Hillson, 1996). An increased frequency of stress markers at the onset of agriculture has also been reported in the Eastern Mediterranean (Angel, 1984; Smith et al., 1984) and Western Asia (Rathbun, 1984).

Also regarding prehistoric Asia, where rice farming spread, there has been discussion about the impact of the introduction of agriculture on the health status of the people. For example, in Vietnam and Thailand in continental Southeast Asia, the

			Early rice	e farmers			Hunter-		Fisher's exact test		
	Hen	nudu	Tianluoshan		Subtotal		gatherers		Farmers' subtotal vs. Hunter-gatherers		
	Ν		N		Ν		N		<i>p</i> -value		
Juvenile	1/3	33.3	0/1	0.0	1/4	25.0	-	-	-		
Adult	0/2	0.0	0/5	0.0	0/7	0.0	3/24	12.5	1.000		
Total	1/5	20.0	0/6	0.0	1/11	9.1	3/24	12.5	1.000		

TABLE 2 Frequency of cribra orbitalia (CO).

N: Number of skulls with CO/Total number of skulls with orbital roof.

adoption of agriculture has been speculated to be accompanied by a decline in oral health, as evidenced by the increased frequency of dental caries and antemortem tooth loss (AMTL) during the Neolithic period (Willis and Oxenham, 2013; Oxenham et al., 2018). Even in the Far Eastern Japanese Archipelago, the frequency of dental caries increased in the Yayoi period, when rice farming was introduced, than in the earlier hunter-gatherer society of the Jomon period (Fujita, 1995; Fujita, 2009). Additionally, the frequency of LEH decreased or was similar in the Yayoi and subsequent Kofun periods compared to the Jomon period (Yamamoto, 1988; Temple, 2010). However, continental Southeast Asia and the Japanese Archipelago are areas where the established ricefarming culture spread from continental East Asia, and not the places of origin of rice farming. The paleopathological characteristics of early farming groups have not been elucidated in the lower reaches of the Yangtze River, where rice farming originated.

This study aims to clarify the health status of Neolithic early rice farmers in areas of rice farming origin. To this end, we investigated the occurrence of stress markers such as cribra orbitalia (CO), LEH, periosteal reaction, and indicators of oral health such as dental caries and AMTL in the human remains from the Neolithic Hemudu Culture excavated from the Hemudu and Tianluoshan sites in the lower reaches of the Yangtze River. This study provides new insights into the health status of early ricefarming populations in East Asia based on paleopathological studies.

2 Materials and methods

2.1 Early rice farmers' remains from the Neolithic Hemudu culture

The human remains of early rice farmers used as materials in this study included eight burial human skeletons from the Hemudu site and 14 from the Tianluoshan site, with a total of 22 burial skeletons (Table 1). In addition, 12 scattered human remains excavated from the Tianluoshan site were used as materials (mentioned later). The Hemudu and Tianluoshan sites are located in Zhejiang Province in southern China, and both are from the Hemudu culture-7,000-5,500 years ago (Figure 1).

The Hemudu site was discovered in 1973 and excavations were conducted between 1973 and 1978 (Zhejiang, 2003). Eight human skeletons (one adult female, two adult males, and five juveniles of unknown sex) were found during the excavations and stored at the Hemudu Site Museum. The skulls of one adult male and one adult female were cleaned, and the results of the morphological research have been reported (Zhejiang, 2003). However, the post-cranial trunk and limb parts of these two skeletons were not exhumed, and the entire grave pits were preserved in the museum. The other six whole skeletons were not exhumed; all grave pits were preserved in the museum, and their anthropological reports have not been published. In this study, we examined eight human skeletons for CO, LEH, dental caries, and AMTL. However, some parts of the skull that were in an earth-encased state could not be adequately observed. In addition, the postcranial parts were not investigated for periosteal reactions because it was impossible to remove them from the soil of the grave pit.

The Tianluoshan site, located 7 km from the Hemudu site, was discovered in 2001 and has been continuously excavated since 2004. Its archaeological results have been reported by Nakamura (2010b) and Matsui and Kikuchi (2016); however, no anthropological reports of excavated human remains have been published. In this study, 14 buried human skeletons (four adult females, five adult males, and five juveniles of unknown sex) stored at the Tianluoshan site museum, and 12 scattered human remains (one adult mandible, two adult right femurs, one adult left femur, one adult left tibia, and seven permanent teeth) that were considered to have originated from individuals other than those in these burials, were examined for the presence of CO, LEH, periosteal reaction, dental caries, and AMTL.

2.2 Hunter-gatherers' remains from the early holocene southern East Asia

There were no hunter-gatherer sites in the lower reaches of the Yangtze River, from which many human remains were excavated. Therefore, the human skeletons of non-agricultural hunter-gatherers in the Early Holocene, approximately 9,000 to 7,000 years ago, from the Huiyaotian and Liyupo sites in Guangxi Province, Southern China (Matsumura et al., 2017), were used as comparative materials for early farmers in this study (Figure 1). The Huiyaotian and Liyupo sites, as well as the

			Early ric	e farmers		Hunter-		Fisher's exact test	
	Hen	nudu	Tianlu	Tianluoshan Subtotal		gatherers		Farmers' subtotal vs. Hunter-gatherers	
	Ν		N		Ν		N		<i>p</i> -value
LEH in UI1	2/3	66.7	2/2	100.0	4/5	80.0	14/19	73.7	1.000
LEH in LC	2/3	66.7	4/4	100.0	6/7	85.7	20/40	50.0	0.112

TABLE 3 Frequency of linear enamel hypoplasia (LEH).

N: Number of teeth with LEH/Total number of teeth remains, UI1: Upper first incisor, LC: Lower canine.

Hemudu and Tianluoshan sites, belong to the warm humid climate (Cfa) category of southern East Asia in the Köppen climate classification. They share similarities with the Hemudu cultural sites in being located in a plain area along the lower reaches of a large river. Paleopathological investigations of 91 hunter-gatherer human skeletons from the Huiyaotian and Liyupo sites were conducted by one of the authors of this study (JS) using the same methods as those used in this study (Sawada, 2017a; Sawada, 2017b; Sawada et al., 2017).

2.3 Age and sex estimation

The age of juvenile human remains was estimated based on the tooth formation status (Smith, 1991), eruption status (Ubelaker, 1999), and the fusion status of the epiphyseal ends of the limb bones (Maresh, 1970; Fazekas and Kosa, 1978; Cunningham et al., 2016). The age of adult remains was estimated based on the closure status of the cranial sutures (White et al., 2012) and the metamorphosis of the pubic symphysis surface (Brooks and Suchey, 1990).

The sex of the human remains was estimated from the shape of the adult coxal bone and skull, based on the sex determination method in White et al. (2012).

2.4 Paleopathological investigation

2.4.1 Cribra orbitalia (CO)

CO is a pathological condition characterized by porotic or sieve-like lesions in the supraorbital bone. CO is caused by nutrient deficiencies, such as vitamin B12, vitamin C, or iron-deficiency, anemia, mainly during growth (Walker et al., 2009; Oxenham and Cavill, 2010), and has been used in studies of ancient human populations (Larsen, 1995). The identification criteria for CO were based on those described by Nathan and Haas (1966) and Hirata (1988). The presence of CO was confirmed by naked-eye observations, and individuals with CO in either or both orbits were considered to have CO.

2.4.2 Linear enamel hypoplasia (LEH)

LEH is an enamel thickness defect that results from physiological stress during crown formation. This disease is regarded as a relatively sensitive and nonspecific indicator of stress (Goodman et al., 1980; Nikiforuk G and Fraser, 1981; Goodman and Rose, 1990). The upper first incisors (UI1) and lower canines (LC) were observed using a 10x magnifying lens under an LED light. According to Goodman and Rose (1990) and Hillson (1996), a groove or row of small pits parallel to the perikymata on the dental crown surface is considered LEH. To align the conditions for observation of the crowns, teeth with more than half of their original crown height were investigated, and LEHs that appeared below half of their original crown height were recorded.

2.4.3 Periosteal reaction

Adult femoral and tibial diaphyses remaining over half of the original length were used for the investigation or periosteal reaction. The criteria summarized by Weston (2008) were used to determine the presence of a periosteal reaction in the diaphysis.

2.4.4 Dental caries

The presence or absence of dental caries was investigated in all the erupted permanent teeth. Caries were identified according to the classification criteria commonly used in dentistry (C1: lesions mainly confined to the crown enamel; C2: dentin affected, but not the pulp cavity; C3: lesions reaching the pulp cavity; and C4: only the tooth root remained). Since it is sometimes difficult to identify C1 when observing archaeological human remains, C2 or higher, which can be determined with certainty as caries, was recorded as caries. The presence of dental caries was confirmed by observation using a 10x magnifying lens.

2.4.5 Antemortem tooth loss (AMTL)

The alveoli of all permanent teeth were examined for each tooth socket to determine whether the tooth had lost while alive. AMTL was determined based on alveolar conditions according to Ortner (2003).

One of the authors (JS) collected all paleopathological data to prevent inter-observer errors.

2.5 Statistical analyses

Fisher's exact test was used to examine any statistically significant difference in the frequency of occurrence of each paleopathological characteristic between early farmers and hunter-gatherers. The significance level was set at p < 0.05. Statistical analyses were conducted using the SPSS software package for Macintosh, version 25.0.0 (IBM).

3 Results

Because of the small number of human remains of both sexes identified in the materials of the early rice farmers of the

TABLE 4 Frequency of occurrence of periosteal reaction.

	Tianluoshan ea	rly rice farmers	Hunt gathe		Fisher's exact test		
				rers	Farmers vs. Hunter-gatherers		
	Ν		Ν		<i>p</i> -value		
Femur	1/12	8.3	0/76	0.0	0.136		
Tibia	3/9	33.3	4/71	5.6	0.028		
Total	4/21	19.0	4/147	2.7	0.009		

N: Number of adult bones with periosteal reaction/Total number of adult bones.

The left and right were combined.

TABLE 5 Frequency of dental caries.

			Early rice	farmers	Hunter- gatherers		Fisher's exact test				
	Hem	udu	Tianlu	oshan	n Subtotal		gatherers		Farmers' subtotal vs. Hunter-gatherers		
	N		N		Ν		N %		<i>p</i> -value		
Permanent tooth	3/20	15.0	4/36	11.1	7/56	12.5	45/820	5.5	0.041		

N: Number of erupted permanent teeth with caries/Total number of erupted permanent teeth.

Hemudu culture, the sexes were combined and analyzed as a whole. Fisher's exact test of sex composition showed no statistically significant difference between early farmers (five females and seven males) and hunter-gatherers (38 females and 36 males).

3.1 CO

The occurrence ratio of CO in juveniles of early rice farmers was 25.0%, and that in adults was 0.0% (Table 2). By contrast, the incidence of CO among hunter-gatherers was 12.5% in adults (no juveniles with preserved supraorbital walls). Fisher's exact test was used to examine any statistical difference in the frequency of CO occurrence between farmers and hunter-gatherers. No significant difference was found at the 0.05 level.

3.2 LEH

The occurrence rates of LEH in early rice farmers in UI1 and LC were 80.0% and 85.7%, respectively. The occurrence rates of LEH in the hunter-gatherer population were 73.7% and 50% in the UI1 and LC, respectively (Table 3). Fisher's exact test showed no statistically significant differences between farmers and hunter-gatherers at the 0.05 level.

3.3 Periosteal reaction

As mentioned above, examining the limb bones from the Hemudu site was impossible; therefore, only the bones from the Tianluoshan site were used to analyze the periosteal reaction. Periosteal reactions observed in these materials were mild. The occurrence ratio of periosteal reactions in the adult lower limb long bones of the farmers (19.0%) tended to be higher than that of the hunter-gatherers (2.7%), and Fisher's exact test showed a statistically significant difference (Table 4).

3.4 Dental caries

The percentage of dental caries in the total number of erupted permanent teeth was 12.5% in early rice farmers compared to 5.5% in hunter-gatherers, a statistically significant difference at the 0.05 level in Fisher's exact test (Table 5). The dental caries ratio of the Huiyaotian and Liyupo hunter-gatherers used for comparison in this study tended to be similar to the low caries rates of modern hunter-gatherer groups (Turner, 1979).

3.5 AMTL

The occurrence rate of AMTL in the early rice farmers, calculated based on the number of permanent tooth sockets, was 7.8%. This tended to be higher than that in hunter-gatherers (4.8%), although no statistically significant difference was detected (Table 6).

4 Discussion

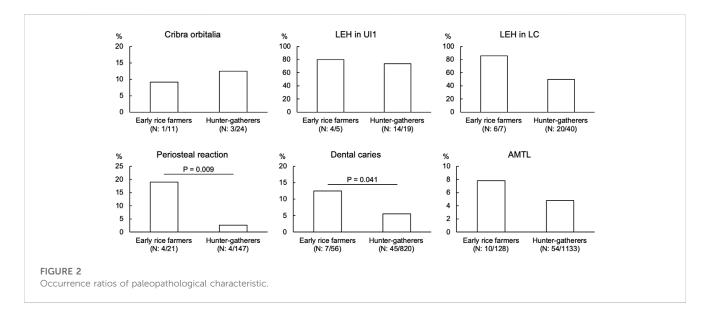
4.1 Stress markers: CO, LEH and periosteal reaction

No statistically significant difference in the frequencies of CO and LEH were found between early rice farmers of the Hemudu culture and hunter-gatherer groups in southern China. Although our findings are preliminary owing to the small sample size of early rice farmers,

TABLE 6 Frequency of AMTL.

			Early rice	e farmer	Hunter-		Fisher's exact test		
	Hemudu		Tianluoshan Subtotal		gatherers		Farmers' subtotal vs. Hunter-gatherers		
	N		N		N		N %		<i>p</i> -value
Socket for AMTL	0/62	0.0	10/66	15.2	10/128	7.8	54/1133	4.8	0.138

N: Number of sockets for AMTL/Total number of sockets in adult maxilla and mandible remains.



there was no evidence that the health status of early rice farmers was appreciably different from that of hunter-gatherers. While it is thought that health declined after the introduction of agriculture in North America and the Mediterranean (Goodman et al., 1980; Angel, 1984; Smith et al., 1984; Larsen, 1995), such changes may not have occurred in the Neolithic societies of East Asia that began rice farming. In this regard, examining the characteristics of people's livelihoods during Hemudu culture may be necessary. Zooarchaeological studies suggest that the people of the Hemudu culture consumed a variety of mammals and fish acquired through hunting and fishing, as well as domestic animals, such as pigs and geese (Matsui and Kikuchi 2003; Eda et al., 2022). A diversified food acquisition strategy that did not rely heavily on rice farming may have contributed to a stable food supply and prevented a significant decline in the health status of the people during the Hemudu culture period.

However, for periosteal reactions in adult bone, the occurrence ratio in the early rice farmers was significantly higher than that in the hunter-gatherer group (Figure 2). The periosteal reaction is an early response of the bone to the effects of disease or trauma, and is considered an indicator of non-specific stress (Larsen, 1999; Waldron, 2021). Although it is difficult to extrapolate a specific causative lesion from the periosteal reaction alone (Weston, 2008), unlike LEH, which is derived from stress in infancy, the periosteal reaction in adult bones is probably derived from stress at a later age. The high incidence of periosteal reactions in early rice farmers may be related to infections caused by long hours of farming in rice fields with high mechanical stress, or noxious insects such as schistosomes (Ross et al., 2013) and mosquitoes that are easily contacted in the aquatic environment. Future studies on human bones for paleopathological features associated with infection and whether DNA from infection remains in human bones will allow for a more specific discussion of the etiology of periosteal reactions.

4.2 Oral health: dental caries and AMTL

The occurrence ratios of dental caries and AMTL in people of the Hemudu culture tended to be higher than those in the huntergatherer group (Figure 2). These results suggest that early ricefarming societies in the lower reaches of the Yangtze River had factors that contributed to oral hygiene deterioration. In general, the occurrence of dental caries is related to factors such as caries pathogens (e.g., mutans), dietary characteristics (sugar and sticky foods), dental habits, and lifestyle (Hillson, 1996; Hillson, 2008; Waldron, 2021). Temple and Larsen (2007) reported that a ricebased diet is responsible for dental caries in rice-farming populations. However, Willis and Oxenham (2013), who presented data on the decline in oral health during the introduction of agriculture in Southeast Asia, are critical of the view that rice is the main cause of dental caries, based on a review by WHO (2003), which stated that "rice has low potential cariogenicity." Willis and Oxenham (2013) highlighted sexual differences in the quality of oral hygiene and physiological factors that lead to poor oral hygiene in females (especially during pregnancy).

We could not examine sexual differences because the sample size was too small to allow sex determination owing to the large number of juvenile and fragmentary human bone materials that are difficult to determine. We hope that future excavations will increase the amount of human material from early farming groups in the lower reaches of the Yangtze River and advance the study of sexual differences in the occurrence ratios of paleopathological features.

5 Conclusion and future direction

The frequencies of occurrence of stress markers and oral pathological features in early rice farmers in the lower reaches of the Yangtze River and hunter-gatherers in the early Holocene in southern China were examined. The results showed that: 1) the occurrence ratios of CO and LEH were not significantly different between early rice farmers and hunter-gatherers, and 2) the occurrence ratios of dental caries, AMTL, and adult periosteal reactions were higher in early rice farmers. Based on these findings, we hypothesized that early rice-farming societies during the Hemudu culture had a diversified subsistence strategy that did not rely too heavily on rice as a food resource, that work in rice fields and its environment brought new stressors that were different from those of hunter-gatherer subsistence, and that the diet of ricefarming societies led to a decline in oral health. However, the sample size of early rice farmers used in this study was small; therefore, additional data will be needed in the future to verify the validity of the views presented here.

Problematized as an "Osteological Paradox" (Wood et al., 1992; Wright and Yoder, 2003; DeWitte and Stojanowski, 2015; Pilloud and Schwitalla, 2020), if one died at the time of stress, the stress could not leave traces in the bones. Therefore, a group's high or low frequency of stress markers may not simply reflect the society's low or high health status. For example, a high occurrence of stress markers but a low percentage of severe stress markers might indicate that severely stressed individuals died, rather than the environment being less susceptible to severe stress. Although the sample size was small, and the proportion of severe cases could not be examined in this study, such an examination is necessary when additional materials are available. In addition, it is expected that a combination of paleopathological analyses and paleo-demographic studies of life expectancy will provide valuable insights. The frequency of CO, periosteal reactions, and dental caries, which have been reported to change with age (Larsen, 1995; Waldron, 2021), were only discussed in two categories, adults and juveniles, owing to the scarcity of materials. Future studies on the human remains of rice farmers, including those from the lower reaches of the Yangtze River after the Hemudu culture (e.g., Okazaki and Takamuku, 2019; Okazaki et al., 2021), will help resolve these issues and clarify people's health status in the development of rice-farming societies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

JS, GS, WH, SK, MY, and SN contributed to the conception and design of the study. JS, SK, and FS contributed to the organization and analyses of the materials. JS wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/feart.2023.1225928/ full#supplementary-material.

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