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**Growth response of dental tissues to developmental stress  
in the domestic pig (*Sus scrofa*)**

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3 **Title:** Growth response of dental tissues to developmental stress in the domestic pig  
4 (*Sus scrofa*)  
5  
6  
7

8 **Abbreviated title:** dental tissues and stress in pigs  
9

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**ABSTRACT**

**Objectives:** To compare relative response of enamel, dentin and bone to developmental stressors between attritional and catastrophic mortality assemblages of pigs.

**Materials and Methods:** Heads from 70 *Sus scrofa* of known sex, weight and age comprising an Attritional sample of 50 Sick Pen pigs that died prematurely vs. 20 Control pigs slaughtered at six months (Catastrophic assemblage). Hard tissue changes (alveolar bone thinning), abnormal bone formation (Harris lines) and remodeling (auditory bullae) were recorded. Areas and volumes of coronal enamel and dentin were recorded from microCT scans with Avizo 6.3 and Geomagic Wrap.

**Results:** Attritional and Catastrophic assemblages are metrically indistinguishable. Ages at death and tissue measures in the Sick Pen (SP) pigs are differentially distributed, necessitating partition into developmental outcome cohorts. SP 'late death' pigs are of lesser physiological maturity than expected, free of disease, with large dental tissue dimensions, comparable to 'Controls'. SP 'early death' pigs have 5% less dentin and enamel and chronic bone infection. Older cohorts of the SP 'early deaths' mortality assemblage show progressively reduced enamel. SP pigs show dental evidence of reduced bone mass in the maxilla.

**Discussion:** Bone, dentin and enamel tissues, each, respond distinctively to developmental stressors. Bone mass evinces malnutrition not disease. Both dental tissue reduction and abnormal bone formation link to chronic infection.

Paradoxically, reduced dentin mass signals lower survivorship while reduced enamel signals enhanced survivorship. Meaningful comparison of Attritional and

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3 Catastrophic assemblages necessitates recognition of developmental outcome  
4 cohorts, stratified by age at death and physiological maturity, to reveal  
5  
6 heterogeneity of survivorship, tissue measures and lesions.  
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9  
10 **Key words:** enamel, dentin, pathology, survivorship, osteological paradox  
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## 14 **Introduction**

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16  
17 A generation of bioarchaeologists has grappled with the provoking article by  
18  
19 Wood et al (1992) that expressed fundamental concerns with the meaning of hard  
20  
21 tissue lesions in archaeological skeletons for reconstructing health in a once-living  
22  
23 assemblage (DeWitte and Stojanowski 2015). The challenge of the osteological  
24  
25 paradox, paraphrased simplistically as ‘bad health makes for good skeletons’ or -  
26  
27 equally- ‘sick skeletons signal a healthy response’, has been distilled to the  
28  
29 realization that we need to link hard tissue lesions, convincingly, to survivorship  
30  
31 (Temple and Goodman 2014). Doing so is indeed a challenge. However, the  
32  
33 emergence of sophisticated and nuanced methods applied to archaeological  
34  
35 assemblages suggest these difficulties can be overcome. For example, the linkage of  
36  
37 famine-related deaths to paleo-pathological markers suggests that linear enamel  
38  
39 hypoplasia signals heightened frailty while bone pathologies (e.g., cribra orbitalia)  
40  
41 do not (Yaussy and DeWitte 2018). The authors conclude that not all skeletal or  
42  
43 dental lesions are equally sensitive or reliable gauges of frailty.  
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50 Skeletal and dental lesions reflect the response of the individual to stressors.  
51  
52 Stress, per se, is not directly measurable in hard tissues (Hillson 2017). Most  
53  
54 developmental hard tissue defects are non-specific “generalized” responses to any  
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3 number and variety of stressors (Goodman and Rose 1990); consequently, use of the  
4 term 'stress' is often simply a frank acknowledgment of ignorance as to etiology.  
5  
6 Consequently, we define stress simply as physiological disruption (Temple and  
7  
8 Goodman 2014) sufficient to affect hard tissue formation.  
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11  
12 In our view, in the absence of any ability to measure actual reproductive  
13 success in skeletal samples of individuals, the best measure of biological fitness is  
14 staying alive. The growing organism, faced with developmental stressors is, in effect,  
15 faced with the decision of whether to grow or conserve. Naturally, there are  
16 consequences for the individual.  
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24 "...humans differentially allocate energy budgets to essential tissue  
25 growth and maintenance during periods of stress. However, the process  
26 is not without consequence as these energetic allocations reduce  
27 investment in future growth and maintenance as well as other functions  
28 such as reproduction and disease resistance... These individuals are  
29 frequently stunted in size, reach reproductive maturity at earlier ages  
30 and die younger"(Temple and Goodman 2014) (p. 189).  
31  
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35 In this study, we examine the link between survivorship and developmental  
36 stress early in life using two cohorts of domestic pigs (*Sus scrofa*) whose ages at  
37 death are known and whose cranio-dental features preserve a record of a variety of  
38 developmental stressors (some of which overlapped in time with dental crown  
39 formation). The first cohort is those that died prematurely (hereafter referred to as  
40 Sick Pen (SP) pigs, and are thus considered to have experienced developmental  
41 stress. The second cohort is a Control group that survived until normal slaughter  
42 age. We determine whether pigs with more, or more severe, lesions die at younger  
43 ages and whether their hard tissues (bone, enamel and dentin mass) are metrically  
44 affected in ways consistent with survivorship (Temple and Goodman 2014). Here  
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3 we will use the phrases 'tissue volume' and 'tissue mass' interchangeably. Firstly, we  
4  
5 will briefly review studies of the effect of environmental stressors on tooth size as  
6  
7 well as the challenge of the osteological paradox.  
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9

#### 10 *Dental tissue volumes and developmental stressors*

11  
12 As estimates of the heritability of tooth size have reduced over the years, the  
13  
14 potential of dental tissues to reflect environmental influence has correspondingly  
15  
16 increased. Tooth size has long been an important subject of study in dentistry and  
17  
18 anthropology. Orthodontists require knowledge of the predictability of tooth size  
19  
20 (Osborne et al. 1958; Townsend and Brown 1978; Kabban et al. 2001) while  
21  
22 anthropologists use tooth size for phylogenetic reconstructions and biological  
23  
24 distance analysis (Pilloud and Kenyhercz 2016). However, over the past half  
25  
26 century, estimates of the genetic contribution to tooth size have dropped from 0.9  
27  
28 (Garn et al. 1965) to roughly 0.64 (Osborne et al. 1958; Townsend and Brown 1978;  
29  
30 Townsend et al. 2009). Environmental effects are now thought to account for about  
31  
32 one-third of variation in tooth size; thereby providing a metrical means of studying  
33  
34 the impact of social, nutritional and medical factors as these vary among human  
35  
36 populations over space and time.  
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43 There are numerous studies of the effect of mal-, under and improved  
44  
45 nutrition on tooth size in humans and animals (Shaw and Griffiths 1963; Keene  
46  
47 1971; DiOrio et al. 1973; Guagliardo 1982; Harris et al. 2001). In such studies, dental  
48  
49 crowns are usually treated as homogenous structures (Potter et al. 1968; Kabban et  
50  
51 al. 2001), with no concern for potential differences in tissue response to  
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53 developmental stress, even though enamel and dentin develop from different germ  
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3 layers over contrasting periods of time (Osborn 1981). This perspective may derive  
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5 from studies such as Holloway et al.'s containing the assertion that smaller molars in  
6  
7 the offspring of mother rats fed a 'low protein: high sucrose' diet, nevertheless,  
8  
9 showed no difference in enamel thickness (1961). However, the plentiful literature  
10  
11 on enamel thinning, arising from studies of enamel hypoplasia, (Boyde 1970; El-  
12  
13 Najjar et al. 1978; Goodman and Armelagos 1985; Suckling et al. 1986; Goodman  
14  
15 and Armelagos 1988; Goodman and Rose 1990; Goodman et al. 1991; Skinner and  
16  
17 Goodman 1992; Eckhardt and Protch von Zieten 1993; Blakey et al. 1994; Ensor and  
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19 Irish 1995; Duray 1996; Guatelli-Steinberg 1998; Goodman and Song 1999; Lukacs  
20  
21 et al. 2001; King et al. 2002; Chollet and Teaford 2010; Guatelli-Steinberg et al.  
22  
23 2012; McGrath et al. 2015; Hillson 2017) suggests that the comparative response of  
24  
25 enamel to stress should be re-evaluated. For example, a decrease in porcine enamel  
26  
27 thickness in the area of 'depression' enamel defects has been reported (Witzel et al.  
28  
29 2008). Also, enamel thickness is an oft-recognized character in hominine  
30  
31 systematics and dietary reconstructions (Martin 1985; Grine and Martin 1988;  
32  
33 Olejniczak et al. 2008; Smith et al. 2012; Skinner et al. 2015). Similarly, a decrease in  
34  
35 dentin volume, producing 'coronal waisting' has been linked to nutritional stress in  
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37 chimpanzee infants (Skinner et al. 2012). Consequently, the potential for enamel  
38  
39 and dentin to respond sensitively and independently to stressors needs to be  
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41 assessed.

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50 Our earlier study, related to this one, found that sick pigs exhibit thinning  
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52 and fenestration of the molar tooth crypt wall associated with enamel defects  
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54 (Skinner et al. 2014). Inherent in this conclusion is the assumption that tooth  
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3 formation is less sensitive to developmental stress than is bone formation. The  
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5 current study, using the same cohort of animals, asks whether dentin and enamel,  
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7 also, differ in their response to stress.  
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10 *A consideration of attritional and catastrophic assemblages*

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12 The living population may be the phenomenon of interest but is preserved only  
13  
14 rarely, due to catastrophic events such as flash floods, war, or massacre that  
15  
16 preserve all individuals (Wilson 1988; Margerison and Knusel 2002). A catastrophic  
17  
18 assemblage is created by an event in which all individuals of a living assemblage die  
19  
20 (and are preserved) without reference to host characteristics such as age, sex and  
21  
22 frailty. For example, comparison of two catastrophic assemblages of culled  
23  
24 elephants with an attritional assemblage of drought driven die-off of elephants, near  
25  
26 a dwindling water source, acknowledged that all three populations, sampled in  
27  
28 death, likely differed (Conybeare and Haynes 1984). Specifically, one should expect  
29  
30 to observe sub-groups within an attritional assemblage, which reflect heterogeneity  
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32 of thanatic factors or time. The goal of this study is to record and understand  
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34 heterogeneity of an attritional assemblage so as to allow meaningful comparison  
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36 with a catastrophic assemblage.  
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43 A notable study of the difference between those that die and those that  
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45 survive was made long ago by Bumpus in his study of sparrows exposed to a snow,  
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47 rain and sleet storm: "...the former (survivors) are shorter and weigh less (i.e., are of  
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49 smaller body), they have longer wing bones, longer legs, longer sternums and  
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51 greater brain capacity" (Bumpus 1898)(p.213). Later re-analysis reported a bi-  
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53 modal distribution of measures among non-surviving females (Johnston et al. 1972).  
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3 While Bumpus' influential study is noted as an early recognition of stabilizing  
4 selection, more importantly for our purposes, the death assemblage he described  
5 reflects heterogeneity of host vulnerability. In addition to variation in host  
6 vulnerability, an attritional assemblage is composed of individuals who differ from  
7 the living assemblage in exposure and response to stressors over time. Not  
8 surprisingly, an attritional assemblage may misrepresent the live population  
9 (Conybeare and Haynes 1984).

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20 This study compares dental tissue volumes in two assemblages: a) pigs that  
21 died while in a sick pen; versus b) slaughter age (ca. 6 months) pigs that were  
22 sufficiently heavy (about 109 kg) and healthy to go to slaughter. We consider these  
23 to be Attritional and Catastrophic assemblages, respectively. The Sick Pen pigs form  
24 a mortality cohort whose deaths occurred at any time between about three and  
25 seven months of age. Causes of death are not known but can be assumed to be of  
26 several kinds (McGee and Martin 1995); that is, there is heterogeneity of stressors  
27 (not mutually exclusive) some of which may have been chronic while others were  
28 acute, leading to immediate death. Consequently it would be simplistic to treat the  
29 Sick Pen sample as a homogenous entity. For the Catastrophic sample, cause of  
30 death is a known, single event - not several. Any negative events they had  
31 experienced earlier, including, perhaps, time in the Sick Pen, were insufficient to  
32 cause them to die.

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50 The objectives of this study are: a) to measure relative response of forming  
51 tissue types (bone, enamel, dentin) to developmental stressors, within and between  
52 cohorts of sick pen (SP) and Control (Control) domestic pigs (*Sus scrofa*); b) examine  
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3 whether there is link between tissue mass and survivorship; and c) to evaluate  
4  
5 etiological influences on reduction of tissue mass.  
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8 **(Table 1 about here)**  
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## 10 **Materials and Methods**

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12 The sample of pigs used in this study (Table 1) has been reported in detail  
13  
14 previously (Skinner et al. 2014). . No animals were subject to experimental  
15  
16 procedures; nor were their deaths linked to this research. All animals died or were  
17  
18 slaughtered as part of the normal operations of hog production independent of the  
19  
20 conception or conduct of this research.  
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23  
24 Briefly, pigs were obtained from a hog supplier in the Fraser River valley of  
25  
26 British Columbia, Canada. The heads of 50 successive natural fatalities (termed Sick  
27  
28 Pen pigs) were procured, deep frozen, and de-fleshed for comparison with 20 pigs,  
29  
30 from the same source, which survived to slaughter age (termed Control pigs). All  
31  
32 animals were exposed to the same husbandry practices (Supplementary Materials).  
33  
34 Weight and age at death of the mortality cohort animals were recorded. No records  
35  
36 were kept of which were littermates, nor of size at birth. None was likely to have  
37  
38 been a runt (Widdowson 1971) since newly-born pigs with birth weights of less  
39  
40 than approximately one kg are not kept  
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### 43 *Heterogeneity in the Sick Pen assemblage: Developmental Outcomes*

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47 Marked variation in developmental fate of these animals necessitated the  
48  
49 creation of mortality sub-groups in order to make meaningful comparisons of  
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51 dental measures both within and between the attritional and catastrophic  
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53 assemblages (Fig. 1).  
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3 Figure 2 illustrates the relationship of weight and age at death or slaughter age for  
4 our sample in comparison to the Garth Standards (Carr 1998). Some SP pigs (green  
5 squares in Figure 2) failed to reach a minimum weight (85 kg) sufficient for  
6 slaughter despite remaining in the Sick Pen for weeks and months beyond normal  
7 slaughter age (ca. 6 months) presumably because of permanent damage to vital  
8 tissues. These pigs are classified as Sick Pen 'late deaths'. Such animals may have  
9 been switched into and out of the Sick Pen more than once in the hopes they would  
10 grow sufficiently for slaughter.  
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22 The remaining Sick Pen pigs died prior to normal slaughter age, anywhere  
23 between 0.22 to 0.43 years. These pigs are classified as Sick Pen 'early deaths'. Sick  
24 Pen 'early death' pigs were further subdivided into four groups based on age and  
25 physiological maturity. The latter evaluation is based on the averaged rank position  
26 of each pig in terms of three seriated physiological variables: dental formation (from  
27 radiographs), skull length, and weight at death (acknowledging that the last  
28 measure may have changed dramatically in the days leading up to death). We term  
29 this an overall Developmental Rank. The four groups are created from a simple  
30 dichotomization of age at death (above and below median age) and Developmental  
31 Rank (above and below mid-rank). These groups are deemed to reflect decreasing  
32 'felt stress' and ordered as follows: low age/low rank (SP 1); low age/high rank (SP  
33 2); high age/low rank (SP 3); high age/high rank (SP 4). These are conceptualized as  
34 'developmental outcomes'; they are used as analytical units to test for heterogeneity  
35 within the Attritional assemblage.  
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#### 54 *Dental measures*

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3 Dental formation for the maxillary and mandibular cheek teeth was recorded  
4 radiographically with a Fisher Portable 200 x-ray machine using standard  
5 procedures. Simple measures of the cranium, as well as mesio-distal length and  
6 bucco-lingual width for the third and fourth deciduous molars, were taken with  
7 Mitutoyo digital calipers. Mandibular first molar (usually left) tissue volumes were  
8 measured from microCT scans (Diondo, 130 kV, 130 mA, 0.5mm brass filter, 3060  
9 projections, 2 frame averaging, isometric voxel resolution of 20-25 microns).

10  
11  
12 Reconstructed TIFF image stacks of each first molar tooth were filtered with  
13 a macro that utilizes a three-dimensional median and a mean of least variance filters  
14 with a kernel size of 1 to 3 (Wollny et al. 2013). Filtering facilitates the manual  
15 segmentation of dental tissues and improves tissue grey-scale homogeneity, while  
16 preserving the morphology of dental structures. The filtered image stacks were  
17 imported into Avizo 6.3 and segmented semi-automatically, after which enamel  
18 volumes for each tooth were calculated from the segmentation.

19  
20  
21 Because cracks may display the same greyscale values as dentin, the enamel-  
22 dentin junction was extracted as a triangulated surface file (PLY format) and  
23 processed in Geomagic Wrap to remove any surface triangles that represent cracks;  
24 after which surface area measurements of the models were taken. Dentin volume  
25 was measured in Geomagic Wrap by sealing the bottom of each EDJ surface model  
26 with a consideration for the shape of the cervix.

27  
28  
29 In this study we evaluate the metrics of two dental tissues: enamel and  
30 dentin. Their apposition starts at the EDJ proceeding in opposite directions. Dentin  
31 volume is closely related to the size of the EDJ while enamel thickness is a function  
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3 of the amount and duration of secretion by ameloblasts. These may be considered as  
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5 two independent heterochronological processes (Kono 2004). In that we judge  
6  
7 volume measures to be more generally more informative of cellular function than  
8  
9 are more traditional linear variables, we created four direct measures and four  
10  
11 indirect measures; as follows;  
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13

#### 14 Primary variables

15  
16 Enamel cap volume ( $\text{mm}^3$ ): tissue segmented digitally above the enamel-  
17  
18 dentin junction  
19

20  
21 Coronal dentin volume ( $\text{mm}^3$ ): pulp chamber is incorporated in this  
22  
23 dimension  
24  
25

26  
27 Enamel-dentin junction (EDJ) surface area ( $\text{mm}^2$ ): area of convoluted contact  
28  
29 between enamel and dentin  
30

31  
32 Cervical area ( $\text{mm}^2$ ): horizontal plane at most cervical extent of enamel  
33

#### 34 Derived variables

35  
36 Enamel/dentin volume ratio: dimensionless index intended to detect relative  
37  
38 sensitivity to stress of the two tissue types  
39

40  
41 Average enamel thickness (mm): volume  $\div$  EDJ area  
42

43  
44 Average coronal dentin thickness (mm): volume  $\div$  EDJ area  
45

46  
47 EDJ area  $\div$  cervical area: dimensionless index intended to detect cervical  
48  
49 constriction, if any, arising between birth and crown completion  
50  
51 (ca. 3 months).  
52

#### 53 *Radiography:*

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3 In addition to assessing tooth formation, radiographs were taken of mandibular  
4 rami to evaluate disturbed bone growth (Harris lines and radiolucencies) and of  
5 fractured cranio-facial bones (Skinner et al. 2014).  
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10 *Enamel wear:*

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12 We expect Sick Pen pigs to have reduced dental tissues and reduced survivorship. In  
13 our study, enamel wear is a confounding variable in that longer-lived animals can be  
14 expected to have more worn teeth. If, despite this likelihood, it can be shown that  
15 reduced survivorship is significantly linked to reduced tissue mass, then the finding  
16 is strengthened; in other words the study design is conservative. Including animals  
17 with worn teeth increases the probability of Type II error (failing to detect a real  
18 difference), a disadvantage offset by an increase in statistical power. Enamel wear is  
19 recorded as none; trace faceting (indicating gingival eruption) and dentin exposure  
20 on one or more cups, visible in microCT images, indicating the progressive  
21 attainment of occlusal eruption. Most of the Control and Sick Pen 'late deaths' were  
22 sufficiently old that their first molars showed some (albeit slight) dentin exposure.  
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38 *Predictions and statistical analysis:*

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40 Markers of developmental stress are recorded in Table 1. We have no *a priori* reason  
41 to expect enamel and dentin to differ quantitatively in response to stress. We  
42 predict that tissue volumes will be greatest on average in Control pigs and least in  
43 the lowest age at death/lowest developmental rank cohort among the Sick Pen pigs.  
44  
45 However, in that crown formation is completed prior to death in our sample, there  
46 will possibly be some individuals in the Attritional assemblage who had experienced  
47 little or no developmental stress during crown formation but who died suddenly.  
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3 Specifically, we ask whether dental tissue volumes (enamel and dentin),  
4  
5 created in the first three months of a pig's life are linked to survivorship in the  
6  
7 succeeding months. If the answer is 'no', tissue volumes will not differ among  
8  
9 developmental outcome cohorts in the Attritional assemblage nor in comparison of  
10  
11 the latter to the Control/slaughter-age pigs. If yes, tissue volumes will differ  
12  
13 significantly between early and later deaths in the Attritional assemblage; and we  
14  
15 can ask in what direction (increase or decrease) do the volumes change.  
16  
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19  
20 Evaluation of differences among the developmental outcome groups was  
21  
22 performed with the non-parametric median test, which tests whether the groups  
23  
24 are sampled from a population in which the median of the test variable is the same.  
25  
26 The alternative, Kruskal-Wallis test is sensitive to outliers and less suitable for our  
27  
28 data. Alpha was set at 0.05. Strictly speaking, in that we do not consider it  
29  
30 biologically realistic to expect developmental stress to increase dental tissue  
31  
32 volumes, we could have used one-tailed tests of significance, since our prediction is  
33  
34 that the mortality cohort will have lessened tissue volumes; however, we felt it  
35  
36 would be more conservative to use two-tailed tests.  
37  
38  
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42

### 43 **Results**

44  
45 Figure 3 shows the relationship of age at death to Developmental Rank and  
46  
47 illustrates the distribution of SP 'early death' cohorts (weight and physiological  
48  
49 maturation are not recorded for individual Control pigs). As may be seen, Sick Pen  
50  
51 'late deaths', despite being markedly older, have physiological ranks noticeably less  
52  
53 than expected in comparison to the trend for the Sick Pen 'early death' pigs; in other  
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2  
3 words, as a group, 'late death' pigs are dentally immature, small-headed, and/or  
4  
5 light-weight.  
6

#### 7 *Comparative development at birth*

8  
9  
10 While weights were not recorded for these animals at birth we do have tooth  
11  
12 dimensions for teeth formed prenatally and those formed both prenatally and  
13  
14 postnatally (Skinner et al. 2014) which are not part of this particular study of lower  
15  
16 first molars whose crown formation is almost entirely (ca. 90%) postnatal (based  
17  
18 on location of the neonatal line visible in a thin section). Table 2 compares dental  
19  
20 areas for deciduous lower third molars (prenatal) and deciduous upper fourth  
21  
22 molars (both). It can be concluded that the Sick Pen and Control pigs start out with  
23  
24 similar tooth sizes at birth but later-forming teeth come to differ postnatally.  
25  
26  
27

28  
29 **(Table 2 about here)**  
30

#### 31 *Sex differences*

32  
33 There are equal numbers of males and females in the Control sample while 20 of our  
34  
35 50 Sick Pen pigs are males. The distribution of sexes between the two Groups (Table  
36  
37 3) does not differ significantly (Pearson's Chi Square=0.583, Fisher's Exact Test  
38  
39 P=0.594).  
40  
41

42  
43 Mann-Whitney tests indicated no sex differences in dental volumes and areas  
44  
45 in the sample as a whole or among the separate groups (similar results were  
46  
47 obtained with parametric tests). Consequently, later analyses lump the sexes.  
48  
49

50 **(Table 3 about here)**  
51

#### 52 *Comparison of assemblages*

1  
2  
3 A simple comparison of the two assemblages shows only one statistically significant  
4 difference; that being the derived variable of average dentin thickness (Table 4).  
5  
6 Simply put, with only one exception, the Attritional and Catastrophic assemblages  
7  
8 are statistically indistinguishable. However, it is noteworthy that in every primary  
9  
10 measure, the Sick Pen tissues are smaller and more variable than are those of the  
11  
12 Controls. These observations suggest there may be masked heterogeneity in the Sick  
13  
14 Pen assemblage.  
15  
16  
17  
18

19  
20 **(Table 4 about here)**

21  
22 *Heterogeneity within Sick Pen assemblage*

23  
24 Tissues masses are compared among the Sick Pen 'early deaths', 'late deaths' and  
25  
26 Controls (Table 5). The 'late deaths' cohort has the largest tissue masses and the  
27  
28 'early deaths' group of cohorts, the smallest; Controls have middling values. Given  
29  
30 this situation, it is appropriate to ask whether the Sick Pen 'early deaths' are  
31  
32 statistically distinguishable from either of the other cohorts.  
33  
34  
35

36  
37 **(Table 5 about here)**

38  
39 In a comparison within the Sick Pen assemblage, statistically significant  
40  
41 differences in dental measure (ca. 12%) are observed between the 'early' and 'late  
42  
43 death' groups in dentin volume and area of the cervix (Table 5). Interestingly, the  
44  
45 ratio of enamel volume to dentin volume is higher in the SP 'early deaths'. Clearly, all  
46  
47 three variables reflect coronal dentin space, which is reduced in those pigs that died  
48  
49 before slaughter age.  
50  
51

52  
53 In a comparison between the 'SP early deaths' and 'Control' pigs (i.e.,  
54  
55 excluding the 'late death' cohort), the former group of cohorts show significantly  
56  
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1  
2  
3 reduced (ca. 5%) dentin volume and average dentin thickness and, consistently,  
4 heightened ratio of enamel to dentin volume (Table 5); again, all are attributable to  
5  
6 reduced coronal dentin in the Attritional mortality cohorts.  
7  
8  
9

10 Evaluation of enamel volume and related variables is problematic due to  
11 small samples, occasioned by enamel wear in the 'late deaths' cohort. The Sick Pen  
12 'early deaths' show a statistically non-significant reduction in enamel of about 5%  
13 compared to the other groups.  
14  
15  
16  
17  
18

### 19 *Comparison of developmental outcome cohorts*

20  
21 A finer-grained analysis of median values of tissue masses among developmental  
22 outcome cohorts indicates that, with the exception of coronal dentin mass, median  
23 values of all primary and secondary variable measures vary significantly among  
24 cohorts (Table 6). A graphical depiction of these results is shown in Figures 5 thru  
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In terms of the primary variables, enamel cap volume (Fig. 5) and EDJ surface  
area (Fig. 7) decline with survivorship among the 'early deaths' and contrast with  
the 'late death' Sick Pen pigs ( $P=0.038$  and  $0.024$ , respectively). Volume of coronal  
dentin does not vary significantly among developmental cohorts ( $P=0.297$ ) but is  
greatest in the SP 'late deaths' (Fig. 6). Area of the cervix (Fig. 8) is significantly  
smaller in the SP 'early deaths' compared to the 'late deaths' ( $P=0.048$ ). The derived  
variables confirm these impressions, with the relative proportion of enamel to  
dentin (Fig. 9) and average enamel thickness (Fig. 10) declining with age at death  
among the 'early deaths' ( $P=0.0004$  and  $0.028$ , respectively). Interestingly, average  
dentin thickness (Fig. 11) increases significantly among developmental cohorts  
ranged from 'worst' to 'best' outcome ( $P=0.004$ ); a result which can come about

1  
2  
3 from reducing EDJ area and/or increasing dentin volume. Cervical constriction (Fig.  
4 12) is most marked in the two low age Sick Pen cohorts (P=0.043). Finally and  
5 notably, median values of tissue measures in 'late death' pigs exceed those of the  
6 Control pigs for all primary variables. In conclusion, analysis by developmental  
7 cohorts reveals marked heterogeneity of both enamel and dentin tissue mass  
8 measurements among developmental outcome cohorts.  
9

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16  
17 **(Table 6 about here)**  
18

19 *Survivorship*

20 The Control pigs were all slaughtered at about the same stage of development.  
21  
22 Consequently the following analysis compares tissue masses and age at death  
23 among developmental outcome cohorts excluding Controls whose individual age at  
24 death are not known. The bottom panel of each of Figures 5 thru 12 shows the  
25 relationship. For most variables the tissue measure changes with age at death in the  
26 Sick Pen 'early deaths' while the 'late deaths' cohort's position is offset and clearly  
27 does not continue the former group's trend.  
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38 The most striking contrast in the analysis of all six cohorts together,  
39 described above, is the behavior of the enamel versus dentin tissues. Enamel volume  
40 is least in the longest surviving Sick Pen 'early deaths' cohort while measures of  
41 dentin mass either remain constant (volume) or increase (cervix area) in the longer-  
42 lived assemblages. The derived measures remain consistent with the pattern shown  
43 by the primary variables. These impressions-that the Sick Pen 'early deaths' cohorts  
44 as a group show heterogeneity-are examined next.  
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54 *Heterogeneity within the Sick Pen 'early deaths' grouping*  
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3 This grouping is the largest ( $n \leq 42$ ) and has the greatest potential for providing  
4 insights into the response of dental tissues to stress. However, when broken down  
5 into four separate cohorts the samples are small and the patterns need to be  
6 scrutinized for consistency (Table 7).  
7  
8  
9  
10  
11

12 **(Table 7 about here)**  
13

14  
15 Fundamentally, enamel measures (including EDJ surface area) decline with  
16 increased survivorship while dentin measures remain fairly constant or increase  
17 non-significantly. These trends are shown most clearly by the reduction of enamel  
18 to dentin volume ratio (Fig. 9). Average dentin thickness increases with  
19 survivorship, which seems to be driven by the marked (but not statistically  
20 significant) reduction in EDJ surface area with increased age at death (Fig. 7).  
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29 The decline of enamel measures with increased survivorship holds even in  
30 pigs with no wear at all. There is a decline in enamel volume ranging between 10  
31 and 16% compared among wear groups (Fig. 13).  
32  
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34  
35

36 *Bone mass and developmental outcome*  
37

38 We assess bone mass through a reconsideration of 'crypt fenestration', previously  
39 reported (Skinner et al. 2014), occurring over the forming last molar crypt area of  
40 the pig maxilla among these pigs. The Sick Pen assemblage showed 82% with bone  
41 thinning (crypt fenestrations) compared to 55% in the Controls. In a cross-tabs  
42 analysis, equal proportions (roughly 85%) of both SP 'early' and 'late deaths' show  
43 crypt fenestrations (Chi square=0.087,  $P=0.768$ ). Similarly, crypt fenestrations are  
44 evenly distributed among the four cohorts comprising the SP 'early deaths' (Chi  
45 square =4.987,  $P=0.173$ ). It can be concluded that bone thinning is more common in  
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3 the Sick Pen assemblage as a whole but otherwise does not vary with developmental  
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5 outcome. Bone thinning is common to Sick Pen 'early' and 'late deaths' groups, both  
6  
7 of which share a pattern of growth faltering (Fig. 2).  
8  
9

#### 10 *Summary of results on hard tissue analysis*

11  
12 1. In terms of measures of both enamel and dentin, Controls are virtually  
13  
14 indistinguishable from the SP mortality assemblage due to marked heterogeneity  
15  
16 among developmental outcome cohorts. Sick Pen 'late deaths', which as a group  
17  
18 lived beyond customary slaughter age, show significantly larger dentin-related  
19  
20 measures in comparison to both 'early deaths' and Controls.  
21  
22

23  
24 2. In terms of the relationship of dental measures to survivorship, SP 'early deaths'  
25  
26 show significantly declining enamel volume and, not surprisingly, decreasing  
27  
28 enamel to dentin volume ratio. Both enamel and dentin thicknesses increase  
29  
30 significantly with age at death, which can be attributed to the non-statistically  
31  
32 significant reducing EDJ surface area. EDJ surface area as a variable behaves more  
33  
34 like enamel than it does like dentin. This makes sense since enamel extension  
35  
36 dictates in part the size of the EDJ.  
37  
38

39  
40 3. Maxillary bone mass in the last molar crypt area is more reduced in the Sick Pen  
41  
42 mortality assemblage than in the Control pigs.  
43  
44

#### 45 *Etiological influences on tissue mass*

46  
47 Given the observed patterns of metrical change or stasis in tissue measures, we turn  
48  
49 now to a consideration of probable causes. We will examine growth attainment,  
50  
51 bone formation and hard tissue lesions. All the Sick Pen pigs are light for their ages  
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53 (Fig. 2). It can be assumed that growth faltering has occurred, as further attested by  
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3 the evidence from crypt fenestrations. Although food quality and ration were tightly  
4 controlled for our sample, malnutrition might occur through social competition or,  
5 more likely in the case of husbanded pigs, through disease. For example, infection  
6 can produce malnutrition through diarrhea and anorexia, so clearly the two  
7 stressors are not mutually exclusive. However, some of the pigs show clear evidence  
8 of infection-particularly, inflamed and highly remodeled auditory bullae. In our  
9 experience, pigs affected with chronic middle ear infection thrive (somewhat),  
10 continue to eat, but are slow growers.  
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22 Our approach to elucidating etiology is to compare tissue volumes among  
23 three groups: those pigs with bone formation problems, those with infection (with  
24 or without problems of bone formation) and those with no evidence of hard tissue  
25 pathology. Table 1 lists a variety of hard tissue responses by the pigs to stressors. It  
26 is presumed that all the fractured facial bones (with two exceptions-pigs 5 and 9-  
27 which have a well-healed zygomatic and coronoid process fracture, respectively)  
28 occurred after first molar crown formation was completed since the broken edges  
29 show little or no remodeling. All fractures are ignored for purposes of analyzing  
30 tissue mass. Similarly, there are a few instances of infection on crowns or roots that  
31 are clearly post-eruptive in timing. These are excluded from the 'infected' sample in  
32 the following analyses of tissue mass. For analytical purposes here, we have chosen  
33 to divide the pigs into the following non-mutually exclusive categories (Table 8):  
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- 50 a) Pigs with no hard tissue lesions
- 51  
52 b) Bone formation lesions; i.e., mandibular ramus abnormalities such as  
53 Harris lines and radiolucent borders (Skinner et al. 2014) (excluding  
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3 facial fractures). Pigs with abnormal rami plus infection are placed in the  
4  
5 next grouping.  
6

- 7  
8 c) Pigs with well-established hard and soft tissue changes judged to be due  
9  
10 to infection (with or without problems of bone formation) (including  
11  
12 remodeled auditory bullae, bone plaques, eroded TMJ, dental and soft  
13  
14 tissue abscesses).  
15

16  
17 **(Tables 8 and 9 about here)**  
18

19  
20 It can be seen in Table 8, which describes tissue measures for all three groups, that  
21  
22 the presence of infection only significantly reduces dentin volume and EDJ surface  
23  
24 area. A simple dichotomization of the sample into uninfected versus infected  
25  
26 animals shows that the presence of infection is associated, in addition, with a  
27  
28 reduction in area of the cervix (Table 9).  
29

30  
31 The reduction in enamel cap volume of about 11% in the animals with  
32  
33 chronic infection is not quite statistically significant (two-tailed  $P=0.091$ ). The  
34  
35 inclusion of pigs with worn enamel on their first molars increases sample size to the  
36  
37 point where the ratio of median enamel mass in infected to uninfected pigs parallels  
38  
39 that for dentin (Fig. 14). As noted earlier, the comparability of enamel volume ratios  
40  
41 in unworn and worn teeth, boils down to whether infected animals have more worn  
42  
43 teeth than they should. Average wear score (number of affected cusps, ranging from  
44  
45 0 to 4) is 1.9 in non-infected animals and 1.4 in infected animals, i.e., infected  
46  
47 animals had less worn teeth than the uninfected animals. This implies that, if  
48  
49 anything, inclusion of worn teeth would act to reduce enamel cap volumes more in  
50  
51 the non-infected animals. Thus, the result-that infected animals showed smaller  
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3 enamel caps and dentin measures-is believable. We conclude that a marked  
4  
5 biological significance is being signaled in which infection is the likely cause of  
6  
7 differences in tissue mass.  
8  
9

10 *Disease, bone formation, and survivorship*

11  
12 We turn now to a consideration of whether animals with hard tissue lesions  
13  
14 (diseased) show also reduced bone mass. We test this notion through examination  
15  
16 of animals, with and without infection, who showed crypt fenestration (bone) and  
17  
18 crypt fenestration enamel defects (CFEDs-dental hypoplasia due to crypt  
19  
20 fenestration (Skinner et al. 2016)). Crypt fenestrations, reflecting bone thinning, are  
21  
22 observed in 75% (9/12) of infected pigs and 76% (44/58) of uninfected pigs while  
23  
24 CFEDs are found in 75% (9/12) of infected pigs and 84% (49/58) of uninfected pigs.  
25  
26 The differences are not statistically significant (data not shown). An examination of  
27  
28 those pigs with diseased auditory bullae (see below) shows that the two pigs with  
29  
30 most severely affected bullae do not show crypt fenestrations, further negating the  
31  
32 view that bone mass is affected by chronic disease in these pigs. By sharp contrast,  
33  
34 abnormal bone formation (as shown by Harris lines and radiolucencies in the  
35  
36 mandibular rami) is seen in 66% (38/58) of uninfected animals but 92% of infected  
37  
38 animals (Likelihood ratio=3.911, P=0.048). In this case, disease is linked to  
39  
40 abnormal bone growth.  
41  
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47  
48 Well-established bone infection takes several forms in these pigs but  
49  
50 inflamed auditory bullae are the most striking (Fig. 15). Severity of the latter was  
51  
52 judged on the basis of number and size of foramina, cloacae, swelling, shell-like  
53  
54 formation, and bi-laterality. The relationship between age at death and dental  
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3 tissues (ratio of enamel to dentin) as well as severity of affected bullae is shown in  
4  
5 Fig. 16. As survivorship increases, the relative amount of enamel decreases and the  
6  
7 severity of disease declines. It seems likely that disease is a contributor to earlier  
8  
9 death and enamel thinning. The likely agents for infection of the auditory bullae are  
10  
11 oral/aural bacteria (Olson 1981).  
12  
13

14  
15 While, as seen, bone mass does not seem to link to disease, bone formation  
16  
17 does. Likewise, diseased animals show a reduction in enamel and dentin of about 11  
18  
19 and 9%, respectively (Table 9, Fig. 14).  
20  
21

## 22 23 24 **Discussion**

25  
26 We acknowledge that our sample sizes, especially for sub-divided mortality  
27  
28 cohorts, are small. We had not anticipated the observed complexity of tissue  
29  
30 responses to stress and, hence our interpretations are cautious. Nevertheless, the  
31  
32 general approach of drawing upon readily-available domestic farm animals, whose  
33  
34 individual development is closely monitored by cost-conscious producers, we would  
35  
36 argue is an almost untapped resource to address theoretical issues in skeletal  
37  
38 biology (Skinner 2017). The observed tissue reductions noted above are small in  
39  
40 absolute terms but in relative terms (ca. 10%) are comparatively quite large; e.g.,  
41  
42 permanent stature reduction resulting from the notoriously severe 'Dutch famine'  
43  
44 amounted to about 2% (Portrait et al. 2017).  
45  
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50  
51 Here we will revert to using the terms 'Attritional' and 'Catastrophic' as one  
52  
53 of our primary goals in this research was to see if dental tissue masses differed  
54  
55 between a natural mortality cohort and a living assemblage that had died  
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3 catastrophically. At the outset of this study, our expectation was that pigs, which had  
4 experienced sufficient developmental stress to kill them, would show reduced tissue  
5 volumes. In a crude comparison of the two assemblages (Sick Pen and Control pigs),  
6 this is not true. Fundamentally, in explanation of this finding, the Sick Pen  
7 assemblage is markedly heterogeneous both in terms of age at death and in  
8 measurements of dental tissues. Those Sick Pen pigs who lingered past customary  
9 slaughter age (so-called 'late deaths') had large coronal dentin volume exceeding  
10 that of Control pigs. Enamel measures did not differ among the three groups.  
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22           However, with the analytical creation of six developmental cohorts based on  
23 the combination of age at death and physiological maturation, it became clear that,  
24 counter-intuitively, enamel volume declined with increasing survivorship among  
25 the four Sick Pen 'early death' cohorts. We will return to this anomaly shortly.  
26  
27 Dentin showed no such pattern. Estimation of bone mass from crypt fenestration  
28 indicates that crypt wall thinning is more common in the mortality cohort but does  
29 not vary within this assemblage. It can be seen that each of the three tissue types  
30 (enamel, dentin, bone) responds differently to developmental stress.  
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41           In the hope of understanding these contrasts, recourse was made to disease  
42 history. Chronic disease, as exemplified most dramatically by florid remodeling of  
43 auditory bullae, is assumed here to have prevailed during dental crown tissue and  
44 bone formation. Bone mass is not linked to disease in this study. However, the  
45 presence of disease links significantly with abnormal bone formation and a  
46 reduction of dental tissue volumes (enamel ca. 11%, dentin ca. 9%). More severe  
47 chronic infection is linked to a relative decrease in enamel volume and in  
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3 survivorship. Significantly, neither the 'late deaths' nor Controls show infection of  
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5 this nature.  
6  
7

8         The decline of enamel volume with increased survivorship among cohorts  
9  
10 within a mortality assemblage is unexpected and requires consideration. As noted in  
11  
12 this study, enamel thinning is linked to chronic infection. A cardinal sign of  
13  
14 inflammation is fever (Kreshover and Clough 1953) and a common explanation for  
15  
16 enamel thinning in the form known as enamel hypoplasia is fever (El-Najjar et al.  
17  
18 1978). It would seem that enamel thinning is a positive sign (increased  
19  
20 survivorship) but also signals the presence of disease. This conundrum can be  
21  
22 resolved by recourse to the literature on whether fever is adaptive (Kluger 1986).  
23  
24 This is considered an old dispute (Romanovsky and Szekely 1998) which has been  
25  
26 largely resolved in the sense that fever is now considered an adaptive  
27  
28 thermoregulatory response which, despite being metabolically costly, often leads to  
29  
30 enhanced immune response, inhibition of a pathogen (Hart 1988) and which confers  
31  
32 a survival benefit. Fever acts as a systemic alert system for surveillance of invading  
33  
34 pathogens by enhancing the immune system (Elliot et al. 2002; Evans et al. 2015).  
35  
36 The latter authors conclude that the survival benefit conferred on the host  
37  
38 outweighs the metabolic cost of fever.  
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45         In terms of the response of enamel to fever, it has been shown  
46  
47 experimentally, by injecting turpentine into rats in order to produce a fever, that it  
48  
49 is the fever itself not the disease that produces enamel defects (Tung et al. 2006).  
50  
51 Also enamel is more sensitive to fever than is dentin (Kreshover and Clough 1953;  
52  
53 Ryyänänen et al. 2014). The decline in relative proportion of enamel to dentine  
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3 shown in Figure 16 conforms with experimental evidence that the ratio of enamel to  
4  
5 dentin height in developing mouse molars reduces with duration of fever (Ryynänen  
6  
7 et al. 2014).  
8  
9

10 Notably, older animals from the Attritional mortality and Catastrophic  
11  
12 assemblages have no evidence of chronic infection. By inference 'early deaths' are  
13  
14 linked to infection. In our study, infection is associated with reduced dental  
15  
16 volumes. One would expect the thinnest enamel in the youngest deaths. This is not  
17  
18 so. Longer-lived 'early death' pigs have thinner enamel. In that enamel formation is  
19  
20 completed before death, their advantage arose during crown formation when  
21  
22 combatting infection, presumably through the benefits of fever which, inadvertently,  
23  
24 acted to reduced enamel.  
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29 One of the motives for undertaking this study was to assess whether enamel  
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31 thickness could be used as a proxy for developmental well-being much as stature is  
32  
33 used in human growth studies. Just as there are myriad factors that can affect  
34  
35 growth in stature, ranging from genetics to malnutrition and disease, we hoped that  
36  
37 enamel thickness could be a powerful summation of developmental experience. If  
38  
39 so, we could draw upon insights taken from the literature of growth studies,  
40  
41 commencing some two centuries ago, which has so enriched our understanding of  
42  
43 human biology (Bogin 1999; King and Ulijaszek 1999) and guided our approaches to  
44  
45 epidemiology (Steckel 1995). Our zeal has been tempered by the results of this  
46  
47 study which suggest that in domestic pigs, subject to developmental stress, each oral  
48  
49 hard tissue has a somewhat different story to tell. The presence of chronic disease,  
50  
51 likely accompanied by bouts of fever, is a key factor. Simply put, abnormal bone  
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3 formation and reduced dentin mass are linked to reduced survivorship while  
4  
5 enamel reduction is linked to enhanced survivorship.  
6

7  
8 The other motive for this study was to determine if a domestic pig model  
9  
10 could contribute to the important debate subsumed under the rubric 'osteological  
11  
12 paradox' which we summarized earlier. We acknowledge that, whatever the findings  
13  
14 of our research, we have to be cautious about generalizing the results to other  
15  
16 species. Domestic pigs are selected and husbanded to gain weight extremely fast  
17  
18 and efficiently. It is not likely that their tissue biology, diseases and relative  
19  
20 survivorship can be applied directly to ancient humans.  
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23

24 In our study, we have the advantage of knowing, with precision, age at death  
25  
26 which allows us to test for differentially distributed measures of dental tissues and  
27  
28 to evaluate the role of chronic disease. It is very clear from our results that the  
29  
30 Attritional assemblage is much worse off developmentally and in terms of  
31  
32 survivorship than are the pigs that make it to slaughter. However, in terms of dental  
33  
34 tissue measurements, the two groupings are indistinguishable, simply because the  
35  
36 Attritional assemblage shows marked heterogeneity in survivorship (early and late  
37  
38 death groups) with notably larger coronal dimensions in the latter cohort. In that  
39  
40 the Control cohort shows intermediate tissue measures, this cohort may be a  
41  
42 product of stabilizing selection.  
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47 While most of the Sick Pen 'early death' pigs show problems of size and hard  
48  
49 tissue formation, a notable component (16%) of the Attritional assemblage (the Sick  
50  
51 Pen 'late deaths'), if we may generalize, have healthy-looking, large hard tissues, and  
52  
53 survived longer but, nevertheless, still died. Whether such laggards are a typical  
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3 component of most Attritional assemblages awaits confirmation. In sum, most of our  
4  
5 'early death' domestic pigs show compromised hard tissues, signaling real problems  
6  
7 of survivorship. The 'late deaths' are developmentally retarded, healthy-looking and  
8  
9 survived a little longer before becoming part of a mortality cohort. In pigs,  
10  
11 avoidance of disease, or the ability to overcome infection, is key to longer survival.  
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14  
15 In terms of the 'osteological paradox', relatively lesion-free and large-toothed  
16  
17 cohorts are found in both the Attritional and Catastrophic assemblages. Short-lived  
18  
19 pigs, as a group, tend to show chronic infection, abnormal bone formation and  
20  
21 reduced volumes of dentin and enamel. However, diminished enamel per se is  
22  
23 clearly linked to enhanced survivorship among pigs inferred to have been  
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25 experiencing fever.  
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### 31 **Conclusions**

32  
33 All three oral hard tissues (bone, dentin and enamel) respond in their own  
34  
35 characteristic way to developmental stressors. We can link clear signs of infection to  
36  
37 dental tissue reduction and abnormal bone formation. Bone mass is evincing  
38  
39 primarily malnutrition not disease. Both enamel and dentin are reduced (about  
40  
41 10%) in response to long-standing disease during crown formation. Paradoxically,  
42  
43 reduced dentin signals lower survivorship while reduced enamel signals enhanced  
44  
45 survivorship. Extrapolating from pigs, we conclude that meaningful comparison of  
46  
47 attritional and catastrophic assemblages necessitates the recognition of  
48  
49 developmental outcome cohorts to reveal heterogeneity of survivorship, tissue  
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51 measures and lesions.  
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Table 1. Composition of sample separated by group (Control and Sick Pen) and sex, seriated by age at death with hard and soft tissue abnormalities indicated.

					Hard tissue abnormality									Soft tissue
					Skeletal					Dental				
Group	Sex	Pig ID <sup>1</sup>	Age (yrs) <sup>1</sup>	Wt (kg) <sup>1</sup>	Crypt fenestration	Facial fracture	Bulla/TMJ infection	Man. ramus abnormal	Bone plaque	CFED on M1 <sup>2</sup>	Root constriction	Cr/Root pitting	abscess	abscess
Control	F	53	.49	108	X					no				
		54	.49	108						X				
		55	.49	108						X				
		59	.49	108	X					X				
		61	.49	108	X					X		X		
		62	.49	108	X					X				
		63	.49	108	X			X		no	X	X		
		64	.49	108						no	X			
		69	.49	108				X		X		X		
		70	.49	108						no				
	M	51	.49	108						X				
		52	.49	108	X			X		no				
		56	.49	108						X				
		57	.49	108	X					X				
		58	.49	108	X					X				
		60	.49	108				X		no				
		65	.49	108						no				
		66	.49	108	X					X				
		67	.49	108	X			X		no		X		
		68	.49	108	X			X		no				
Sick Pen	F	12	.25	31.8	X			X						
		13	.25	29.6	X			X						
		36	.25	22.7	X	X	X	X						X
		18	.27	18.2	X	X		X						
		2	.27	31.8	X							X		
		3	.27	31.8	X			X						
		40	.27	25.0	X			X						
		11	.28	40.9	X			X						
		28	.28	45.5	X			X						
		8	.28	38.6	X			X				X		
		34	.30	59.1		X	X	X		no		X		
		22	.31	38.6			X			X				
		23	.31	40.9	X									
		38	.31	40.9	X			X		no				
		46	.33	36.4	X			X						
		48	.33	54.6				X		X	X			
		6	.33	50.0	X			X		X				X
		7	.33	45.5	X			X		X				
		47	.34	38.6			X	X		no		X		
		1	.35	40.9		X		X		no				
		15	.39	50.0				X		no				
		29	.39	56.8	X		X	X	X	no				
		25	.42	63.6	X			X		X				
		35	.42	63.6	X			X		X				

		26	.43	77.3	X			X		X		X		
		19	.50	45.5	X			X		X	X			
		33	.53	68.2	X			X		X				
		41	.58	45.5	X			X		X	X			
		42	.62	79.6	X					X		X		
		39	.66	68.2		X		X		X	X			
	M	30	.22	18.2	X			X						
		16	.25	31.8	X			X						
		37	.25	22.7	X			X				X		
		21	.28	31.8	X			X			X			
		31	.30	18.2	X	X					X			
		20	.31	45.5	X		X	X		X	X			
		24	.33	45.5	X					X				
		27	.33	50.0	X	X	X	X		X				
		45	.35	50.0	X			X						
		49	.35	54.6	X		X	X		no			X	
		9	.35	47.7	X	X <sup>3</sup>	X	X		X				
		17	.38	50.0	X	X		X		X	X	X		
		50	.38	43.2	X		X	X		no	X		X	
		43	.39	54.6		X <sup>4</sup>				X				
		5	.39	61.4	X	X <sup>3</sup>		X			X			
		32	.44	59.1	X		X	X		no	X			
		44	.45	72.7	X					X	X			
		4	.50	63.6	X			X		X				
		10	.58	77.3	X			X		X				
		14	.59	95.5				X		X				

1. Ages at death and weight for Control pigs presumed to be normal age at slaughter weight
2. Crypt fenestration enamel defect (Skinner et al. 2014; Skinner et al. 2016)
3. Healing or healed fracture
4. Maggots in mouth

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2  
3 Table 2. Deciduous molar tooth area (mm<sup>2</sup>) for molars that form prenatally versus  
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5 those that form both prenatally and postnatally compared between all Sick Pen pigs  
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7 and Control pigs.  
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<u>Group</u>	<u>Tooth type</u>	<u>N</u>	<u>DM3 area</u>	<u>SD</u>	<u>'t'-value</u>	<u>Prob.</u>
Sick Pen	Lo DM3 <sup>1</sup>	50	49.4	4.6	-0.452	0.653
Control		20	50.0	5.0		
Sick Pen	Up DM4 <sup>1</sup>	49	140.0	13.3	-2.773	0.007
Control		20	149.6	12.7		

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19 1. Lo=lower, Up=upper, D=deciduous, M=molar  
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Table 3. Male and female unworn enamel and dentin volumes in lower first molars:

Sick Pen 'early death' pigs, Sick Pen 'late death' pigs and Control (there are no unworn male enamel crowns among the 'late deaths' group)

Primary measure	Group	Sex	N	Mean	SD	Median	Mann-Whitney <sup>1</sup> Z	Prob
Enamel Volume <sup>2</sup>	All	Male	8	313.9	55.1	297.3	-0.722	0.470
		Female	18	319.5	29.5	323.8		
EDJ area		Male	30	422.4	46.2	423.8	-0.332	0.740
		Female	40	424.2	32.4	424.3		
Dentin volume		Male	30	617.0	86.7	606.7	-.166	0.868
		Female	40	615.0	59.1	612.6		
Cervical area		Male	28	138.3	15.2	135.1	-1.148	0.251
		Female	38	141.9	11.3	140.9		
Enamel volume	Cohorts	Sick Pen						
		Male	8	313.9	55.1	297.3	-0.722	0.470
		Female	18	319.5	29.5	323.8		
		Early deaths						
		SP						
		Male	0					
EDJ area	SP	Late deaths						
		Female	0					
		Control						
		Male	0					
Dentin volume	SP	Female	0					
		Male	17	418.5	52.0	403.9	-0.756	0.450
		Female	25	420.7	31.2	418.7		
		Early deaths						
		SP						
		Male	3	436.7	37.5	432.7	-0.447	0.655
Female	5	451.4	43.3	464.3				
Cervical area	Control	Male	10	424.7	40.6	427.8	-0.492	0.623
		Female	10	419.6	25.7	423.4		
		Male	17	583.5	73.6	571.7	-1.294	0.196
		Female	25	603.4	53.3	607.8		
Enamel volume	SP	Male	3	669.3	121.2	695.6	-0.149	0.881
		Female	5	662.4	75.3	679.3		
		Control						
		Male	10	658.2	80.0	661.5	-1.134	0.257
		Female	10	620.5	58.2	606.4		
		Cervical area	SP	Male	15	133.2	14.9	134.2
Female	23			138.9	10.1	134.6		
Early deaths								
SP								
Male	3			145.8	19.1	153.4	-0.149	0.881
Female	5			151.8	10.6	152.2		
EDJ area	Control	Male	10	143.7	12.9	142.8	-0.076	0.940
		Female	10	143.7	12.0	142.1		

1. Parametric tests gave the same results as non-parametric tests
2. Enamel volumes are for molars with no wear

Table 4. Comparison of dental tissue measures between assemblages

Measure		Assemblage				Statistical test	
		Sick Pen	CV	Control	CV	M-W Z	Prob.
Enamel cap volume	n	50		20			
	Mean	916.8		950.1			
	SD	102.0	11.1	99.2	10.4		
	Median	920.7		948.4		-1.092	0.275
Coronal dentin volume	n	50		20			
	Mean	606.5		639.4			
	SD	70.5	11.6	70.8	11.1		
	Median	604.8		628.6		-1.625	0.104
EDJ junction surface area	n	50		20			
	Mean	424.0		422.1			
	SD	40.9	9.6	33.2	7.9		
	Median	420.7		427.8		-0.228	0.820
Cervical area	n	46		20			
	Mean	138.9		143.7			
	SD	13.4	9.6	12.1	8.4		
	Median	135.1		142.2		-1.305	0.192
Enamel/dentin volume ratio	n	50		19			
	Mean	0.51		0.49			
	SD	0.05	10.2	0.05	9.8		
	Median	0.51		0.49		-1.599	0.110
Average enamel thickness	n	50		20			
	Mean	0.73		0.73			
	SD	0.05	6.8	0.05	6.8		
	Median	0.73		0.74		-0.176	0.860
Average dentin thickness	n	50		20			
	Mean	1.43		1.51			
	SD	.08	5.6	0.08	5.3		
	Median	1.42		1.51		-3.614	0.0003
Cervical constriction	n	46		20			
	Mean	3.02		2.94			
	SD	0.16	5.3	0.16	5.4		
	Median	3.03		2.96		-1.702	0.089

Table 5. Ratios (in **bold face**) of median dental tissue Primary measures of Sick Pen 'early deaths' or Sick Pen 'late deaths' to Control sample (values for enamel based on only teeth with no or, at most, one cusp with dentine exposure)

	Sick Pen 'early deaths'				Sick Pen 'late deaths'			Control	
	<u>N</u>	<u>Median</u>	<u>Ratio to Late deaths</u>	<u>Ratio to Control</u>	<u>N</u>	<u>Median</u>	<u>Ratio to Control</u>	<u>N</u>	<u>Median</u>
Enamel volume	36	313.1 <sup>1,9</sup>	<b>.95</b>	<b>.94</b>	4	328.0 <sup>1</sup>	<b>.99</b>	12	332.7 <sup>9</sup>
EDJ area	42	417.4 <sup>2,10</sup>	<b>.93</b>	<b>.98</b>	8	450.1 <sup>2</sup>	<b>1.05</b>	20	427.8 <sup>10</sup>
Dentin volume	42	597.9 <sup>3,11</sup>	<b>.87</b>	<b>.95</b>	8	687.5 <sup>3</sup>	<b>1.09</b>	20	628.6 <sup>11</sup>
Cervix area	42	134.4 <sup>4,12</sup>	<b>.88</b>	<b>.95</b>	8	152.8 <sup>4</sup>	<b>1.07</b>	20	142.2 <sup>12</sup>
E/D ratio	36	0.53 <sup>5,13</sup>	<b>1.10</b>	<b>1.04</b>	4	0.48 <sup>5</sup>	<b>.94</b>	19	0.51 <sup>13</sup>
Enam thickness	36	.75 <sup>6,14</sup>	<b>1.03</b>	<b>.99</b>	4	0.73 <sup>6</sup>	<b>.96</b>	20	0.76 <sup>14</sup>
Dentin thickness	42	1.42 <sup>7,15</sup>	<b>.97</b>	<b>.94</b>	8	1.47 <sup>7</sup>	<b>.97</b>	20	1.51 <sup>15</sup>
Cerv. constriction	42	3.03 <sup>8,16</sup>	<b>1.01</b>	<b>1.02</b>	8	3.01 <sup>8</sup>	<b>1.02</b>	20	2.96 <sup>16</sup>

1. M-W Z=-0.135, P=0.892
2. M-W Z=-1.773, P=0.076
3. M-W Z=-2.143, P=0.032
4. M-W Z=-2.290, P=0.022
5. M-W Z=-2.570, P=0.006
6. M-W Z=-1.610, P=0.107
7. M-W Z=-1.799, P=0.072
8. M-W Z=-0.725, P=0.469
9. M-W Z=-0.279, P=0.781
10. M-W Z=-0.685, P=0.493
11. M-W Z=-2.108, P=0.035
12. M-W Z=-1.890, P=0.059
13. M-W Z=-2.134, P=0.033
14. M-W Z=-0.143, P=0.886
15. M-W Z=-3.975, P=0.0001
16. M-W Z=-1.750, P=0.080

Table 6. Comparison of dental tissue measurements (median test) among all six developmental cohorts ranging from theoretical worst to best outcome.

Measure		Sick Pen 'early deaths'						Control	Median test	
		Developmental outcomes from theoretical worst (1) to best (6)							Chi square	Prob.
		1	2	3	4	5	6			
Enamel cap volume	N	14	12	11	5	8	20			
	Mean	335.8	298.9	298.6	286.8	313.6	310.7			
	SD	36.7	33.5	25.4	35.2	52.7	37.9			
	Median	329.2	298.2	291.6	275.7	328.0	307.0	11.794	0.038	
Coronal dentin volume	N	14	12	11	5	8	20			
	Mean	608.9	578.3	586.8	617.1	665.0	639.4			
	SD	67.7	66.7	50.5	62.2	86.4	70.8			
	Median	610.3	568.7	610.7	581.6	687.5	628.6	6.091	0.297	
EDJ junction surface area	N	14	12	11	5	8	20			
	Mean	440.5	410.0	406.0	416.0	445.7	422.1			
	SD	46.6	36.1	28.4	40.8	39.1	33.2			
	Median	438.0	404.4	413.0	403.9	450.1	628.6	12.959	0.024	
Cervical area	N	10	12	11	5	8	20			
	Mean	137.5	133.6	137.8	139.7	149.6	143.7			
	SD	16.8	12.2	8.8	11.4	13.4	12.1			
	Median	133.6	132.7	138.2	135.3	152.8	142.2	11.191	0.048	
Enamel/dentin volume ratio	N	14	12	11	5	8	19			
	Mean	0.55	0.52	0.51	0.47	0.47	0.49			
	SD	0.04	0.04	0.02	0.04	0.03	0.05			
	Median	0.55	0.53	0.51	0.48	0.47	0.49	22.516	.0004	
Average enamel thickness	N	14	12	11	5	8	20			
	Mean	0.76	0.73	0.74	0.69	0.70	0.73			
	SD	0.04	0.05	0.04	0.04	0.07	0.05			
	Median	0.76	0.73	0.73	0.71	0.73	0.74	12.574	0.028	
Average dentin thickness	N	14	12	11	5	8	20			
	Mean	1.38	1.41	1.44	1.48	1.49	1.51			
	SD	0.07	0.06	0.05	0.04	0.10	0.08			
	Median	1.38	1.40	1.44	1.47	1.47	1.51	17.534	0.004	
Cervical constriction	N	10	12	11	5	8	20			
	Mean	3.10	3.07	2.95	2.97	2.99	2.94			
	SD	0.13	0.17	0.18	0.09	0.17	0.16			
	Median	3.15	3.15	2.91	2.99	3.01	2.96	11.473	0.043	

Table 7. Evaluation (median tests) of heterogeneity within the Sick Pen 'early deaths' group

<u>Measure</u>	Sick Pen 'early deaths'								Median Test	
	From theoretical worst to best outcome								<u>Chi square</u>	<u>Prob.</u>
	1		2		3		4			
Low age/low rank <u>N</u>	<u>Median</u>	low age/high rank <u>N</u>	<u>Median</u>	High age/low rank <u>N</u>	<u>Median</u>	High age/high rank <u>N</u>	<u>Median</u>			
Enamel cap volume	14	329.2	12	298.2	11	291.6		275.7	11.094	0.011
Coronal dentin volume	14	610.3	12	568.7	11	610.7		581.6	2.767	0.429
EDJ junction surface area	14	438.0	12	404.4	11	413.0		403.9	3.923	0.270
Cervical area	10	133.6	12	132.7	11	138.2		135.3	5.783	0.123
Enamel/dentin volume ratio	14	0.564	12	0.525	11	0.505		0.481	12.177	0.007
Average enamel thickness	14	0.76	12	0.73	11	0.73		0.71	10.925	0.012
Average dentin thickness	14	1.38	12	1.40	11	1.44		1.47	12.177	0.007
Cervical constriction	10	3.11	12	3.15	11	2.91		2.99	7.006	0.072

Table 8. Tissue mass compared among pigs with no hard tissue lesions, pigs with disturbed growth of the mandibular rami, and pigs with well-established bone or soft tissue lesions (thought to have formed during crown formation) (statistically significant differences in **bold**).

	Measure	Pathology	N	Mean	SD	Median	Kruskal Wallis	
							Chi square	Prob
Unworn Enamel <sup>1</sup>	Enamel volume	No lesions	4	323.3	18.5	317.8	0.970	0.616
		Bone formation	20	320.1	37.0	318.0		
		Infections	9	305.1	39.2	314.7		
Unworn and worn	Enamel volume	No lesions	21	307.2	33.6	308.6	3.604	0.165
		Bone formation	37	317.0	41.0	319.8		
		Infections	12	295.9	37.5	274.9		
	EDJ area	No lesions	21	422.3	34.5	426.0	7.318	<b>0.026</b>
		Bone formation	37	432.3	39.6	428.6		
		Infections	12	398.1	32.9	389.9		
	Dentin volume	No lesions	21	624.5	68.7	602.6	6.837	<b>0.033</b>
		Bone formation	37	626.9	72.7	620.2		
		Infections	12	566.6	55.7	562.1		
Cervix area	No lesions	21	143.2	12.8	141.5	4.899	0.086	
	Bone formation	34	141.1	13.7	142.2			
	Infections	11	132.5	8.9	132.1			

1. includes teeth with 'trace' enamel wear; i.e., faceting

Table 9. Dental tissue masses compared between pigs with and without well-established hard tissue lesions judged to be of infectious origin (statistically significant differences in **bold**).

	Measure	Pathology	N	Mean	SD	Median	Mann-Whitney test		
							Z	Prob	% reduction
Unworn <u>Enamel</u> <sup>1</sup>	Enamel volume	No infection	24	320.6	34.3	318.0	-0.970	0.332	1.0
		Infection	9	305.1	39.2	314.7			
Unworn and worn	Enamel volume	No infection	58	313.4	38.5	310.5	-1.691	0.091	11.5
		Infection	12	295.9	37.5	274.9			
	EDJ area	No infection	58	428.7	37.8	427.0	-2.548	<b>0.011</b>	8.7
		Infection	12	398.1	32.9	389.9			
	Dentin volume	No infection	58	626.1	70.7	615.8	-2.602	<b>0.009</b>	8.7
		Infection	12	566.6	55.7	562.1			
Cervix area	No infection	55	141.9	13.3	141.8	-2.159	<b>0.031</b>	6.8	
	Infection	11	132.5	8.9	132.1				

1. includes teeth with 'trace' enamel faceting

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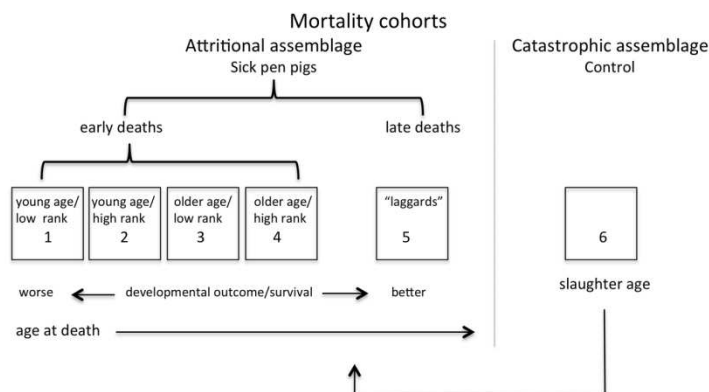


Figure 1. Organization and terms for analysis of dental measures of mortality cohort sub-groups. Sick pen pigs died naturally and are termed the Attritional assemblage. Control pigs are those that survived to slaughter age and are termed the Catastrophic assemblage. The term 'rank' refers to relative physiological maturity based on combined measures of dental formation, skull length and weight at death (see text for further details). Note that some Sick pen pigs survived for a while beyond normal age for slaughter; these are considered 'late deaths'.

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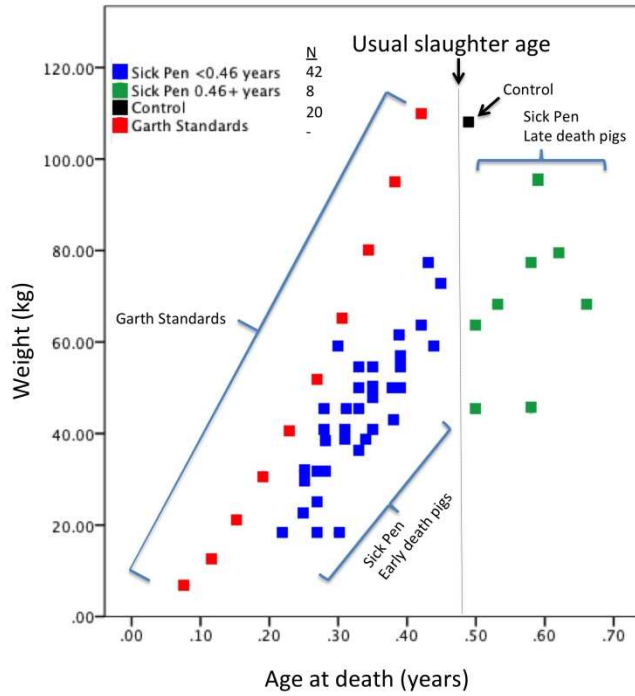


Figure 2. Relationship of body weight at death and age at death among Sick Pen 'early death' pigs, Sick Pen 'late death' pigs, and Control pigs compared to a standard. Pigs are slaughtered for consumption at a typical age (180 days) and weight (108 kg). Note that all Sick Pen pigs are light for age; and 'late deaths' clearly form a separate cluster.

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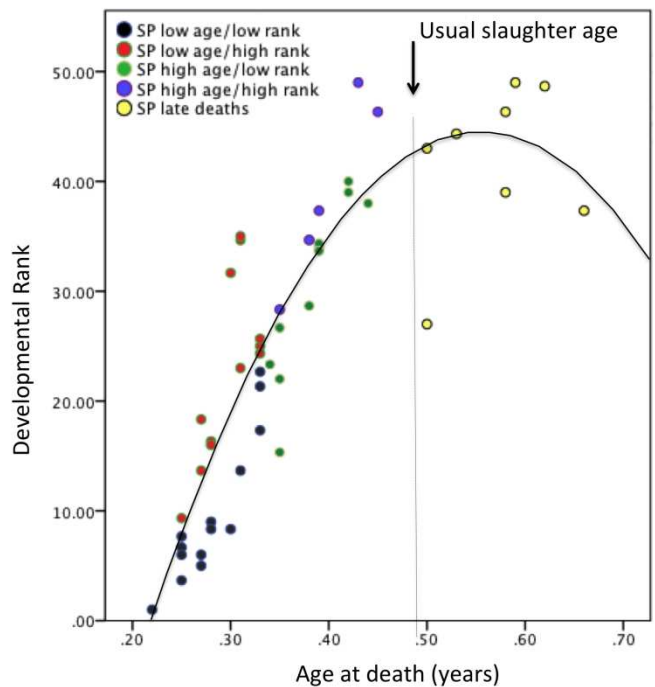


Figure 3. Relationship of Developmental Rank (average seriated maturity markers: dental formation, skull length, and body weight) to age at death. Control animals are not included as their individual weights and age at death are unknown. The distinctiveness of the Sick Pen 'late deaths' is accentuated here; they are developmentally retarded for their ages.

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Figure 4. Screenshots of the youngest and oldest pig lower first molars in our study; Sick Pen pig 30 (aged 0.22 years, right molar crown complete but not fully mineralized) and 39 (aged 0.66 years, a 'late death' pig left molar crown), respectively.

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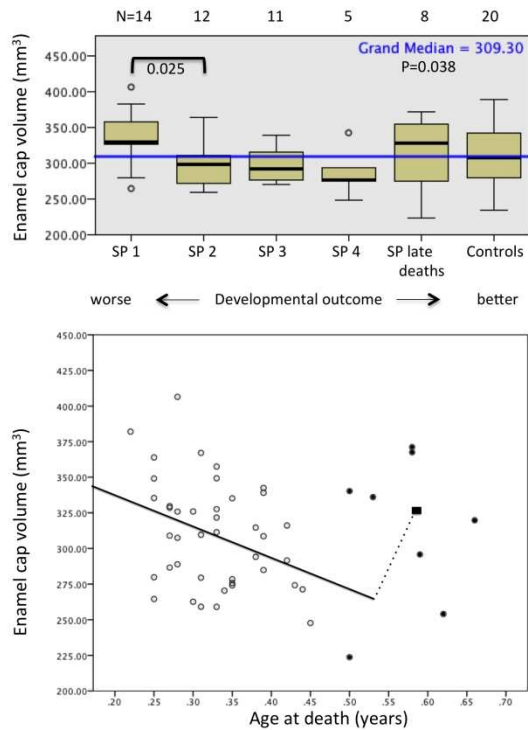


Figure 5. Enamel cap volume: comparison of median values among cohorts ranged from theoretical worst to best developmental outcome. Probability value indicates whether differences in medians are statistically significant (alpha is 0.05). Significant pairwise post hoc differences are shown. Lower panel shows heterogeneity of the Sick Pen assemblage by graphing the relationship of dental measure to survivorship among just the Sick Pen 'early deaths' in comparison to the 'late deaths' cohort whose median age and tissue measure value are indicated by the black square. Progressively older Sick Pen 'early death' pigs show diminishing volumes with age at death while the Sick Pen 'late deaths' show even larger values than the Controls.

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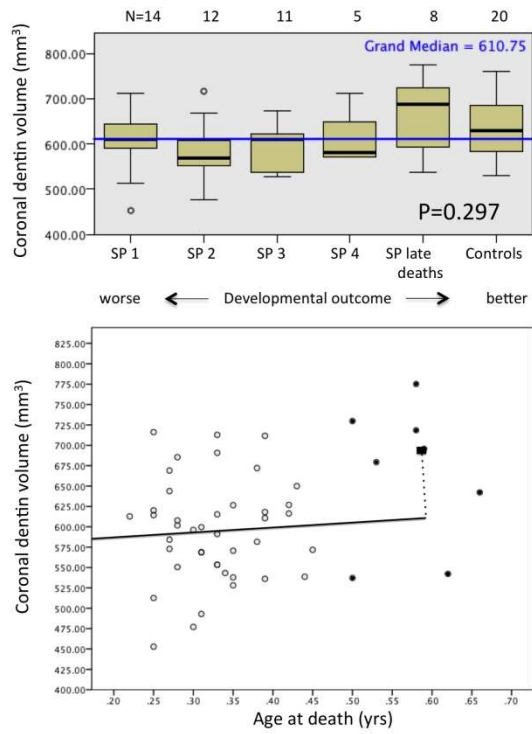


Figure 6. Coronal dentin mass compared among developmental outcome cohorts (as for Figure 5). There are no significant differences overall although large size of the Sick Pen 'late deaths' coronal dentin is evident.

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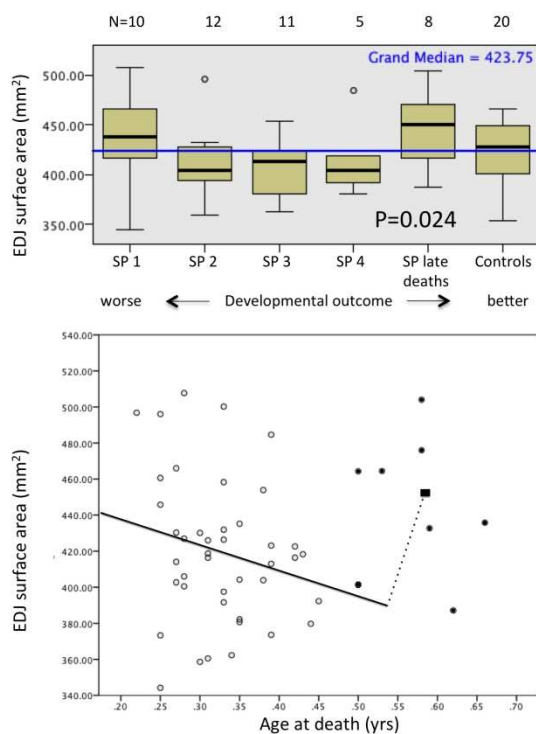


Figure 7. Surface area of the enamel-dentin junction compared among developmental outcome cohorts (as for Figure 4). Overall, median values differ significantly, which is driven by a decline in surface area among the SP 'early death' cohorts. Large size of the 'late deaths' cohort is noteworthy as is the similarity of the pattern of this variable to that of enamel (Fig. 5) rather than dentin (Fig. 6).

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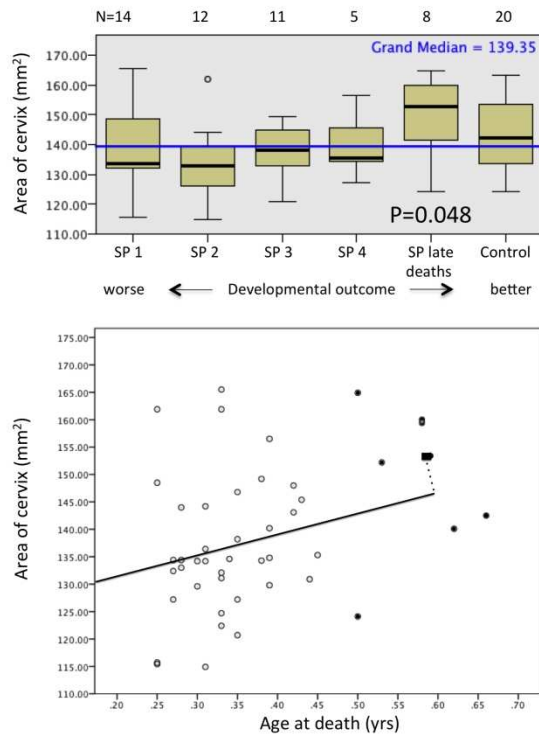


Figure 8. Area of the cervix compared among developmental outcome cohorts (as for Figure 5). There is a statistically significant difference among medians, which seems to be driven by the comparatively small cervixes of the SP 'early death' cohorts.

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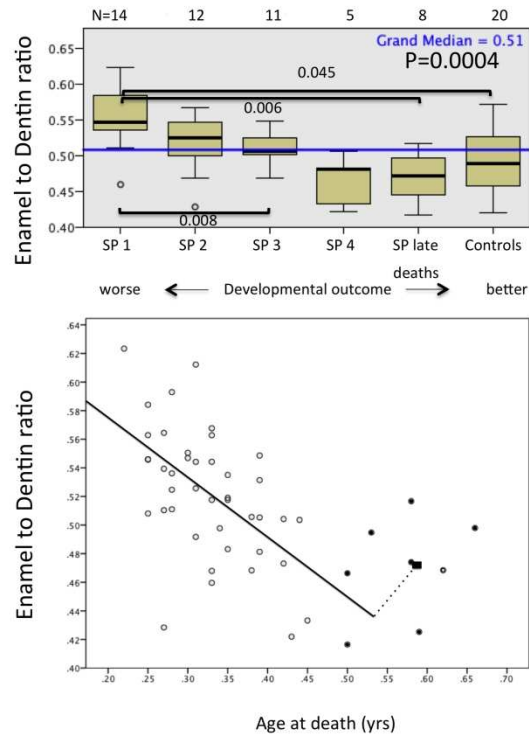


Figure 9. Ratio of enamel volume to that of dentin compared among developmental outcome cohorts (as for Figure 5). The strong statistical trend is a function of slightly increasing dentine volume combined with significantly decreasing enamel volume with increasing age at death in the Sick Pen 'early death' cohorts.

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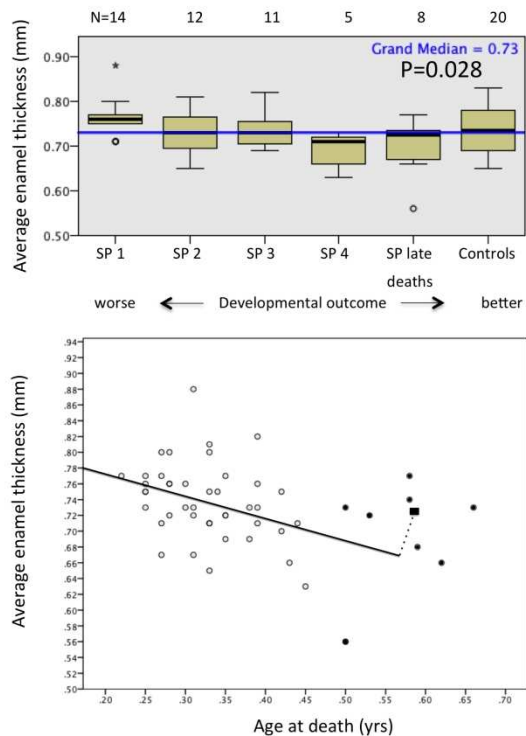


Figure 10. Average enamel thickness compared among developmental outcome cohorts (as for Figure 5). The behavior of this variable is a function of relative volume and surface area of the EDJ. The decline in enamel thickness shown among the Sick Pen 'early deaths' cohorts in the bottom panel, while statistically significant, reflects simply enamel cap volume reduction in this group.

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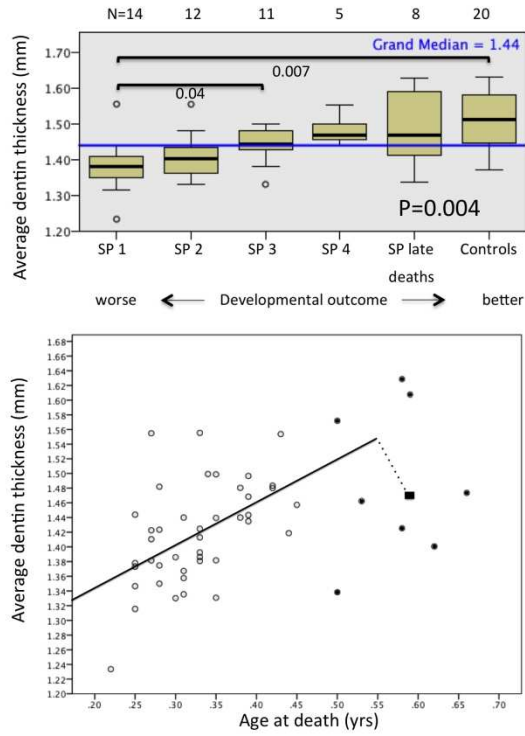


Figure 11. Average dentin thickness compared among developmental outcome cohorts (as for Figure 5). The behavior of this variable is a function of relative volume and surface area of the EDJ. Dentin is progressively thicker in longer-surviving cohorts.

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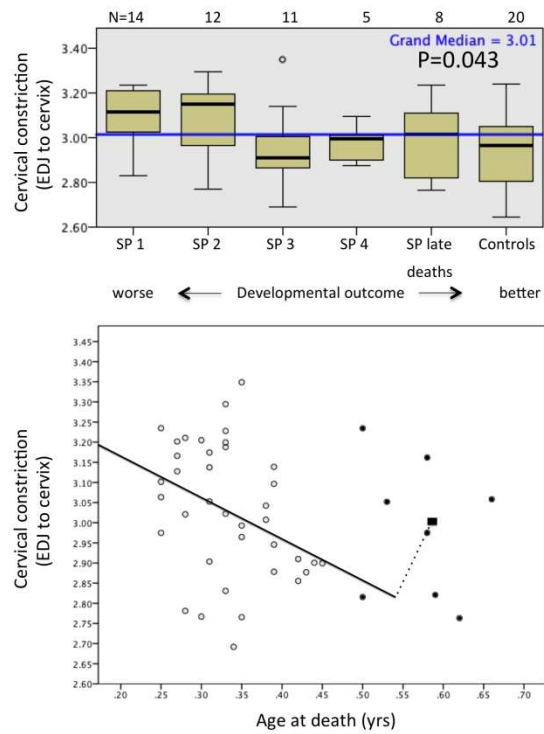


Figure 12. Cervical constriction (EDJ area/Cervix area) compared among developmental outcome cohorts (as for Figure 5). There is a statistically significant difference overall among medians driven by a tendency for shorter-lived pigs to have more constricted cervixes.

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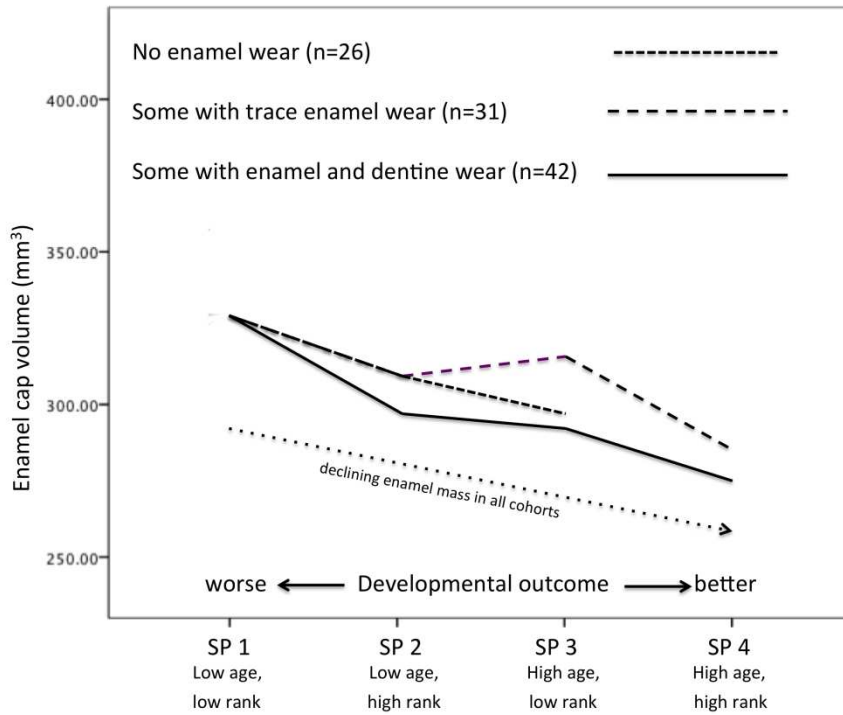


Figure 13. Enamel cap volume compared among Sick Pen 'early death' cohorts illustrating the decline in enamel volume with increasing developmental rank regardless of the potential impact of occlusal wear on this variable.

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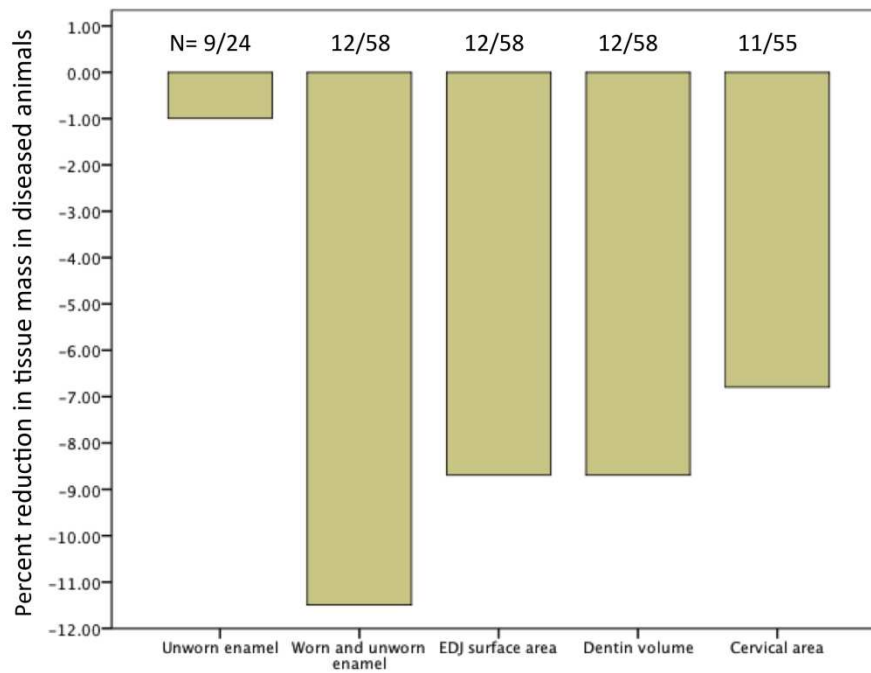


Figure 14. Percent decrement in tissue measure compared between animals with and without well-established hard tissue lesions. Sample sizes are shown for affected and unaffected animals. All coronal measures are reduced in animals with chronic disease.

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Fig 49 age=0.35 years, severity=2

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47 age=0.34 years, severity=6



34 age=0.30 years, severity=6



Figure 15. Examples of abnormal remodeling of auditory bullae. Severity score 6 is the most severe. Note shell formation in Fig 47 and lack of involvement of its left bulla. Note Fig 34 with very severe bilateral involvement and its early age at death (Fig. 16).

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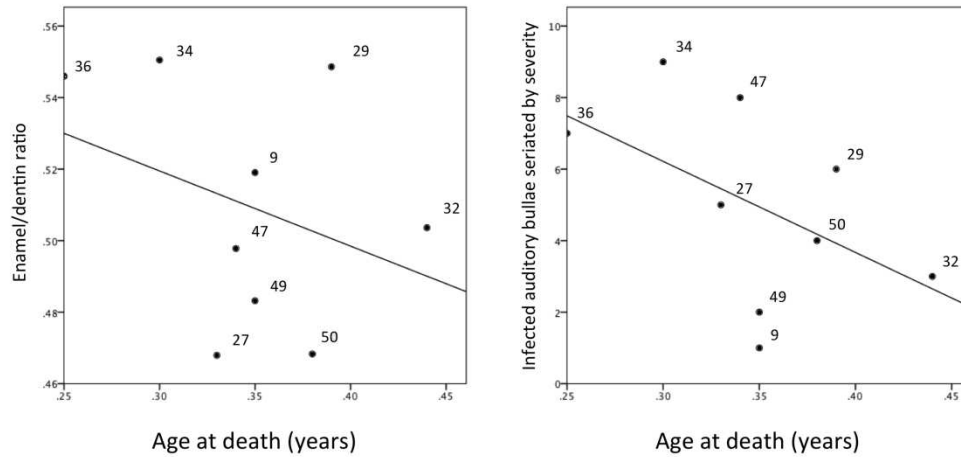


Figure 16. Relationship of enamel/dentin ratio to age at death in those pigs with abnormal auditory bullae. Note that enamel and coronal dentin formation are completed before death, implying that the disease probably existed before crown completion. Both severity of bone remodeling and enamel thickness decline with increasing survivorship.

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