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Determining the age classes of free-ranging female nilgai (*Boselaphus tragocamelus*) in southern Texas, USA

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Abstract

Age classification methods have not been evaluated for free-ranging nilgai antelope (Boselaphus tragocamelus) either on their native or introduced ranges of southern Texas, USA. Our objectives were to 1) determine if cementum annuli aging was a feasible method for aging nilgai, 2) measure the crown-lingual crest heights of nilgai molariform teeth to assess if these measurements were correlated to the cementum-annuli age of nilgai, and 3) develop nilgai age classes based on visual observations of tooth eruption and wear patterns. Six commercial nilgai meat harvests were conducted via helicopter from May-September 2018-2021 across 3 properties. We collected data from 225 harvested nilgai (n = 213 females and n = 12 juvenile males). We found significant relationships between cementum annuli ages and the crown-lingual crest heights of nilgai teeth (n = 63). However, the relationship was weak given the low R-squared values (0.23-0.46), suggesting that the age of female nilgai does not explain a large amount of variation among tooth heights (i.e., wear). From nilgai teeth samples, we documented 13 tooth eruption stages and 6 age classes. Female nilgai age classes presented provide land managers with the basic tools needed to assess population age structures. On the Indian subcontinent, our method could be used to evaluate age-biased predation on free-ranging nilgai.

Keywords: aging, cementum annuli, nilgai antelope, tooth eruption, tooth wear.

Methods to determine age classifications have been established for many ungulates. Some of these techniques include evaluating tooth eruptions and wear patterns (Severinghaus, 1949; Dow & Wright, 1962; Gee *et al.*, 2002), correlating dental crown heights to known-age samples (Klein *et al.*, 1981, Morrison & Whitridge, 1997; Steele, 2002), and counting cementum annuli in the central incisor, including notation of deciduous incisors (Keiss, 1969; Lockard, 1972, Miller, 1974). These methods require evaluation of dentition through live animal or carcass handling and data recording. However, age classification methods have not been evaluated for free-ranging nilgai antelope (*Boselaphus tragocamelus*) either on their native (*i.e.*, India, Pakistan, and Nepal) or introduced ranges of southern Texas, USA.

As part of East Foundation's Exotic Ungulate Control Program, 150–300 female or juvenile male nilgai are removed annually. This is accomplished through commercial meat harvests from a helicopter by a wild meat vendor (www.brokenar-rowranch.com). Following aerial harvests, carcasses are prepared for cold shipping on site; offal, skins, and other inedible carcass parts are recycled on the ranches. This provided us with the unique opportunity to obtain and evaluate dentition records of free-ranging nilgai and further develop techniques to determine age classifications.

Our objectives were to 1) determine if cementum annuli aging was a feasible method for aging nilgai, 2) measure the crown-lingual crest heights of nilgai molariform teeth to assess if these measurements were correlated to the cementum-annuli age of nilgai (in the absence of known-age nilgai), and 3) develop nilgai age classes based on visual observations of tooth eruption and wear patterns.

Our study occurred following commercial nilgai meat harvests on 3 ranches in southern Texas, USA, including portions of Kenedy and Willacy counties. These properties included East Foundation's El Sauz and Santa Rosa ranches, and the Norias Division of King Ranch® (Table 1). Six commercial meat harvests were conducted via helicopter from May–September 2018–2021 across the three properties. We collected data from 225 harvested nilgai (n = 213 females and n = 12 juvenile males). We collected dentition photos capturing the buccal, lingual, and occlusal views of the incisors and the right mandibular premolars and molars. Additionally, we collected the two central incisors (I1), including deciduous I1 for young animals, for cementum annuli analyses as well as the lower right mandible. If the lower right mandible was damaged, we collected the lower left mandible (n = 3).

We shipped incisors (n = 225) to Matson's Laboratory in Manhattan, Montana, USA for cementum annuli analyses (www.matsonslab.com). The laboratory used its aging model for pronghorn (*Antilocapra americana*) because aging models have not been developed for nilgai teeth and the annular patterns of pronghorn teeth most closely matched those of nilgai.

Evaluating the mandibles, we used digital calipers (AccuMAS-TER™ Digital Caliper 7410, ± 0.02 mm, Carson City, NV, USA) to measure the combined crown and lingual crest heights of the second and third premolars (P2 and P3, respectively) and the first and second molars (M1 and M2, respectively; Figure 1) immediately following removal of the mandibles so that the gingivae were still present. We used premolars and molars because these teeth wear against their upper counterparts throughout the animals' life, as opposed to incisors which, in bovids and cervids, only occur on the mandible (Spinage, 1973; Klein et al., 1981). We defined the crown-lingual crest measurement as the minimum distance between the interalveolar septa of the mandible and the tip of the lingual crest on the lingual surface of the posterior cusp of each tooth (Steele, 2002). We sorted individuals by age based on cementum annuli results. We then performed least squares linear regression for each tooth type (P2, P3, M1, and M2; IBM Corporation 2020, SPSS version 27.0, Armonk, NY, USA). For these analyses, we considered tooth measurements as the dependent variable and cementum annuli age as the independent variable on samples measured from 2020-2021 (n = 63).

Lastly, using dentition photos (Canon EOS Rebel T7 DSLR, Canon U.S.A., Inc., Melville, New York, USA) and mandibles (used in instances with low photo quality), we evaluated tooth replacement and wear patterns relative to the cementum annuli determined ages. Specifically, for younger nilgai samples we determined the different stages of tooth replacement that occurred and correlated that with the cementum annuli age for each individual. Also, for older nilgai samples we determined the dental features that provided the best estimates for the ages of individuals. We used wear on the lingual crest of the molars and premolars, similar to other free-ranging ungulates (Severinghaus, 1949; Dow *et al.*, 1962; Keiss, 1969; Foley *et al.*, 2021). We assessed these criteria with a single observer for all samples.

We evaluated 219 of 225 harvested nilgai for cementum annuli because incisor roots were broken on 6 samples and could not be used. Of the samples from juvenile animals (all with deciduous I_1) no annuli were found and we determined these nilgai to be <1 year old. This included all 12 males that were sampled; consequently, all following results pertain to female nilgai. We found samples with the first set of permanent incisors, but not all permanent teeth (n = 6) to have one annulus and we determined these nilgais to be one year old. Premolars from these samples possessed little tartar build up, indicating these teeth had recently erupted. We assigned samples to the two-year-old category (n = 33) that had 2 annuli; only 2 of these nilgai did not have all their permanent teeth. We assigned the age of \geq 3-years-old to samples with corresponding annuli and all permanent teeth.

We found significant relationships between cementum annuli ages and the crown-lingual crest heights of nilgai teeth (n = 63). Specifically, for P_2 (R^2 = 0.23, $F_{1.60}$ = 17.82, P < 0.001), for P_3 (R^2 = 0.46, $F_{1.61}$ =50.97, P < 0.001), for M_1 (R^2 = 0.45, $F_{1.61}$ = 49.07, P < 0.001), and for M_2 (R^2 = 0.30, $F_{1.61}$ = 26.45, R < 0.001; Figure 2). The negative coefficient values for each tooth type represent a negative relationship, which was expected because tooth wear increases with age and thus crest height diminishes. However, the relationship was weak given the low R-squared values (0.23–0.46), suggesting that age of female nilgai does not explain a large amount of the total variation among tooth heights (i.e., wear).



Figure 1. Left mandible of adult female nilgai (Boselaphus tragocamelus, sampled from 2018–2021 on 3 ranches in southern Texas, USA) in the lingual view demonstrating the range of the crown-lingual crest heights (shown as an orange line) which is defined as the distance between the interalveolar septa of the mandible and the tip of the lingual crest on the lingual surface off the posterior cusp on the P₂, P₃, M₁, and M₂.

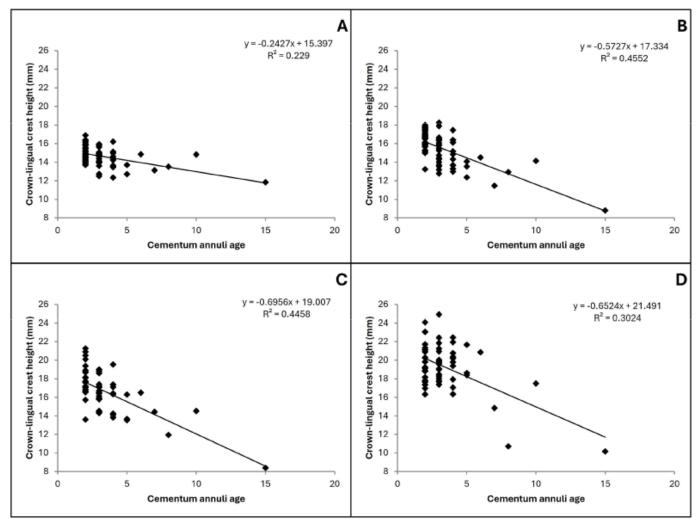


Figure 2. Correlation between the crown-lingual crest heights and the cementum annuli age of sampled female nilgai (n = 63; Boselaphus tragocamelus) from 2020–2021 on 3 ranches in southern Texas, USA. A) is for P2, B) is for P3, C) is for M1, and D) is for M2.

From nilgai teeth samples, we documented 13 tooth eruption stages (Table 2). Stages 1 and 2 possessed all four sets of deciduous incisors with the M, beginning to erupt in 33 individuals, and the M, beginning to erupt in 22 individuals. All nilgai within these stages had no annuli present, indicating that these animals were <1 year old. Stage 3 had permanent I₁, with the P₂, M₄, and M₂ beginning to erupt. Stage 4 was equal to stage 3 with the addition of P₁ and P₂ beginning to erupt. Stage 5 had permanent I₁, P₁, P₂, M₁, and M₂, with P3 erupting. Stage 6 was equal to stage 5 with the addition of M3 beginning to erupt. Stage 7 had permanent I_1 , P_1 , P_2 , M_1 , and M_2 with I_2 and M_3 beginning to erupt; however, P_3 was still deciduous. Stage 8 is equal to stage 7, with the addition of P_3 beginning to erupt. In stage 9 there were permanent I_1 , I_2 , P_1 , P_2 , M_1 , and M_2 , with P_3 and M_3 erupting. Stage 10 samples possessed the same permanent teeth as stage 9, with I3 and M3 erupting; I4 and P3 were deciduous. Stage 11 was equal to stage 10, with the addition of I4 erupting. Stage 12 had permanent I_1 , I_2 , I_3 , P_1 , P_2 , M_1 , and M_2 , with I_4 , P_3 , and M_3 in the final eruption stages. Stage 13 samples possessed all permanent teeth except for M₂, which is in the final stage of eruption. All nilgai in stages 3-13 had one annulus in the cementum.

We found the width of the enamel and dentine on the occlusal surface of the mandibles to be unreliable for determining age of nilgai. Although white-tailed deer (*Odocoileus virginianus*) possess wider enamel than dentine at young ages (Cooper *et al.*, 2013), nilgai have wider dentine than enamel at all stages of

adulthood. However, there was a clear pattern of deterioration that occurs on the lingual crest heights as nilgai mature, which provides a visual basis for creating broad age classes for female nilgai relative to cementum annuli ages (Figures S1–S4).

The method of using cementum annuli to age wildlife can be highly accurate (80-95%) in many ungulate species, including pronghorn, white-tailed deer, blacked-tailed deer (O. hemionus columbianus), caribou (Rangifer tarandus), elk (Cervus canadensis), mule deer (O. h.), bison (Bison bison), moose (Alces alces), and sheep (Ovis aries; www.matsonslab. com). Nilgai incisors have not been analyzed for cementum annuli prior to our study. Although some uncertainty in this method remains until known-age samples are tested, the overall consistency of cementum annuli results compared with eruption patterns for age classes 1 and 2 suggests that this method is accurate (>80%) for female nilgai. There were some variations in cementum annuli results and tooth eruption patterns as shown by the 6 individuals that were aged by cementum annuli as 1-year old but possessed all adult teeth. However, this approach can be especially useful for land managers that regularly conduct recreational or commercial meat harvests of nilgai because they can collect incisors from many nilgai in a short period of time for cementum annuli testing. This is not an ideal method for fast, field-based age determination. However, it provides biologists with a framework to develop visual field-based aging methods for female nilgai (see Figures S1-S4).

Table 1. General habitat characteristics of East Foundation's El Sauz and Santa Rosa ranches, and the Norias Division of King Ranch® in southern Texas, USA where nilgai antelope (*Boselaphus tragocamelus*) were sampled via helicopter from May–September, 2018–2021 (Snelgrove *et al.*, 2013).

Property	Hectares	Primary Soil Complex	Primary Vegetative Cover			
El Sauz	10,984	Mustang	Mesquite-Granjeno Parks			
Santa Rosa	7,545	Willamar	Mesquite-Granjeno Parks			
Norias	96,315	Sarita	Live Oak Woods/ Parks			

Table 2. Thirteen unique tooth eruption stages related to cementum age from nilgai (*Boselaphus tragocamelus*) samples from 2018–2021 on 3 ranches in southern Texas, USA.

Tooth eruption and replacementa												
Eruption stage	Incisors				Premolars			Molars				
	I1	12	13	I4	P1	P2	Р3	M1	M2	М3	n	Cementum annuli age
1	D	D	D	D	D	D	D	(P)	A	A	33	<1
2	D	D	D	D	D	D	D	P	(P)	A	22	<1
3	P	D	D	D	D	(P)	D	P	P	A	8	1
4	P	D	D	D	((P))	((P))	D	P	P	A	7	1
5	P	D	D	D	P	P	D	P	P	A	3	1
6	P	D	D	D	P	P	((P))	P	P	(P)	1	1
7	P	(P)	D	D	P	P	D	P	P	(P)	4	1
8	P	(P)	D	D	P	P	((P))	P	Р	(P)	7	1
9	P	P	D	D	P	P	((P))	P	Р	(P)	5	1
10	P	P	(P)	D	P	P	D	P	Р	(P)	3	1
11	Р	P	(P)	(P)	P	((P))	D	P	Р	(P)	1	1
12	Р	P	P	((P))	P	P	((P))	P	Р	((P))	9	1
13	P	P	P	P	P	P	P	P	Р	((P))	5	1

^a Key: "A" = absent, "D" = deciduous teeth, "(P)" = permanent teeth that are in the early eruption stage, "((P))" = permanent teeth that are in the late eruption stage, and "P" = fully erupted dentition.

The crown-lingual crest heights consistently decreased with age, but the overall variation (up to 8 mm differences among tooth measurements in the same age class) in tooth heights indicates this method was not reliable for age determination of nilgai. This method is documented as accurate (>80%) in archeological studies (Klein *et al.*, 1981; Morrison & Whitridge, 1997; Steele, 2002); however, Gee *et al.* (2002) utilized this method on freshly harvested white-tailed deer and concluded that it was not accurate for age determination in that species.

Aging ungulates by tooth replacement and wear patterns is a widely used technique. For many species, including white-tailed deer (Severinghaus, 1949), mule deer (Robinette *et al.*, 1957), and elk (Qunmby & Gaab, 1957), eruption patterns have been determined to be accurate (≥95%) for aging individuals ≤2 years old. However, many studies caution that the accuracy of aging wildlife by tooth wear declines as the animal gets older and can be highly subjective (Severinghaus, 1949, Hamlin *et al.*, 2000, Gee *et al.*, 2002, Nussey *et al.*, 2007, Foley *et al.*, 2021). It is for this reason that we created broader age classes for nilgai as opposed to exact year classes (see Figures S1–S4).

Factors that influence tooth wear in ungulates include low abundance of high-quality food, poor habitat quality, grazing of vegetation close to the ground, and resource competition (Kojola et al., 1998; Nussey et al., 2007). Ungulates living in poor quality habitats may experience shortages of high-quality food and this can lead to an increase in resource competition (Kojola et al., 1998). Consequently, individuals will have to rely on larger amounts of low-quality vegetation which often consists of coarse forage that can increase the rate at which molars wear down (Nussey et al., 2007). Nilgai are intermediate feeders with their diet consisting of 66% grass, 25% herbaceous species (forbs), and 15% browse in southern Texas (Sheffield, 1983). Texas commonly experiences drought conditions which reduces the growth of nutritious grasses and forbs. This often leads many ungulates to increase browse use which consists of coarser material that has a greater potential to cause tooth wear (Foley et al., 2021).

The rate of wear can also differ between sexes within a species because of differences in size, habitat preference, and foraging behavior (Van Deelen *et al.*, 2000; Loe *et al.*, 2003; Nussey *et*

al., 2007). Many ungulates are sexually dimorphic, with males typically being larger than females. Therefore, males would require more food intake to support their large body weights which could cause teeth to wear faster than in females (Loe et al., 2003; Nussey et al., 2007). Van Deelen et al., (2000) demonstrated this in white-tailed deer where teeth of male deer showed more visible wear than those of female deer. Nilgai are sexually dimorphic, with mature males being larger than females (Leslie, 2007); therefore, rate of wear documented in our study of female nilgai may not be representative of male nilgai. We recommend further research to determine tooth wear rates and patterns between male and female nilgai.

Female nilgai age classes presented in our study provide land managers with the basic tools needed to assess population age structures across southern Texas and perhaps their native range. Nilgai have high reproductive rates (Granger et al., 2024). Having the ability to determine female nilgai ages will allow biologists to assess at what rate their populations are changing (increasing, decreasing, or stable). By determining age structures of female nilgai populations, managers will be able to make informed decisions regarding population control or harvest measures to keep nilgai populations under control and prevent future property damage and the potential spread of livestock and wildlife disease vectors. On the Indian subcontinent, our method could be used to evaluate age-biased predation of free-ranging nilgai.

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CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

DATA AVAILABILITY

The original contributions presented in the study are included in the manuscript; further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

This study involved carcasses only. All aspects of animal capture and handling were consistent with protocols established by the American Society of Mammalogists (Sikes $\it et al., 2016$) and Texas A&M University-Kingsville.

iTHENTICATE

Software shows a high overlap (>50%) with the first author's Master's thesis; however, the overlapping document is only archived on internet and not published as a peer-reviewed article.

AUTHOR CONTRIBUTIONS

Conceptualization, C.D.H, L.R.S. and T.A.C.; methodology, M.M.G., C.D.H., H.L.P.-B. and T.A.C.; validation, C.D.H. and L.R.S.; formal analyses, M.M.G., C.D.H., S.E.H. and H.L.P.-B.; investigation, M.M.G., C.D.H. and L.R.S.; resources, L.R.S. and T.A.C.; writing-original draft preparation, M.M.G. and C.D.H.; writing-review and editing, S.E.H, H.L.P.-B., L.R.S and T.A.C.; supervision, C.D.H.; project administration, T.A.C. All authors have read and agreed to the published version of the manuscript.

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