EVALUATION OF NEW TECHNOLOGIES FOR ESTIMATING AGE OF WHITE-TAILED DEER BY TOOTH CHARACTERISTICS

by

JEREMY MICHAEL MEARES

(Under the Direction of Karl V. Miller and Robert J. Warren)

ABSTRACT

Despite considerable previous research, there remains a need for an objective, reliable method for estimating age of harvested white-tailed deer (*Odocoileus virginianus*). Using digital photographs and GIS, I measured dentine and enamel widths on molars of known-aged, wild deer from South Carolina to objectively evaluate the original tooth wear and replacement technique. I found that objective measurements of the dentine:enamel ratio of the molars could not separate among age classes. To improve predictability, I used K nearest neighbor (KNN) analyses to evaluate combinations of tooth wear measurements. Using KNN analysis, I achieved an overall accuracy rate of 54.4% for placing deer into year age class categories 2.5, 3.5, and 4.5. I also examined the use of near-infrared spectroscopy to estimate age on a sample of mandibles from captive deer at the University of Georgia ranging in age from 1.5-6.5 years. I used readings from molars to generate a regression relationship with age that produced an unreliable predictive relationship with age. My results suggest that subjective age estimates from biologists are more accurate than multivariate analyses of precise tooth measurements.

INDEX WORDS: Age, GIS, K nearest neighbor, Near-infrared spectroscopy, *Odocoileus virginianus*, White-tailed deer
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DEDICATION

I dedicate my thesis to all of my family. Mom and Dad, I can never say thank you enough for everything you both have done for me. Mom, I want to thank you for always being positive and supportive no matter what the situation is, or how dire the outcome seems to be. Dad, you and grandpa are the reason I have pursued this profession. Both of you instilled a passion for all things wild at a very young age and strengthened and supported that passion throughout my youth. Being raised around the hunting camp has given me a firm grasp on what really matters in this life and those are memories and lessons I would not trade for anything in this world. Jenna, you are a constant source of entertainment and drama, which sometimes is a welcome distraction. I hope you stay on track and realize your potential to do great things. Glen, Wanda, and Buddy I thank you for always providing a place where I can pursue my outdoor passions which was much needed throughout this process. Leslie, you have been my rock through this whole process. You have endured many late nights and stress-filled months throughout this journey. I can truly say that I do not know where I would be without you in my life. I was set to leave ABAC with only a two-year degree until I met you and somehow I found my way to Athens. I want to thank you for your enduring love, patience and for your tolerance of all my outdoor passions. I thank GOD everyday for allowing me to call you my wife.
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CHAPTER 1
INTRODUCTION, LITERATURE REVIEW
AND THESIS FORMAT

Introduction

Strategic management of deer (Odocoileus spp.) populations is dependent on age-specific data (Severinghaus 1949) because biotic potential of individuals within a population varies by age (Alexander 1958). Once assigned to age classes, biological data are used for comparing population trends over time, and to develop predictive models. In addition, the growing popularity of quality deer management (QDM) has prompted many deer herd managers to establish age-based harvest criteria, designed to recruit more males into older age classes (i.e., ≥ 3.5 years old). Without accurate age estimates for individuals, deer herd managers cannot evaluate current deer population status, nor recognize when their management goals have been achieved.

Tooth Wear and Replacement and Cementum Annuli Techniques

The most commonly used technique to place individual deer into age-class categories involves patterns of eruption and wear of mandibular teeth (Sauer 1984, Hamlin et al. 2000). The tooth replacement and wear technique of estimating age of white-tailed deer (O. virginianus) is based on a study by Severinghaus (1949). Width of the dentine (soft, darker inner core of a tooth) in relation to the thickness of the enamel (hard, white outer coat of a tooth) indicate wear patterns and are keys to separating age classes. The study by Severinghaus (1949) in New York included 127 age records from 81 individual deer. Fifty-six deer were examined once, 14 deer were examined twice, five deer were examined three times, three deer were examined four times,
two deer were examined five times, and one deer was examined six times. In that study, 101 age records on deer $\leq$ 2 years of age, and 26 age records on deer $>2$ years of age were collected. Information on tooth eruption and wear were collected on live specimens. Measurements taken included degrees of wear (slight, moderate, heavy) and eruption points or crests through the gum (Severinghaus 1949).

Another commonly used method of estimating the age of deer is the cementum annuli technique. This technique involves counting the annual rings in a stained cross-section of the roots of a deer’s first incisor (Gilbert 1966). The permanent first incisors of white-tailed deer are fully erupted by 6 to 7 months of age (Severinghaus 1949). Other studies using cementum annuli to estimate age of whitetails involved examination of the molar teeth (Ransom 1966), which was found to be too variable to provide reliable estimates. Gilbert’s (1966) technique involved the following steps: (1) decalcification of the incisor in 30% formic acid for 96 hours, (2) immersion in Cellusolve for 24 hours, (3) three paraffin baths for 3, 2, and 2 hours each, (4) longitudinal sectioning of the tooth at 10 microns thick using a microtome, and (5) standard staining using Delafield’s hematoxylin for a maximum staining time of 30 minutes. Gilbert (1966) showed that deposition of cementum begins prior to eruption, which causes the first restriction in visible cementum growth to occur during the first fall and winter of a deer’s life (Gilbert 1966).

In the study by Gilbert (1966), the technique was tested against only 10 known-age deer from Michigan. However, he did not indicate how accurate the technique was when compared to the known-age samples. Gilbert also stated that this technique should be applicable throughout the northern range of the whitetail. Later studies (Cook and Hart 1979, Jacobson and Reiner 1989) compared results of the cementum technique to known-age deer from the southern U.S.
These researchers found varying accuracy rates among biologists’ abilities to estimate age using the Severinghaus technique, as well as variable accuracy rates from cementum analysis.

Several studies have compared the accuracy of the tooth wear and replacement and the cementum annuli techniques for estimating the age of deer. Based on a collection of 682 mandibles that were assumed to be correctly assigned to age classes by cementum annuli analysis, Gilbert and Stolt (1970) reported tooth-wear characteristics exhibited too much variation for reliable and accurate age estimation. Using a collection of 55 known-age mandibles from New York ranging from 1 to 6.5 years, Sauer (1971) reported that 84% of the sample was correctly classified using cementum annuli analyses, whereas tooth wear was inadequate for assigning ages beyond 1 year, 7 months.

In Texas, Cook and Hart (1979) used marked, free-ranging, known-age deer from two wildlife management areas to compare age estimates provided by biologists and technicians using tooth wear and replacement versus those attained from cementum annuli analysis. Biologists and technicians correctly estimated ages for 66.7% of the samples (N = 242), with most errors being an overestimate of age. For the 4.5-year-old age class they were 35.5% of estimates were correct and 46.5% of estimates were correct for the 5.5-year-old class. Ages of a subset of the collection (n = 25) were estimated by cementum annuli analysis, and only 16.0% (4) of the incisors were correctly assigned. Of the 21 incorrect ages assigned, 90.5% (19) were underestimated. The authors state that although cementum annuli can provide accurate age estimates in some areas, the technique should be evaluated using known-age samples from local areas.

A similar study in Mississippi (Hackett et al. 1979) compared age estimates based on tooth wear and replacement and cementum annuli analysis of free-ranging deer at state-operated
check stations \((n = 212)\). Incisors were collected from deer 1.5 years old and older. Only 17.3% of the deer considered yearlings by tooth wear and replacement were classified as yearlings by cementum analysis. All deer identified as fawns by tooth wear (5) were classified as yearlings by cementum annuli. These discrepancies in classifying the fawn and yearling age classes raise questions regarding the use of cementum annuli for estimating deer age in Mississippi (Hackett et al. 1979). The authors suggested that whitetails in Mississippi might deposit more than one cementum layer per year, implying that deer experience more than one nutritional stress period per year. Castle et al. (1979) showed that fluctuations in seasonal body and organ weights of Mississippi deer may signal stress periods during late summer and late winter, which may lead to deposition of double annuli. For this reason, cementum annuli may be a questionable method for estimating age of deer in the southeastern U.S.

Shackleford (1981, unpublished data) examined 34 captive, known-age and 32 wild white-tailed deer in Arkansas and compared the accuracies of eight potential techniques for estimating age—tooth wear and replacement, molar tooth ratio, incisor ratio, dental cementum, mandible, diastema and tooth-row relationships, degree of ossification of leg bones, eye lens weight, and quantitative analysis of lens protein. Accuracy of the wear and replacement technique was 77.1%. Based on their results, the researchers modified the original tooth wear and replacement technique by classifying deer into the following categories: 0.5 years, 1.5 years, 2.5-4.5 years, 5.5-6.5 years and >6.5 years. Accuracy of age estimation using cementum annuli was 31.4% for the known-age specimens (Shackleford 1981, unpublished data).

Jacobson and Reiner (1989) also compared accuracy of tooth wear and replacement vs. cementum annuli on 94 free-ranging deer (tagged as fawns) from Mississippi, eight free-ranging deer (tagged as fawns) from the Radford Army Ammunition Plant in Virginia, and 48 captive
deer from Mississippi State University. Using these samples, 98 known-age jawbones were compiled to test the age estimation accuracy of 55 biologists from the southeastern United States. However, the authors did not specify what criteria they used to select mandibles for their test. Overall estimates from tooth wear and replacement and cementum annuli analysis provided similar results. However, accuracy was highly dependent on deer age. Tooth wear and replacement was more accurate for age classes \( \leq 3.5 \) years old and cementum annuli analysis was more accurate for age classes \( >3.5 \) years old. Age estimates according to tooth wear and replacement were highly variable among biologists, and most tended to underestimate the age of deer \( >3.5 \) years old. Using the mode response, biologists correctly aged 71.4% of the jawbones, but overall estimates on all jaws were 62.6% correct. Ages of a subsample of 76 incisors were estimated via cementum annuli analysis resulting in a correct estimate for 71% of the sample. For deer \( >3.5 \) years-of-age, both methods tended to underestimate the actual age. The authors concluded that most southeastern U.S. biologists were able to obtain acceptable results using the tooth wear and replacement method (Jacobson and Reiner 1989).

Mitchell and Smith (1991) reported an overall accuracy rate of 46% from a sample of 69 known-age mandibles from the southeastern United States using the tooth wear and replacement technique. The range of ages from this sample was 0.5 to \( \geq 6.5 \) years. No trends in overestimation or underestimation of particular age classes were seen. The authors also tested the cementum annuli technique and reported an accuracy rate of 41% (Mitchell and Smith 1991).

McCullough (1996) examined the tooth cementum aging technique at varying deer densities for two deer populations. One population consisted of black-tailed deer (\( O. \) hemionus columbianus) in California and the other was a population of whitetails on the George Reserve in Michigan. Data for the George Reserve herd from the years 1952 through 1971 showed no
apparent problems in age estimates from measurements of cementum annuli (McCullough 1979). During this period, the population density ranged from 9.9-19.2 deer/km². However, following a population reduction that reduced densities to 2.2 deer/km² (McCullough 1982, 1983), cementum annuli became progressively more obscure. Subsequently, as population densities again increased, annuli again became apparent (McCullough 1996). Similarly, the herd in California was reduced from about 25 deer/km² to approximately 10 deer/km². Cementum annuli layers were not evident in incisoriform teeth and were obscure in the molariform teeth after the herd reduction (McCullough and Beier 1986). Therefore, at lower deer densities, cementum annuli deposition seemed to become unreliable. These results suggest that in severely reduced populations, such as following epizootics or severe winters, inconsistent deposition of cementum annuli may occur even in strongly seasonal climates (McCullough 1996).

Hamlin et al. (2000) tested biologists in Montana and Washington to determine their ability to correctly estimate age of white-tailed deer and mule deer using tooth wear and replacement. Six biologists were selected for the study, and they correctly assigned ages to 23.8% to 66.7% of 21 known-age whitetail samples. The ranges for mule deer were 54.7% to 71.7%. Cementum annuli-based age estimates for whitetails and mule deer were 92.6% and 85.1% correct, respectively (Hamlin et al. 2000).

Gee et al. (2002) attempted to validate or adjust the tooth wear and replacement technique using 106 known-age white-tailed deer from south-central Oklahoma. They confidently placed deer into fawn, yearling, and adult age classes. They also tested 34 biologists from the Southeast using the known-age samples from Oklahoma and dental casts and reported accuracy rates of approximately 40% for deer ≥2.5 years old. Buccal and lingual crests of molar
teeth were measured and compared but did not allow for better distinction among years (Gee et al. 2002).

Some research has detected sexual differences in wear patterns of deer teeth. Van Deelen et al. (2000) proposed that morphology and wear of the molariform teeth of adult whitetails differ by sex. Sex-based differences may be reflective of differing life history strategies for males and females. Natural selection favors males that grow quickly and attain dominance early, while selection favors longevity in females (Sauer 1984, Van Deelen et al. 2000). Van Deelen et al. (2000) suggest that age estimation based on tooth replacement and wear should be calibrated using local, known-age samples from both sexes.

Marchinton et al. (2003) suggested that age estimation errors might partially explain why some deer populations appeared unresponsive to QDM management strategies, which protect young males. These errors possibly are a result of poor training of biologists and an error in the labeling of a widely used diagram describing the tooth wear patterns of the first molars in 3.5-year-old deer. The error first appeared in the second edition of Wildlife Investigational Techniques (Taber 1963) and has been repeated in subsequent editions. The labeling in the diagram indicates that the dentine line in the crests of the first and second molars is wider than the enamel for the 3.5–year-old age class. If biologists use the technique as described in the manual, they would underestimate the age of most 4.5-year-old deer by one year, and might also underestimate the age of 3.5 year olds as well (Marchinton et al. 2003).

All studies to date have either compared biologists’ age estimates to known-age deer or estimates to cementum annuli estimates. No previous study has attempted to objectively quantify the Severinghaus (1949) technique as originally stated by taking precise measurements of key tooth wear patterns described as indicative for a given age class.
Near-infrared Spectroscopy

Near-infrared spectroscopy (NIRS) has primarily been used in deer management to investigate diet quality and habitat selection (Leite and Stuth 1994). This technology gained most of its recognition from the agricultural arena, where it has been used as a nondestructive method for rapid, accurate, and precise evaluations of the chemical composition and associated feeding value of forages. Each of the major organic components of a sample has specific absorption characteristics in the near infrared region of the spectrum. These characteristics determine diffuse reflectance, which allows for an assessment of composition. Spectra are created from heat-induced, asymmetric stretching vibrations of hydrogen bonds in the functional groups of molecules from the samples being analyzed (Marten et al. 1989). When frequencies of incidental light equal frequencies of vibrational waves, they are absorbed, while other frequencies are reflected or transmitted (Foley et al. 1998).

Recently, the use of NIRS has spread into the ecology field as a means of assessing plant composition, animal tissues, and aspects of animal foraging behavior. Using NIRS, the concentrations of certain materials in feces have been used to assess nutritional status in grazing animals, including white-tailed deer. Also through fecal analysis, NIRS has shown potential for identifying tick infestation, pregnancy, gender, and animal species (Dryden 2003).

Many times in ecological experiments, constraints such as time and available resources to perform required analyses can impact experimental design. This technology can alleviate some constraints by providing a fast, non-destructive and quantitative analysis of a wide range of organic components of plant and animal tissues. Near infrared spectra are dependent upon the quantity and type of C–H, N–H, and O–H bonds in the samples being analyzed, which are the primary components of the organic materials in plant and animal tissues. These spectral features
are then combined with reliable compositional analyses of the sample in a predictive statistical model, which is used to predict the composition of unknown samples. The predictions made by NIRS are determined in part by the accuracy and precision of the reference values for the calibration data set. NIRS also can be used to distinguish among complex mixtures and to identify compounds affecting characteristics of interest (Foley et al. 1998).

To obtain accurate and precise measures using NIRS, an accurate calibration procedure is essential. The core of a calibration procedure is to ensure that the range of spectral variation in the entire population is captured in the samples chosen for developing the calibration model. The whole population is ranked according to distance from the average spectrum. Preceding ranking, several mathematical treatments can be applied to the spectra to highlight only data of importance. These treatments generally include scatter correction using standard normal variate procedures and mathematical derivative transformations (Foley et al. 1998).

After the population has been assessed in this manner, samples with extreme spectra or those with very similar spectra are removed, allowing for the remaining spectra to represent a defined level of spectral variation. Once the appropriate number of samples has been selected, traditional compositional analyses are performed on the subset. The analysis used is dependent upon the component of interest. Following the analyses, the actual model is created by developing a regression equation between the spectral absorbencies and the laboratory analyses (Shenk and Westerhaus 1991, 1993; Foley et al. 1998). This procedure can involve a variety of multivariate regression procedures that may include multiple linear regressions, principal components regression, and partial least squares regression (Foley et al. 1998). A popular procedure for validating the calibration set is called cross validation, in which the population is
randomly divided into a small number of groups for which a prediction is made of the values for one group based on calibrations from the remaining groups (Shenk and Westerhaus 1993).

Due to its growing use for analyzing organic materials, I felt that NIRS might be a valuable, new technique for predicting deer age based on a relationship between chemical composition of teeth and known deer age. The dentine portion of a deer’s tooth is approximately 80% inorganic material, while the remaining 20% is mostly collagen. Given this, I felt NIRS was a valid technique given its ability for rapid and repeatable measures of the chemical makeup of samples with little to no need for sample preparation.

Objectives and Thesis Format

There is a great need for a standardized, reliable method for estimating the age of whitetailed deer. Studies in the whitetail’s northern range suggest that cementum annuli analysis is the better method, while studies done in the southern range suggest the tooth wear and replacement method yields better results. Both methods possess inherent sources of error and variability based on geographic location and wear patterns. However, the accuracy of the tooth wear and replacement technique as described by Severinghaus (1949) has not been assessed objectively. Most studies have compared the two techniques, or evaluated the biologists’ application of tooth wear and replacement.

Using molariform teeth from known-aged deer, I evaluated the use of dentine and enamel width measurements from digital photographs and the use of NIRS as techniques for estimating deer age. The purpose of this study was to standardize and improve tooth-based age estimate techniques using new technologies. I tested two methods, the first being GIS-based measurements of dentine and enamel widths on molar teeth. The second method tested was
NIRS to establish a predictable relationship between chemical composition in molar teeth and known deer age.

This thesis is divided into four chapters. Chapter 1 provides background information on methods for age estimation of white-tailed deer using tooth characteristics, the inherent sources of error associated with these methods, and previous studies that have assessed the accuracy and precision of the methods mentioned. Chapter 2 is in manuscript form (to be submitted to the Wildlife Society Bulletin) and is the main body of the thesis. Chapter 3 is an evaluation of the use of NIRS for predicting age of white-tailed deer. Chapter 4 summarizes my findings, presents management implications, and identifies needs for further research.

**Literature Cited**


CHAPTER 2

AN OBJECTIVE, QUANTITATIVE EVALUATION OF THE SEVERINGHAUS METHOD FOR ESTIMATING AGE OF WHITE-TAILED DEER

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Abstract

Most wildlife biologists use the tooth wear and replacement method to estimate age of harvested white-tailed deer (Odocoileus virginianus). However, its subjectivity is sometimes questioned and there remains a need for an objective, quantitative application of the method. Previous studies have focused on biologists’ application of the technique, but none has truly attempted to objectively quantify the technique based on tooth wear criteria. Using digital photographs and ArcView 3.2a, we measured dentine and enamel widths on molars of 91 wild, known-aged deer from South Carolina to objectively evaluate the tooth wear and replacement technique as presented by Severinghaus (1949). We found that objective measurements of the dentine:enamel ratio of the molariform teeth as originally described could not separate among age classes due to high variability within age classes. To improve predictability, we used a nonparametric, discriminant statistical analysis technique (K nearest neighbor [KNN]) to evaluate a combination of tooth wear measurements. Using KNN analysis, we were able to correctly classify 71.4% of 2.5-year-olds, 25.0% of 3.5-year-olds, and 66.7% of 4.5-year-olds (n = 67) for an overall accuracy rate of 54.4%. Based on our analyses and previous studies, it appears the experienced perspective of a well-trained observer may actually increase the predictability of the tooth wear and replacement technique for aging deer.

Introduction

Strategic management of deer (Odocoileus spp.) populations is dependent on age-specific data (Severinghaus 1949) because biotic potential of individuals within a population varies by age (Alexander 1958). In addition, the popularity of quality deer management has
motivated many deer herd managers to establish age-based harvest criteria, designed to recruit more males into older age classes (≥3.5 years old). Without accurate age estimates for individuals, deer herd managers cannot evaluate current deer population status, nor recognize when their management goals have been achieved. Deer herd managers have long used visual assessment of tooth wear and replacement as described by Severinghaus (1949) for estimating deer age. A second, less-widely used method is the cementum annuli technique (Gilbert 1966). This technique involves counting the annual rings in a stained cross-section of the roots of a deer’s first incisor.

Following the seminal studies by Severinghaus (1949) and Gilbert (1966), several researchers compared the tooth wear and replacement and cementum annuli methods for estimating age of deer. Gilbert and Stolt (1970) reported tooth-wear characteristics exhibited too much variation for reliable and accurate age estimation among older-aged animals. Their conclusions were based on a collection of 682 mandibles that age was assumed to be correctly assigned by cementum annuli analysis. This conclusion is somewhat questionable considering known-aged animals were not used to validate their claims. Using a collection of 55 known-age mandibles from New York ranging in age from 1 to 6.5 years, Sauer (1971) reported that 84% of the sample was correctly classified using cementum annuli analyses, whereas tooth wear was inadequate for assigning ages beyond 1 year, 7 months.

In Texas, Cook and Hart (1979) used marked, free-ranging, known-age deer from two wildlife management areas to compare age estimates provided by biologists and technicians using tooth wear and replacement versus those attained from cementum annuli analysis. Biologists and technicians correctly estimated ages for 66.7% of the samples (N = 242), and most errors were the result of overestimating age. For the 4.5-year-old age class they were
35.5% correct and 46.5% correct for the 5.5-year-old class. The ages of a subset of the collection (n=25) were estimated by cementum annuli analysis, and only 16.0% (4) of the incisors were correctly assigned. Of the 21 incorrect ages assigned, 90.5% (19) were underestimated. The authors state that although cementum annuli can provide accurate age estimates in some areas, the technique should be evaluated using known-age samples from local areas.

Jacobson and Reiner (1989) also compared accuracy of tooth wear and replacement vs. cementum annuli on 94 free-ranging deer (tagged as fawns) from Mississippi, eight free-ranging deer (tagged as fawns) from the Radford Army Ammunition Plant in Virginia, and 48 captive deer from Mississippi State University. Using these samples, 98 known-age jawbones were compiled to test the age estimation accuracy of 55 biologists from the southeastern United States. However, it was not stated what criteria were used to select the mandibles used in the test. Overall estimates from tooth wear and replacement and cementum annuli analysis provided similar results. However, accuracy was highly dependent on deer age. Tooth wear and replacement was more accurate for age classes \( \leq 3.5 \) years old and cementum annuli was more accurate for age classes \( > 3.5 \) years old. Age estimates according to tooth wear and replacement were highly variable among biologists, and most tended to underestimate the age of deer \( > 3.5 \) years old. Using the mode response, biologists correctly aged 71.4% of the jawbones, but overall estimates on all jaws were 62.6% correct. Ages of a subsample of 76 incisors were estimated via cementum annuli analysis resulting in a correct estimate for 71% of the sample. For deer \( > 3.5 \) years-of-age, both methods tended to underestimate the actual age. The authors concluded that most southeastern biologists were able to obtain acceptable results using the tooth wear and replacement method (Jacobson and Reiner 1989).
Mitchell and Smith (1991) reported an overall accuracy rate of 46% from a sample of 69 known-age mandibles from the southeastern United States using the tooth wear and replacement technique. The range of ages from this sample was 0.5 to $\geq 6.5$ years. No trends in overestimation or underestimation of particular age classes were seen. The authors also tested the cementum annuli technique and reported an accuracy rate of 41% (Mitchell and Smith 1991).

Hamlin et al. (2000) tested biologists in Montana and Washington to determine their ability to correctly estimate age of white-tailed deer and mule deer using tooth wear and replacement. Six biologists were selected for the study, and they correctly assigned ages to 23.8% to 66.7% of 21 known-age whitetail samples. The ranges for mule deer were 54.7% to 71.7%. Cementum annuli-based age estimates for whitetails and mule deer were 92.6% and 85.1% correct, respectively (Hamlin et al. 2000).

Gee et al. (2002) attempted to validate or adjust the tooth wear and replacement technique using 106 known-age white-tailed deer from south-central Oklahoma. They confidently placed deer into fawn, yearling and adult age classes. They also tested 34 biologists from the Southeast using the known-age samples from Oklahoma and dental casts and reported accuracy rates of approximately 40% for deer $\geq 2.5$ years old. Buccal and lingual crests of molar teeth were measured and compared but did not allow for better distinction among years (Gee et al. 2002).

Our study differs from those previously mentioned in that we quantitatively measured the variables found in the Severinghaus (1949) technique (i.e., dentine and enamel widths), which allowed for an objective evaluation of this method. To achieve this we used ArcView GIS and digital photography to take precise and accurate measurements of dentine and enamel. A second
step to this study was the use of discriminant analysis to evaluate measures of dentine and enamel widths and their ability to better predict deer age.

**Methods**

We used mandibles obtained from known-aged, wild, hunter-harvested deer collected by the South Carolina Department of Natural Resources. These deer were captured as either fawns or yearlings and were collected from Hampton, Jasper, and Williamsburg Counties. Ages of deer in the sample that provided sufficient numbers of representatives per age class ranged from 1.5-4.5 years old ($n = 91$).

We photographed mandibles with a 4.0-megapixel digital camera mounted to a tripod at 44.5 cm directly above each mandible. Before photographing, we removed debris from the teeth using a dental pick and compressed air, and added a metric ruler to the view for size reference. Photographs were imported into ArcView 3.2a (ESRI, Redlands, CA) to facilitate accurate and precise measurement of tooth characteristics. Once in ArcView, an individual theme was created for each tooth, which allowed editing of individual teeth. We used the X-Tools extension to calculate dentine and enamel widths based on a reference scale for each image. Enamel measurements were taken on the occlusal surface of each molar (i.e., widths from each side of the dentine were summed to obtain a total enamel measurement). We measured widths of lingual enamel, lingual dentine, buccal enamel, and buccal dentine on each sample in hundredths of a millimeter. These data were exported into Excel (Microsoft Corp., Redmond, WA) to facilitate age-class comparisons.

We examined several comparisons between tooth measurements and known age. These comparisons included the ratio of dentine to enamel on the lingual and buccal crests of the molariform teeth for each age class to objectively quantify the Severinghaus (1949) technique
based on the criteria described for each age category. We also studied individual dentine widths on the lingual crests of molars 1, 2, and 3 and the third cusp of molar 3 and assessed their association with age classes because traditionally these have been areas of emphasis used by biologists to aid in separating 2.5-, 3.5-, and 4.5-year-old deer.

In an attempt to increase classification accuracy, we conducted a nonparametric discriminant analysis (K-nearest neighbor classification with cross-validation [KNN]; Cover and Hart 1967, Hand 1982) of combined tooth measurements in a predictive model. The KNN analysis is used to predict a response of an observation using a nonparametric estimate of the response distribution of its K nearest neighbors and does not require an assumption of multivariate normality. However, it assumes that characteristics of members of the same class should be similar and, thus, observations closer together in covariate space belong to the same class (Peterson 1998). This analysis was conducted in SAS 8.2 (Cary, NC) using dentine:enamel ratios on the lingual and buccal crests of molars 1, 2, and 3 from deer 2.5-4.5 years old (n=67).

Results

We found that dentine:enamel ratios on the lingual crest of the molariform teeth were ineffective at separating the 2.5-, 3.5-, and 4.5-year-old age classes and there was much overlap in ranges among age classes (Figure 1). The original criteria described by Severinghaus (1949) for the lingual crest of molar 1 stated that dentine widths do not become wider than enamel widths until age 3.5. On the lingual crest of molar 1, we saw dentine:enamel ratios >1 for the 1.5-year-old age class. We did not include the 1.5-year-old age class in the analyses because this age class can be identified using tooth replacement criteria and we could not obtain sufficient measurements from molar 3 for comparisons. Except lingual and buccal ratios of molar 1, we
identified a progressive increase in mean dentine:enamel ratio as age increased; however, the great amount of overlap prohibited accurate age classification. According to Severinghaus (1949), dentine widths on the buccal crest of molars 1 and 2 were not to exceed the enamel widths until age 3.5. We found that dentine:enamel ratios on the buccal crests of molars 1 and 2 were >1 for the 2.5-year-old age class. Although we observed some separation among age classes by examining means and standard errors of the ratios (e.g., on lingual and buccal dentine:enamel ratios of molar 3), the distribution of the data suggested a type of nonparametric discriminant analysis would be needed to assess the ability of the dentine:enamel ratios to predict deer age.

Because of the distribution of our data, we used KNN classification to attempt to create a predictive model based on ratios of dentine:enamel at an optimal $K$ value of 14. The model was poor at distinguishing 3.5-year-old deer, misclassifying equal numbers of deer as 2.5 and 4.5 years old (Table 1). The KNN classification indicated that tooth measurements yielded an overall accuracy rate of 54.4%. To increase classification accuracy, we combined the 3.5- and 4.5-year-old age classes. The overall accuracy rate following this combination increased to 73.8% (correctly classified 77.5% of 2.5-year-olds and 86.1% of deer $\geq$3.5 years old) at an optimal $K$ value of 11.
Figure 1. Dentine:enamel ratios from individual mandibles with means (periods), standard errors (boxes), and ranges (bars) for lingual and buccal crests of molars 1, 2, and 3 from the South Carolina known-aged sample of deer ages 2.5–4.5 years (n = 67).
Table 1. Correct age classification percentages and associated error rates using K nearest
neighbor analysis with cross-validation of dentine:enamel ratios from South Carolina known-age
mandibles of deer ages 2.5-4.5 years (n = 67).

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>% Classified into age class</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>2.5</td>
<td>21</td>
<td>71.4</td>
<td>19.1</td>
</tr>
<tr>
<td>3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28</td>
<td>35.7</td>
<td>25.0</td>
</tr>
<tr>
<td>4.5</td>
<td>18</td>
<td>0.00</td>
<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> One ratio classified by SAS as “other” constituted 3.6%
Discussion

When objectively quantified using digital photographs and ArcView 3.2a, the Severinghaus (1949) technique was a poor predictor of deer age. Specific delineation of dentine and enamel widths can be somewhat of an art depending on the orientation of the mandible when photographed. According to the Severinghaus technique, the width of dentine in relation to the width of the enamel on the lingual crest of the first molar should separate the 2.5- and 3.5-year-old age classes. However, we found significant overlap in the dentine to enamel ratio between these 2 age classes. Similarly, dentine width in relation to enamel width on the lingual crest of molar 2 is critical to separating the 3.5- and 4.5-year-old age classes. Again, we found this criterion to be unreliable. However, previous research has reported acceptable levels of accuracy obtained from biologists’ estimates of known-age deer using the Severinghaus (1949) technique, suggesting that the subjective evaluation by well-trained observers may increase the accuracy of the tooth wear and replacement technique over strict application of the technique. Another factor that may influence biologists’ accuracy rates is differing shades of dentine. The colors of dentine seen on the mandibles used in this study covered a spectrum from a light tan coloration to almost black. Thus the utility of the technique itself may be dependent on the subjective and simultaneous evaluation of multiple tooth wear criteria and the ability to interpret varying shades of dentine by experienced observers.

Using the KNN discriminant analysis, we were able to classify 2.5-year-old deer with reasonable accuracy (71.4%). However, the 3.5-year-old age class was classified correctly only 25% of the time. The error in this age class was split evenly between misclassifying 3.5-year-olds as 2.5-year-olds and 3.5-year-olds as 4.5-year-olds. When the 3.5- and 4.5-year-old age classes were combined, accuracy rates increased. However, combining the 3.5- and 4.5-year-old
age classes was not a desirable option, given the limitations on the range of ages from this sample. This limitation may have influenced the percentage correctly classified into the $\geq 3.5$-year-old age class. Considering the variability observed among individuals, it is probable that the percent correctly classified into this age class would be reduced if mandibles from older deer were present in our sample. The overall accuracy rate we observed using the KNN analysis (54.4%) was slightly better than results reported using tooth wear and replacement by Mitchell and Smith (1991) and Gee et. al (2002) at 46% and 40% (for deer $\geq 2.5$ years old), respectively, but lower than what was reported by Cook and Hart (1979) and Jacobson and Reiner (1989) at 66.7% and 62.6%, respectively. Our results also fall in between accuracy rates reported from cementum annuli analyses in previous research. Cook and Hart (1979), Jacobson and Reiner (1989), Mitchell and Smith (1991), and Hamlin et al. (2000) reported accuracy rates from the cementum annuli technique of 16%, 71%, 41%, and 92.6% respectively.

Management Implications

Based on previous research, accuracy rates obtained from biologists’ estimates using tooth wear and replacement and cementum annuli analysis of known-age samples have been variable. Jacobson and Reiner (1989) found no significant difference between accuracy rates obtained by southeastern U.S. biologists vs. those from cementum analysis. The authors stated that the tooth wear and replacement was the better method for deer $\leq 3.5$ years old, whereas cementum annuli gave better results for deer $>3.5$ years. Differing results were reported by Mitchell and Smith (1991) and Gee et al. (2002), where accuracy rates were 46% (for all deer) and 40% (for ages $\geq 2.5$ years old), respectively. Based on our results, strict application of the guidelines described by Severinghaus (1949) results in an unacceptably high error rate in
estimation. In addition, the subjective estimates of well-trained biologists are of similar accuracy as our multivariate analyses of objective measurements of dentine and enamel.

As deer populations continue to expand and hunter objectives change, deer management in many areas of the United States is undergoing dramatic changes. A shift towards quality deer management has increased the importance of age-related data. Because deer age affects body growth, antler quality, fertility of does, and sex ratios of offspring, age-specific criteria must be established on a localized basis (Verme 1983, Sauer 1984, Miller and Marchinton 1995). In some areas, managers have experienced difficulties in achieving a higher proportion of older bucks (i.e., \( \geq 3.5 \) years old) in a herd. It is possible that these difficulties could be associated with age estimation errors rather than lack of response to management strategies.

This study provides insight into the accuracy of the tooth wear and replacement technique as originally described. The variability of biologists’ accuracy rates reported from previous studies (Cook and Hart 1979, Jacobson and Reiner 1989, Mitchell and Smith 1991, Gee et al. 2002) may not be failures of the biologists in their application of the technique, but rather inaccuracies and variability in the technique itself. Until there is a more reliable method for determining age of white-tailed deer, managers should confine the use of the tooth wear and replacement technique to determination of fawn, yearling, and adult age categories as stated by Gee et al. (2002).

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Literature Cited


CHAPTER 3:

THE INABILITY OF NEAR-INFRARED SPECTROSCOPY ANALYSIS OF TEETH TO ESTIMATE AGE OF WHITE-TAILED DEER

Abstract

Wildlife biologists often need to estimate age of white-tailed deer (*Odocoileus virginianus*), but available techniques are subjective or require long processing times. Near-infrared spectroscopy (NIRS) has been used to assess livestock forage quality, composition of plant and animal tissue, and wood quality. Because the dentine portion of a deer’s tooth is composed of both organic and inorganic material, we hypothesized that NIRS could be used to estimate age of deer. We examined teeth from known-aged, captive deer from the University of Georgia (UGA; *n* = 63) ranging in age from 1.5-6.5 years. We used readings on the molars of jaws to generate a regression relationship with age. In an effort to better depict variation among samples, we assigned deer to month-specific age categories. The calibration model for molar 3 was exceptionally weak, so we excluded the predictive values for that tooth. The results for the other two molars were highly variable and produced an unreliable predictive relationship with age (*R*² = 0.02 and SEP = 29.56 months for molar 1; *R*² = 0.26 and SEP = 21.69 months for molar 2). Although this technique was not a reliable method for estimating age of white-tailed deer, we feel all new technologies showing promise should be investigated using reliable known-age mandibles.

Introduction

Near-infrared spectroscopy (NIRS) has been used in the analysis of forage quality for livestock feeds. This method of forage analysis is a nondestructive, instrument-based method for rapid, accurate, and precise evaluations of the chemical composition and associated feeding value attributes of forages. Each of the major organic components of a sample has specific absorption characteristics in the near infrared region of the spectrum. These characteristics
determine diffuse reflectance, which allows for an assessment of composition. Spectra are created from heat-induced, asymmetric stretching vibrations of hydrogen bonds in the functional groups of molecules from the samples being analyzed (1). When frequencies of incidental light equal frequencies of vibrational waves, they are absorbed, while other frequencies are reflected or transmitted (2).

Recently, ecological applications of NIRS technology have included assessment of plant composition, animal tissues, aspects of animal foraging behavior, and parasite loads. Using NIRS, the concentrations of certain materials in feces have been used to assess nutritional status in grazing animals, including white-tailed deer. Also through fecal analysis, NIRS has shown potential for identifying tick infestation, pregnancy, gender, and animal species (3).

Time and available resources often constrain the design of ecological experiments; NIRS technology has the potential to provide a fast, nondestructive, and quantitative analysis of a wide range of organic components of plant and animal tissues. Near infrared spectra are dependent upon the quantity and type of C–H, N–H, and O–H bonds in the samples being analyzed, which are the primary components of the organic materials in plant and animal tissues. These spectral features are then combined with reliable compositional analyses of known samples in a statistical model, which is used to predict the composition of unknown samples. The predictions made by NIRS are determined in part by the accuracy and precision of the reference values for the calibration data set. NIRS also can be used to distinguish among complex mixtures and to identify compounds affecting characteristics of interest (2).

An accurate calibration procedure is essential to obtain accurate and precise measures using NIRS. The core of a calibration procedure is to ensure that the range of spectral variation in the entire population is captured in the samples chosen for calibration. The whole population
is ranked according to distance from the average spectrum, however this method is not used in all cases. Preceding ranking, scatter correction using standard normal variate procedures and mathematical derivative transformations (2) are used to highlight data of importance. After the population has been assessed, samples with extreme spectra or very similar spectra are removed, allowing the remaining spectra to represent a defined level of spectral variation. Once the appropriate number of samples has been selected, traditional compositional analyses are performed. Subsequently, the actual model is created by using multiple linear regression, principal components regression, or partial least squares regression between the spectral absorbencies and the laboratory analyses (3,4). Cross validation of the calibration often is used by dividing the population into a small number of groups, for which predictions are made for one group based on calibrations from the remaining groups (5).

The dentine portion of a deer’s tooth is approximately 80% inorganic material, while the remaining 20% is mostly collagen. Therefore, we tested whether NIRS was a valid technique for rapidly and reliably estimating age of deer based on the chemical composition of teeth, with little need for sample preparation.

**Methods**

We examined compositional changes in teeth in relation to known deer age using NIRS analysis of a sample of mandibles from captive deer from UGA \((n = 63)\). We used the sample of captive deer for our NIRS analysis due to the controlled environment of captivity. Also, in an effort to better depict variation among samples, we assigned deer to month-specific age categories.
Spectra were collected from the molars of individual mandibles using an Ocean Optics, Inc. (Dunedin, FL) NIR512 instrument. A fiber optic probe was connected to both the spectrometer and the accompanying light source; the end of the probe was placed 5 mm from the tooth surface. Absorption spectra were collected for each of the three molars.

Statistical analyses were performed on the spectra using partial least-squares (PLS) regression (6). Cross-validation was used to evaluate classification accuracy. Data were examined using the raw spectra plus with the mathematical treatments first derivative, second derivative, and multiplicative scatter correction [MSC]. These data were separated into two distinct data sets—the calibration and the validation (prediction) sets. The calibration data set for molars 1 and 2 contained 40 mandibles, while the set for molar 3 contained 38 samples. The prediction data sets for molars 1 and 2 contained 18 and 17 mandibles, respectively. Calibrations were created and then tested to predict ages of deer not included in the calibration, which allowed for an unbiased assessment of the applicability of a calibration. Calibration and prediction results were presented as the coefficient of determination ($R^2$) when the optimal number of PLS factors was used along with standard error of calibration (SEC), standard error of cross-validation (SECV), and standard error of prediction (SEP) (The Unscrambler, Camo, Woodbridge, NJ). The standard error of cross-validation was used to identify and prevent overfitting the model.

**Results and Discussion**

The calibration model for molar 3 was weak, so we excluded the predictive values for that tooth. Using PLS regression, we found a weak and unpredictable relationship between spectral readings and known age on each of the molar teeth (Table I). The data treatment that
produced the best results was MSC. Using the first and second derivatives yielded weak calibration models, thus predictive values using these treatments also were excluded.

In the analysis of the mandibles from UGA, this technique proved to be a poor predictor of deer age. This technique’s predictive abilities were less than what has been reported for biologists’ subjective age estimates using tooth wear and replacement as described by Severinghaus (1949) (7). In addition, we are unsure what compositional changes in the teeth were being identified using NIRS, thus making the value of this technique for aging deer questionable, at the current time.

Although NIRS technology has been used extensively in livestock forage analysis, and more recently in wood quality assessment, its value to the field of wildlife management is still being investigated. Recently NIRS has been used in the deer management profession to evaluate diet and parasite loads (3). We felt that investigating new techniques that show initial promise in their ability to predict age, like NIRS, are crucial to finding a more reliable method for estimating age of deer. Until a method is discovered that eliminates subjectivity and long processing time, researchers must strive to find new techniques as well as reliable, known-age samples to validate these techniques.
Table I. Regression statistics for near-infrared spectroscopy (NIRS) results on molars 1, 2, and 3 from the captive University of Georgia sample of deer using multiplicative scatter corrected (MSC) treated near-infrared spectra.

<table>
<thead>
<tr>
<th>Molar</th>
<th>Factors&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Calibration</th>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>R²</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>40</td>
<td>0.76</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>40</td>
<td>0.78</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>38</td>
<td>0.31</td>
</tr>
</tbody>
</table>

<sup>a</sup> Optimal number of factors from partial least-squares (PLS) regression analysis.

<sup>b</sup> SEC - standard error of calibration, SECV - standard error of cross-validation, and SEP - standard error of prediction.
Literature Cited


CHAPTER 4

SUMMARY AND IMPLICATIONS FOR ESTIMATING DEER AGE USING TOOTH CHARACTERISTICS
Summary

Age-related data are crucial to evaluate the current and past status of deer populations and to plan herd management strategies. Realizing this importance, researchers have attempted to refine existing techniques for estimating age of deer or develop new ones. However, current deer aging techniques still do not provide reliable age predictions across geographical regions. Much has been theorized about factors that may influence a deer’s tooth wear patterns. However, it would be virtually impossible to design a study that encompasses all these factors. Still, when new age estimation techniques show promise, they should be tested with known-aged samples across different populations to identify important sources of variation.

My study was designed to objectively and quantitatively evaluate the tooth wear and replacement technique by recording precise measurements of dentine and enamel widths and testing relationships between those morphological measurements and known deer age using discriminant analysis. I used ArcView 3.2a to measure dentine and enamel widths from each of the three molariform teeth. I then compared the tooth measurements to specific age-related wear patterns as stated by Severinghaus (1949). I found no distinguishing differences in dentine and enamel widths among age classes based on 91 mandibles from known-aged deer from South Carolina. Based on previous studies that tested the accuracy of trained biologists, I believe the tooth wear and replacement technique when combined with the experience of well-trained observers may provide more reliable age estimates than does precise measurements of dentine and enamel widths. This implies that subjectivity when coupled with experience and training improves predictability.

I also tested the value of near-infrared spectroscopy as a method for aging deer by their teeth. Initially, the near-infrared technology showed promise, in that it is designed to detect
subtle changes in the organic material of a sample. After testing the captive samples, I found a weak relationship with low levels of predictability between infrared spectra and known age. Although the near-infrared technology did not provide a better technique for estimating age of deer, only by investigating new technologies can we hope to eventually discover a more reliable technique.

**Implications for Estimating Deer Age Using Tooth Characteristics**

As deer populations continue to expand and hunter objectives change, deer management in much of the United States is undergoing dramatic changes. A shift towards quality deer management has increased the importance of age-related data. Because deer age affects body growth, antler quality, fertility of does, and sex ratios of offspring, age-specific criteria must be established on a localized basis (Verme 1983; Sauer 1984; Miller and Marchinton 1995). In some areas, managers have experienced difficulties in achieving a higher proportion of older bucks (i.e., \( \geq 3.5 \) years old) in a herd. It is possible that these difficulties could be associated with age estimation errors rather than lack of response to management strategies.

This study provides insight into the accuracy of the tooth wear and replacement technique when applied as originally stated. My results suggest that managers should use caution when estimating age using strict tooth wear and replacement guidelines (i.e., the ability of a well-trained observer to evaluate multiple tooth wear factors at once may provide more accurate results). By using the Severinghaus (1949) technique, I found that using dentine:enamel ratios on the molar teeth were inadequate at estimating deer age beyond age 1.5. This is similar to the conclusions of Gee et al. (2002) in that they could correctly classify deer into three age categories: fawn, yearling, and adult. The discriminant analysis allowed me to correctly classify
71% of 2.5-year-old deer. However beyond age 2.5, this technique became unreliable, yielding an overall accuracy rate of 54%. The accuracy rate I observed was slightly better than biologists’ estimates seen in Mitchell and Smith (1991) and Gee et al. (2002), but lower than what was reported by Cook and Hart (1979) and Jacobson and Reiner (1989). It may be that until there is a more reliable method for determining age of white-tailed deer, managers should not use the tooth wear and replacement technique for age estimation beyond the general categories of fawn, yearling, and adult as stated by Gee et al. (2002).

**Literature Cited**


