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MECHANICAL PROPERTIES OF DIET AND ITS EFFECT ON ORAL HEALTH

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MECHANICAL PROPERTIES OF DIET AND ITS EFFECT ON ORAL HEALTH

by

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Bachelor of Science
George Mason University, 2010

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Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in

Biomedical Science

School of Medicine

University of South Carolina

2014

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ACKNOWLEDGEMENTS

I would never have been able to finish my dissertation without the guidance of my committee members, help from friends, and support from my family members. I would like to express my deepest gratitude to my advisor, Dr. Adam Hartstone-Rose, for his guidance throughout the process.

I would like to thank my committee members Dr. Edie Goldsmith and Dr. Chandrashekhar Patel for their time and valuable advice.

Also special thanks goes to Tyler Antonelli and Hannah Selvey for collecting valuable data and always being there to help. My research would not have been possible without their help.

I would also like to thank my mom, sister and boyfriend for always supporting and encouraging me.

ABSTRACT

The predominant diet fed to captive carnivores consists of ground meat formulated to provide full nutritional requirements. However, this ground meat diet completely lacks the mechanical properties such as toughness, hardness and stiffness of the foods the animals would eat in the wild. The goal of this research was to evaluate the effect of mechanical properties of diet on oral health by comparing prevalence of periodontal disease and calculus accumulation in wild and captive felids. One-way ANOVA analysis of variance indicated that these differences are statistically significant (P<.0001). The results of this study indicate that diet texture is a significant factor in oral health of felids. Also there is a significant correlation between oral health and overall health of lions and tigers.

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CHAPTER 1

Introduction & Background

The composition of the diet of captive carnivores is of great interest to animal curators, keepers and veterinarians. However, that interest has focused almost entirely on the nutritional composition of the diet (Haberstroh et al., 1984) and there is little consideration given to the mechanical properties such as toughness, hardness and stiffness of the foods. While the incorporation of bones as dietary supplements is a growing trend for captive carnivores, the predominant diet that captive carnivores are fed consists of ground meat (Haberstroh et al., 1984). While this diet contains all of the nutrients found in a natural diet, it does not simulate the ingestive or masticatory challenges that wild carnivores face. In particular, the lack of bone and connective tissue manipulation in the captive diet may lead to unnatural dental health issues (Skibiel, Trevino, & Naugher, 2007). Numerous studies in domestic animals, as reviewed in Watson (1994) and Logan (2006) have demonstrated that mechanical attributes of the diet appear to affect calculus buildup and oral health in general. Thorough examination of diets of captive felids and their wild conspecifics, with a focus on the mechanical properties of the diet and their effects on the oral health remains to be carried out.

In this study, I will investigate oral health of captive and wild felids by comparing degree of calculus build-up and periodontal disease and finally correlate these markers of oral health to a proxy of overall health.

Background

Several factors may affect the pathogenesis of dental abnormalities such as genetics, environment, injuries, development, infection and nutrition (Wiggs & Bloom,

2003). My research will focus on the nutrition aspect and how diet affects the oral health in captive versus wild felids. Studies have shown that the mechanical properties of diet may have a distinct effect on an animal's dental health and cranial morphology (O'Regan & Kitchener, 2005).

Experimental Findings

A study conducted on dogs and cats showed that food composition and texture could directly affect the oral health through maintenance of tissue integrity, stimulation of salivary flow and cleaning of the tooth surfaces by appropriate abrasive action (Watson, 1994). The texture of food has been long thought to affect the oral health of dogs and cats (Logan, 2006). Several studies have proven that excessive calculus accumulation; gingivitis and periodontal disease have been noted in animals on a soft diet. A study conducted by (Vosburgh, 1982) on timber wolves showed that diet texture is a significant factor in the oral health of these captive wolves. The wolves fed a hard, dry diet exhibited significantly lower levels of plaque accumulation than those fed a soft, moist, meat-based diet (Vosburgh, 1982). Dry diet is associated with less gingival pathology because the soft textured diet, does not provide adequate abrasive action to clean the teeth (Haberstroh et al., 1984).

Another study conducted on ferrets showed the importance of diet and texture on oral health. Ferrets were used for this study because they exhibit similar patterns of plaque and calculus accumulation leading to periodontal disease as seen in other domestic carnivores. The findings showed that ferrets kept on a soft diet such as bread and milk developed periodontal disease within 8-12 weeks. The addition of bones to these diets

prevented periodontal disease and even caused a decrease in calculus accumulation (Verstraete, 2003). Numerous other studies conducted on different types of animals showed similar relationship between diet and oral health. Since my study is based on analyzing periodontal disease and calculus build-up in captive and wild felids it is important to have a basic understanding of dental terminologies and general anatomy of teeth.

Dental Anatomy

Although the teeth of felids can vary in shape, size and functions, the constituents and structure of all teeth are similar. A tooth comprises of crown, which is the anatomical area of teeth usually covered by enamel, and roots, which is covered by the cementum. The junction where the enamel of the crown and the cementum of the root converge is called the cementoenamel junction (CEJ). (Logan, 2006)

The periodontium provides the support necessary to maintain healthy functions of teeth. It consists of gingiva, periodontal ligament, alveolar bone and cementum (Mariotti, 2007). The gingiva is the soft tissue oral mucosa that covers the alveolar bone of the jaws and surrounds the necks of the teeth (Mariotti, 2007) . In healthy mammals, normal gingiva covers the alveolar bone and tooth root to a level just below the area where enamel meets the root surface (Niemiec, 2008a). The periodontal ligament is composed of a complex vascular and highly cellular connective tissue that surrounds the tooth root and connects it to the inner wall of the alveolar bone (Newman, 2006). The alveolar bone surrounds the teeth and it is a tooth dependent structure; it creates the socket that the tooth sits in and is connected to the tooth via the periodontal ligament (Newman, 2006) .

It is formed with the eruption of the teeth and generally reabsorbed when the teeth is removed (Gioso & Carvalho, 2005).

Problems with the periodontium arise once accumulation of food particles and bacteria form a substance called plaque that sticks to the surface of the tooth (Gioso & Carvalho, 2005). Plaque is a microbial biofilm, consisting of an organized community of cooperating microorganism on surface of the teeth (Albuquerque et al., 2012). If plaque is not removed by mechanical cleaning, minerals in the saliva harden the plaque into dental calculus. As plaque and calculus spread under the gingival line it causes irritation to the gingiva and leads to an inflammatory condition known as gingivitis (Campbell, 2007). Gingivitis is the mildest form of periodontal disease and it does not affect the underlying supporting structures of the teeth and is usually reversible (Pihlstrom, Michalowicz, & Johnson, 2005). This early stage of inflammation, if left untreated, will lead to periodontal disease (Gioso & Carvalho, 2005) .

Dental Formulae of Felids

A typical felid has a total of thirty teeth. Generally their dental formulae is written as I = 3/3 C=1/1 P=3/2 M=1/1 where I, C, P and M stand for Incisors, Canines, Premolars and Molars respectively and the number on either side of the "/" stand for the number of teeth of that kind in each upper and lower quadrant respectively (Wiggs & Bloom, 2003). The generalized Felids dental row (Figure1.1) can be broken into four functional units: the incisors, canines, premolars and molars.
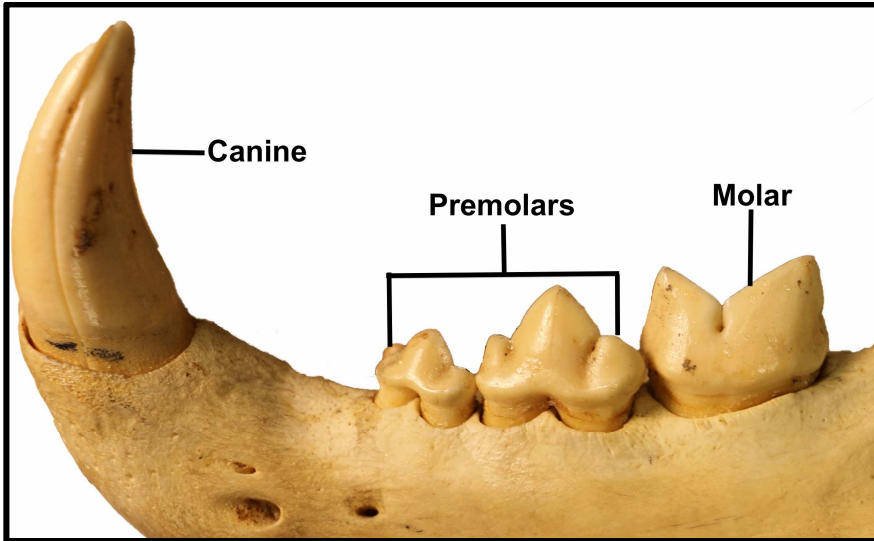
Figure.1.1: The Mandibular Tooth Row of *Panthera tigris*
 USNM 253289

Dental Calculus

Dental calculus can be defined as calcified or calcifying deposits that are attached to the surface of teeth (Newman, 2006) . Calculus can be classified into two groups depending on the location. Supra-gingival calculus is located above the gingival margin and sub-gingival calculus located below the gingival margin (Roberts-Harry & Clerehugh, 2000). Calculus or tartar are usually formed as a result of the calcification of dental plaque or biofilm (Busscher, 2004).

According to Vosburgh (1982), plaque a precursor of calculus tends to accumulate at the opening of salivary duct near the maxillary premolar or where the tongue does not regularly contact the tooth surface. This could explain why the front of the canines exhibit little plaque and calculus build-up.

Calculus is mostly made up of inorganic compound, consisting of crystalline salts and the organic portion of dental calculus consists of proteins, carbohydrates and lipids. A layer of active plaque usually covers calculus, plaque comprises of living bacteria and as the active layers of plaque mineralizes, and new calculus is formed. Calculus formation results in displacing epithelium around the gum line, allowing bacteria from the plaque to reach the alveolar bone. (Greene, Kuba, & Irish, 2005)

Periodontal Disease

In veterinary medicine periodontal disease is the most prevalent disease in domestic carnivores and is found in approximately 80% of dogs aged 2 years or older (Niemiec, 2008b). Periodontal diseases are pathological processes that affect the tissues surrounding the teeth. Previous names for periodontal diseases includes gum disease, pyorrhea, and periodontitis (Mariotti, 2007). Periodontal disease (PD) can be referred as a group of inflammatory diseases caused by bacterial plaque in the periodontium (Albuquerque et al., 2012).

Periodontal disease can be broadly divided into two groups, gingivitis and periodontitis. Gingivitis is the reversible stage of periodontal disease and can be treated with thorough plaque removal whereas periodontitis is more severe (Logan, 2006). Periodontitis can be referred to as the destructive form of periodontal disease and it is characterized by several alterations in periodontal tissues such as inflammation and loss of the periodontal attachment (Mariotti, 2007). The hallmarks for periodontitis are alveolar bone loss, periodontal pocketing, gingival inflammation and attachment loss (Mariotti, 2007).

Plaque deposits present in cases of untreated gingivitis will form calculus. As this calculus builds under the gingiva, the bacteria in the sub-gingival plaque set in motion a cycle of damage to the supporting tissues around the tooth. Once periodontal disease begins, pockets will form where the teeth meet the gingiva and bone fostering bacterial growth. This growth can lead to bone loss, tissue destruction or tooth loss. (Campbell, 2007)
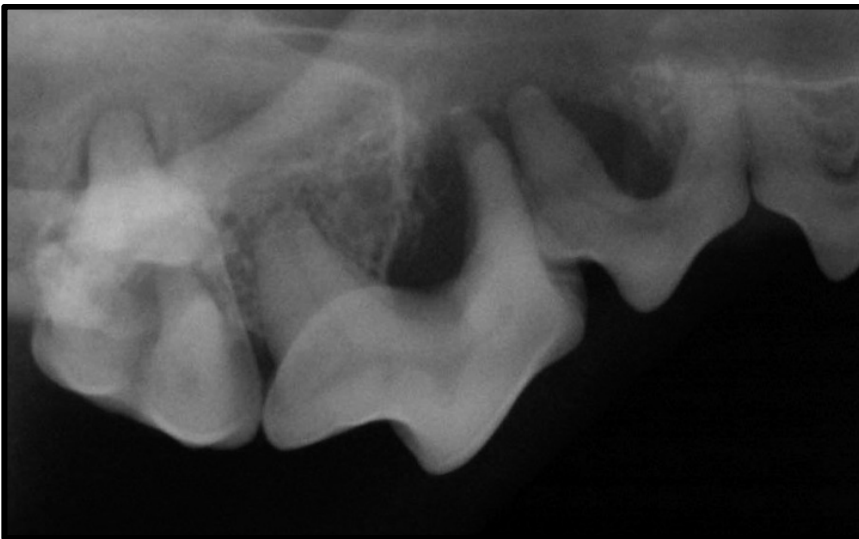
Figure 1.2: Intraoral Bisecting-angle Dental Radiograph of a Dog modified from (Niemiec, 2008b).

This clinical radiograph of the maxillary right premolars reveals severe periodontium loss and reabsorption of alveolar bone associated with periodontal disease. This is indicative of severe periodontal disease (Niemiec, 2008b) .

For this study we used skulls of captive and wild felids to score for periodontal disease and dental calculus. Since periodontal disease affects the alveolar bone (Figure 1.2 and 1.3), skulls were scored for periodontal disease by measuring the distance the alveolar ridge degraded from its original position, combined with sizes of the holes in

the alveolar bone, refer to Chapter II for a detailed scoring technique which was used to measure dental calculus and periodontal disease.

Although a combination of dental plaque and dental calculus is the primary cause of periodontal disease, we should keep in mind several additional factors that may contribute to periodontal disease such as malocclusions, absence of oral hygiene, teeth crowding and genetics (Albuquerque et al., 2012).

Effects of aging on oral disease

When comparing wild and captive felids we should keep in mind how age affects calculus and periodontal disease. Improved veterinary care in zoos has resulted in an increased longevity in captive felids (Longley, 2011). According to several sources (Albuquerque et al., 2012; Gawor et al., 2006; Patterson, Neiburger, & Kasiki, 2003; Sone, Koyasu, & Oda, 2004; Watson, 1994) the prevalence and severity of periodontal disease is age related. One of the limitations of this study was that we did not know the age of our specimen when scoring for periodontal disease and dental calculus.

One might argue our results to be skewed due to age restriction but a study by Gawor (2006) on the influence of diet on oral health of cats and dogs showed that even after they adjusted for age, the mean oral health index was significantly higher in cats and dogs fed soft food compared with those fed dry or mixed food. These results clearly indicate that feeding a dry food diet has a positive influence on oral health (Gawor et al., 2006)

Furthermore, we have found clearly young specimens with advanced oral health diseases (Fig. 1.4) demonstrating that although age might be a confounding factor in this study it is not necessarily the main correlate of dental disease.
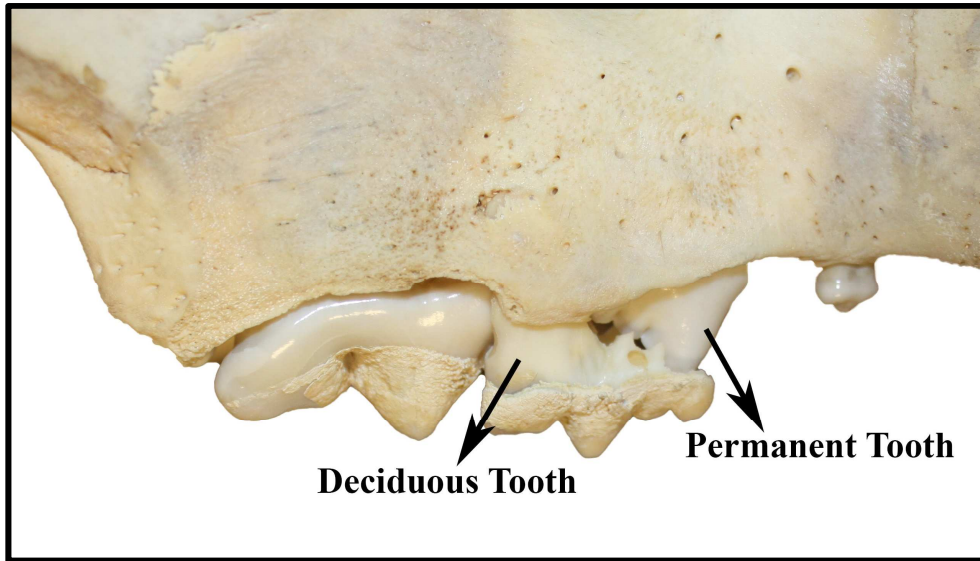
Figure 1.3: Severe Calculus on Maxillary Premolars of a Juvenille Tiger. Catalog number: USNM 396272

Note the deciduous premolar being replaced by the permanent premolar. This specimen was studied for graphical purposes though only adult specimens are included in the statistical analyses.

Specific Aims and Hypotheses

The overall goal of this study is to assess the effect of mechanical properties of diet on oral health. This goal is achieved by determining if differences in periodontal disease and dental calculus exist between wild and captive felids. Multiple hypotheses relating oral health to captivity and overall health will be tested.

Aim 1: To determine if the incidence of dental calculus and periodontal disease is higher in captive felids.

Studies on various animals as discussed in this paper, have shown that excessive calculus accumulation and periodontal disease have been noted in animals on a soft diet.

*Experimental Hypothesis 1***:** Because the predominant diet that captive carnivores are fed consists of ground meat (Haberstroh et al., 1984), it is predicted that captive felids will have higher incidence of dental calculus and periodontal disease compared to the wild felids.

Aim 2: To determine the correlation between oral health and overall health.

Since gingivitis and early stages of periodontal disease is both curable and preventable, evidence showing that it is an independent risk factor leading to other systemic diseases and condition would be of great importance in public health (Campbell, 2007).

*Experimental Hypothesis 2***:** Higher PC2 scores, which is a measure of skull deformity is correlated to higher periodontal disease and dental calculus.

Aim 3: To determine prevalence of calculus and periodontal disease in the posterior versus anterior dentition.

Carnivores display a diverse array of teeth, all of which are presumed to be adapted for certain functions, such as slicing flesh, killing its prey, cracking into bones and chewing (VanValkenburgh, 1996). Van Valkenburgh (1996) describes the general dentition of carnivores as being comprised of three regions: the grasping incisors, penetrating canines and food processing cheek teeth. For this study we want to see if there is a significant

interactions between the anterior and posterior teeth. Since the function differs in anterior versus posterior teeth, will this affect the degree of periodontal disease and calculus buildup? When scoring the skulls at the museum, we noticed higher incidence of calculus on the posterior teeth, when compared to the anterior teeth and an opposite trend was observed when scoring for periodontal disease.

***Experimental Hypothesis 3*:** Higher prevalence of calculus will be observed in the posterior teeth and higher prevalence of periodontal disease will be observed in the anterior teeth.

CHAPTER 2

Methods

Sample

The present study will focus primarily on lions (*Panthera leo*) and tigers

(*P. tigris*) (Table 2.1) though data were also collected on other carnivores as well

(Table 2.2) for future analysis.

Table 2.1: Focal Sample (N = 83)

Common Name	Species	Captive (Zoo)	Wild	Unknown
Lion	*Panthera leo*	n = 22	n = 25	n = 0
Tiger	*Panthera tigris*	n = 23	n = 13	n = 0
	TOTAL	45	38	83

Table 2.2 Other preliminary Sample (N = 133)

Common Name	Species	Captive (Zoo)	Wild	Unknown
Jaguar	*Panthera onca*	n = 5	n = 4	n = 2
Leopard	*Panthera pardus*	n = 7	n = 6	n = 0
Snow Leopard	*Panthera uncia*	n = 7	n = 1	n = 1
Clouded Leopard	*Neofelis nebulosa*	n = 4	n = 0	n = 0
American Black Bear	*Ursus americanus*	n = 8	n = 3	n = 0
Brown Bear	*Ursus arctos*	n = 10	n = 1	n = 2
California Sea Lion	***Zalophus californianus***	**n = 16**	**n = 2**	**n = 0**
	TOTAL	57	17	5

For the sake of this paper, "wild" refers to individuals who did not live in captivity and "captive" refers to individuals who resided in zoos or animal rescue facilities at the time of their deaths. Although their captivity status at birth is unknown for most of the specimens, since importation of wild animals has historically been rare, we believe that most if not all were born in captivity. In this study, only adult specimens were used. Samples were obtained from collections at the American Museum of Natural History (AMNH) the Smithsonian (USNM) and the research collection of Dr. Hartstone-Rose (University of South Carolina School of Medicine). These specimens originated at the Bronx Zoo, Central Park Zoo, New York Zoo, New York Zoo Society, New York Zoo Gardens, New York Park Commission, National Zoological Park (Smithsonian), Toledo Zoological Society, Academy of Natural Science, Barnum and Bailey Circus, Prospect Park Zoo, and the Carolina Tiger Rescue.

All specimens were evaluated and scored for calculus and periodontal disease by carefully examining each skull and assessing the dental arcade in six sections upper and lower anterior teeth (canines and incisors), left and right cheek teeth (premolars and molars). Figure 2.1 depicts how the mandibular (2.1 – A) and maxillary (2.1 – B) teeth were categorized into six sections and each of the six regions was scored for calculus and periodontal disease. After dividing the upper and lower teeth into six sections, each section was given a score of 0 to 5 for calculus build-up and periodontal disease according to the index provided in Table 2.3 and 2.4 respectively.

Figure 2.1 - A: shows the lower teeth divided into three sections

Lower Anterior Teeth

Lower Left Cheek Teeth

Lower Right Cheek Teeth
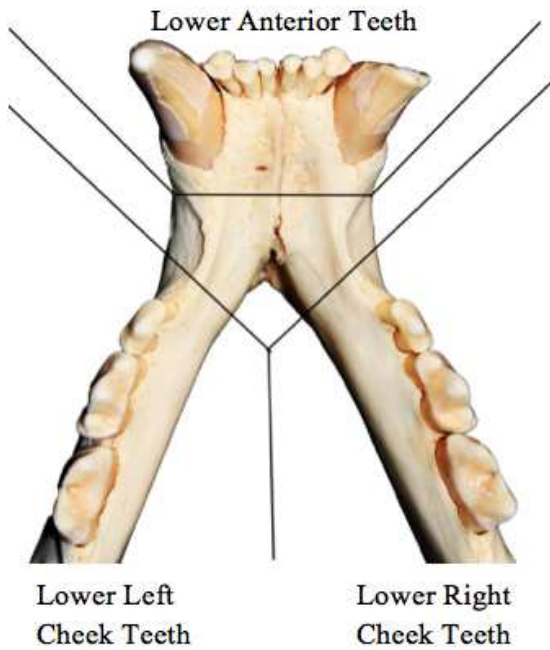
Fig 2.1 - B: shows the upper teeth divided into three sections

Upper Anterior Teeth

Upper Right Cheek Teeth

Upper Left Cheek Teeth
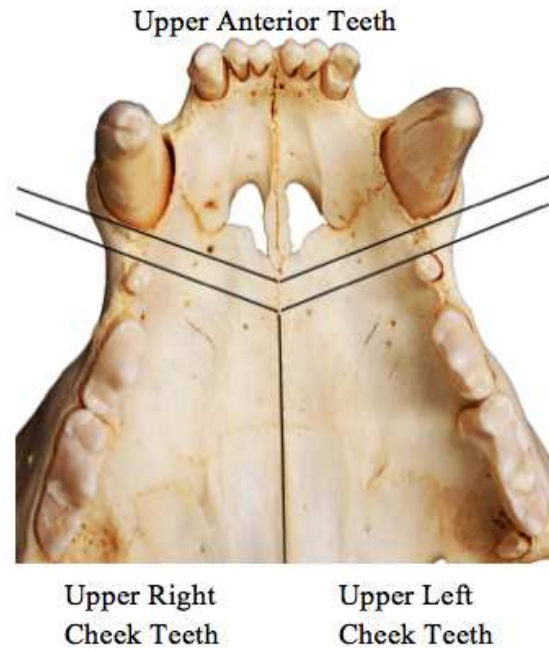
Figure 2.1: Sectioning of Mandibular (a) and Maxillary (b) teeth.

In this study, the score of any given section in the calculus and periodontal index (Table 2.3 and Table 2.4) tend to reflect the amalgamated score for each of the six sections as a whole rather than scoring for each individual tooth. This eliminates the discrepancies that may arise due to individual missing or damaged teeth.

2.1. Calculus Scoring

To score for dental calculus we observed amounts of calculus covering the crown of all teeth and each of the six sections were scored according to the calculus index below (Table 2.3).

Table 2.3: Calculus Index

Calculus Scores	Observations
0	No evidence of calculus (Figure 2.2-A)
1	1 – 10% of the crown of the teeth is covered by calculus (Figure 2.2-B)
2	10 – 25% of the crown of the teeth is covered by calculus (Figure 2.2-C)
3	25 – 50% of the crown of the teeth is covered by calculus (Figure 2.2-D).
4	50 – 75% of the crown of the teeth is covered by calculus and/or minor caries (Figure 2.2-E).
5	75 – 100% of the crown of the teeth is covered by calculus OR thickened calculus and/or major caries (Figure 2.2-F).

Figure 2.2 illustrates varying degrees of calculus build-up for the lower left cheek teeth. A score of 0 as seen in figure 2.2-a shows no evidence of calculus. Figure 2.2-b we see about 10 percent of the crown covered with calculus in the lower left molar. Figure 2.2-c received a score of 2 because approximately 15 percent of the lower left molar was covered with calculus. In figure 2.2-d we see about 50 percent of the lower left pre-molar covered with calculus. Figure 2.2-e received a score of 4 because of the visible calculus on the lower left premolar and caries seen in the molar. Finally figure 2.2-f received a score of 5 because of thick calculus surrounding the molar and major caries observed in the lower left pre-molar. On the right side of each figure we see a magnified view of the calculus build-up.
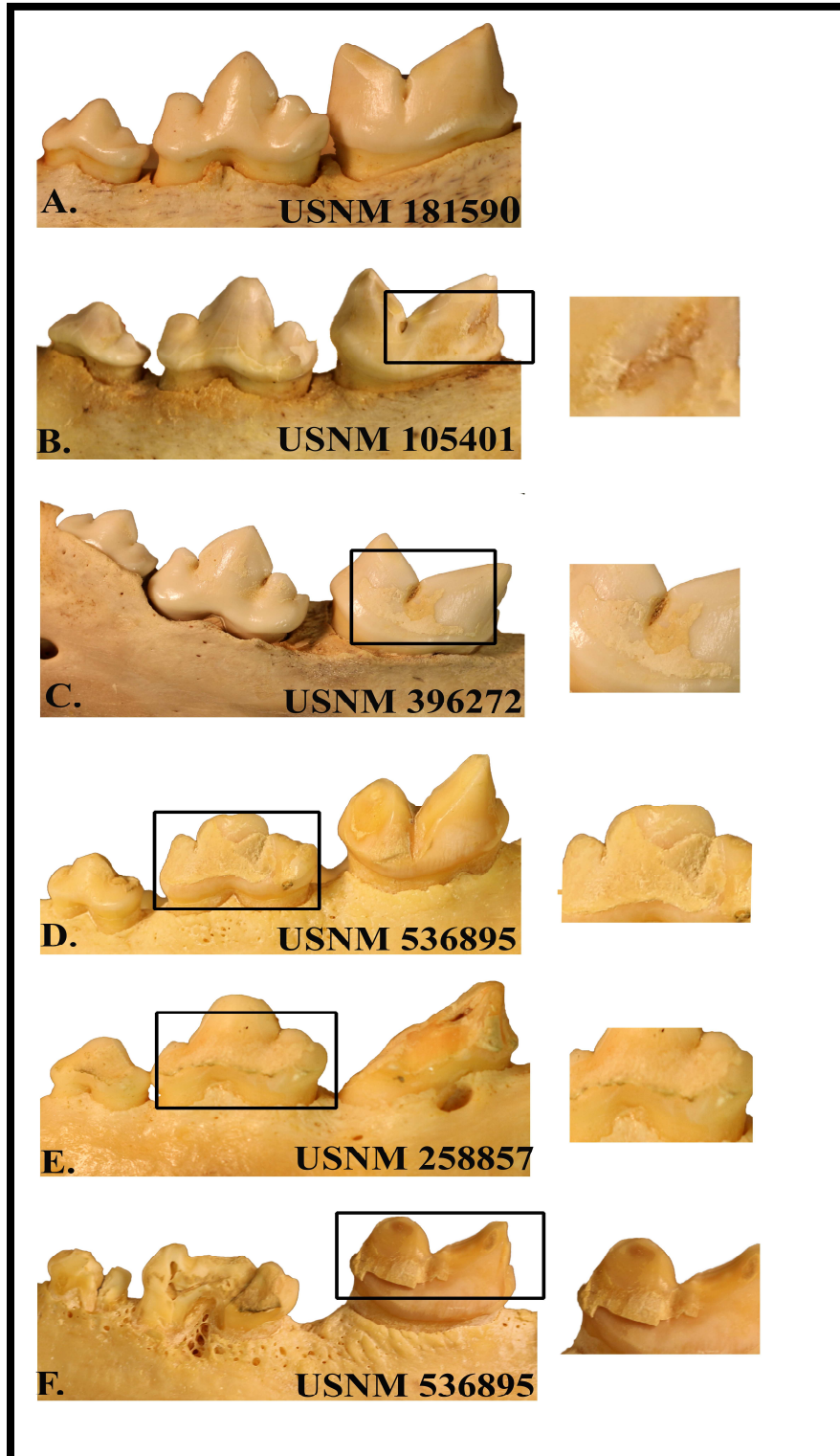
Figure 2.2: Comparison of calculus score

2.2. Periodontal Disease Scoring

Skulls were scored for periodontal disease by measuring the distance the alveolar ridge degraded from its original position combined with abscess scoring technique. This scoring technique relies on the size of any holes in the alveolar bone created by abscess damage, and a score for the entire skull may be based on the number of abscess damaged sites. Below is the index used to score for periodontal disease (Table 2.4).

Table 2.4: Periodontal Index Score

Periodontal Scores	Observations
0	The bone within 5mm of gum line has no visible porosity (Figure 2.3-A).
1	The bone within 5mm of gum line has noticeable porosity see figure 3-b below (Figure 2.3-B).
2	The bone within 5mm of gum line has excessive porosity OR the bone within 1cm of the gum line has noticeable porosity (Figure 2.3-C).
3	The bone within 1cm of the gum line has excessive porosity OR bone remodeling without excessive porosity (Figure 2.3-D).
4	Openings in the alveolar bone >1mm, but <2mm and/or evidence of abscess with bone remodeling (Figure 2.3-E).
5	Multi-millimeter openings in the alveolar bone and/or tooth loss (Figure 2.3-F).

A. USNM 253289

B. USNM 251789

C. USNM 399556

D. USNM 282303

E. USNM 399566

F. USNM 539999
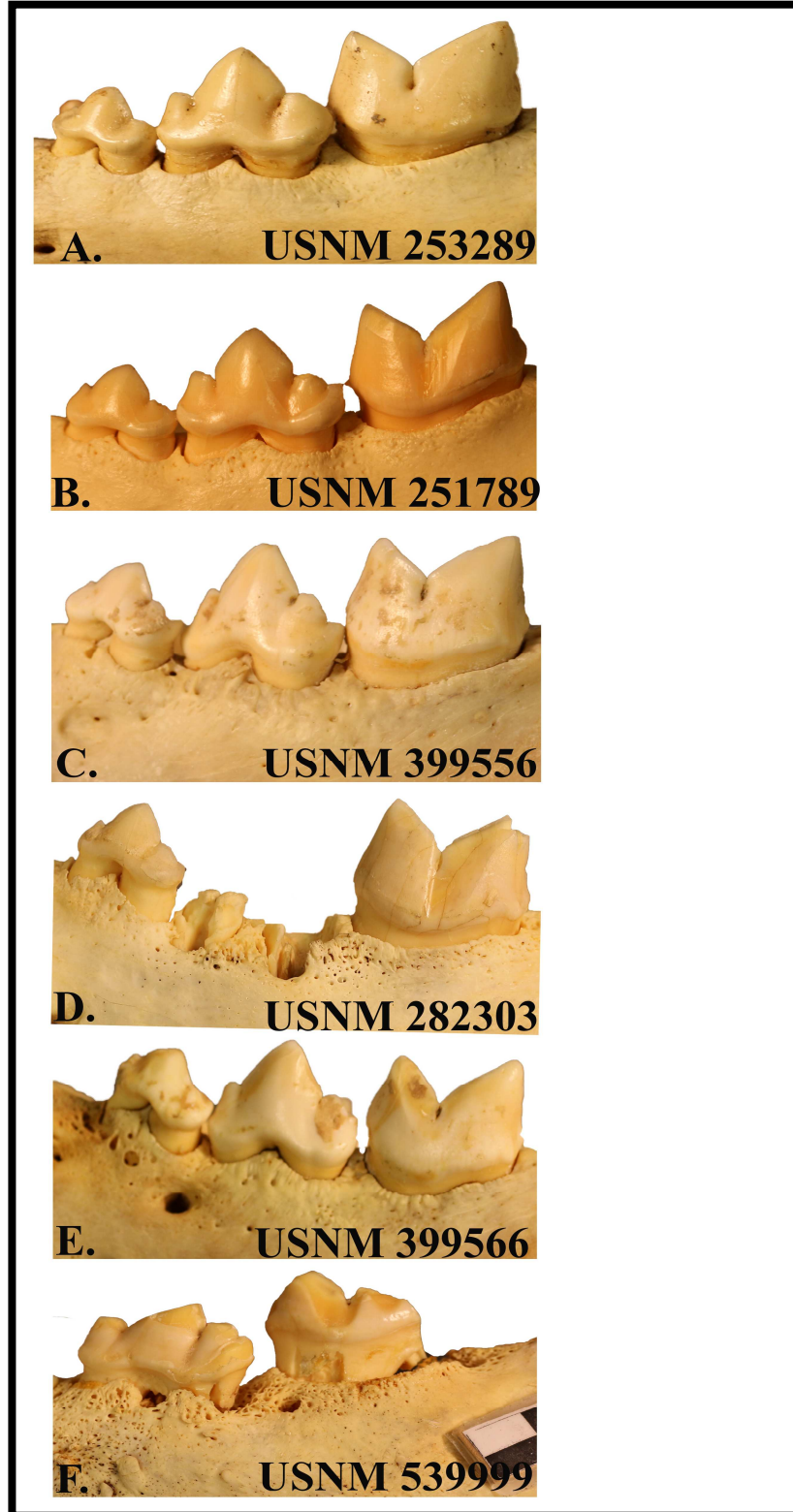
Figure 2.3: Comparison of Periodontal Disease.

Figure 2.3 illustrates varying stages of periodontal disease in the left posterior mandibular dentition. A score of 0 as seen in figure 2.3-a, shows no evidence of periodontal disease because of no visible porosity and the alveolar bone is within 5mm of gum line. In figure 2.3-b there are noticeable porosity in the alveolar bone around the premolar and molar teeth. Figure 2.3-c received a score of 2 because of excessive and noticeable porosity along the two premolars and molar teeth. In figure 2.3-d we see the alveolar bone within 1 cm of the gum line and has excessive porosity. Figure 2.3-e received a score of 4 because of the openings in the alveolar bone >1mm, but <2mm surround the premolars. Finally figure 2.3-f received an over all score of 5 for the lower left check teeth because of multi-millimeter openings in the alveolar bone and tooth loss.

2.3. Principle Component Analyses (PCA)

Principle component analyses is a statistical procedure that applies data to a new coordinate system, which separates the data that differs the most as the first principal component and the subsequent variation as second principal component, third principal component and so on. Hence PCA is used to show the strongest factor driving the variation across a population. An important component of my research was to correlate oral health to overall health. This was accomplished by taking advantage of another study that was conducted in the research lab of Dr. Adam Hartstone-Rose where the cranial morphology of wild versus captive felids were compared.

The cranial morphology study examined the effect of differences in mechanical diet across captive and wild populations of lions and tigers with three-dimensional

geometric morphometric examination of their skulls and analyzed these samples with PCA to statistically discern the results across a sample of eighty-one specimens, with each specimen being comprised of forty-three landmarks. According to the PCA the second greatest source of variation (PC2) across the population was driven by captivity as seen in figure 2.4 (Hartstone-Rose, Selvey, Villari, Atwell, & Schmidt, 2014). For this study we compared the PC2 scores to periodontal disease and calculus build-up to see if the variables were correlated. Table 2.5 shows the markers that were used to generate all graphs.
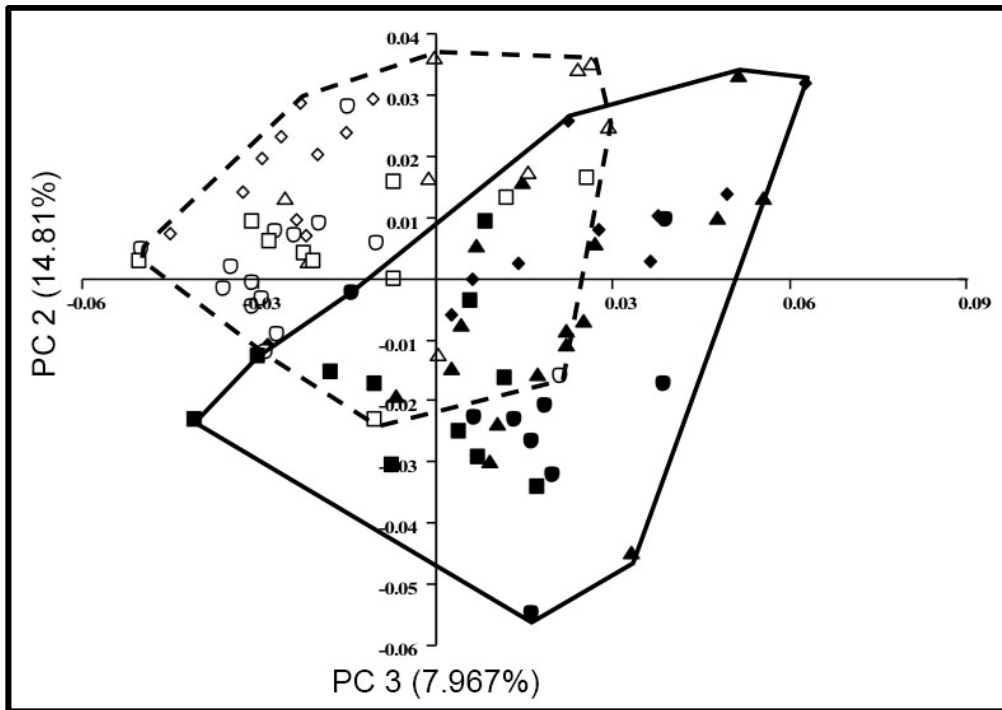
Figure 2.4. PCA output with second principal component against third.

Minimum convex lines describe captive (solid) and wild (dashed).
See table 2.5 for marker description.

Table 2.5: Marker Key For All Graphs

▲	**P. *tigris*, Male, Captive**
■	P.*tigris*, Female, Captive
◆	P.*leo*, Male, Captive
●	P.*leo*, Female, Captive
△	P.*tigris*, Male, Wild
□	P.*tigris*, Female, Wild
◇	P.*leo*, Male, Wild
○	P.*leo*, Female, Wild

2.4. Statistical Analysis

JMP statistical software was used to analyze our data. We used mean and
maximum values of periodontal disease and calculus build-up to look for general trends
such as: how the mean and maximum values change with captivity statuses, PC2 scores
and regions of the teeth. We used t-test analysis and compared the captivity statuses to
mean periodontitis score, maximum periodontitis scores, mean calculus scores and
maximum calculus score to determine if captive felids have higher periodontal and
calculus scores. One-way ANOVA was used to get the mean of wild versus the mean of

captive felids. P-value was used to determine if the zoo animals had statistically significant higher periodontal disease than wild animals.

The main assumption used throughout our results section is that the samples have been independently drawn from their populations. The null hypothesis is that the population from which the samples are obtained is the same.

CHAPTER 3

Results

Initial Observations

When scoring the skulls we observed, that the occurrence and magnitude of periodontal and calculus scores were much higher in captive carnivores compared to the wild carnivores. We also noticed higher calculus scores on the posterior teeth when compared to the anterior teeth and saw an opposite trend for periodontal disease.

2.1. Effect of Captivity Status on Periodontal Disease and Calculus

To test our hypothesis, captive carnivores will have higher incidence of calculus and periodontal disease we performed a t-test analyses comparing the mean and maximum periodontal and mean and maximum calculus scores relative to captivity statuses. On our x-axis we have wild and zoo felids and on our y-axis we have periodontal and calculus scores. Box plots in red are used for identifying outliers and for comparing distributions. Mean Periodontal Disease, Maximum Periodontal Disease, Mean Calculus and Maximum Calculus (table 3.2) all significantly separate captive and wild populations. Table 3.2 shows that captive felids have significantly higher ($P <$ .0001) mean periodontal disease, maximum periodontal disease, mean calculus and maximum calculus.

Table 3.2: One-way ANOVA Analysis of Oral Health and Captivity Status.

Variable	Mean Zoo	SD Zoo	Mean Wild	SD Wild	P -Value
Mean Periodontal Disease	1.62	0.75	0.39	0.53	<.0001
Maximum Periodontal Disease	3.08	1.4	1	1.09	<.0001
Mean Calculus	1.05	0.66	0.08	0.21	<.0001
Maximum Calculus	1.77	1.33	0.21	0.49	<.0001

Figure 3.1: The mean and maximum periodontal score and mean and maximum calculus score statistically separates captive and wild carnivores. On the right side of the graph we have the captive felids (Z) and on the left we have the wild (W) felids. The box plot in red shows the average of mean periodontal disease for wild and captive felids. The average values of mean and maximum periodontal disease for wild felids = 0.39 +/- 0.53, 1 +/- 1.09 and captive felids = 1.62 +/- 0.75, 3.08 +/- 1.4 respectively. The average values of mean and maximum dental calculus for wild felids = 0.08 +/- 0.21, 0.21 +/- 0.49 and captive felids = 1.05 +/- 0.66, 1.77 +/- 1.33 (see Table 3.2). The P value of <.0001 suggest that zoo animals have significantly higher mean and maximum periodontal disease and mean and maximum calculus scores (Table 3.2 and Figure 3.1).
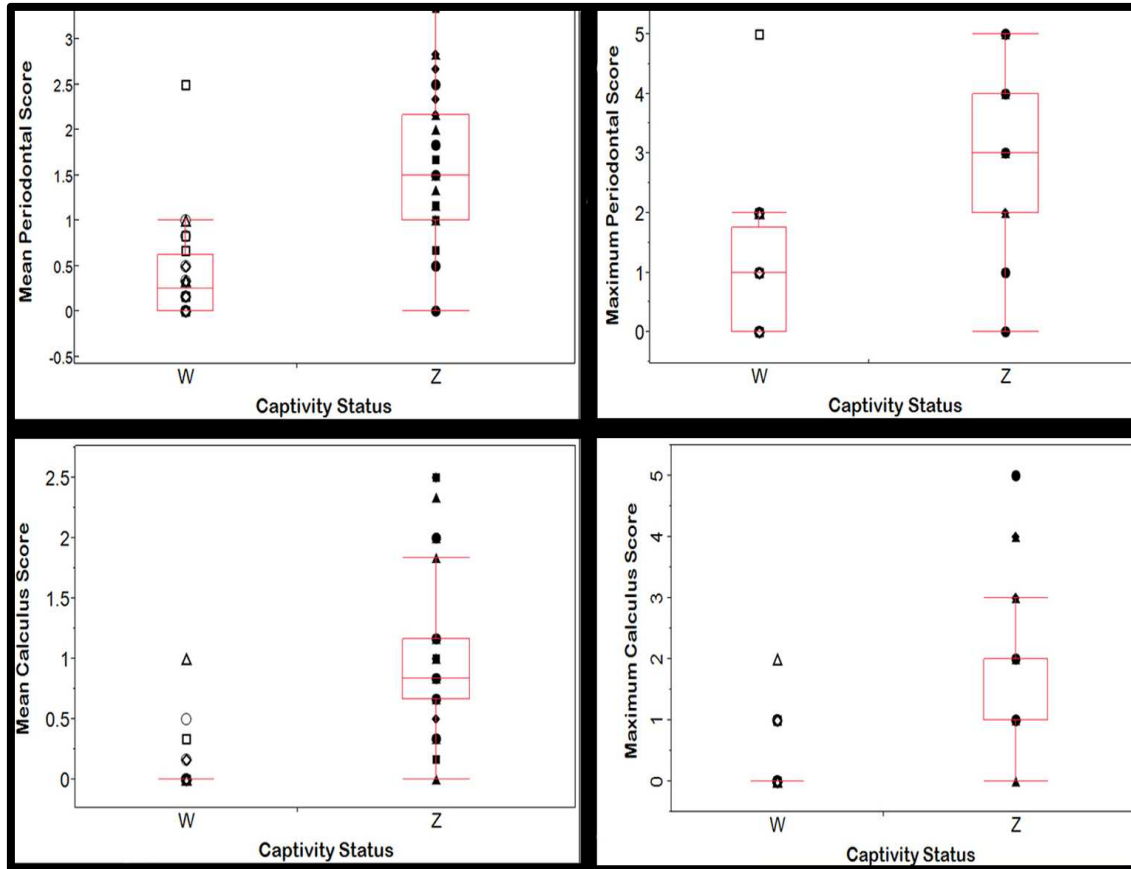
Figure 3.1: Periodontal and Calculus Scores versus Captivity Status. Symbols described in Table 3.1.

2. 2. *Correlation of Oral Health to Overall Health*

In order to find a correlation of oral health to overall health, we compared PC2 scores (measure of skull deformity or captivity coefficient) to mean/maximum periodontal disease, and mean/maximum calculus scores by using bivariate analysis on JMP statistical software. On our x-axis we have PC2 scores of wild and captive tigers and lions and on the y-axis we have mean and maximum periodontal disease and dental calculus. For this research it was difficult to determine which of the two variables (PC2 scores and Oral Health Scores) is the independent and dependent variable. To avoid any

errors we used Reduced Major Axis regression (RMA) instead of Ordinary Least Square (OLS) since RMA incorporates an assumption that there is error in x, which is our independent variable. Table 3.3 outlines the descriptive statistic for mean/maximum periodontal and calculus scores versus PC2 and shows that the relationship between oral health and overall health is statistically significant P <. 05. Higher PC2 scores, which is a measure of skull deformity is correlated to higher periodontal disease and dental calculus.

Table 3.3: Descriptive statistics for RMA regressions against the PC2 scores.

y-variable	Slope	y-intercept	r	Lower CL	Upper CL	P-Value
Mean Periodontal	34.04	1.09	0.42	18.22	63.61	>0.0006
Maximum Periodontal	61.84	2.19	0.48	37.52	101.94	>.0001
Mean Calculus	26.65	0.63	0.41	13.84	51.33	>0.0009
Maximum Calculus	49.03	1.11	0.33	19.59	122.71	>0.0076

Figure 3.2: A bivariate fit of mean and maximum periodontal score and mean and maximum dental calculus score against PC2 (captivity coefficient) shows a positive slope. This positive slope shows that there is a direct relationship between PC2 scores and oral health. The relationship between the mean and maximum periodontal score and mean and maximum calculus score versus PC2 is statistically significant (table 3.3).
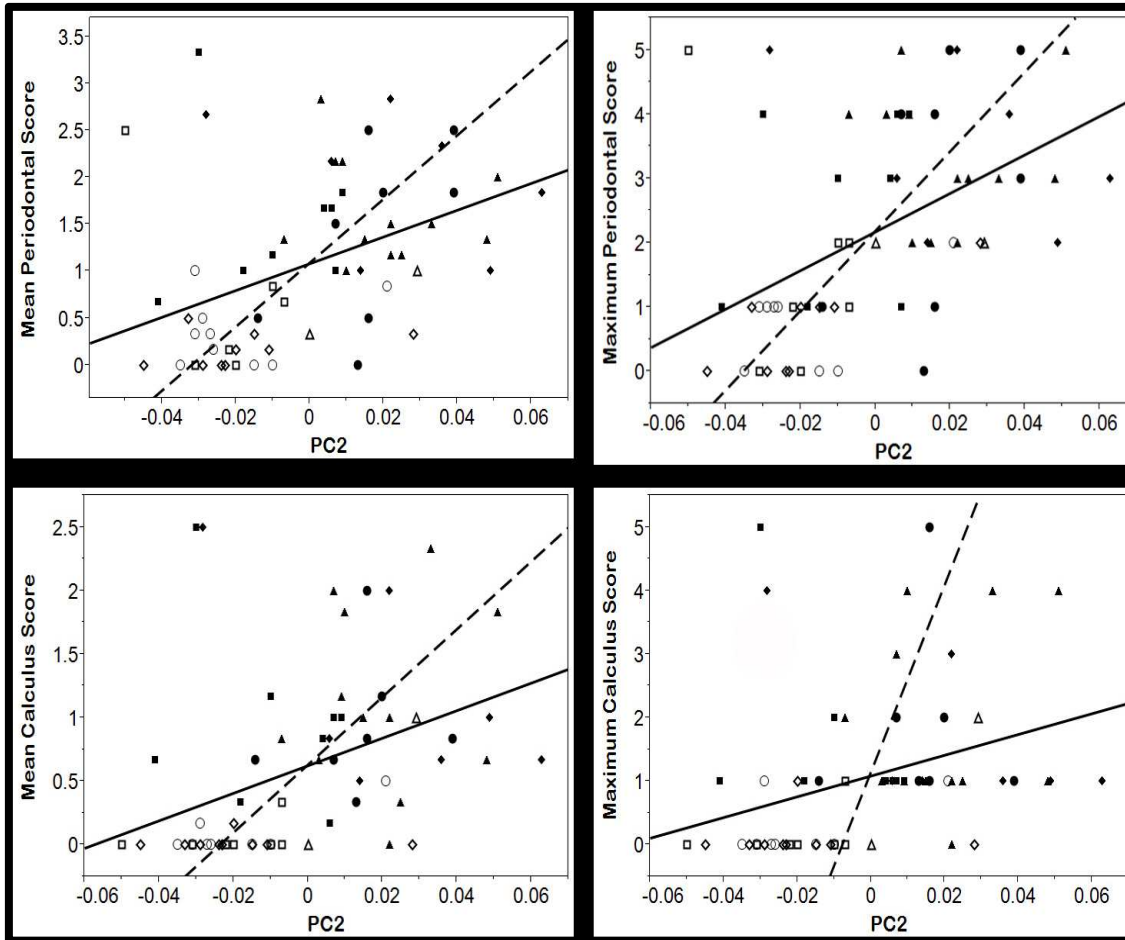
Figure 3.2: Periodontal and Calculus Scores versus PC2. Symbols described in Table 3.1.

3.3 Comparison of Posterior versus Anterior Dentition

Bivariate analysis on JMP statistical software was used to analyze the correlation between posterior and anterior dentition (see Figure 3.3). P<.0001 suggest that the relationship between posterior and anterior teeth are statistically significant and they are highly correlated. Positive slope suggest that there is a direct relationship between the two regions. Since the slope for mean posterior calculus (1.64) is greater than 1, we can say that calculus accumulation will be greater in the posterior teeth when compared to the

anterior teeth and a slope of 0.70 (see Table 3.4) suggest that there will be higher prevalence of periodontal disease in the anterior teeth.

Table 3.4: Descriptive statistics for RMA regressions against anterior dentition

y-variable	Slope	y-intercept	R2	Lower CL	Upper CL	P-Value
Mean Posterior Periodontal Score	0.70	-0.24	0.38	0.50	0.98	<.0001
Mean Posterior Calculus Score	1.64	0.14	0.35	1.14	2.366	<.0001
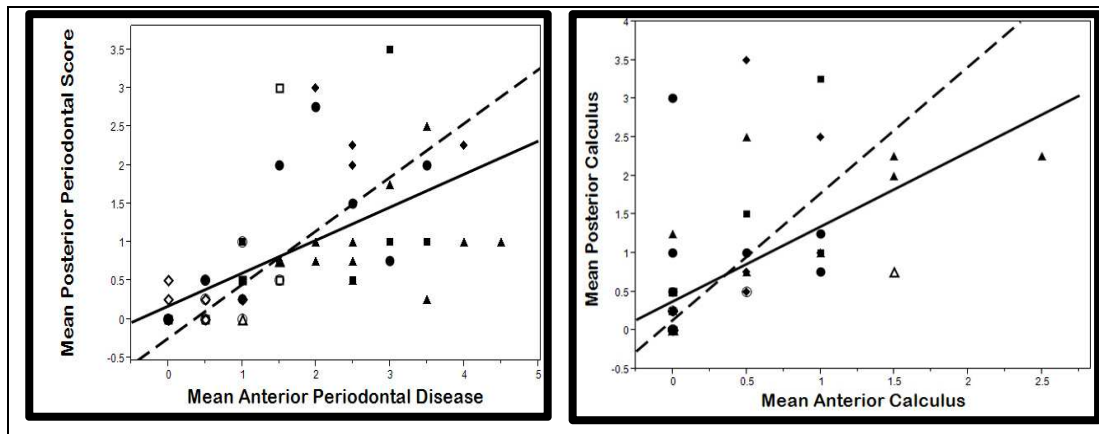
Figure 3.3: Anterior versus Posterior Teeth. Symbols listed in Table 2.5.

3.4. Influence of Sex and Species on Oral Health

In order to study the influence of sex and species on periodontal and calculus scores, we performed a one-way ANOVA analysis on JMP statistical software. Species (Tiger versus Lion), sex (Male versus Female) and captivity (Wild versus Captive) were graphed on our x-axis and mean/maximum periodontal and calculus scores were graphed

on our y-axis. When analyzing all eight variables, the p-value for almost all our responses were greater than .05 suggesting that these variables are not statistically significant. The only response that was significant (p-value = 0.038) was the analysis of maximum periodontal disease by species. The analysis showed that Tigers have slightly higher maximum periodontal score. This variation could be due to random chance. To conclude tigers have slightly high maximum periodontal disease but all the other variables score equally across sexes and species.

3.5. *Presence and Absence of Calculus and Periodontal Disease*

A simple yet important analysis of this research was to calculate the total percent of wild and captive specimens that have zero or minor (a max score of zero or one) calculus and periodontal disease.

Table 3.5: Presence and Absence of Calculus and Periodontal in Wild Felids.

Variables	% Score of 0	% Score between 0-1
Mean Periodontal Score	36%	89%
Maximum Periodontal Score	36%	75%
Mean Calculus Score	82%	96%
Maximum Calculus Score	82%	96%

Table 3.6: Presence and Absence of Calculus and Periodontal in Captive Felids.

Variables	% Score of 0	% Score between 0-1
Mean Periodontal Score	3%	12%
Maximum Periodontal Score	3%	17%
Mean Calculus Score	3%	54%
Maximum Calculus Score	3%	66%

In (Table 3.5 and 3.6) we can see that a score of zero or one is more common in wild felids when compared to captive felids meaning that felids in wild have lower prevalence of periodontal disease and calculus accumulation.

CHAPTER 4

Discussion

Effect of Captivity on Oral Health

The goal of this research was to evaluate the effect of mechanical properties of diet on oral health by comparing wild and captive felids. Several studies showed that consistency of the diet plays a major role in the occurrence of periodontal disease and calculus accumulation.

The result of my research indicates that diet and texture plays a significant role in the oral health of captive lions and tigers. Our first aim was to determine if the incidence of dental calculus and periodontal disease was higher in captive felids. Since captive carnivores in the zoo are fed a soft diet and if captive felids have higher incidence of oral disease, we could correlate soft diet to dental health. As expected when comparing wild and zoo felids, we see higher incidence of maximum/mean periodontal and calculus scores in captive tigers and lions (Table 3.2). Figure 3.1 clearly shows that the average periodontal and calculus scores were much higher in captive felids when compared to their wild counterparts.

The results of this research supports our first hypothesis, captive felids on soft diet, have higher incidence of dental calculus and periodontal disease when compared to the wild felids. This is probably due to lack of abrasive action that usually accompanies chewing on bones or connective tissues. Also soft diets tend to produce more bacterial plaque than firm diets resulting in an increased calculus accumulation and eventually gingivitis or periodontitis (Fagan, 1980).

Periodontal Disease is not the only complication that can arise due to lack of mechanical properties of diet. Lack of texture in diets can lead to malocclusion, which can have serious consequences for an animal's overall health and oral health. Malocclusion could lead to animals unable to graze effectively or catch and kill its prey. Also mechanical properties of an animal's food may have a distinct effect on its cranial morphology (O'Regan & Kitchener, 2005).

Effect of oral health on overall health

The possibility of damage to other organs and tissues, as a consequence of periodontal disease has been a major topic of study for a long time. Although changes in nutritional aspect of the diet have arguably improved the health of cats and dogs, periodontal disease remains a serious problem (Gawor et al., 2006). Periodontal disease is estimated to affect approximately 75% of the people in the United States, and 20 to 30% of the adults suffer moderate to severe forms of periodontal disease (Campbell, 2007). Historically dental caries and periodontal disease have been considered the most important global oral health burdens (Petersen, 2008). Hence it is important to study the pathogenesis of periodontal disease and its effect on overall health.

Scientists and dental professionals have long suspected associations between oral health and systemic health. In people, an association has been established between periodontal disease and diabetes, cardiovascular disease and adverse pregnancy (Logan, 2006). Although the nature of the relationship is not fully understood, researches in this field clearly point to a connection between periodontal and systemic health (Campbell, 2007). In order to determine the effects of oral health on overall health, we compared

mean/maximum periodontal and calculus scores to the cranial deformation axis from our lab's other study that correlated with the captivity status of these same specimens (PC2 scores). The results of that comparison showed that there is a direct relationship between cranial deformation and oral health. An increase in cranial deformation scores is correlated to an increase in periodontal disease and calculus accumulation (Table 3.3 and Figure 3.2).

The results of this study supports our second hypothesis, higher cranial deformation scores is correlated to higher periodontal disease and dental calculus and the correlation is statistically significant (P<.0001), indicating that skull abnormality is related to periodontal disease. There are many risk factors associated with periodontal disease. Progression to periodontitis can be prevented by proper oral hygiene and effective plaque control. Although there is no direct evidence linking periodontal disease to overall health, researches continues to confirm that periodontitis is strongly associated with other systemic conditions (Campbell, 2007).

Dental Health of Anterior versus Posterior Dentition

Wild and captive felids depend on teeth for survival. Van Valkenburgh (1996) described the general dentition of felids as being comprised of three regions, the grasping incisors, penetrating canines and food processing cheek teeth. Different teeth of carnivores provide different functions. The incisors are used for grasping and tearing, the canines are used for capturing and killing preys and the premolars and molars are used primarily for grinding and chewing (Logan, 2006). Since the functions of these teeth differ, our third aim was to determine the prevalence of calculus and periodontal disease in posterior versus anterior dentition.

Bivariate analysis was used to test if variation exists in posterior versus anterior dentition of captive and wild lions and tigers due to differences in functions of the teeth. Our results support our third hypothesis, proving that the relationship between posterior and anterior teeth are statistically significant and they are highly correlated P<.0001 (Table 3.4). The slope in figure 3.3 showed that calculus buildup was greater in the posterior teeth when compared to the anterior teeth. The opposite trend was seen for periodontal disease, higher prevalence of periodontal disease was noted in the anterior teeth when compared to the posterior teeth. Since carnivores primarily use their anterior teeth for grasping and tearing, this action may damage the gingiva, leading to inflammation and if not treated could lead to periodontal disease, this could explain why periodontal disease is more prevalent on the anterior teeth. Higher prevalence of calculus accumulation on the posterior teeth could be due to lack of regular tongue contact on the tooth surface. Also plaque tends to accumulate at the openings of salivary duct near the maxillary premolar (Vosburgh, 1982).

Other Findings

One-way ANOVA analysis was performed in order to study the influence of sex and species (lions and tigers) on periodontal and calculus scores. The results of this study showed that the influence of sex and species on oral health were not statistically significant (P>.05). Sex and species had no effect on the oral health of wild and captive felids and there were no significant correlation. The only response that was significant (p-value = 0.038) was the analysis of maximum periodontal disease by species. The analysis showed that tigers have slightly higher maximum periodontal score when compared to lions. This variation as

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explained earlier could be due to random chance. To conclude tigers have slightly higher maximum periodontal disease when compared to lions but overall, the other variables score equally across sexes and species.

Another important findings of this research were to calculate the total percent of wild and captive specimens that have zero or minor (a max score of zero or one) calculus and periodontal disease. Table 3.5 and 3.6 shows that 3% of captive specimens have zero mean calculus and mean periodontal disease respectively, compared to 82% and 36% for those same metrics for wild specimens. When considering specimens with only the most mild evidence of both metrics (scores of 0 or 1), then the difference is more stark: 66% and 17% for maximum calculus and periodontal disease scores in captive animals versus 96% and 75% for the same metrics in the wild specimens."

Limitations

One of the limitations of this study was that we did not know the exact age of our specimen when scoring for periodontal disease and dental calculus, especially knowing the age of wild felids was challenging. We learned that improved veterinary care in zoos has resulted in an increased longevity in captive felids and according to several studies, the prevalence and severity of periodontal disease is age related (Longley, 2011). Although Figure 1.4 depicts severe calculus on a juvenile Tiger suggests that age might not necessarily be the main correlate of dental disease.

Another potential caveat is that some zoos may practice regular dental care, which may result in some captive carnivores to have lower prevalence of periodontal disease and calculus accumulation. Also overcrowding of teeth may increase accumulation of calculus and require additional care to maintain healthy gingiva.

We should also keep in mind that there are other factors that can affect periodontal disease such as malocclusions, absence of oral hygiene, diet, environment and genetics (Albuquerque et al., 2012).

Broader Impacts

The results of the research should give an indication of which physical aspects of diets are most important to oral health. This study could lead to recommendations for improvement in captive animal diets from a mechanical perspective. Management of dental disorders provides animals with a higher quality of life, extends their life span and improves breeding capabilities (van Foreest, 1993).

Most importantly this is a study that shows, yet again, another link between oral health and overall health (as represented by the cranial deformation score). Understanding the possible relationships between periodontal health and other systemic conditions could increase oral home efforts in hopes of improving overall health.

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