FLORIDA MUSEUM OF NATURAL HISTORY™

BULLETIN

METHODS OF ASSESSING HEALTH AND DIET OF FLORIDA PANTHERS (*Puma concolor*) USING MUSEUM SPECIMENS

PART I: Osteology as a Means of Assessing Florida Panther Health

Laurie Wilkins, Julie M. Allen, Joan Coltrain, Shelly Flanagin, Terry D. Allen, & David L. Reed[1]

PART II. Stable Isotope Geochemistry: A Method to Evaluate the Diet of Florida Panthers (Puma concolor) Using Museum Specimens

Julie M. Allen, Joan Coltrain, Laurie Wilkins, Shelly Flanagin, & David L. Reed

Vol. 47, No. 3, pp. 73-108 2007

UNIVERSITY OF FLORIDA GAINESVILLE

The **FLORIDA MUSEUM OF NATURAL HISTORY** is Florida's state museum of natural history, dedicated to understanding, preserving, and interpreting biological diversity and cultural heritage.

The **BULLETIN OF THE FLORIDA MUSEUM OF NATURAL HISTORY** is a peer-reviewed publication that publishes the results of original research in zoology, botany, paleontology, and archaeology. Address all inquiries to the Managing Editor of the Bulletin. Numbers of the Bulletin are published at irregular intervals. Specific volumes are not necessarily completed in any one year. The end of a volume will be noted at the foot of the first page of the last issue in that volume.

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ISSN: 0071-6154

Publication Date: November 30, 2007

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Key Words: methods, museum specimens, osteology, health, stable isotopes, diet, Florida panther, *Puma concolor*

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Wilkins, L, J. M. Allen, J. Coltrain, S. Flanagin, T. D. Allen, and D. L. Reed. 2007. Methods of assessing health and diet of Florida panthers (*Puma concolor*) using museum specimens. Part I. Osteology as a method of assessing Florida panther health. Bull. Florida Museum of Nat. Hist. 47(3), Pt. I: 73-97

Allen, J.M., J. Coltrain, L. Wilkins, S. Flanagin, and D.L. Reed. 2007. Methods of assessing health and diet of Florida panthers (*Puma concolor*) using museum specimens. Part II. Stable isotope geochemistry: A method to evaluate the diet of Florida panthers. Bull. Florida Museum of Nat. Hist. 47(3), PT. II: 98-108

METHODS OF ASSESSING HEALTH AND DIET OF FLORIDA PANTHERS (*Puma concolor*) USING MUSEUM SPECIMENS

PREFACE

Natural history museum specimens provide a valuable resource to examine aspects of the biology of the living animal long after its death, which is particularly useful for large carnivores that are difficult to study or may be endangered. Voucher specimens record changes in animal populations over time and increase in value as new data, new technologies, or new research applications emerge. For example, animal pelts may now be analyzed for the environmental toxins they store, and skeletons provide a permanent historic record of diet, illness, trauma, or past injury. These in turn reflect physical and physiological processes or environmental conditions affecting the population, and they may be important indicators of dietary deficiencies, environmental toxins, or inbreeding. Knowledge of such processes contributes to management and conservation decisions. Likewise, museum specimens collected over time may show the positive effects of management activities on threatened populations.

The Florida Museum of Natural History (FLMNH) collection of Florida panther specimens *(Puma concolor,* previously *coryi)* acquired through a cooperative salvage program with the Florida Fish and Wildlife Conservation Commission (FWC), represents a unique and valuable assemblage of the large, endangered carnivore. Over 140 specimens of skins, skulls, and skeletons exist from 1950 to the present, and they include every panther that has been recovered since the early 1980s - the beginning of the FWC capture and monitoring program (Belden et al. 1988). These provide a permanent historical record of morphologic, genetic, and demographic changes in the panther population over the past 25 years. Specimens record biomedical conditions that existed but were not necessarily detected *in vivo* by researchers. More importantly, the collection covers the time period before and after genetic intervention and habitat management strategies began for this endangered species.

Previous studies of museum specimens facilitated the description of phenotypic traits unique to the Florida population of puma, which was relatively unknown until a surviving population was discovered more than 25 years ago in south Florida (Belden 1978). Among these unique characteristics are the highly inflated nasals of the cranium, or "roman nose," that had been described by Young (1946) as a distinctive feature of the Florida population, and which later distinguished the panthers of the Big Cypress and western Florida from the genetically distinct Everglades population (O'Brien et al. 1990; Wilkins et al. 1997). In addition, museum specimens dating back to the late-1800s revealed a high frequency of kinked tails and cowlicks (whorls of hair) found in the mid-dorsal region (Belden 1986; O'Brien et al. l990; Wilkins et al. 1997). These anomalies have now become accepted indicators of the highly inbred condition of the original Florida panther population, which together with a high incidence of cryptorchid males, congenital heart defects, and elevated pathogen/parasite loads revealed a highly stressed and at-risk population (Roelke et al. 1993). With the onset of the genetic out-breeding program in 1995, the frequency of kinks, cowlicks and the more deleterious expressions of inbreeding in Florida panthers have decreased, but continue to be carefully monitored and investigated (Land et al. 2005, Pimm et al. 2006, M. Roelke, pers.comm.).

Along with increased health risks due to inbreeding, there is evidence that panther health has varied through time among regions in Florida, possibly due to diet and prey density (Roelke 1990). Likewise, past morphological studies have shown that Florida panthers exhibit a greater-than-expected incidence of skeletal trauma, dental fracture, developmental abnormalities, and osteopathologies when compared to a wild, presumably healthy population of puma from other regions of the country (Duckler & Van Valkenburgh 1998a,b). External skeletal pathologies include arthritis, infection, trauma, and bone lesions of unknown cause. A single internal morphological feature prominent in that analysis was the presence of Harris Lines (HLs), also known as growth arrest lines, which are visible as transverse lines of bone deposition in radiographs of long bones. Generally, HLs can occur in long bones of humans and other mammals, and they are believed to represent renewed growth after a period of interruption (Park 1964; Wing & Brown 1979). Their appearance has been linked to nutritional deficiencies, infectious agents, and/or environmental toxins, all of which are documented threats to panther survival (Roelke 1990; Roelke et al. 1993a,b; Facemire & Guillette 1994; Facemire et al. 1995, Pimm et al. 2006).

Herein we present results of two studies of the FLMNH's collection of Florida panthers. In Part I, we expand earlier investigations by Duckler and Van Valkenburgh (1998a,b) examining osteopathologies and HLs as health indicators. In Part II we analyze stable carbon and nitrogen isotope ratios in Florida panther bone collagen to investigate dietary trends. The sample includes specimens from a range of locations and time periods, and some animals born after the out-breeding initiative in 1995, in which Texas females were introduced into Florida to supplement the existing Florida panther population. Citations for references in the Preface are found in the Literature Cited section of Part I.

- Laurie Wilkins, Julie M. Allen, and David L. Reed, Florida Museum of Natural History.

PART I: OSTEOLOGY AS A MEANS OF ASSESSING FLORIDA PANTHER HEALTH

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ABSTRACT

Conservation efforts to reverse the negative effects of inbreeding in an isolated population of Florida panthers (*Puma concolor coryi*) resulted in the release of eight Texas females into Florida in 1995 (Seal 1994; Johnson et al. 1998). Since that time, Florida panthers have shown increased productivity, range expansion, and the reversal of a suite of deleterious morphological and physiological effects of inbreeding. (Land et al. 2005, Pimm et al. 2006). Previously described bone pathologies in the Florida panther may result from a compromised immune system due to inbreeding, poor health related to diet and nutrition, or the presence of previously undetected pathogenic diseases. We examine the current collection of 140 post-cranial skeletons to determine the frequency of trauma, infection, arthritis, and incidence of Harris Lines. Harris lines, visible from X-rays of long bones, represent a cessation of growth due to a major episode of starvation or illness. We compare the population born before and after 1995 to examine changes over a time line that includes genetic, biomedical, and management interventions. Our data support earlier findings that there are idiopathic bone pathologies that exist in the Florida population, and we explore possible causes. Multivariate analysis reveals that Harris Lines and osteopathologies increase with age, and those pathologies affect males more than females, and both show increases after two years of age. There is a reduction in the number and severity of osteopathologies in panthers born after 1995; however, the demographics of our population (as represented in the museum sample) have shifted from an "aged" population to one that includes a disproportionately large number of young animals (<2 years old). It is likely that more than one biological process is operating to produce this result, and the study of osteological material alone cannot provide definitive diagnoses. Advanced studies in the pathology of human arthritis offer intriguing insights, and we expect at least some of their findings to have application in wildlife disease studies. Our results, together with the rich resource of archival material, leads to new cooperative research opportunities between museums, wildlife biologists, and wildlife veterinarians in the efforts to improve the conservation status of Florida panthers.

INTRODUCTION

Post-mortem skeletal remains reveal a history of activity, injury or traumatic events in the life of an animal. This is expressed by scars, malformations, unusual lesions, or excessive bone deposition as animals overcome infections, disease, or injury in life. Unusual or abnormal osteological features that have been described in panthers include arthritis, evidence of infection, trauma and bone lesions of unknown cause. Harris Lines (HLs), internal osteological features, were also recorded in high frequency in panthers. Duckler and Van Valkenburgh (1998a) showed that 69% of Florida panthers (N=51) exhibited at least one post-cranial osteopatholgy compared to 46% in a sample of pumas (N=26) from other locations. In the same study, the prevalence of HLs in Florida panthers was significantly greater (56.9%) than that of non-Florida pumas (27%). They conjectured that the elevated incidence of HLs was due to more regular episodes of poor nutrition, perhaps exacerbated by health problems associated with inbreeding.

Harris Lines are visible in radiographs as dense lines of bone deposition in long bones of humans and other mammals (Fig. 1). They record episodes of arrested bone growth in young individuals (Park 1964), and have been experimentally induced in rats, rabbits, pigs and dogs by starvation, selective nutrient deficiency, and bacterial inoculation (Harris 1933; Wolbach 1947; Park & Richter 1953; Platt & Stewart 1962; Mays 1995). It was these experimental studies that linked the formation of HLs, with the occurrence of a physical stress (Grolleau-Raoux et al. 1997). In humans, HLs have been associated with episodes of childhood illness (for

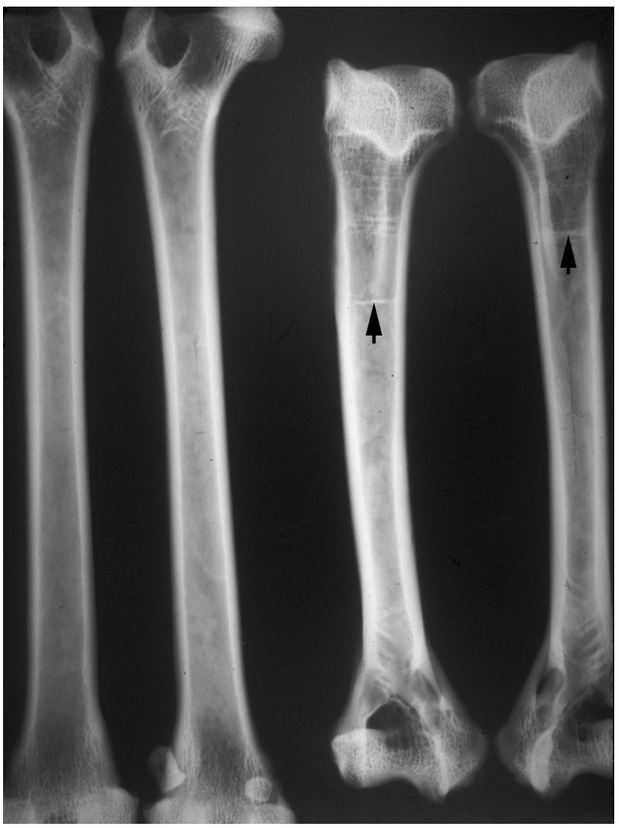


Figure 1: X-ray of humerii of Florida panther 15 , UF24563) showing Harris Lines. Harris Lines are transverse lines of bone density that represent a period of arrested growth followed by renewed growth.

review, see Garn et al. 1968). As a result, they have been used extensively in historical and archeological studies to characterize the health of human populations (Macchiarelli et al 1994; McHenry 1968; Rathbun 1987).

Current interpretations differ on the significance of HL formation. Some believe that HLs form as stress lines during accelerated growth spurts rather than as indicators of past illness, trauma, or malnutrition (Alfonso et al. 2005). The alternative explanation—that HLs represent renewed bone growth upon recovery from a serious illness—remains well supported (Mays 1995), although it has been shown that there is often no one-to-one correspondence between episodes of disease or nutritional deficiency and HL formation (Marshall 1968). Medical monitoring of Florida panthers during the 1980s showed cats to be in variable health declining southward towards the Everglades National Park (ENP) and the Fakahatchee Strand State Preserve (FSSP), a condition associated with the type and abundance of prey (Roelke 1990), and supported by food habit studies. Scat analysis showed the panthers living north of Alligator Alley were killing predominantly large prey (white-tailed deer and feral hogs), while those panthers living in the FSSP were taking a large number of small prey (raccoon and armadillo) (Roelke et al. 1986, Maehr et al. 1990). A relationship between diet, health, and presence of HLs may exist in this stressed population, but it is complex and requires further investigation.

Studies on the incidence of osteological abnormalities in a wild mammal population are rare, and frequently involve the description of a single or few specimens. This is often true because post-cranial skeletons were not archived, and are not available for study. The panther specimens in the FLMNH mammal collection today number more than 140 individuals, including those salvaged through mid-2006. This provides an unparalleled opportunity to expand the earlier study of morphology conducted by Duckler and Van Valkenburgh (1998a,b) and to further explore the nature of osteopathology in natural populations. The literature on bone pathology in mammals is vast, but often relates to veterinary studies on domestic animals; terminology and the diagnosis of skeletal abnormalities from skeletal assemblages is difficult and often confusing.

Arthritis is defined by the presence of osteophytes, which are bone spicules that develop around joint margins or sites of injury and represent the body's attempt to repair an injury. Arthritis involves proliferation of bone rather than loss of bone as in osteoporosis. The degree of osteophytosis in humans and animals can vary considerably—from single spicules, a ridge of new growth along an area of muscle attachment, to a massive outgrowth of bone. The presence of osteophytes is a common indicator of osteoarthritis (OA) or degenerative often age-related joint disease (DJD), which is common in humans (Rogers & Waldron 1995), wild mammals (Fox 1939; Greer et al. 1977) and domestic animals (Jubb et al. 1985). Degenerative joint disease is a progressive condition in which the articular cartilage is slowly degraded and the surrounding bone reacts by producing osteophytes. The disease affects many animal species, and is common in domestic breeds of cats. However, the presence of osteophytes may be associated with other conditions, as 'arthritis' is a general term that includes a broad spectrum of disorders or diseases that has been extensively studied in humans, and is beginning to emerge as a field of study in other mammals, as we will discuss in greater detail later.

In past studies, joint disorders have been broadly separated into two categories, non-inflammatory and inflammatory. Osteoarthritis (OA), or DJD, including joint disease resulting from trauma and developmental, metabolic, or dietary causes is generally considered to be non-inflammatory (Turnbull & Cowan 1999). Factors

cited in pathogenesis of DJD include genetics, abnormal joint alignment, excessive stress, trauma, local inflammation, and hormonal influences (McKeag 1992; Lane & Buckwalter 1993; O'Connor & Brandt 1993). Inflammatory arthritis includes a variety of disorders that typically involve reactive bone formation and fall under the general term spondyloarthropathy (SpA). One particular form of SpA is reactive arthritis (ReA), not to be confused with another type of arthritis, rheumatoid arthritis (RA). In humans (ReA) is mediated by a variety of infectious organisms to which panthers may also be susceptible. Variable manifestations of SpA include asymmetrical, pauciarticular (involving less than five joints), peripheral (appendicular) joint erosions and fusion, and axial (spine and pelvis) joint inflammations (Resnick & Niwayama 1988; Rothschild & Martin 1993).

The use of the term DJD is widespread in the both the wildlife and veterinary medicine literature but the descriptive terminology is confusing as its symptoms overlap with that of SpA. Non-inflammatory disorders can often lead to secondary inflammations, and inflammatory joint disorders commonly result in secondary, often severe degenerative changes (Turnbull & Cowan 1999). According to Rothschild et al. (1998, 2001), it is the more inflammatory form of arthritis, (namely SpA), that is the more likely condition in wild mammal populations, including large cats, whereas DJD is more often associated with domestic breeds and zoo animals. SpA has been described in hyenas, bears, canids, non-human primates, elephants, large felids, and mammalian and non-mammalian fossils (Rothschild & Rothschild 1994; Rothschild & Woods 1989, 1991, 1992; Rothschild et al. 1993, 1998, 2001). Turnbull and Cowan (1999) described synovial joint diseases in wild cetaceans that included both degenerative and infectious manifestations.

Medical management, genetic augmentation, and intervention to increase prey species may have contributed to improved health of Florida panthers over the years, which could reduce the expression of health-related skeletal anomalies. Our objective was to review earlier studies, investigate a larger sample of cats from a longer time period, and quantify the observed skeletal features to determine if there have been any changes in the frequencies found by Duckler and Van Valkenburgh (1998a). Further, using multivariate statistical methodology, we explore the interrelationship of HLs, skeletal anomalies observed per individual, and the degree of severity of those anomalies, and to what extent these vary by age, sex and habitat. Any general deviation from normal bone development is referred to as an Abnormal Osteological Feature (AOF), to distinguish it from the many specific types of pathologies that exist in nature and to have a consistent and easy reference. We hope to learn more about the causes of HLs and AOFs, or at least the conditions under which they form, utilizing a large data set and ample life history information about individual Florida panthers.

Considerable analogy with human arthritis research was used for insight into panther bone pathology, as others have done (Rothschild et al. 2001; Turnbull & Cowan 1999), to reveal new directions in arthritis research, and to search for explanations for the high frequencies of HLs and AOPs. The study of osteological material alone cannot provide a definitive diagnosis; therefore this is a first step to categorize an idiopathic disease process that may exist in the panther population of Florida.

METHODS

HARRIS LINES

To document frequency of HLs in Florida panthers, we X-rayed left and right humerii and femora of 69 males and 43 females including animals that died as recently as 2006. HLs were scored when the density line was perpendicular to the long axis of the bone and extended across the entire shaft. This is a more conservative measure as often HLs need only extend one-fourth to one-half way across the shaft of the bone. Ultimately, only humeral HLs were counted, since femora showed very few. Harris Lines do not always occur symmetrically, so we totaled the number of HLs present in L and R humerii. Age, sex, year of death, and use area were recorded for each specimen (Appendix 1). Our X-rayed individuals overlapped with those studied by Duckler and Van Valkenburgh, but did not completely duplicate their series.

ABNORMAL OSTEOLOGICAL FEATURES

Major skeletal elements of Florida panther specimens in FLMNH collections including 77 males 50 females, and one unknown (n=128) were inspected and abnormal osteological features (AOFs), including healed fractures, arthritis, infection, unusual lesions, malformations, and unknown pathologies were tabulated (Appendix 1). We gained insight into bone pathology by referencing studies in archaeology and forensics (Baker and Brothwell 1980; Buikstra & Ubelaker 1994; Rogers & Waldron 1995), paleopathology (Rothschild & Martin 1993), wildlife and veterinary science, and contemporary studies in human arthritis, as well as consultation with forensic specialists. Our results are reported and compared to the earlier study by Duckler and Van Valkenburgh (1998a,b).

We examined the cranial, axial, pelvic, and appendicular skeleton including the feet, and scored any ab-

normality within each. We recorded the total number of AOFs, being conservative in our estimate. For example, an AOF associated with two or more bones, such as tibia-fibula or humerus-radius-ulna joint was only counted once regardless of the number of affected bones. If there were two lesions on one bone, both would be counted only if they were qualitatively different, or located in a different region of the bone, although we could never be certain of the relationship between any two observations because of possible systemic involvement. The skeleton records a lifetime of insults, and there is no way to know for certain that this scoring system adequately distinguished one event from another. No fractures that were related to the cause of death were recorded; that is broken bones without evidence of bone remodeling.

An overall severity score (S) was assigned to each specimen, ranging from S1-Mild, S2-Moderate, to S3-Severe, depending on the nature and extent of the anomaly. Measures of severity suggest the greater or lesser expression of a condition or the later phase of a disease. While neither of these may have any basis in clinical practice (Rogers & Waldron 1995), they do provide a basis for comparison. A severity score of S3 (the most severe case) was assigned only in cases where bones were broken or the deformation from normal state was extreme, and potentially crippling, the latter having been noted either in life through observations and reports, or during necropsy. Since many skeletons had more than one incident that ranged in severity from S1 to S3, the highest single score was selected, since that one would have the greatest potential threat to the survival of that animal.

PANTHER STUDY GROUPS

In many cases, the ages calculated for Florida panthers were estimates, so age classes were established based on several references, including degree of fusion of cranial sutures and epiphyseal closure, direct comparison with animals of known age, and estimates provided in FWC annual reports (Land et al. 2005). Animals were grouped into five age classes (Class I = <1 year, Class II = 1-2 years, Class III = 2-4 years, Class IV = 4-10 years, and Class V = > 10 years. To gain an understanding of the relationship of health parameters (HLs, AOFs) to regions of Florida (north or south), we assigned a "Use Area"(UA) to each animal based on published reports or, in the case of panthers not radiocollared by the FWC, the location where they were found dead. Use areas were defined UA1) north of Interstate 75 (former SR 84 or Alligator Alley), and UA 2) south of Interstate 75 including southern Big Cypress National Preserve (sBCNP), Fakahatchee Strand State Preserve (FSSP), and the Everglades National Park (ENP)(see map in Part II). Past health and diet studies of panthers frequently delineated three distinct regions by considering ENP a separate region, but sufficient samples did not exist, so ENP cats were grouped into UA2.

ANALYSES

The dependent variables in the study are total number of abnormal osteological features (AOFs), severity index (SI) of AOFs, and HLs. The independent variables were sex, age class and use area. The model used to test the dependent variables included all three independent variables and all possible interactions. A Multivariate Analysis of Variance (MANOVA) was performed simultaneously testing all three dependent variables against the independent variables using SAS (SAS Institute Inc., Cary, NC). Once significance was established with the MANOVA, individual ANOVAs were run on each independent and dependent variable. A student's t was used to find differences between Age Class groups whenever significance was established for that variable. All of the individual ANOVAs were analyzed using JMP version 5.0 Statistical software for the Macintosh (SAS Institute Inc., Cary NC).

The relationships between the three dependent variables (HLs, AOFs, and SI) were analyzed using regression analysis. Animals with no osteopathologies were removed because these animals also had a scoring of zero for the AOF severity index. Therefore, only animals with at least one AOF were used to examine trends between the dependent variables. To determine the effect on HL or AOF frequency resulting from the introduction of Texas cats in 1995, we compared the prevalence of HLs and AOFs of animals that were born prior to 1995 (HL n=63, AOF n = 79), to those born since that time (HL n=49, AOF n = 49) using ANOVA.

RESULTS AND DISCUSSION

The MANOVA testing the differences among total AOFs, Severity of AOFs, and total HLs on sex, age class, and use area shows a significant multivariate effect for sex and age class. This test fails to reveal any significant multivariate effect for use area or any interactions of the three independent variables (Table 1).

HARRIS LINES

The average number of HLs increases with each Age Class (AC) for all animals combined (Fig. 2). There is a continuous increase in number of HLs with each successive AC and overall significant differences were found between the ACs (ANOVA; $_{F4,107}$ = 4.82, p =

Table 1: MANOVA results for Florida panthers from the Florida Museum of Natural History. The MANOVA examined three dependent variables; total AOFs, severity of AOFs, and Harris Lines.

Variables	Wilks' Lambda	F statistic	df	p
Sex	0.90	3.14	3/88	0.0292
Age Class	0.59	4.29	12/233.12	<0.0001
Age Class * Sex	0.83	1.37	12/233.12	0.1789
Use Area	0.98	0.46	3/88	0.7006
Use Area * Sex	0.98	0.50	3/88	0.6859
Age Class * Use Area	0.92	0.61	12/233.12	0.8299
Age Class * Use Area * Sex	0.96	0.28	12/233.12	0.9923

0.0013). A significant increase exists from AC-I to AC-IV as well as from AC-IV to AC-V. There is no difference between males and females with respect to HLs, nor was there any significant difference between the average number of HLs for animals from the north, above SR 84, and those from the south, including sBCNP, FSSP and the ENP (MANOVA results; Table 1).

The apparent continual accumulation of HLs throughout life is an unexpected result. Harris Lines develop at the metaphases during growth before the epiphyseal plate fuses to the long bone, at which point bone growth stops. For panthers, this would be approximately between the ages of three and four years. Because bone is remodeled over time, it is also expected that evidence of HLs would disappear with age as bone continuously remodels, so an older animal should have fewer HLs rather than more. One possibility for the continued visibility of HLs in older animals is that previously formed HLs become easier to detect as cats age, perhaps due to a reduction in the thickness of the bone cortex. This has not been studied, however, it is possible to measure cortical bone thickness in individuals that vary in age to determine if this is the case. (A. Falsetti, C. A. Pound Human Identification Lab, pers. comm.). If it is assumed that HLs are indeed an indication of stress in the population, then a likely explanation is that the older animals in our study experienced greater health-related stress at a younger age than those in the younger age classes, a possibility also noted by Duckler and Van Valkenburgh (1998a). This might suggest that health or living conditions have improved since the mid-1980s. This is consistent with information from diet and prey studies conducted in the mid-1980s in the sBCPR and FSSP that reported that animals in the south were eating fewer deer and more smaller prey, and that the general health of animals living in the Fakahatchee was poor (Roelke et al. 1986, Maehr et al. 1990, Roelke 1990).

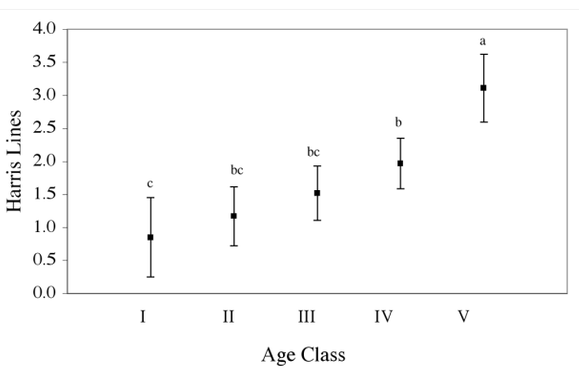


Figure 2: Mean number of Harris Lines for Florida panthers at each age class. Letters represent groups that are significantly different. Sample sizes are; Age class I (n=13), II (n=23), III (n=27), IV (n=31), V (n=18).

This time period during the 1980s also reflects a time when deer hunting in the FSSP was legal, but later banned, and therefore panthers and hunters may have been competing for the same large prey species with the result that panthers in FSSP were eating small prey rather than deer and hog (Roelke 1990). This too is a complex issue because many factors affect prey density. Prior to 1980s there was no intentional management on public lands to increase prey (such as deer) for panthers. During the 1980s, actions were taken to reduce access to public lands, reduce hunting pressure and harvest on white-tailed deer and hogs, reduce the use of hunting dogs, and protect does and fawns (Schortemeyer et al. 1991, Beier et al. 2003). Additional factors affecting deer densities are length and intensity of hydroperiod in the ENP that might affect fawn survival and variable quality of vegetation and soil types (Land 1991, Fleming 1994). Improvements in prey base throughout the range of panthers would contribute to a healthier population.

Contemporary arguments exist that HLs do not form as a result of pathological stress or disease, but rather may reflect accelerated growth during specific

early periods of development (Alfonso et al. 2005). Many of the HLs observed were faint, spatially clustered and appeared more frequently in proximal humerii close to the epiphyseal closure. The clustered nature of the HLs may reflect many individual stresses associated with rapid growth during a brief period of time. It is unlikely that this study will clarify the debate on the cause of HLs in vertebrates at least not without further research. However, it should be noted that serious health incidents nearly always result in transverse lines of considerable density, and such lines tend to remain detectable despite subsequent bone remodeling or advanced age (Park 1964, Maat 1984).

Dense lines in panthers frequently occur in the humerus anywhere from slightly distal to the midpoint up to the upper proximal portion of the limb bone. No dense lines in the lower quadrants were observed. If HLs are caused by pathological stress or nutritional deficiencies, then those events may not occur during the earliest months after birth, but rather after cubs become independent and begin to disperse at approximately 14 months of age. This activity might be more stressful, especially for males who move a greater distance than females, who must compete with resident males in other locations (Maehr et al. 2002). Harris Lines formed in very young animals are more likely to disappear as the bone is continuously remodeled during the earliest months of growth. As pointed out by Duckler and Van Valkenburgh (1998a), HLs are more likely to be retained in the adult skeleton when they form close to the time of epiphyseal closure and growth termination.

Duckler and Van Valkenburgh (1998b) also showed that pumas throughout their range have higher incidence of HLs compared to other extant species, including mule deer *(Odocoileus hemionus*), bobcat (*Lynx rufus)*, and gray wolf (*Canis lupus*). This may be due to the stress to the bone brought about by one or a combination of larger size (compared to the wolves), and the reckless nature of their hunting style, in which leaping over boulders, jumping from trees, and incredible spurts of speed are legendary (Sunquist & Sunquist 2002). However, why Florida panthers have more HLs than puma from other geographic locations remains uncertain. A long history of poor health as a result of diet, as well as deleterious consequences of inbreeding manifested as cardiac defects, and high pathogen/parasite loads increased the potential for illness or disease and HL formation (Duckler and Van Valkenburgh 1998b). This remains a plausible explanation.

A superficial review of the life history of several young cats does not support the conventional view that HLs form as a result of one particular stressor or starvation. For example, Florida panther 22 was captured by FWC at six months old, subsequently abandoned by her mother, and was later recaptured by FWC in a starved and dehydrated condition. She was rehabilitated, released, and again in 3-4 months, as a result of another FWC capture attempt, she was separated from her mother and was recaptured again in a debilitated and dehydrated condition. She was brought into captivity and not released until 2 years later (M. Roelke, pers. comm.). In spite of two traumatic separations from her mother, two bouts of starvation, two capture events, and a two-year period in captivity, there is no evidence of HLs in this individual. Her sibling, FP 23 also captured twice had distinct HLs. Florida panther 8, who later in life was captured and found to be underweight and in poor health, had HLs, but they were in the middle portion of the limbs, indicating that the cause of the HL occurred when she was very young. These examples demonstrate that it is not possible to make a direct correlation between episodes of starvation and the presence of Harris lines, particularly in older individuals.

HLs reportedly form as a result of a variety of infectious diseases, as well as protein or vitamin deficiencies. There is also evidence to suggest that they may form in humans as a result of exposure to mercury and other heavy metals (Raber 1999). A study examining the presence of HLs as they relate to mercury levels recorded in individual panthers is underway (M. Cunningham, pers. comm.).

The importance of HLs as indicators of pathological stress has gone in and out of vogue over the past 50 years. Bone formation and homeostasis is a dynamic process, and many physiological processes affect bone health. Earlier research (Duckler and Van Valkenburgh 1998a) has shown that prior to 1995, Florida panthers had significantly more HLs (56%) than non-Florida populations (27%) (Table 3). We are unable to compare our results directly to theirs since we used total HLs for each animal specimen rather than presence per side. In addition, we did not have the opportunity to examine X-rays of the non-Florida sample. In our results, an ANOVA testing for HL differences between animals born before and after 1995 showed no significant difference ($F_{1,110} = 1.31$ $p = 0.2554$) although there is a slight trend showing a reduction in the average number of HLs of post-1995 animals, (1.53) compared to the pre-1995 animals (1.92). Presence of HLs in Florida panthers has not diminished over time in any significant way and HLs continue to be abundant in all age classes. Future research will investigate the relationship between HLs and variables such as overall health, mercury levels, and specific biomedical conditions reported for panthers.

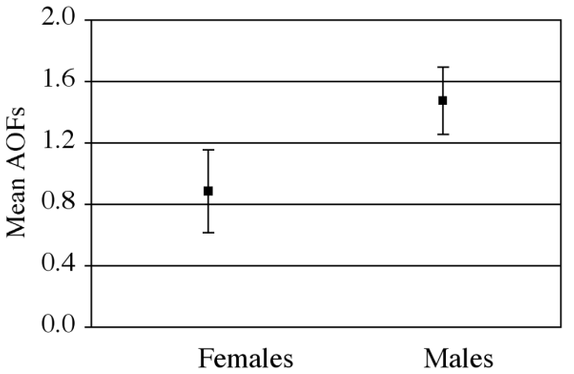


Figure 3: Mean Abnormal Osteological Features (AOFs) for female (n = 50) and male (n = 78) Florida panthers with error bars. Males have significantly more AOFs than females (ANOVA; $F_{1,126} = 4.45$, $p = 0.0369$).

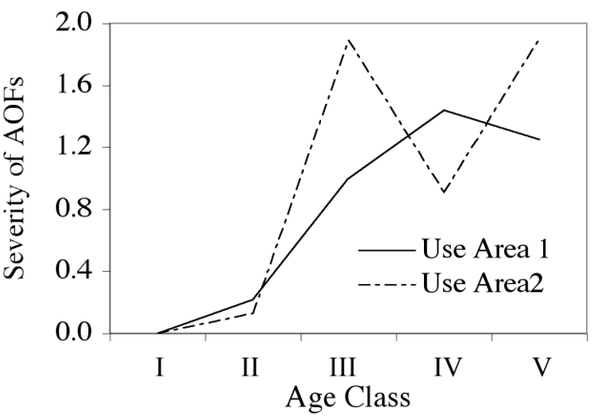


Figure 5: Interaction plot between Florida panthers from the two different use areas. Each point is a mean for each age class. Panthers in age class three show a significant difference between the two use areas (ANOVA $F_{9,116} = 2.55$ $p = 0.043$)

ABNORMAL OSTEOLOGICAL FEATURES

The total number of AOFs was significant for both sex and age class, in the MANOVA analyses (Table 1). Males had more AOFs than females (ANOVA; $F_{1,126} = 4.45$, $p = 0.0369$; Fig. 3), and older animals had more AOFs than younger animals with the total number increasing with each successive age class (ANOVA; $F_{4,123} = 12.84$; $p < 0.0001$; Fig. 4)

There were no significant results in the interaction between any two variables and our dependent variables in the MANOVA. However, when ANOVAs were run for just severity of AOFs, use area, and age class (Fig 5), a significant interaction was found ($F_{9,116} = 2.55$ $p = 0.043$; Fig. 5). These results suggest that a trend exists, but the original MANOVA is not sensitive enough to detect this. Alternatively, it could represent a type I error, but because results are significant it is necessary to mention the possible trend. The severity of the AOFs in Use Area (UA) 2 are more variable than UA 1, but generally animals in this group also have more severe AOFs. Severity (and occurrence) increases between AC-II and AC-III, between ages of approximately two to four. A nearly significant interaction was also found between sex and age class with severity index (SI) of AOF's ($F_{9,115} = 2.36$ $p = 0.057$; Fig. 6). Males and females have a similar low SI in AC-I and AC-II, but males increase dramatically in the severity of AOF's compared to females in AC-III and AC-IV. This corresponds to the period of time that males are dispersing and attempting to establish a territory and home range.

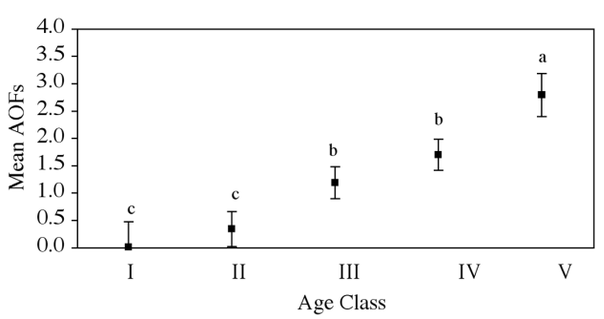


Figure 4: Mean AOFs for Florida panthers for each age class with error bars. Letters represent significant differences between groups. Sample sizes are: Age Class I (n=14), II (n=27), III (n=34), IV (n=35), V (n=18).

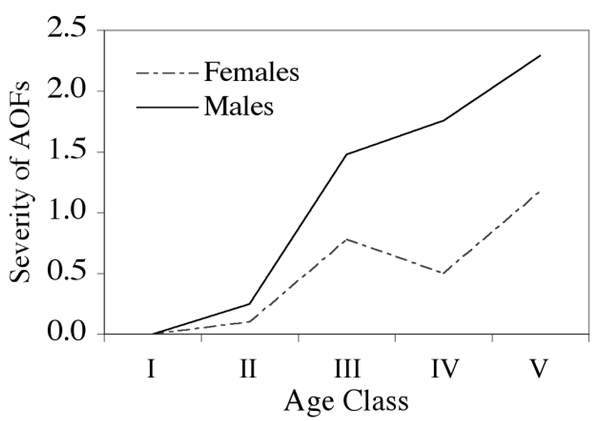


Figure 6: Interaction plot for female and male Florida panthers in each age class. Females and males from the 3rd, 4th and 5th age classes are significantly different from each other (ANOVA $F_{9,115} = 2.37$ $p = 0.057$).

Table 2: Number of Florida panthers in age class for animals born before and after 1995.

Age Class	Pre-1995	Post-1995	Total
1	6	8	14
2	9	18	27
3	21	13	34
4	26	9	35
5	17	1	18

Maehr et al. (2002) describe the process as beginning at approximately 14 months of age and continuing for 7.0 months for females and 9.6 months for males with males' efforts frequently frustrated by insufficient vacant range or range containing no individuals of the opposite sex. Independence and dispersal of young cats can, and probably does, increase food stress as well as encourage, especially in males, male-male conflicts. As males disperse, they also have a greater chance of injury (and death), thus accounting for both the increase in young animals in our collection, and increased evidence of trauma and AOFs.

Our sample consists of animals born prior to 1995 (n=79) and those born since 1995 (n=49; Table 2). There has been a significant reduction of both the number (ANOVA; $F_{1,12} = 13.53$, $p = 0.0003$; Fig. 7) and severity (ANOVA; $F_{1,124} = 6.97$, $p = 0.0093$; data not shown) of AOFs since 1995 in a sample that includes both males and females. There is only one animal in AC- V that was born after 1995 (n = 1; Table 2). This could bias these results because AOFs also increase with age class,

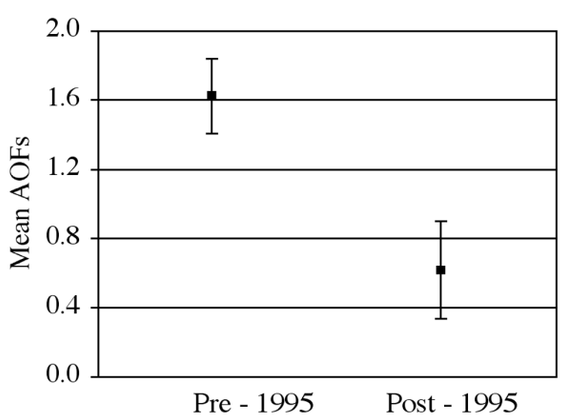


Figure 7: Mean AOFs and error bars for Florida panthers born before 1995 (n = 79) and after 1995 (n = 49). Panthers born after 1995 have significantly fewer AOFs (ANOVA; $F_{1,126} = 13.53$, $p = 0.0003$).

but when we repeat the analysis after removing AC-V, our results remain significant for total AOFs (ANOVA; $F_{1,108} = 9.61$ $p = 0.0025$). This leads us to conclude that there is a trend towards a reduction of AOFs and their severity since 1995.

Although no correlation exists between Harris Lines and total AOFs (Fig. 8A,B), a correlation exists between the number of AOFs and the severity of AOFs (Fig. 8C). This correlation is expected given that an increase in the number of AOFs will increase the probability that at least one of them is severe.

Panthers over the age of two born in Florida over the last 50 years, show a range of mild-to-severe, and potentially debilitating, osteological conditions (Appendix 1). These include trauma (breaks and bite wounds), arthritis, periostitis (disease of the periostium), enthesitis (inflammation of osseous attachment of tendons, ligaments, synovium) and other unidentifiable infections frequently observed in the feet, forearms, and axial skeleton.

The overall incidence of fractures and other trauma remain high when compared to the population of non-Florida panthers analyzed by Duckler and Van Valkenburgh 1998. (Table 3). The overall prevalence of all pathologies in our sample was 60.8% in the pre-1995 sample compared to 69% reported by Duckler and Van Valkenburgh (1998a). However, our scoring protocol differed in that we counted number of AOFs rather than a type and presence/absence per side. This resulted in more incidents of AOFs in our study. We also did not consider HLs in our final totals of AOFs, thereby potentially lowering the score. We preferred to treat HLs separately since there is disagreement as to whether HLs are only associated with illness or injury. Further, we hoped to learn more about the possible relationship of one to the other through these analyses. Regardless of how pathologies were counted, both studies found a greater than 50% incidence of abnormal osteological features in the population of Florida panthers. This is far greater than any published study of abnormal bone development in any wildlife species, although those studies are themselves very rare. Pathologies were less frequent in the post-1995 sample (n=49) with 18 individuals (36.7%) exhibiting one or more pathology compared to 48 (60.8% pre-1995). The fraction of the post-1995 population with pathologies is significantly lower than the pre-1995 sample. However, the proportions of old versus young animals in the two samples vary considerably. There is a 34% increase in animals under two years of age in the post- 1995 sample, and it has already been demonstrated that injuries and other or AOFs accumulate with age (Table 3).

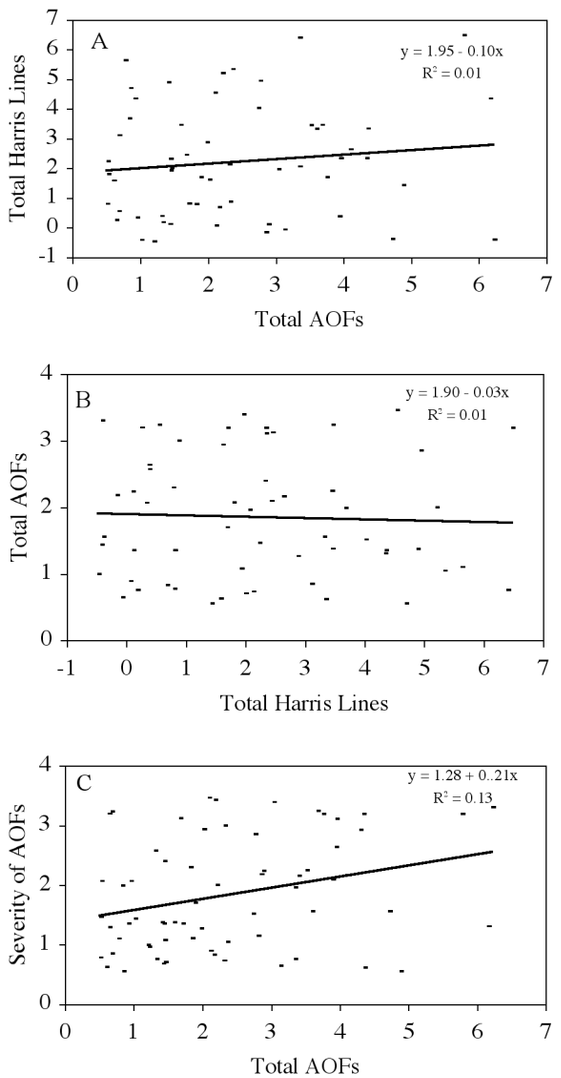


Figure 8: Correlations between total number of Harris Lines (A), total AOFs (B), and severity of AOFs (C) for Florida panthers. Points were jittered (offset) by adding a random number between –1 and 1 to each point so that the points don't lay on top of each other. Animals without any AOFs were removed from the analysis because they also had a zero Severity Index, which would influence the regression line.

The most abundant osteological expression was that of arthritis, which was present in 53.2% (n=42) of the sample of animals born before 1995, but only 30.6% (n=15) of those born after 1995. It might have been easy to dismiss the severe arthritis we observed as age-related, since many of the specimens acquired in the 1980s and early 1990s represent an "aged population" (Ballou et al. 1989; Roelke 1990; Duckler & Van Valkenburgh 1998a). However, arthritis, as the accumulation of osteophytes, even in an incipient form, was present in cats of all ages, except the very youngest (Age Class I). Young animals (those younger than two years of age) did not show significant arthritic lesions, and there are considerably more young animals (53.1%) in our post-1995 sample. Age is a significant factor to be considered in the interpretation of these results, as insults to the skeleton continue to accumulate through life.

Some types of lesions were more prevalent, whereas others were more notable because of their severity. Among the most common AOFs were inflammation and osteophytosis, an inflammatory arthritis, in forelimbs, including elbow and wrist. A 3-year old male (FP 89), reportedly small for his age, had extensive proliferation of bone around the articular surfaces of both the left and right humerus/radius/ulna joints, which was also observed in life as an open wound at the elbow (M. Cunningham, pers. comm.) (Fig. 9). A similar, but even more extreme case is that of FP16 from Dade County. Evidence of osteoarthritis was present in the spine and feet of this 14-year old male, however, all bones of the forelimb showed an advanced joint disease, possibly caused by a broken right radius/ulna, which calcified into a large mass in healing (Fig. 10A). Deep eburnations visible in the trochleae of both humerii and the trochlear notch of both ulnae reflect the loss of the protective cartilage resulting in bone-on-bone contact (Fig. 10B). Long-term instability and/or inflammation were indicated by the excessive bone deposition around both joints suggesting a debilitating and presumably painful condition. In addition, the distal fibulae where they attach to tibiae, both appeared to have been deformed or possibly broken, a condition that is difficult to explain (Fig. 10C).

While these were the most extreme cases, there were other cats with less severe inflammations, but that also involve the olecrenon process, the attachment site of the complex triceps brachii muscle. Often the condition was expressed in both limbs, which might suggest a chronic front limb dysplasia creating the potential for instability, injury and inflammation. Some of these same animals also showed osteophyte formation at distal radius/ulna above the level of the wrist, which may be a related condition. These cases, particularly in the young panther FP89, where there was no obvious evidence of a wound or injury that could result in an infection, alerted us to the possibility that there was a predisposition to inflammation at certain sites. This led us to scrutinize those sites (e.g., elbow joints) where we observed the early signs of inflammation or distortion. One Florida panther (FP 2; UF 20777), shows areas of inflammation at the proximal ulnae, and, both distal left and right ulnae show evidence of injury with subsequent osteophyte formation (Fig. 11). FP 205 (UF26520), approximately 1-1/

Table 3. Number and per cent of individuals with AOFs and Harris Lines in 2 populations of *Puma concolor* over time and a comparison of two studies of Florida panthers. Total individuals[a] (w/combined[3]Osteopathologies/ AOPs)

	Sample (N)	Arthritis	Infection	Trauma	Unknown	Total individuals[a]	Harris Lines[4]	S3 severity[5]	# aged 2 yrs
Previous Study[1]	*Florida*								
Skull	53	0	1 (2%)	1 (2%)	2 (4%)	4 (8%)	0		
Skeleton	51	9 (18%)	4 (8%)	4 (8%)	6 (12%)	35 (69%)	29 (57%)		
	Non-Florida								
Skull	443	0	3 (0.7%)	3 (0.7%)	8 (2%)	14 (3%)	0		
Skeleton	26	3 (12%)	0	4 (15%)	5 (19%)	12 (46%)	7 (27%)		
This Study[2]	*Florida*								
Skull +Skeleton	*AOP/HL*								
Pre-1995	79/63	42 (53.2%)	9 (11.4%)	12 (15.2%)	12 (15.2%)	48 (60.8%)	42 (66.7%)	13 (31.0%)	15 (23.8%)
Post-1995	49/49	15 (30.6%)	1 (2.04%)	5 (10.2%)	3 (6.1%)	18 (36.7%)	29 (59.2%)	5 (17.2%)	26 (53.1%)
Total	128/112					66 (51.6%)	91 (81.3%)		

[1] Duckler and Van Valkenburgh 1998a

[2]The two studies used different parameters in measuring pathology and are not directly comparable; our definition for arthritis was more inclusive of minor skeletal changes

[3-4] Harris Line count in current study is not included in Osteopathology (as it is in the Duckler & Van Valkenburgh study). We do not consider HLs to be strictly
a function of pathology, see text.

[5] The highest measure of severity represents broken bones, advanced debilitating arthritis, or other extreme deviations from normal bone development; the percentage shown
is based on the number of individuals with a severe condition among the total number of number of individual animals expressing one or more pathologies.



Figure 9: Example of severe inflammatory arthritis in ca. 3-year old Florida panther (FP89, UF30064) that died of intraspecific aggression. Left are the right and left humerii, and corresponding ulnae are seen at the right. In life an open wound was observed at the elbow.

2 years old, shows osteophyte formations at the metaphases (Fig. 12). Note that FP205 was not included in the statistical analyses, because he spent time in captivity. However, it is noteworthy that this animal died of an infection due to esophageal laceration, and the osteophyte formation visible in the photograph may be a systemic reaction to that infection.

A second very distinctive osteopathology worth noting was a severely degraded, porous, and rugose distal ulna in panther FP 10 (UF23986), who was killed by another Florida panther. The remnants of a periostitis infection, suggested by a layer of black film that covered most of the cleaned limb bones (Fig. 13) might have been a contributing factor. There were several young animals that exhibited a very porous state in the distal radius/ulna region of the forearm. Without knowing more about the developmental process we cannot say this is abnormal, only that young cats were vulnerable to osteophyte formation and infections in the distal ulna.

Frequencies of fractures and other trauma are high compared to the population of non-Florida panthers and much higher than in other vertebrates such as deer and wolf (Duckler & Van Valkenburgh 1998a,b). Few comparative studies exist of traumatic, degenerative or developmental lesions among wild carnivores or any mammal species. Among the few is a study of wolves (*Canis lupus*) and coyote (*Canis latrans*) in Saskatchewan (Wobeser 1992). Wolves showed a much greater number of broken bones (22.8%), but degenerative joint disease, involving the spinal column and limb joints similar to that reported here, was found in only seven wolves and two coyotes. As with panther FP16 (UF29821), the articular cartilage of a single wolf was eroded from the condyles of both femurs with eburnation of the underlying bone and extensive periarticular osteophytes were present. One would expect a greater number of injuries in carnivores, such as wolves and puma, than other mammals because hunting is a dangerous activity. Although the osteopathologies of non-Florida wild living puma were high (n=12, 46%), they were not as high as those observed in the Florida population (Duckler and Van Valkenburgh 1998a). Now, ten years later with a sample







Figure 10: All bones of forelimb show advanced joint disease in this 14-year old male (FP16, UF29821), probably initiated by the broken right radius/ulna (A). Deep eburnations visible in the trochleae of both humerii and the trochlear notch reflect the loss of the protective cartilage, resulting in bone-on-bone contact (B). Distal fibulae, at the attachment site of tibiae, both appeared to have been deformed or previously broken (C).

twice that of Duckler and Van Valkenburgh's study (1998a), we do see a decline in trauma and arthritis. However, once again, it is necessary to consider the proportionate difference in age classes. Following Duckler and Van Valkenburgh (1998a,b) we have documented a high incidence of injury, arthritis and other pathology associated with the skeletons of Florida panthers. We are not able to attribute them to any specific type of arthritis, since so many have been described (see following discussion of human arthritis studies) but it is apparent that some level of infection is operating, and it is likely that more than one causal agent exists. In several specimens, the degree of osteophytosis alone, whether initially due to an injury with secondary infection, would suggest there is a tendency for a systemic and inflammatory condition to prevail. The consistent involvement of the enthesis of the olecrenon process and the distal radius/ulna suggests abnormal joint alignment or ligamentous instability, particularly because these were often symmetric. This may also cause inflamation. Another consideration is that this front limb joint is more vulnerable to injury since it bears significant stress in many locomotor activities (see McGonagle et al. 2001 below). Thirty-eight panthers have been killed by intraspecific aggression (Land et al. 2005); several of these were severely debilitated.

NEW ARTHRITIS RESEARCH

Before we attempt a discussion of possible causes AOFS, we would like to present a summary of the intensive research in human arthritis during the last two

decades. This provides new information and intriguing insights into the pathogenesis the spondyloarthropathies (SpA), also referred to as arthropathies. References to SpA in wildlife studies are rare, but do exist (Turnbull and Cowan 1999), and recall that they have been identified in museum specimens (Rothschild and his colleagues, op cit.). Spondyloarthpathies include a diverse suite of related conditions in humans, including ankylosing spondylitis, reactive arthritis, psoriatic arthritis, and inflammatory, bowel-disease (Benjamin & McGonagle 2001). Collectively these arthropathies are characterized by inflammatory arthritis, extra-articular inflammation, preceding bacterial infection, seronegativity for rheumatoid factor, and a strong genetic association (HLA-B27) (Dougados et al. 1991; Calin & Taurog 1998). In humans, an important factor associated with the susceptibility of an individual to reactive arthritis is HLA-B27. It appears that B-27 positive individuals are affected more severely, although the pathogenesis is still not fully understood (Toivanen and Toivanen 2004). Today SpA is commonly referred to as enthesopathy because of the involvement of the enthesis, the insertion site of a tendon, ligament, or articular capsule into bone. Enthesitis, the inflammation of an enthesis, is believed to be a unifying concept for SpA (McGonagle et al. 1998). Numerous enthesitic arthritic lesions, some incipient and others grotesquely abundant, were observed at the olecrenon process and distal radius ulna of several young panthers. Enthesitis can accompany many disorders, including traumatic, degenerative, inflammatory, endocrine, and metabolic conditions. In some cases, enthesitis represents the initial or predominant manifestation of disease (Resnick & Niwayama 1983). Furthermore, one aspect of SpA, namely ReA, (reactive arthritis) is now considered in humans to be a disease, triggered by a host of infectious agents including bacteria, parasites, and viruses (Toivanen & Toivanen 2001a,b). The inflammatory expression is the interaction between the infectious agent and the host immune response (Schoen 2000). A hypothesis in the study of psoriatic arthritis is that enthesitis arises at sites of high shear and compression forces, with the additive interaction between mechanical stress, microtrauma, tissue repair mechanisms, and bacterial molecules variably leading to inflammation (McGonagle et al. 2001).

Whether it is possible to extend human research to panthers is as yet unknown. The tendency for front limb inflamation at the enthesis of the humero-radius ulna joint may be due to the biomechanical stresses of the front limbs associated with puma hunting behavior and occasional injury. However, it is also well known that panthers are immuno-compromised and vulnerable



Figure 11: Areas of unusual rugosity or inflamation at the proximal ulnae and osteophyte formation at the distal, possibly due to injury or instability caused by dysplasia (FP 2, UF20777).

to a variety of pathogens (bacterial, viral, and parasitic) possibly as a result of low genetic diversity (Roelke et al. 1993a,b; Glass et al. 1994; Rotstein et al. 2000). Panthers exhibit a variety of congenital abnormalities, such as atrial septic defect and cryptorchidism related to low genetic variability (Beier et al. 2003; Pimm et al. 2006). Elbow dysplasia in dogs is influenced by multifactorial processes including genetic predisposition, and if untreated is known to progress to crippling osteoarthrosis (Pool 2002). Dysplasia and/or the increased susceptibility to osteological inflammation as a result of injury or infection could be another previously unsuspected manifestation of panther inbreeding. Further, inflammations associated with SpA, such as those identified in the el-



Figure 12: 1.5 year old Florida panther (FP 205, UF26520), shows early osteophyte formation at the metaphysis. This animal died from an infection due to esophageal laceration.



Figure 13: Distal ulna is abnormally porous and rugose; possible cause is a perostitis, an infection of the periostium, suggested by a layer of black film that covered most of the cleaned limb bones (FP 10, UF23986).

bow joint of panthers in this population, have been described in other wild mammals, including cetaceans, and are considered to be debilitating and a significant mortality factor if allowed to progress (Turnbull and Cowan 1999).

Veterinary involvement with the panther began in 1983. Since that time, biomedical research to further the understanding of disease, nutrition, and reproductive physiology has been an integral part of the Florida panther recovery efforts (Roelke 1990, Dunbar 1994). Among the many thousands of biological samples taken over the years including blood, urine, skin biopsy, feces, hair, saliva, viral and bacterial culture swabs, one is of particular interest here; namely Feline Syncytia-forming Virus (Roelke, pers. comm.). Feline Syncytia-forming virus (FeSFV) is common in healthy as well as sick domestic cats, but has been reported in conjunction with chronic progressive polyarthritis. Pedersen et al. (1980) reported that in a study 20 domestic cats with CPA between 1.5 and 5.0 years of age, only males were affected, and two distinct forms of the disease were manifested. The following is their actual account:

"The most prevalent form was characterized by osteopenia and periosteal new bone formation surrounding the affected joints. The second form was characterized by severe subchondral marginal erosions, joint instability, and deformities. The periosteal proliferative forms resembled Reiter's arthritis of man, and the deforming type resembled human rheumatoid arthritis. The disease began as tenosynovitis and synovitis, with subsequent changes in the articular cartilage and periosteal bone. Histopathologic changes in these cats were similar to those occurring in both chronic Reiter's and rheumatoid arthritis of man. Chronic progressive polyarthritis of cats was not caused by identifiable bacteria or

mycoplama, but was etiologically linked to feline leukemia virus (FeLV) and feline syncytia-forming virus (FeSFV) infections."

Pedersen et al. (1980) postulated that polyarthritis was an uncommon manifestation of FeSFV that occurred in predisposed male cats. Feline leukemia virus may not have been directly involved in the disease, but may have acted in some way to potentiate the pathogenic effects of FeSFV. Of all panthers tested for FeSFV from 1987-1992, 59% were found to be infected with FeSFV (original number not disclosed; Dunbar 1994). Sixteen of those panthers reside in the FLMNH collection and some of the males showed signs of severe arthritis, whereas females were less affected. Seven males (FP7, 10, 12, 13, 16, 17, and 20), demonstrated the following arthritis or unknown infections: SI of 3 (n=2), SI of 2 (N=4), and SI of 1(N=1), and five of seven males had three or more episodes of arthritis and infection that we would describe as arthritic, infectious, or having a component that would coincide with a description of enthesititis (inflamation in region of enthesis). Of the eight females represented in our collection, most showed no evidence (N=2), or mild arthritis (N=3), moderate infection on the rear feet (N=1), and more extreme episodes were apparent in old females (N=2, FP18 and 21, 11 and 14 years of age, respectively). It is difficult to come to any conclusions based on this small sample, but males had both more AOFs and more severe AOFs. Tests for FeSFV were discontinued after 1994, however, archived tissue samples exist and it may be worthwhile to explore this medium as a possible contributing factor to the high incidences of AOFs in Florida panthers.

Environmental contaminants such as methylmercury might further compromise the animals' ability to resist disease (Roelke et al. 1991). As well, they may be a contributing factor in the formation of Harris Lines and/or other osteological pathologies. Elevated levels of mercury have been reported in Florida panthers (Newman et al. 2005), and the death of at least one animal (FP27, UF 24557) from the Everglades was suspected to be the result of mercury toxicosis. Mercury is known to interfere with bone metabolism and calcium homeostasis (Suzuki et al. 2004), although it is not known if it exacerbates the inflammatory process. Presence of mercury in south Florida environments may also eventually explain the high incidence of Harris Lines. Dense metaphyseal banding has many possible causal agents, but heavy metals including lead and mercury, can induce physiological changes that result in increased calcium deposition (Raber 1999). An examination of whether Mercury plays a role in the high number of osteopathologies in Florida panthers is warranted.

CONCLUSIONS AND RECOMMENDATIONS

We present preliminary results of an exploration into presence and causes of abnormal osteological features, some of which may have a pathogenic basis, with respect to a timeline that includes genetic out-breeding, biomedical monitoring, and habitat and prey-base improvements. Our study demonstrates that HLs accumulate over time throughout all age classes, but there is no difference in the frequency of HLs between males and females, no difference in the number of HLs observed on cats living in the north versus the south, nor is there any significant difference between pre-1995 and post-1995 populations with respect to number of HLs. Harris Lines remain common in Florida panthers, however their cause is enigmatic and somewhat controversial. Food stress, illness, and infection cannot be eliminated as possible causes for HLs. Exposure to heavy metals, including Mercury, are known to cause HLs in humans as well. We cannot eliminate the possibility that at least some of the HLs are a response to accelerated growth at certain times in the growth cycle. Inasmuch as HLs were also frequent in Puma from other geographic regions, and in much higher frequencies than in other large mammals in general. Another plausible explanation for HLs might be high compression forces operating on the forelimbs. As we are able to gather further information about causes of AOFs, we may be better able to understand HLs and their relationship to bone pathology.

Osteopathology, and specifically joint disease, expressed through the formation of arthritic lesions in the forelimbs, of Florida panthers appears to exist out side the normal limits of a healthy population. Both AOF numbers and severity increase with age, with males showing a more dramatic increase than females, which may correspond to the risks that dispersal places on young animals, particularly males. The potential causes of AOFs are multiple, complex, and probably interrelated. Multiple physiological inflammatory responses to a broken bone are likely, as it presents an opportunity for a pathogenic invasion (Woodard & Riser 1991). We have seen that a break can create a systemic response, with equally well developed and even grotesque arthritic lesions on the opposite limb. Of course, a broken bone creates instability, not only in the damaged area but on other joints as well. The forelimb has shown a much higher incidence of osteophytic development than other limbs even though it is sometimes incipient and difficult to detect. High compression forces operating at this joint may account for the frequency of enthesitis or le-

sions observed at both the elbow and wrist joints. Microtrauma can provide an opportunity for bacterial involvement leading to inflamation and subsequent arthritis. At least some of these osteophytic expressions may be due to one or more of the spondeloarthropathies described in the human medical literature. Collectively, these arthropathies are characterized by inflammatory arthritis, preceding bacterial infection, a strong genetic predisposition, and a propensity for inflammation at the sites where tendons, ligaments, and joint capsules attach at the entheses (Dougados et al. 1991 Calin & Taurog 1998). The role that FeSFV plays in the elevated incidents of AOFs is unknown, but provides at least one link to the presence of polyarthritis in this population.

There was a significant interaction effect between Age Class and Use Area in the severity (and occurrence) of AOFs, with a greater severity occurring in Use Area 2. This is consistent with earlier studies implying that animals in the south were less healthy than in the north, but many management strategies to improve prey base have been implemented. We must consider that we are looking at animals of different ages through a time lens of perhaps 50 years, so it is difficult to pinpoint the specific time frame of the interaction.

The prevalence of total AOFs prior to 1995 (60.8%) was significantly higher than in animals that were born after 1995 (36.7%). Arthritis was the most common AOF in both the pre- and post-1995 samples. There has been a decrease in the incidence of pathology over time. The presence of many more young animals in the current population (relative to pre-1995 populations) may bias the results. However, we cannot rule out, and we can hope, that animals living today are healthier, less genetically compromised, and therefore less likely to develop severe pathologies. Whatever gains have been made as a result of increased variability through outbreeding or prey species management could be reversed as the increase in panther numbers create greater competition for habitat and prey, and offspring continue to mate with each other.

Our initial goal of assessing the effect of out-breeding on the health of Florida panthers vis-à-vis their osteology was limited by sample sizes in the post-1995 higher age classes. There was only one animal in AC-V and nine in AC-IV of the post-1995 sample, compared to 17 and 26, respectively, in pre-1995 sample. Our sample sizes in each group were also skewed with 79 pre-1995 and 49 post-1995. We cannot say with certainty that the out-breeding initiative played an important role in the improved health represented by our results as only eight animals known to be the product of the genetic cross between Texas and Florida cats were included in our study. Most other panthers, with the exception of the Everglades cats, showed at least one of the inbred panther characters of kinked tail or cowlick, and therefore our sample included many more of the original inbreed panther stock. Without the benefit of genetic diversity, we are left with the same basic problems of an inbred population: namely lowered disease resistance and reduced vitality (Roelke, et al. 1993a,b).

We have identified that a predisposition for joint disease may exist in Florida panthers and offer this assessment as a first step in developing a protocol to characterize the nature of this disease. That bone is limited in the way it can respond to any particular "insult" may limit the ability to distinguish among diseases when studying museum skeletons. Therefore, we recommend follow-up with studies that include soft tissue analyses, histology, bone density tests and radiology of joints including the forearm and pelvis joints as the latter is often involved in SpA.

There is new information revealed by this study and the potential for new paths of discovery. Recommendations by a panther advisory commission encourages analyses to consider research in toxins, diseases, and panther health, as well as how the prevalence of abnormalities in panthers is correlated with, or interacts with, genetic status (Beier et al. 2003). Our results, together with the rich resource of archival material, leads to new cooperative research opportunities between museums, wildlife biologists, and wildlife veterinarians in the efforts to improve the conservation status of Florida panthers.

ACKNOWLEDGEMENTS

We would like to thank Geordie Duckler and Blair Van Valkenburgh, University of California, Berkley, for their advice and the use of data and photographs in a Florida panther Symposium that gave rise to this paper. Anthony Falsetti, forensic specialist, along with the staff and students of the C. A. Pound Human Identification Laboratory, University of Florida, provided invaluable assistance with X-ray equipment and forensic information. Mark Cunningham, Florida panther veterinarian, and Bambi Ferree, and Darrell Land, all of the Florida Fish and Wildlife Conservation Commission, responded to many requests for panther historical and biomedical information, as did Melody Roelke, National Institute of Health. Forensic anthropologist Suzanne Abel, assisted in our understanding of human and animal osteopathology. Bruce Rothschild, Arthritis Center of Northeast Ohio, and Donald Scott of the Southeastern Arthritis Center, consulted with us on panther specimens, and Donald

Scott reviewed the Osteopathology section of this manuscript. Pre-veterinary student Christopher Gauthier, began the arduous task of documenting skeletal anomalies. Photographs were taken by FLMNH photographer Jeff Gage. Anthropologist John Krigbaum advised on the applicability Harris line analyses to Florida panthers. We would like to thank three anonymous reviewers for their assistance in improving this manuscript.

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Appendix I. Florida Panthers listed by FLMNH number (UFID), Fish and Wildlife Conservation Commission number (FP#), age class, pre or post 1995 group, use area, total number of abnormal osteological features (AOFs), severity of AOFs, specific AOFs of Arthritis (AT), Infection (IN), Unknown Cause (UN), Gross Trauma (GT), and number of Harris Lines.

UF ID	FP#	AGE CLASS	PRE-POST 1995[1]	USE AREA[2]	TOTAL AOFs	OSTEO SEVERITY[3]	AT[4]	IN	UN	GT	TOTAL HARRIS LINES
9789		3	1	N	2	1	AT1		UN		
10424	UCFP 06	3	1	S	1	1		IN			2
11915		2	1	S	0	0					0
11927	UCFP 05	2	1	N	0	0					.
14390	UCFP 02	2	1	S	0	0					.
14699	R.ALLEN	3	1	N	0	0					
16374	6	4	1	S	2	2	AT1			GT	1
18798	3	4	1	S	0	0					3
18847	14	5	1	S	1	2	AT2				4
18944	PCO 047	3	1	N	3	2	AT1		UN		.
19077	CB 17	3	1	N	0	0					
19090		4	1	N	0	0					5
19096	1	5	1	S	4	3	AT1	IN		GT	2
20777	2	5	1	S	4	3	AT2	IN		GT	0
20957	UCFP 13	2	1	S	0	0					.
20958	UCFP 12	4	1	S	0	0					.
20973	UCFP 14	2	1	S	0	0					
22409	7	4	1	S	3	2	AT2				0
22529	4	5	1	S	2	1	AT1				1
23849	UCFP 34	2	2	N	0	0					0
23986	10	2	1	N	5	2	AT1	IN	UN		0
24042	Volusia Co.	4	1	N	4	3	AT1				2
24096	13	4	1	N	1	1	AT1				0
24160		4	1	S	1	1				GT	.
24267	8	5	1	S	1	1		IN			4
24268	PCO 059	4	1	S	1	1		IN			.
24314	20	4	1	N	1	2		IN			2
24315	25	4	1	N	1	1	AT				0
24316	24	4	1	N	0	0					3
24557	27	3	1	S	2	3	AT3			GT	.
24561		3	1	N	4	3	AT1		UN	GT	.
24563	15	4	1	S	0	0					3
24595	33	3	1	N	0	0					0
24611	35	1	1	N	0	0					0
24621	30	2	1	S	0	0					0
24644	39	3	1	S	3	1	AT1		UN		0
24645	UCFP 19	1	1	N	0	0					1
24646	17	4	1	N	3	2	AT2				2
24928	18	5	1	N	6	1	AT1			GT	4
24929	41	3	1	N	0	0					1
24931	37	4	1	N	1	1	AT1				.
25908	76	3	2	S	0	0					2
25914	84	2	2	N	0	0					0
25922	PCO 192	1	1	N	0	0					0
26083	43	3	1	N	0	0					1
26157	28	4	1	N	3	2	AT1		UN		0
26159	29	3	1	N	3	1	AT1				

UF ID	FP#	AGE CLASS	PRE-POST 1995[1]	USE AREA[2]	TOTAL AOFs	OSTEO SEVERITY[3]	AT[4]	IN	UN	GT	TOTAL HARRIS LINES
26161	22	4	1	S	0	0					0
26840	47	2	1	S	0	0					3
26841	44	3	1	S	1	3				GT	0
26842	53	1	1	N	0	0					0
26843	50	3	1	N	1	1	AT2				2
26844	34	4	1	N	1	2	AT1				2
26845	UCFP 21	1	1	N	0	0					.
26856	UCFP 22	3	1	N	0	.					0
26938	UCFP 23	1	1	N	0	0					0
26939	26	4	1	N	2	3	AT3				5
27148	31	5	1	N	2	1	AT1				3
27370	38	4	1	N	4	2	AT1	IN	UN		3
27616	12	5	1	N	4	3	AT3	IN		GT	2
27618	52	3	1	N	0	0					3
27700	42	4	1	S	5	1	AT1		UN		1
28713	UCFP 30	2	1	S	0	0					4
28802	58	3	1	N	0	0					0
28980	40	5	1	S	2	1	AT1				1
29199	UCFP 25	3	1	N	2	1	AT1				0
29242	UCFP 26	3	1	S	4	2	AT2			GT	3
29250	68	4	1	N	2	2	AT3	IN			5
29262	45	4	1	N	4	2	AT3				2
29263	51	4	1	S	2	2	AT2			GT	2
29273	72	3	1	N	2	3	AT3			GT	1
29370	46	4	1	N	4	3	AT2		UN		3
29532	64	3	2	N	1	3				GT	1
29566	UCFP 33	1	2	N	0	0					5
29567	74	3	2	N	0	0					1
29621	36	5	1	S	1	1	AT1				6
29819	63	4	2	N	3	3	AT1			GT	2
29821	16	5	1	S	6	3	AT3		UN		6
29826	80	4	2	N	1	1	AT1	`			0
30022	UCFP 35	2	2	N	0	0					0
30023	UCFP 36	2	2	N	0	0					0
30064	89	3	2	S	2	3	AT3				2
30178	90	2	2	N	3	2	AT1			GT	4
30366	UCFP 39	1	2	S	0	0					1
30367	UCFP 38	2	2	N	0	0					0
30374	UCFP 43	3	2	N	1	2				GT	0
30391	11	5	1	N	2	1	AT1				3
30393	23	5	1	N	3	3	AT1	IN	UN		5
30398	UCFP 40	1	2	S	0	0					3
30399	UCFP 41	2	2	N	0	0					2
30430	UCFP 29	3	1	N	0	0					0
30431	97	2	2	N	0	0					1
30433	UCFP 27	2	2	N	0	0					6
30434	105	4	2	N	3	1	AT1	IN	UN		6
30935	49	5	1	N	0	0					5
30936	UCFP 46	1	2	N	0	0					0
30937	UCFP 45	3	2	N	1	3			UN		0
30938	96	2	2	N	0	0					1
30948	98	4	2	N	0	0					2
30957	111	5	1	N	0	0					2

UF ID	FP#	AGE CLASS	PRE-POST 1995[1]	USE AREA[2]	TOTAL AOFs	OSTEO SEVERITY[3]	AT[4]	IN	UN	GT	TOTAL HARRIS LINES
30958	92	3	2	N	1	1	AT1				2
30959	UCFP 42	2	2	N	0	0					0
30960	32	5	1	N	2	1	AT1				5
31010	UCFP 52	2	2	N	0	0					0
31011	67	4	2	N	0	0					0
31012	UCFP 48	1	2	N	0	0					0
31018	106	3	2	N	1	1	AT1				2
31019	UCFP 49	2	2	N	0	0					0
31020	108	2	2	N	0	0					2
31021	78	3	2	N	1	1	AT1				3
31022	UCFP 54	1	2	N	0	0					1
31023	UCFP 58	1	2	N	0	0					0
31024	UCFP 50	3	2	N	1	1	AT1				5
31025	UCFP 53	3	2	N	0	0					3
31026	82	4	2	N	0	0					1
31101	91	4	2	S0	0						0
31103	UCFP 66	2	2	S0	.						0
31104	K 94	3	2	S1	1		AT1				5
31106	55	5	2	S4	1		AT1		UN		3
31108	UCFP 62	1	2	S0	0						0
31109	UCFP 65	2	2	N	0	0					4
31110	114	2	2	N	0	0					0
31161	UCFP 69	2	2	S1	1		AT1				0
31162	UCFP 63	3	2	S2	3		AT1			GT	2
31163	59	4	1	N	4	2	AT2		UN		3
31165	115	4	2	N	2	1	AT1				2
31182	69	4	2	N	1	1	AT1				1
31183	109	5	1	N	6	3	AT3				0

[1]Designates animal born before (1) or after (2) 1995
[2]Panther used areas to the North or South of Alligator Alley
[3]Measure of Severity (1) slight (2) moderate (3) represents broken bones, advanced debilitating arthritis, or other extreme condition

PART II: STABLE ISOTOPE GEOCHEMISTRY: A METHOD TO EVALUATE THE DIET OF FLORIDA PANTHER

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ABSTRACT

Many of the methods used to determine the diet of large carnivores are costly, time-consuming and may not give a complete picture of diet. Here we discuss the potential for stable-isotope analysis to provide a more complete picture of long-term intake with a pilot study of Florida panthers. We use 20 Florida panthers (*Puma concolor*) from the Florida Museum of Natural History. Our results are consistent with a diet based primarily on deer and hog as other studies have suggested. However, male and female isotope values differ significantly suggesting distinctly different dietary intake. In addition, contrary to findings from studies employing other techniques, no differences are apparent in the isotope signatures of animals from northern versus southern areas. Although the isotope data presented here are preliminary, stable isotope analysis appears to be a useful tool for assessing differences in diet across demographic parameters and will be useful in dietary studies contributing to conservation efforts.

INTRODUCTION

Determining the diet of an animal is costly and time-consuming, particularly with large carnivores that are difficult to track. Although many of the tools used to determine diet provide useful information, most cannot provide a complete picture of long-term dietary patterning. For example, the analysis of scat is biased toward indigestible dietary components. Furthermore, scat analysis examines the most recent meal but does not reveal seasonal or long-term dietary trends unless collection occurs over a long period of time. Another common method, kill-site analysis, requires that the home range of an animal be accurately determined then searched until a carcass is located. This method also tracks short-term dietary intake and is biased towards the carcasses of larger prey taxa that are easier to locate. Finally, observational studies require significant time depth, particularly when studying large nocturnal carnivores, since predation events are difficult to observe. Alternatively, stable isotope analysis does not require a multi-year study, but does provide a long-term dietary signal and can be used with other methods to give a more complete picture of carnivore diets. Here we present stable isotope analysis of museum specimens as a potential source for information that can aid in management strategies of wild carnivores.

Stemming from the adage, 'you are what you eat,' the stable isotopes of carbon and nitrogen are incorporated into animal tissues in predictable ways representative of the isotopic signature of prey taxa (Hobson & Schwarcz 1986; Kelly 2000). Because tissues are created at different rates, an animal's isotope chemistry can track both long- and short-term dietary patterning. For example, hair grows much faster than bone, so examining the isotopic signature of a hair shaft will give a more recent signature of diet than bone collagen, which turns over very slowly providing an average of long-term intake. Thus stable carbon and nitrogen isotope values are commonly used to reconstruct the diets of large carnivores, such as seals and wolves, and other animals (Hobson et al. 1996; Szepanski et al. 1999; Urton & Hobson 2005, Kelly 2000). These values represent the ratio of the heavy to light isotopes for carbon and nitrogen (13C/12C, 15N/14N) written in delta notation ($\delta^{13}C$, $\delta^{15}N$) as parts per million (‰) difference from an internationally recognized standard (PDB for carbon and atmospheric air for nitrogen). The standard is assigned by definition a value of 0‰ and computed as

follows:

Equation 1:

$$\delta^{13}C \text{ or } \delta^{15}N = \frac{Rsample - Rstandard}{Rstandard} \times 1000 ‰$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$

BACKGROUND ON CARBON ($\delta^{13}C‰$)

Stable carbon isotope ratios in bone collagen monitor the ratio of carbon-13 to carbon-12 in the amino acid sequences that make up collagen fibrils, and are representative of diet for the following reasons. When CO_2 is taken up during photosynthesis, metabolic processes alter or fractionate the ratio of $^{13}C/^{12}C$, depleting plant tissues in ^{13}C relative to atmosphere (-7.7‰). The degree of fractionation associated with photosynthesis covaries with the kinetic properties of carbon uptake and enzymatic processes of carbon fixation (Farquhar et al. 1989). In terrestrial plants, two photosynthetic pathways, the C_3 or C_4 pathway, have distinct carbon isotope signatures. Fractionation is contingent upon the photosynthetic pathway the plant uses to metabolize atmospheric CO_2. Cool season grasses, trees, tubers and most bushy plants utilize the C_3 photosynthetic pathway which discriminates heavily against ^{13}C, expressing a mean $\delta^{13}C$ value of -26.7 ± 2.7‰ (n=370) (Cerling et al. 1998). A small set of forbs and all warm-season grasses such as corn (*Zea mays*), common to regions where daytime growing-season temperature exceeds 22°C and precipitation exceeds 25 mm (Ehleringer et al. 1997), use C_4 photosynthesis resulting in less discrimination against ^{13}C and an average $\delta^{13}C$ value of -12.5 ± 1.1‰ (n=455) (Cerling et al. 1998). $\delta^{13}C$ values are passed from producer to consumer leaving a diagnostic signature in both hard and soft tissues that does not covary with the skeletal element analyzed or sex of the sample independent of differences in feeding ecology (Hobson & Schwarcz 1986; Lovell et al. 1986). Fractionation between plants and herbivores is ca. 5‰ and isotopic enrichment between herbivores and each successive trophic level is about 1‰.

Stable carbon isotope analysis is also used to estimate reliance on marine resources in diets lacking a C_4 component. Kinetic processes governing bicarbonate (HCO_3-) formation in seawater fractionate marine bicarbonates approximately 7‰ relative to atmosphere, placing seawater $\delta^{13}C$ values near 0‰ (Chisholm et al. 1982; Tauber 1981). Because submerged marine plants employ a C_3 photosynthetic pathway and derive carbon primarily from seawater bicarbonates, they express mean $\delta^{13}C$ values of -16 to -18‰, approximately 7‰ more positive than terrestrial C_3 plants giving them a distinctive marine label.

BACKGROUND ON NITROGEN ($\delta^{15}N‰$)

Nitrogen isotopes are also incorporated into the food web in predictable ways. Most terrestrial plant taxa obtain nitrogen from soil ammonium (NH_4^+) or nitrate (NO_3^-) and have mean $\delta^{15}N$ values of 3-6‰ with a 0-9‰ range contingent upon temperature and aridity (Pate 1994). Independent of effective moisture, legumes and some epiphytes have symbionts that fix atmospheric nitrogen and accordingly express mean $\delta^{15}N$ values of 1‰, with a ca. –2 to 2‰ range (Evans & Ehleringer 1994; Pate 1994). Herbivores in temperate climates typically exhibit $\delta^{15}N$ values of 6-9‰ while arid-land species and non-obligate drinkers, those that recycle urea, reflect their water-conservation strategies in more positive $\delta^{15}N$ values (Ambrose 1991).

Stable nitrogen isotope analysis follows from the understanding that $^{15}N/^{14}N$ increases by approximately 2-4‰ with each increase in trophic level due primarily to fractionation during urea production, enriching the isotope signature of nitrogen available for protein synthesis (Schoeller 1999). For example, a carnivore eating a diet consisting mostly of herbivorous prey such as deer will exhibit systematically lower nitrogen isotope ratios than a carnivore in the same ecosystem subsisting primarily on omnivorous animals such as pigs and raccoons.

METHODS

STUDY SYSTEM

The Florida panther (*Puma concolor*) is suitable for dietary studies using stable isotope analysis for several reasons. First, the Florida Museum of Natural History houses a large collection of panther, over 140 specimens, providing an excellent resource for bone tissue from animals representing various geographic ranges and temporal periods. In addition, Florida panther diet has been studied using scat and kill-site analysis providing a comparative database. Finally, Florida panthers are endangered, and an understanding of their predation strategies is central to successful management of a declining population.

Maehr et al. (1990) demonstrated using scat and kill site analysis that the main prey of panthers inhabiting the public lands of southwest Florida, including Florida Panther National Wildlife Refuge (FPNWR), Big Cypress National Park (BCNP), Big Cypress Seminole Indian Refuge (BCSIR), and Fakahatchee Strand State Preserve (FSSP), was wild hog (*Sus scrofa*) and white-

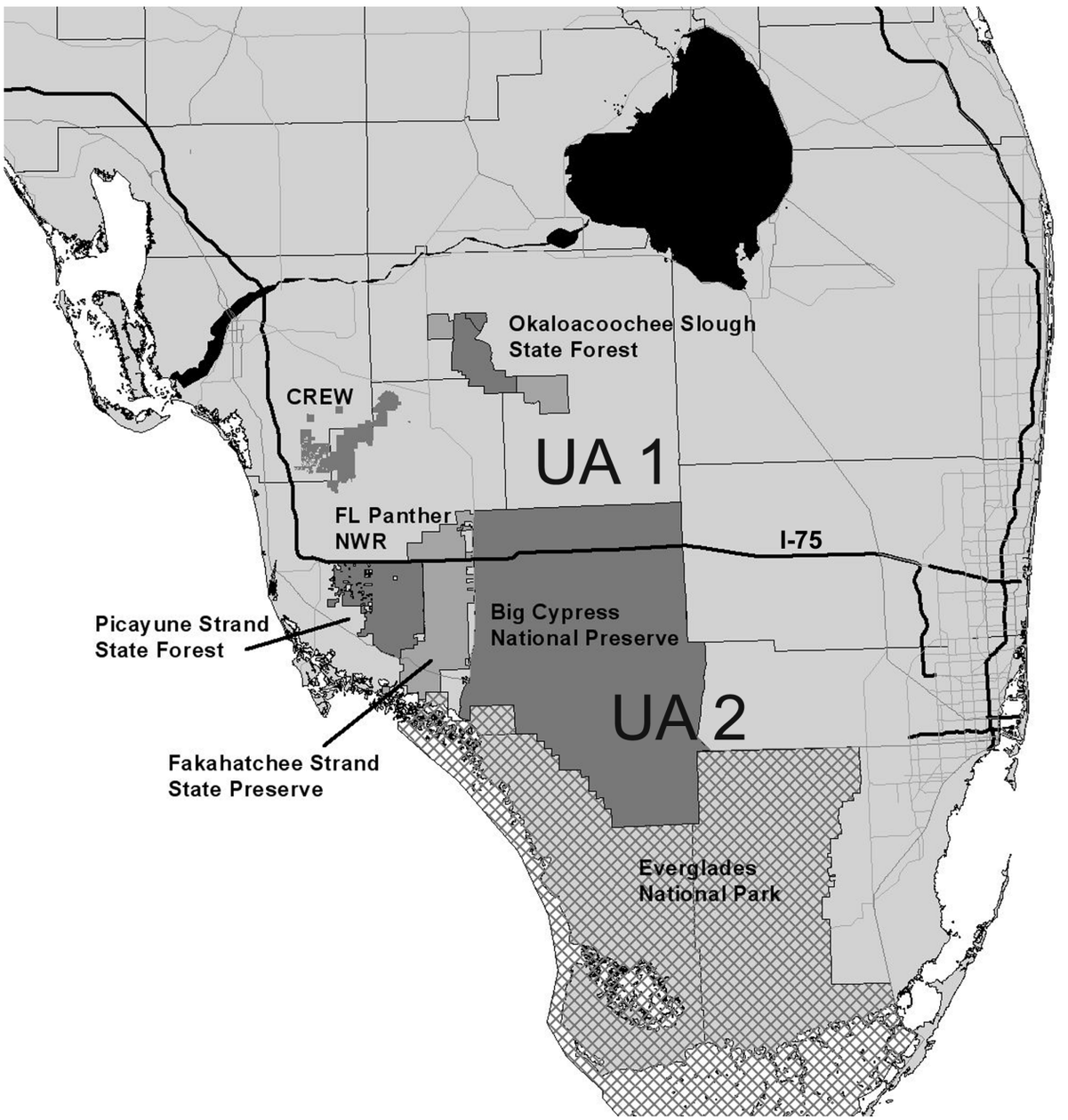


Figure 1: Map of Southern Florida showing the range of the Florida panther and the northern and southern major use areas. The northern use area includes Florida Panther National Wildlife Refuge (FPNWR), Big Cypress Seminole Indian Refuge (BCSIR) not shown, and northern Big Cypress National Park (nBCNP). The southern use area includes southern Big Cypress National Park (sBCNP), Fakahatchee Strand State Preserve (FSSP) and Everglades National Park (ENP). Northern and southern BCNP are separated by I-75. Map was provided by D. Land from FWC.

tailed deer (*Odocoileus virginianus*), followed by raccoon (*Procyon lotor*), armadillo (*Dasypus novemcinctus*), and rabbit (*Sylvilagus spp.)* in order of their abundance in the diet. However, frequencies of both large and small prey varied considerably between the northern and southern portions of BCNP (nBCNP and sBCNP; Figure 1), which crosses a soil boundary. The soils of nBCNP are thought to be better drained and more fertile (Leighty et al. 1954). Here, hogs dominated panther diet making up 58.7% of the biomass consumed, whereas in sBCNP hogs made up merely 22.7% (Maehr et al. 1990, Table 1). In contrast, smaller species (raccoon, armadillo, and rabbit) were present in higher frequencies in the diets of panthers from sBCNP

Table 1: Percent biomass of prey animals consumed by Florida panthers. Values were taken from Maehr et al. (1990), and Dalrymple and Bass (1996) calculated from scat and kill-site analysis.

	Percent Biomass consumed				
	Hog	Deer	Small Mammals	Other	Study
North Big Cypress National Park	58.7	27.0	9.4	4.9	Maehr et al., 1990
South Big Cypress National Park	22.7	43.4	31.8	2.0	Maehr et al., 1990
Everglades National Park	0.7	78.4	18.1	2.4	Dalrymple and Bass 1996

(Maehr et al. 1990). Shortemeyer (1994) reported that south of I-75 deer and hog densities rarely exceed one animal per 100 acres. In Everglades National Park (ENP), deer represented 78.4% of biomass consumed, in part because no hogs are present, (Dalrymple and Bass 1996) but a 67% decrease in deer densities since 1959 has been reported (Fleming et al. 1994) suggesting that deer are more difficult to hunt in ENP. Consequently, the biomass of smaller prey in ENP was 18.2% higher than that of nBCNP (Table 1).

Veterinary studies based on physical condition, body weight, reproductive status, and hematologic and serum values conducted between 1986 and 1990 identified a north/south "health cline." Panthers utilizing land north of State Road 84, including private ranches, the FPNWR, and the Bear Island unit of the BCNP, were in better condition than those in the ENP or FSSP (Roelke 1990). The panthers in sBNCP were also found to be in poorer physical condition, have larger home ranges and lower reproductive output than panthers in the north (Maher et al., 1989a; Maher et al. 1990). McCown (1991) found that panthers in the cypress-dominated southern portion of sBCNP were smaller and less productive than in the Bear Island unit located in nBCNP. Differences in panther diet leading to a north to south gradient of declining health has been attributed to a shift from reliance on hog in the north to deer and smaller prey in the south (Beier et al. 2003). In the most southern part of ENP panther mean body size is small which is thought to be due to low deer densities. One danger the panthers face when feeding on small more aquatic based prey is the potential for mercury ingestion. (Maehr et al. 1990; Iriate et al. 1990; Roelke 1990; Dalrymple & Bass 1996).

Here we report the bone collagen stable carbon and nitrogen isotopes ratios of 20 Florida panthers and 25 prey indivuals. Panthers were chosen across the north/south diet cline to determine if their stable isotope chemistry reflects the apparent dietary differences discussed above. The northern use area included FPNWR, nBCNP and FPSIR; the southern use area was comprised of sBCNP, FSSP and ENP (Figure 1). Only adult panthers were selected for study, and we chose animals with the greatest amount of natural history information in order to relate isotope values to known home ranges and diets. All of the animals were radio-collared and tracked by FWC.

Stable isotope values were also obtained from bone collagen of the five primary prey taxa of Florida panthers gathered from three sources; the FLMNH mammal collection, actual prey remains in panther scats from ENP, and samples provided by Deborah Jansen from panther scats/kill sites in the BCNP. We sampled 10 white-tailed deer (*Odocoileus virginianus*), 11 raccoon (*Procyon lotor*), three rabbit (*Sylvilagus palustris*), two armadillo (*Dasypus novemcintus*), and two feral hogs (*Sus scrofa*).

LAB PROCEDURE

Approximately 500 mg of cortical bone was cleaned of surface contaminants then demineralized whole in 0.6N HCl at 4° C. Sterile ddH_2O was used throughout and glassware was sterilized by heating to 550° C for 3h. The resultant collagen was then rinsed to neutrality, treated with 5% KOH to remove organic contaminants, rinsed to neutrality, and lyophilized. Approximately 100 mg of lyophilized collagen was gelatinized in 5 ml of water (pH 3) for 24 hours at 120° C. Water-soluble and -insoluble phases were separated by filtration and the former was lyophilized and weighed to determine the collagen yield. $\delta^{13}C$ and $\delta^{15}N$ values were determined by flash combustion to produce CO_2 and N_2 and measured against the appropriate reference gas on a Finnigan Delta Plus mass spectrometer with Carlo Erba EA118 CHN interface. Stable isotope measurements and weight percent C and N values were obtained from single sample combustion. All samples met well-established preservation criteria (Ambrose 1990). Analytical precision is ± 0.1‰ for carbon and ± 0.2‰ for nitrogen.

RESULTS AND DISCUSSION

Stable isotope values for panthers and their prey are reported in Table 2. Mean carbon and nitrogen isotope

values for panthers in the northern and southern use areas are not significantly different ($\delta^{13}C$‰, t-test, t=-1.7, df = 11, p=0.10; $\delta^{15}N$, t-test, t=-0.97, df =13, p =0.35; Fig. 2A). However, animals in the north have a wider range of $\delta^{13}C$‰ and $\delta^{15}N$‰ values indicative of a more varied diet.

When sorted by sex, male and female panthers differ significantly in carbon (t-test, t = -3.7, df =17, p = 0.002), and nitrogen isotope values (t =-4.4, df = 15, p = 0.0005; Fig. 2B) indicating likely reliance upon a different suite or combination of prey taxa. Also, the range of male panther isotope values is greater than that of females suggesting a more varied diet in males.

CARBON ($\delta^{13}C$‰) RESULTS

Carbon ($\delta^{13}C$‰) isotope signatures increase by 1‰ with each increase in trophic levels above the primary consumer. $\delta^{13}C$‰ values of deer are consistent with a high C_3 browsing diet and ranged from -24.1‰ to -20.4‰ with an average of -21.9 ± 1.2‰. Feral hog $\delta^{13}C$ values are nested within the range for deer making them indistinguishable isotopically, at -23.2‰ and -21.6‰ with an average of -22.4 ± 1.1‰. Raccoon $\delta^{13}C$‰ values range from -23.9‰ to -16.7‰ with an average of -19.9 ± 2.4‰ indicating a wide range of dietary elements including some with a marine component. Rabbit $\delta^{13}C$‰ values are -21.6‰ to -20.4‰, consistent with a diet of C_3 plant foods. The two armadillos sampled have widely varying $\delta^{13}C$‰ isotope values of -19.9‰ and –15.7‰ suggesting that isotopic diversity is likely high within this species, and that a larger sample of armadillos, as well as raccoon, is needed (Table 2, Fig. 3). Armadillo prey items (invertebrates) may feed on a wide variety of plant material, generating considerable variation in $\delta^{13}C$‰ values.

Male panther $\delta^{13}C$‰ isotope values range from -20.1‰ to -15.6‰ with an average of -18.6 ± 1.5‰ and females from -21.0‰ to -19.1‰ with an average of -20.3 ± 0.7‰ (Table 2, Fig. 3). Three males from the northern use area are outside the range of deer and hog values suggesting a large portion of their diet included other prey taxa, possibly raccoon and armadillo. Two Florida panthers (FP 15 and FP 16) were tracked by Dalrymple and Bass (1996) and have carbon numbers of -19.6‰ and -19.9‰, respectively. Both animals subsisted primarily on deer and hog but it was noted that FP 16 also killed eight raccoons during the tracking.

Table 2**:** Stable carbon and nitrogen isotope values for (A) Florida panthers and (B) their prey species. Florida panthers are listed by Florida Museum of Natural History identification number (UFID) as well as a FP number assigned by the Florida Fish and Wildlife Conservation Commission. The prey taxa are listed by the species, county collected, and UFID for animals sampled from the Florida Museum of Natural History mammal collection.

A. Florida Panthers

UFID	Panther	Sex	$\delta^{13}C$‰	$\delta^{15}N$‰	Use Area
18798	3	F	-21.0	7.9	S
30935	49	F	-20.7	8.0	N
24267	8	F	-20.9	8.1	S
24563	15	F	-19.6	8.1	S
27148	31	F	-19.1	8.1	N
30960	32	F	-21.0	8.2	N
30393	23	F	-20.2	8.3	S
29621	36	F	-20.2	8.5	S
24931	37	M	-20.1	8.3	N
20777	2	M	-19.9	8.4	S
24315	25	M	-18.6	8.4	N
29263	51	M	-18.4	8.4	S
19096	1	M	-19.4	8.5	S
22529	4	M	-18.3	8.7	S
22409	7	M	-20.1	8.9	S
26939	26	M	-18.5	9.0	N
29821	16	M	-19.9	9.3	S
29819	63	M	-17.7	9.3	N
26844	34	M	-16.4	9.3	N
29262	45	M	-15.6	9.8	N

Table 2 continued:

B. Prey Species	County	$d^{13}C‰$	$d^{15}N‰$
Odocoileus virginianus (White-tailed Deer)			
	Dade	-20.9	3.7
UFID 24907	Collier	-24.1	4.2
	Dade	-20.4	4.8
UFID 9512	Collier	-22.2	4.9
UFID 22583	Collier	-22.7	5.2
UFID 22582	Collier	-23.1	5.8
UFID 9508	Collier	-21.0	6.2
	Collier	-20.4	6.2
	Dade	-21.9	6.4
	Collier	-22.0	8.8
Average		**-21.9**	**5.6**
Procyon lotor (Raccoon)			
UFID 25949	Dade	-21.7	6.5
	Dade	-23.0	7.2
UFID 22569	Dade	-21.0	7.6
UFID 10192	Collier	-17.8	8.2
	Collier	-20.2	8.5
UFID 24012	Collier	-16.7	8.5
	Collier	-17.7	8.6
	Dade	-19.4	8.8
	Dade	-20.1	9.1
UFID 10089	Collier	-17.0	9.2
UFID 4611	Broward	-23.9	10.7
Average		**-19.9**	**8.4**
Sus scrofa (Feral Hog)			
	Dade	-21.6	4.8
	Dade	-23.2	6.5
Average		**-22.4**	**5.7**
Sylvilagus palustris (Rabbit)			
	Dade	-20.8	1.2
	Dade	-20.4	1.8
	Dade	-21.6	2.9
Average		**-20.9**	**2.0**
Dasypus novemcintus (Armadillo)			
	Dade	-19.9	7.1
	Collier	-15.7	7.2
Average		**-17.8**	**7.15**

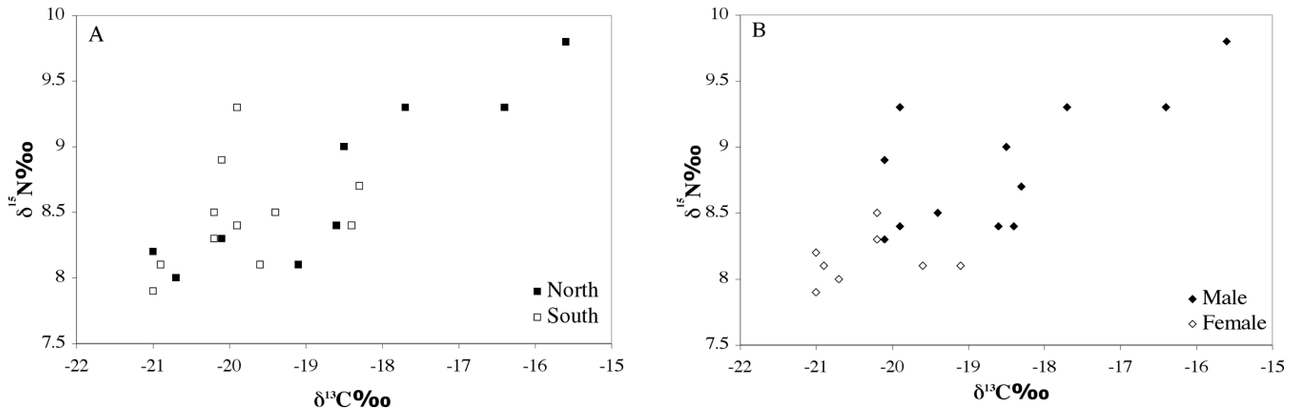


Figure 2: Stable carbon ($\delta^{13}C‰$) and nitrogen ($\delta^{15}N‰$) isotope ratios for 20 Florida panthers from the Florida Museum of Natural History. There was no significant difference between animals in the north (n = 9)and southern (n =11) use areas (A). A significant difference was found between male (n = 12) and female (n = 8) panthers (B).

NITROGEN ($\delta^{15}N‰$) RESULTS

When nitrogen isotope signatures ($\delta^{15}N‰$) are plotted (Fig. 3), panthers have higher $\delta^{15}N‰$ signatures than most of their prey taxa as expected. Nitrogen isotope signatures increase 2-4‰ with each step up the food web, and values for animals at each trophic level met this expectation. Rabbits from the Everglades have the lowest nitrogen values, with a range of 1.2‰ – 2.9‰, n=3), perhaps due to the use of agricultural fertilizers (SWFMD 1992). Fertilizers are depleted in ^{15}N with $\delta^{15}N‰$ values ranging from -0.5 to 0.3‰ (Schwertl et al. 2005). It is also possible, that vegetation in the diets of the sampled rabbits was depleted by high levels of nitrogen-fixing bacteria in agricultural runoff (Craft and Richardson 1998). If so, further sampling of rabbits may show distinct differences between rabbit isotope values in various micro-environmental zones within southern Florida.

Deer $\delta^{15}N‰$ isotope signatures are highly variable ranging from 3.7‰ – 8.8‰ with an average of 5.6 + 1.4‰ (n=10); the lowest value (3.7‰) is from a Dade County specimen. Feral hog $\delta^{15}N‰$ values again fell within the range of deer at 4.8‰ and 6.5‰with an average of 5.7 ± 1.2‰ (n=2) making their influence on panther $\delta^{15}N‰$ indistinguishable isotopically from that of deer. Armadillo $\delta^{15}N‰$ values are 7.1‰ and 7.2‰ (n=2) slightly higher than deer (5.6‰) as expected given their insectivorous diet (Taber 1945). Raccoon $\delta^{15}N‰$ values range from 6.5‰ – 10.7‰ with an average of 8.4 ± 1.1‰ (n=11) representing a varied omnivorous diet (Whitney 1931). Enriched raccoon $\delta^{15}N‰$ values suggest the input of marine resources such as sea turtle eggs, crustaceans, bivalves and other marine invertebrates.

Male panther $\delta^{15}N‰$ values range from 8.3‰ – 9.8‰ (n=12) with an average of 8.9 ± 0.5‰ and female panther $\delta^{15}N‰$ values from 7.9‰ – 8.5‰ (n=8) with

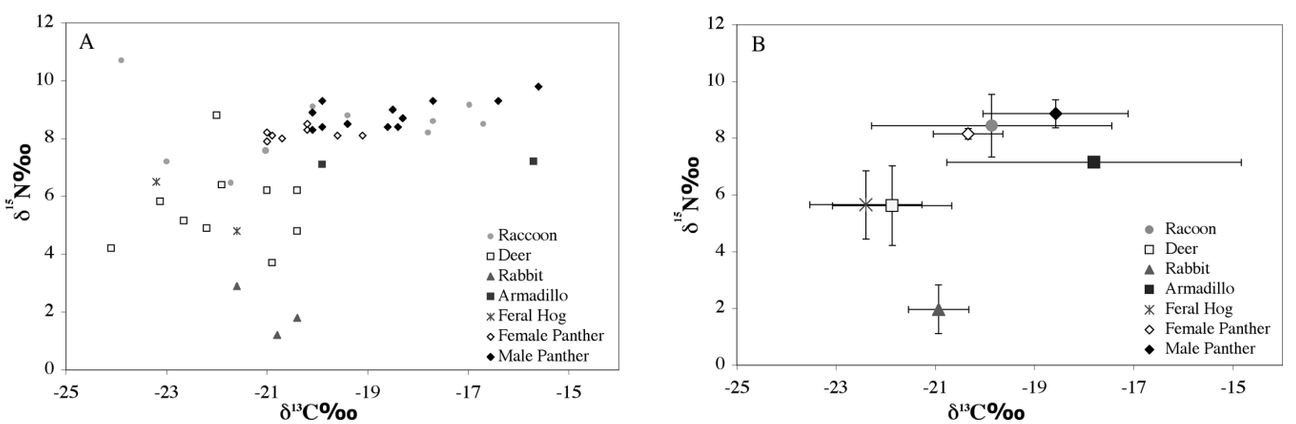


Figure 3: Stable carbon ($\delta^{13}C‰$) and nitrogen ($\delta^{15}N‰$) isotope ratios for 20 Florida panthers and common prey species. Part A includes all data points and B the averages; bars represent one standard deviation.

an average of 8.1 ± 0.2‰. These values are consistent with a diet primarily based on deer and hogs, as reported in earlier dietary studies (Maehr et al. 1990; Dalrymple & Bass 1996). Everglades panthers 15 and 16 (UF24563 and UF29821) were tracked by Dalrymple and Bass (1996) who reported that FP 15 ate 14 deer, one marsh rabbit and one opossum during the follows; FP 15 had a nitrogen value of 8.1‰. FP 16 had the most varied diet in the Dalrymple and Bass study, seven deer, five hogs, 11 alligators, eight raccoons, one marsh rabbit, one otter, one bobcat and exhibits a nitrogen isotope signature of 9.3‰. This nitrogen value is indicative of a diet high in high- trophic-level prey taxa, such as alligator, raccoon and bobcat, in keeping with the findings of Dalrymple and Bass (1996). Bone collagen isotope values average dietary intake over several years, perhaps the lifetime of an adult panther, and tracking is likely to capture predation events over a narrower window of time. These results then suggest that FL panther 15 had a diet high in trophic level taxa for a long period of time.

CONCLUSIONS AND RECOMMENDATIONS

Our stable isotope analyses are consistent with the expectation that deer and feral hog are primary dietary elements. Our results have also revealed interesting differences in male and female panther diets, but further sampling of panthers and prey taxa is required to substantiate sex-based dietary patterning. The most likely prey base for female panthers is deer, hog and rabbits with an emphasis on deer and hog indicated by their nitrogen values. Male panthers had wider ranges of δ^{13}C‰ and δ^{15}N‰ isotope values indicating a more varied diet. It is possible that the more varied diet in males is related to the fact that males have larger home ranges (Maher et al. 2002; Jansen pers. com.). It is possible that males may wander out of suitable deer or hog habitat in defense of a large home range necessitating the addition of other prey species. Also, the simple act of moving across large geographic distances may cause male panthers to come into contact with a more diverse assemblage of prey species. Alternatively, it is possible that defending large home ranges makes it inefficient to remain at kill-sites of very large prey (deer or hog) as long as females. The pronounced differences in male and female panther isotope values should be investigated further.

In particular, male panthers in the north have carbon values that appear more representative of raccoon and armadillo values. A dietary cline has been reported with panthers in the north feeding primarily on feral hog whereas those in the south feed primarily on deer and small mammals (Beier et al. 2003). We found no differences isotopically between the diets of northern and southern panthers, and show no significant difference between deer and hog isotope signatures, thus cannot speak to this issue in greater detail. A larger sample of deer and hogs may reveal differences in their isotopic signatures, given the omnivorous diet of feral hogs. Three male panthers in the northern study area appear to have relied heavily on small mammals, a result not previously found in other studies. Two of the three, FP 45 (UF29262) and FP 63 (UF29819), were likely killed by other panthers. These results suggest heavy competition between panthers in the north for prey or mating opportunity. The third panther, FP 34 (UF26844), died of a bacterial infection. All three panthers had Harris lines and other osteopathologies, which are suggestive of overall health problems (Wilkins et al. 2007). It is possible that stable isotope chemistry can assist in identifying sick animals that were not able to hunt efficiently and were thus forced to eat smaller prey. Similarly, stable isotopes may reveal areas in which prey densities are low and panthers are competing heavily for food. Sampling many more animals in each area may help to differentiate between these two issues. Our results, although preliminary, suggest that stable isotopes may reveal important dietary differences among Florida panthers and prove to be a useful management tool.

ACKNOWLEDGEMENTS

Anthropologist John Krigbaum advised on the applicability of isotope analyses to Florida panthers. We would like to thank Deborah Jansen for providing important samples for this research. This work was supported in part by the Florida Fish and Wildlife Conservation Commission.

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EPILOGUE

Natural history museums are charged with the mission of archiving the world's biodiversity. As threats to the world's biodiversity increase it is becoming apparent that the only place to view or study certain organisms will be within natural history collections. Cooperation between natural history museums and the agencies that monitor threatened wildlife can facilitate the archival of specimens from seriously threatened species. In the case of Florida panthers, the Florida Museum of Natural History has cooperated with the Florida Fish and Wildlife Conservation Commission, The National Park Service, and US Fish and Wildlife Service to recover, process, and archive skeletal, genetic, and other material from every dead Florida panther recovered during the last 20 years. In so doing, the FLMNH now has an impressive collection of over 140 specimens of Florida panther.

Archival material such as that maintained in natural history collections provides a unique opportunity to examine a population over time and link health parameters and dietary shifts to life history parameters such as age, parentage, genetic profile and/or habitat. In the case of small, highly inbred populations like Florida panthers, it is important to note that we cannot foresee all of the health threats to the population without the benefit of having many specimens to examine. Similarly, we cannot foresee all of the tools and technologies that will be available to study these museum specimens. As such, it is important that we maintain a safely archived, permanent repository for Florida panthers and other critically endangered organisms.

In studying the osteology and osteopathology of over 140 individual Florida Panthers, Wilkins et al. (2007, Part I) were able to not only examine the correlates of bone abnormalities that have been well documented, but they also noticed new findings relating to systemic infections that have yet to be studied in detail. Allen et al. (2007, Part II) examined the diet of Florida panthers with stable isotope geochemistry, which has not yet been applied to Florida panthers. This study was able to show significant differences in the diet of male and female panthers, which had never been noted before in the literature. Both studies demonstrate the value of natural history collections to the conservation and maintenance of critically endangered species.