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**The effect of captivity on the oral health of the critically endangered black-footed ferret
(*Mustela nigripes*)**

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Abstract

Black-footed ferrets (*Mustela nigripes* (Audubon and Bachman, 1851)), a North American species of mustelid, faced near extinction after westward expansion during the 20th century destroyed a majority of the population of prairie dogs, their primary food source. Fearing extinction of the black-footed ferret, the U.S. Fish and Wildlife Service captured the entire population between 1985 and 1987 and began a captive breeding program. While in captivity the fertility and genetic diversity of the species was closely monitored, however there is little information about other health consequences of this breeding program. For instance, the black-footed ferrets have been fed a diet that is very different than what they consume in the wild. How did the composition of this diet affect the oral health of these animals? An analysis of wild and captive black-footed ferret dentition reveals that calculus accumulation and periodontal diseases occurred with greater severity in captive black-footed ferrets, suggesting that such oral pathologies arose from the unnaturally soft diet fed to them. These findings offer insight into how mechanical properties of diet can affect oral health and how these dietary properties should be considered, not only in regard to the health of black-footed ferrets, but to the health of all mammals including humans.

Key Words: *Mustela nigripes*, Black-footed ferret, Calculus, Periodontal disease, Captivity

Introduction

The black-footed ferret (*Mustela nigripes* (Audubon and Bachman, 1851)) is a hypercarnivore (a species that specializes in the consumption of vertebrate flesh) in the weasel family, Mustelidae (Hillman and Clark 1980). In the wild, approximately 90% of its diet is composed of prairie dogs (Genus *Cynomys* Rafinesque, 1817) with the other 10% comprising mostly other small rodents and, to a lesser extent, lagomorphs (Hillman and Clark 1980; Campbell III et al. 1987). *Cynomys* also provides *M. nigripes* with shelter, as black-footed ferrets primarily reside in burrows dug by this prey species (Campbell III et al. 1987; Jachowski et al. 2011). Because of this close association, the *M. nigripes* geographic distribution has historically been limited to areas with large *Cynomys* populations, including the states of Montana, South Dakota, North Dakota, Wyoming, Colorado, Nebraska, Oklahoma, New Mexico, and the southern parts of Saskatchewan, Canada (Cahalane 1954; Hillman and Clark 1980; Clark 1994; Vargas et al. 1996). This area has decreased dramatically in size over the last century as human expansion of farmland and intentional poisoning has greatly reduced *Cynomys* populations (Cahalane 1954; Hillman and Clark 1980; Clark 1994). Coupled with this loss, a predisposition to sylvatic plague (*Yersinia pestis* (Yersin, 1894)) and a strong susceptibility to canine distemper (Budd 1981) caused the wild *M. nigripes* population to decline to near-extinction levels by the 1960s (Cahalane 1954; Hillman and Clark 1980).

In 1964, a small population of black-footed ferrets was found in Mellette County, South Dakota. These individuals were believed to be the last existing black-footed ferrets, causing *M. nigripes* to be added to the first U.S. endangered species list in 1967 (Henderson et al. 1974; Clark 1994; Vargas et al. 1996). The last ferret captured from this population died in 1979 and

the species was believed to be extinct until a population was discovered in 1981 near Meeteetse, Wyoming (Forrest et al. 1988; Vargas et al. 1996). The U.S. Fish and Wildlife Service captured these 18 remaining black-footed ferrets (7 male and 11 female) by 1987 (Ballou and Oakleaf 1989; Wisely et al. 2002) to begin captive breeding as part of the Black-Footed Ferret Recovery Act developed in 1978. The U.S. Fish and Wildlife Service partnered with several zoos including the Smithsonian's National Zoo to begin breeding and re-introducing ferrets into the wild (Marinari et al. 2006). From 1985 until 2000, ferrets handled by the U.S. Fish and Wildlife Service at the Sybille Wildlife Research and Conservation Education Unit were fed a soft diet which consisted of 40% skinned, eviscerated, headless ground rabbit or prairie dog, and 60% commercial mink chow pellets, with freshly killed hamsters being supplied once per week (Vargas and Anderson 1996*a,b*). Females that had recently whelped were given this diet with added water, making their food even softer (Vargas and Anderson 1996*b*). Other captivity centers also supplied this "60/40" diet for their own captive *M. nigripes* (Marinari et al. 2006). Prior studies have documented that soft diets such as this are particularly dangerous to the oral health of carnivorous animals (King and Glover 1945; Watson 1994). This diet was altered during the mid-1990s to adjust nutritional components following concerns that the initial diet contained excessive polyunsaturated fatty acids and was eventually replaced by the horse-based "Toronto Zoo Diet" (Marinari et al. 2006). While this diet was found to be nutritionally superior to the 60/40 diet, it probably was not an improvement in terms of preventing calculus buildup or the development of periodontal disease.

Dental calculus is formed by the calcification of dental plaque by the minerals - mostly calcium phosphate salts (White 1997) - present in saliva (Niemiec 2008*a*). Calculus can be described as supragingival, or calculus that develops on the tooth surface, or as subgingival,

which extends under the free gingival margin (Niemic 2008a). Supragingival calculus is known to cause gingival recession (Busscher et al. 2004), which in turn allows adhesion of subgingival calculus to the newly exposed surfaces of the tooth (White 1997). This new growth can lead to chronic inflammation of periodontal tissue and periodontal disease (Busscher et al. 2004). Once periodontal disease has developed, the effects from supragingival calculus become minimal in comparison to the effects derived from subgingival calculus (Niemic 2008a).

Periodontal disease, any disorder that affects the tissues that surround or support the teeth (Pihlstrom et al. 2005), is the most common disease in small mammals, occurring in a majority of domestic pets (Watson 1994). A report published by Eroshin and colleagues (2010) found that 65.3% of sampled adult domestic ferrets, or European polecats (*M. putorius furo* (L., 1758)), a close relative of the black-footed ferret (Marinari et al. 2006), suffered from some degree of periodontal disease. Periodontal disease is categorized in two stages, with the latter being irreversible (Niemic 2008b). The first stage, gingivitis, is described as inflammation to the gingival region (Niemic 2008b). The latter stage, periodontitis, is a disease of the deeper supporting structures of the tooth that is not reversible unless the affected region is removed entirely (Niemic 2008b). Periodontal disease affects the attachment apparatuses of the teeth and, if left untreated, can ultimately lead to tooth loss (Pihlstrom et al. 2005).

Studies conducted by King and Glover (1945) found within a population of ferrets that supplementing an oatmeal or cereal based diet with short pieces of rib bone not only prevented the accumulation of calculus, but could also be used to remove calculus that had previously developed. With both the cereal and oatmeal based diets, all ferrets tested were found to have heavy calculus growth except those also fed either hard lumps of dog biscuits, rib bone striped of attachments (periosteal, muscular, and tendinous), or rib bone plus attachments. Ferrets whose

diet was altered with dog biscuits or rib bone minus attachments “showed relatively light development” of calculus and all ferrets fed rib bone with attachments “remained free from tartar and gum disease” at the conclusion of the 5 month study (King and Glover 1945). Other ferrets in this experiment were fed either wet dog biscuits or rib bone that was finely crushed and these specimens developed calculus similar to the basal diet suggesting that the texture of the rib bone not the nutritional content of it was preventing buildup of calculus (King and Glover 1945). Watson (1994) observed this relationship in dogs during a study in which he found that dogs fed softer diets developed plaque buildup faster and over a larger area than dogs that were fed tougher diets.

The main goal of this study is to determine whether or not calculus deposits and evidence of periodontal disease occur with greater frequency and severity in captive versus wild black-footed ferrets. Previous research would suggest that the unnaturally soft diet fed to these animals in captivity would result in increased cases of both ailments (Colyer 1936; King and Glover 1945). On a broader scale, this study will focus on the relationship between diet and the oral health of the black-footed ferrets in the U.S. Fish and Wildlife breeding program to determine the effect of the captive diet on the oral health of these animals.

One secondary goal of this report is to find if the dietary change implemented in the program in 2000 had an effect on the oral health of the black-footed ferrets. The diet was changed for the purpose of increasing nutritional value (Marinari et al. 2006), but how did this dietary change affect the oral health of the animals?

Materials and Methods

All of the adult *Mustela nigripes* skulls from the National Museum of Natural History (specimens catalogued as USNM; Smithsonian, DC) included in the study for a total of 261 USNM specimens. Each of the specimens belonged to one of four groups: 1) wild specimens accessioned prior to the modern reintroduction campaign, 2) specimens that were captive prior to the Black-Footed Ferret Recovery Act between 1888 and 1925, 3) the captive bred specimens fed the 60/40 diet from 1985-2000 – “Recovery Phase I”, and 4) the captive specimens fed the Toronto Zoo Diet from the breeding program from 2000 until the most recent accessions – “Recovery Phase II” (Table 1). To increase the sample size of the wild and “early captive” groups, all ten *M. nigripes* specimens from the American Museum of Natural History (AMNH; NY) were added to the sample.

[Insert Table 1 Here]

The dentition of each specimen was evaluated in four regions: maxillary right, maxillary left, mandibular right, and mandibular left. Each region was scored independently of the others for calculus, periodontal disease, and dental wear.

Calculus can be identified as a rough white buildup of calcified plaque on the crown of the tooth, and its extent was evaluated using a method described from previous research (Colyer 1936). A calculus index score from 0-2 was assigned to each dental quadrant based on calculus buildup severity (Table 2, Fig. 1).

[Insert Table 2 Here]

Periodontal disease severity was determined using a scale adjusted from a previous study (Kapoor et al. submitted¹). That scale ranged from 0-5 and was used for larger (pantherine)

¹ Kapoor, V., Antonelli, T., Parkinson, J., and Hartstone-Rose, A. Oral health correlates of captivity.

specimens. This scale was reduced to a 0-2 scale for the smaller *M. nigripes* (Table 3, Fig. 1).

Photoshop CC (Adobe Systems, Inc.) was used to alter coloring on Figure 1 to provide clarity.

[Insert Table 3 Here]

[Insert Figure 1 Here]

Dental wear score accounts for the extent to which the teeth have been worn due to tooth-to-tooth contact and abrasion of food during mastication from an unworn state to the state that the teeth are in at the time of measurement (Church 2007). Dental wear was scored on a scale from 0-5 (Table 4, Fig., 2).

[Insert Table 4 Here]

[Insert Figure 2 Here]

Scales used in other studies (Kaidonis et al. 1998) measured the length of wear from an unworn state. However, because of intraspecific variation in tooth size, we regard a qualitative evaluation of tooth wear more useful than a quantified one. While other studies have evaluated tooth wear in more specific regions of the mouth (e.g., nine regions in Elgart 2010), *M. nigripes* teeth tend to wear at approximately the same rate and therefore more summation is justified (Elgart 2010).

The data collected from the three scores was analyzed using JMP (version 10.0.2, SAS Institute, Inc.) and was analyzed using fit X-by-Y graphs where “group” served as the independent variable and max calculus, mean calculus, max periodontal, mean periodontal, max wear, and mean wear all served as dependent variables. Each of these graphs was then subjected to an all pairs, Tukey-Kramer HSD analysis to determine similarity. Both sex and overall captivity status were analyzed against each of the six dependent measurements aforementioned.

These were analyzed in JMP also but results were obtained from a Wilcoxon Rank Sums test. All results were considered statistically significant for $\alpha < 0.05$ and highly significant for $\alpha < 0.01$.

Results

A comparison of the mean calculus scores for each group (Fig. 3) shows highly significant differences (all $p < 0.0001$) in the mean score of the three captive *M. nigripes* groups and the mean score of the wild *M. nigripes*, with the captive groups all having higher average calculus scores than the wild group. Among the three captive *M. nigripes* groups, there was no significant difference between the early captive group and the Recovery Phase I group, but there was a highly significant difference between the early captive group and the Recovery Phase II group. However, there was a highly significant difference in mean score between the two Recovery Phase captive groups due to the Recovery Phase II group (mean=0.94) scoring a much greater score in mean calculus than the Recovery Phase I group (mean=0.33). The comparison of maximum calculus score to group (not shown) returned similar results with one exception: there was no significant difference between the early captive group and the Recovery Phase II group.

[Insert Figure 3 Here]

A comparison of the mean periodontal scores across the groups found less disparity (Fig. 4). The wild group was again highly significantly different than the Recovery Phase I group; however, unlike the calculus means, there was no statistical difference between the wild group (mean=0.35) and the Recovery Phase II group (mean=0.50) in terms of porosity resulting from periodontal disease. There was even more overlap between the wild group and the early captive group. The early captive group exhibited the largest variance of any group, making it

indistinguishable from any other groups in terms of mean periodontal score. The maximum periodontal disease comparison found identical results to the mean periodontal analysis.

[Insert Figure 4 Here]

When comparing wear score as a function of Group (Fig. 5) between the four groups, the captive Recovery Phase I group exhibited the lowest mean score among the groups (mean=1.67). Though not statistically significantly different ($p=0.2019$) from the wild group (mean=1.98), the score was well below the mean scores received from the early captive group (mean=2.75) and the Recovery Phase II group (mean=2.66). The wild group was distinguishable from only the Recovery Phase II group ($p=0.0128$). Of all the groups, the early captives had the highest mean score but also a greater level of variance than any other group.

[Insert Figure 5 Here]

None of the scores were statistically separable by sex (Table 5); thus, male and female specimens were indistinguishable by these oral health measures. In contrast, wild and captive specimens were significantly different in terms of both calculus and periodontal disease with captive specimens having much greater scores than those of the wild specimens. There was no significant difference between the wear scores of the three captive groups.

[Insert Table 5 Here]

There are significant relationships between wear scores (both mean and maximum) and oral health scores for the captive but not wild specimens (Table 6). This lack of significance could be due to the relatively low calculus scores in wild specimens. These relationships can be observed in bivariate plots (Figs. 6 and 7).

[Insert Table 6 Here]

[Insert Figure 6 Here]

[Insert Figure 7 Here]

Discussion

The black-footed ferret (*Mustela nigripes*) was brought back from near extinction in the 20th century. During the intensive breeding effort that resulted from the Black-Footed Ferret Recovery Act, the rightful husbandry focus was almost exclusively on fertility and disease prevention, namely canine distemper and the sylvatic plague (Barnes 1993; Vargas et al. 2000; Wimsatt et al. 2004). The state of oral health in this species has been almost entirely undocumented.

Previous studies have shown that a lack of obdurate elements in a diet can lead to increased calculus development in the oral cavity (King and Glover 1945; Watson 1994; Kapoor et al. submitted), and the current analysis supports this claim. Records indicate that ferrets in the early stages of the captive breeding program were fed an unnaturally soft diet compared to the natural diet of the species (Marinari et al. 2006). As a result, the prevalence of calculus and periodontal disease in these captive specimens was significantly more severe than their wild counterparts. Qualitatively, one of us (JJO) has noticed that the progression of periodontal disease in the palates of captive specimens typically originates in the pocket formed by the m1 and the p4, which are essentially perpendicular to each other. This atypical tooth morphology may make ferrets more susceptible to periodontal disease because it creates a pocket where soft food can easily lodge. The higher quantitative scores are also a clear indication that captivity had a strong effect on the captive black-footed ferrets. Calculus results from the early captive phase support this conclusion. The effect of captivity on periodontal disease is slightly less evident. One captive group (the Recovery Phase I group) did score significantly higher than the wild

group, again suggesting that captivity lead to the demise of oral health in subjects. Though the other captive groups were not statistically significantly different, the wild group does have a lower mean periodontal disease score than either group.

Although the diet of the ferrets was changed in 2000 to increase the nutritional benefits of the diet (Marinari et al. 2006), the current analyses suggest that this dietary change did not improve the prevention of calculus development in the oral cavity of the ferrets. On the contrary, the specimens from after this diet shift have significantly higher calculus scores, perhaps suggesting that the new diet is actually worse for the oral health. However, while calculus scores significantly increased after the diet change, periodontal scores decreased slightly – though not significantly so from periodontal scores from the Recovery Phase I specimens.

Another important finding was that calculus and periodontal disease scores did not drop significantly from the earlier captive animals (i.e., those held before 1960) relative to those of Recovery phases. Such findings seem to indicate that little improvement was made to the mechanical properties of the diet over the span of nearly a century. This finding is not surprising from a periodontal standpoint where the periodontal mean score of the early captives was not considerably different from even the wild specimens. However, concern should be raised in terms of calculus development. Calculus among the Recovery Phase I individuals did drop from the early captive period, but not significantly so. And the mean calculus score actually increased significantly from the early captive group to the Recovery Phase II group.

Unfortunately, we could not control for the effect of age on the oral health scores. The increased scores observed among the captive groups could have resulted from longer life spans in captivity, thus allowing the captive ferrets more time to develop oral problems. While the ages of at least some of the captive individuals are attainable (by reconciling the notes between the

breeding facilities and museum collections to identify specific individuals), the precise ages of the wild ferrets cannot be known. However, because teeth are not regenerative, wear can be considered a rough proxy for age (Stander 1997; Renaud 2005). Though it is likely that teeth wear at different rates in captive and wild animals with notably different diets, wear is still unidirectional and therefore relative within samples. Although there is a slight positive relationship concerning periodontal disease between all sampled ferrets and wild ferrets, the correlation is not nearly as strong between the wild ferrets and all sampled ferrets as it is between the captive ferrets and all sampled ferrets. Thus ferrets on a natural diet experience less accumulation of calculus as their teeth wear (i.e., it ages) and only slightly more periodontal disease with tooth wear. Therefore, the more positive slope of the captive ferrets suggests that captivity causes ferrets to score higher in both calculus and periodontal disease than wild ferrets at a similar wear score.

Future research will aim to expand on a previous study (Wisely et al. 2002) focused on determining if the mechanical properties of diet affect health more systemically or cause cranial morphological changes resulting from unnatural mastication by the studied specimens. This analysis suggests that the mechanical properties of diet are affected in captivity. An informative future study might test the physical properties of the captive and wild diets and correlate the toughness of diet with dental scores recorded in this analysis. Further work could also help determine if the oral health effects investigated in this analysis result in systemic problems in black-footed ferrets, specifically issues concerning cranial morphology. Based on this project, there is reason to believe that the composition of diet could affect the development of masticatory muscles and alter cranial measurements.

In this paper, we have confirmed that the calculus and periodontal disease scoring systems developed for similar projects (e.g., Kapoor et al. submitted) are appropriate methods for measuring oral health problems in *M. nigripes*. Likewise, the wear scale that was created for the purpose of this experiment is also an effective tool for measuring dental wear in specimens and has applications in studies beyond the present one. All three scales can be adapted to measure oral health in other mammalian species with minimal adjustments.

If not for the Black-Footed Ferret Recovery Act, this species would almost certainly be extinct and thus the captive breeding program must be viewed as a remarkable success. Now that the population is relatively stable, this study suggests that mechanical, not just nutritional, properties of diet should be considered in order to maintain oral health in these and other captive mammals.

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References

- Ballou, J.D., and Oakleaf, R. 1989. Demographic and genetic captive-breeding recommendations for black-footed ferrets. *Conservation biology and the black-footed ferret. Edited by U.S. Seal, E.T. Thorne, M.A. Bogan, and S.H. Anderson.* Yale University Press, New Haven. pp. 247-269.
- Barnes, A.M. 1993. A review of plague and its relevance to prairie dog populations and the black-footed ferret. US Fish and Wildlife Service Biological Rep. No. 13, U.S. Fish and Wildlife Service, Washington, D.C.
- Budd, J. 1981. Distemper. *In Infectious Diseases of Wild Mammals. Edited by J.W. Davis, L.H. Karstad, and D.O. Trainer.* Iowa State University Press, Ames. pp. 31-44.
- Busscher, H.J., White, D.J., Kamminga-Rasker, H.J., and van der Mei, H.C. 2004. A surface physicochemical rationale for calculus formation in the oral cavity. *J. Cryst. Growth*, **261**(1): 87-92. doi:10.1016/j.jcrysgro.2003.09.012.
- Cahalane, V.H. 1954. Status of the black-footed ferret. *J. Mammal.* **35**(3): 418-424. doi:10.2307/1375967.
- Campbell III, T.M., Clark, T.W., Richardson, L., Forrest, S.C., and Houston, B.R. 1987. Food habits of Wyoming black-footed ferrets. *Am. Midl. Nat.* **117** (1): 208-210. doi:10.2307/2425722
- Church, B. 2007. Ferret dentition and pathology. *In Ferret husbandry, medicine and surgery 2nd edition. Edited by J.H. Lewington.* Saunders Elsevier, Edinburgh. pp. 467-485.
- Clark, T. 1994. Restoration of the endangered black-footed ferret: a 20-year overview. *In Restoration of endangered species: conceptual issues, planning and implementation. Edited by M.L. Bowles and C.J. Whelan.* Cambridge University Press, New York. pp. 272-297.
- Colyer, S.F. 1936. Variations and diseases of the teeth of animals. Cambridge University Press, New York, N.Y.

- Elgart, A.A. 2010. Dental wear, wear rate, and dental disease in the african apes. *Am. J. Primatol.* **72**(6): 481-491. doi: 10.1002/ajp.20797.
- Eroshin, V.V., Reiter, A.M., Rosenthal, K., Fordham, M., Latney, L., Brown, S., and Lewis, J.R. 2010. Oral examination results in rescued ferrets: clinical findings. *J. Vet. Dent.* **28**(1): 8-15. Available from <http://www.ncbi.nlm.nih.gov/pubmed/21696122> [accessed 22 June 2015].
- Forrest, S.C., Biggins, D.E., Richardson, L., Clark, T.W., Campbell III, T.M., Fagerstone, K.A., and Thorne, E.T. 1988. Population attributes for the black-footed ferret (*Mustela nigripes*) at Meeteetse, Wyoming, 1981-1985. *J. Mammal.* **69**(2): 261-273. doi:10.2307/1381377.
- Henderson, F.R., Springer, P.F., and Adrian, R. 1974. The black footed ferret in South Dakota. Technical Bulletin No. 4, South Dakota Department of Game, Fish and Parks, Pierre, S.D.
- Hillman, C.N., and Clark, T.W. 1980. *Mustela nigripes*. *Mamm. Species*, No. **126**: 1-3. Available from http://digitalcommons.unl.edu/icwdm_usdanwrc/1629/ [accessed 22 June 2015].
- Jachowski, D.S., Millsbaugh, J.J., Biggins, D.E., Livieri, T.M., Matchett, M.R., and Rittenhouse, C.D. 2011. Resource selection by black-footed ferrets in South Dakota and Montana. *Nat. Areas J.* **31**(3): 218-225. doi:<http://dx.doi.org/10.3375/043.031.0304>.
- Kaidonis, J., Richards, L., Townsend, G., and Tansley, G. 1998. Wear of human enamel: a quantitative in vitro assessment. *J. Dent. Res.* **77**(12): 1983-1990. doi: 10.1177/00220345980770120601
- King, J., and Glover, R. 1945. The relative effects of dietary constituents and other factors upon calculus formation and gingival disease in the ferret. *J. Pathol. Bacteriol.* **57**(3): 353-362. doi:10.1002/path.1700570309
- Marinari, P.E., Kreeger, J.S., Roelle, J., Miller, B., Godbey, J., and Biggins, D. 2006. An adaptive management approach for black-footed ferrets in captivity. *In* *Recovery of the black-*

- footed ferret: progress and continuing challenges. Proceedings of the Symposium on the Status of the Black-footed Ferret and Its Habitat, Fort Collins, CO., January 28-29, 2004. U.S. Geological Survey, Reston, VA. pp. 23-27.
- Niemiec, B.A. 2008a. Oral pathology. *Top. Companion Anim. Med.* **23**(2): 59-71.
doi:10.1053/j.tcam.2008.02.002
- Niemiec, B.A. 2008b. Periodontal disease. *Top. Companion Anim. Med.* **23**(2): 72-80.
doi:10.1053/j.tcam.2008.02.003
- Pihlstrom, B.L., Michalowicz, B.S., and Johnson, N.W. 2005. Periodontal diseases. *The Lancet*, **366**(9499): 1809-1820. doi:http://dx.doi.org/10.1016/S0140-6736(05)67728-8.
- Renaud, S. 2005. First upper molar and mandible shape of wood mice (*Apodemus sylvaticus*) from northern Germany: ageing, habitat and insularity. *Mamm. Biol.* **70**(3): 157-170.
- Stander, P.E. 1997. Field age determination of leopards by tooth wear. *Afr. J. Ecol.* **35**(2): 156-161. doi:10.1016/j.mambio.2004.10.004
- Vargas, A., and Anderson, S.H. 1996a. Effects of diet on captive black - footed ferret (*Mustela nigripes*) food preference. *Zoo Biol.* **15**(2): 105-113. doi:10.1002/(SICI)1098-2361(1996)15:2<105::AID-ZOO1>3.0.CO;2-F
- Vargas, A., and Anderson, S.H. 1996b. Growth and physical development of captive-raised black-footed ferrets (*Mustela nigripes*). *Am. Midl. Nat.* **135**(1): 43-52. doi: 10.2307/2426870.
- Vargas, A., Gober, P., Lockhard, M., and Marinari, P. 2000. Black-footed ferrets: recovering an endangered species in an endangered habitat. *In* *Mustelids in a Modern World: Management and Conservation Aspects of Small Carnivore: Human Interactions*. Edited by H.I. Griffiths. Backhuys, London. pp. 97-105.

- Vargas, A., Lockhart, M., Marinari, P., and Gober, P. 1996. The reintroduction process: Black-footed Ferrets as a case study. *In* Proceedings for the American Zoo and Aquarium Association Western Regional Conference, Denver, CO., 15-19 May 1996. pp. 829-834.
- Watson, A.D.J. 1994. Diet and periodontal disease in dogs and cats. *Aust. Vet. J.* **71**(10): 313-318. doi:10.1111/j.1751-0813.1994.tb00905.x
- White, D.J. 1997. Dental calculus: recent insights into occurrence, formation, prevention, removal and oral health effects of supragingival and subgingival deposits. *Eur. J. Oral Sci.* **105**(5): 508-522. doi:10.1111/j.1600-0722.1997.tb00238.x.
- Wimsatt, J., Biggins, D.E., Williams, E.S., and Becerra, V.M. 2004. The quest for a safe and effective canine distemper virus vaccine for black-footed ferrets. *In* Recovery of the black-footed ferret: progress and continuing challenges. Proceedings of the Symposium on the Status of the Black-footed Ferret and Its Habitat, Fort Collins, CO., Fort Collins, CO., January 28-29, 2004. U.S. Geological Survey, Reston, VA. pp. 248-266.
- Wisely, S., Ososky, J., and Buskirk, S. 2002. Morphological changes to black-footed ferrets (*Mustela nigripes*) resulting from captivity. *Can. J. Zool.* **80**(9): 1562-1568. doi:10.1139/z02-160

Table 1. Sample Specimen Distribution of Black-Footed Ferrets (*Mustela nigripes*).

Group	Male	Female	Unknown*	Total
Wild	30	20	18	68
Early Captive	8	3	1	12
Recovery Phase I	53	58	46	157
Recovery Phase II	18	16	0	34
Total	109	97	65	271

*Sex was determined by museum records, some of which were not recorded.

Draft

Table 2. Calculus Index.

CALCULUS INDEX SCORE	OBSERVATIONS
0	Less than 10% of the crowns of the teeth is covered by calculus
1	10 - 50% of the crowns of the teeth is covered by calculus
2	More than 50% of the crowns of the teeth is covered by calculus

Draft

Table 3. Periodontal Index.

PERIODONTITIS INDEX SCORE	OBSERVATIONS
0	The bone along the gum line has no visible porosity.
1	The bone along the gum line has noticeable porosity.
2	The bone along the gum line has excessive porosity OR porosity is noticeable at a substantial distance from the gum line OR tooth loss has occurred as a result of porosity.

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Table 4. Description of Dental Wear Scores.

WEAR INDEX SCORE	OBSERVATIONS
0	Anterior and medial cusps of M1 remain pointed. P2 and P3 cusps remain pointed.
1	Medial cusp on M1 remains pointed, anterior cusp is rounded. P3 cusp is pointed, P2 cusp is rounded
2	Medial cusp on M1 is rounded yet remains larger than anterior cusp. Both P2 and P3 cusps are rounded
3	P2 is plateau shaped, P3 remains rounded but is of equal or lesser height than the M1 anterior cusp. Meeting of anterior and medial cusps on M1 is rounded.
4	Anterior cusp on M1 is no longer evident. P2 and P3 cusps are either flat or plateau shaped. Medial cusp of M1 is barely noticeable.
5	Cusps no longer evident on any teeth

Table 5. Chi² Approximation for each of the six analyzed measurements for both sex and captivity status (wild vs. captive).

Measurement	Sex	Overall Captivity Status
Max Calculus Score	0.2827	<.0001***
Mean Calculus Score	0.5239	<.0001***
Max Periodontal Score	0.4003	.0011*
Mean Periodontal Score	0.1605	.0239*
Max Wear Score	0.2283	0.9197
Mean Wear Score	0.1126	0.7761

* = 0.05 > P > 0.01

** = 0.01 > P > 0.001

*** = P < 0.001

Table 6. P-scores of calculus and periodontal scores as dependent variables of wear scores.

	Wear Mean		Wear Max	
	Captive	Wild	Captive	Wild
Calculus Mean	<0.0001	0.0916	<0.0001	0.2166
Calculus Max	<0.0001	0.4072	<0.0001	0.8061
Periodontal Mean	<0.0001	0.2039	<0.0001	0.4250
Periodontal Max	<0.0001	0.0414	<0.0001	0.0322

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Figure Captions

Fig. 1. Visual Representation of our Calculus and Periodontal Scales in Black-Footed Ferrets (*Mustela nigripes*). The C0 specimen is USNM 349715, C1 is USNM 93M046, C2 is USNM 399113, P0 is USNM A22427, P1 is USNM 188454, and P2 is USNM 592554.

Fig. 2. Visual Representation of Wear Scale. From top to bottom: USNM 251453, USNM 349715, USNM 188454, USNM A30064, USNM 199731, USNM 241014.

Fig. 3. Mean Calculus Scores of the Six Dental Regions as a Factor of Group

Fig. 4. Mean Periodontal Scores of the Six Dental Regions as a Factor of Group

Fig. 5. Mean Wear Scores of the Six Dental Regions as a Factor of Group

Fig. 6. Mean Calculus Score as a Factor of Mean Wear Score. Solid line represents linear fit for all black-footed ferrets (*Mustela nigripes*), dotted line represents captive ferrets, and the dashed line represents wild ferrets. Individual wild specimens are denoted by a solid circle, early captives by an open circle, Recovery Phase I by a solid diamond, and Recovery Phase II by an open diamond.

Fig. 7. Periodontal Mean Score as a Factor of Wear Mean Score. Solid line represents linear fit for all black-footed ferrets (*Mustela nigripes*), dotted line represents captive ferrets, and the dashed line represents wild ferrets. Individual wild specimens are denoted by a solid circle, early captives by an open circle, Recovery Phase I by a solid diamond, and Recovery Phase II by an open diamond.

Fig. 1. Visual Representation of our Calculus and Periodontal Scales in Black-Footed Ferrets (*Mustela nigripes*). The C0 specimen is USNM 349715, C1 is USNM 93M046, C2 is USNM 399113, P0 is USNM A22427, P1 is USNM 188454, and P2 is USNM 592554.
152x92mm (300 x 300 DPI)

Fig. 2. Visual Representation of Wear Scale. From top to bottom: USNM 251453, USNM 349715, USNM 188454, USNM A30064, USNM 199731, USNM 241014.
50x129mm (300 x 300 DPI)

Fig. 3. Mean Calculus Scores of the Six Dental Regions as a Factor of Group
188x135mm (300 x 300 DPI)

Fig. 4. Mean Periodontal Scores of the Six Dental Regions as a Factor of Group
177x135mm (300 x 300 DPI)

Fig. 5. Mean Wear Scores of the Six Dental Regions as a Factor of Group
172x135mm (300 x 300 DPI)

Fig. 6. Mean Calculus Score as a Factor of Mean Wear Score. Solid line represents linear fit for all black-footed ferrets (*Mustela nigripes*), dotted line represents captive ferrets, and the dashed line represents wild ferrets. Individual wild specimens are denoted by a solid circle, early captives by an open circle, Recovery Phase I by a solid diamond, and Recovery Phase II by an open diamond.

140x110mm (300 x 300 DPI)

Fig. 7. Periodontal Mean Score as a Factor of Wear Mean Score. Solid line represents linear fit for all black-footed ferrets (*Mustela nigripes*), dotted line represents captive ferrets, and the dashed line represents wild ferrets. Individual wild specimens are denoted by a solid circle, early captives by an open circle, Recovery Phase I by a solid diamond, and Recovery Phase II by an open diamond.

140x110mm (300 x 300 DPI)