

**FLORIDA PANTHER BIOMEDICAL INVESTIGATIONS**  
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\_\_\_\_\_NOTE: The author chose to use the following preexisting report for these proceedings.

FINAL PERFORMANCE REPORT

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Abstract: Veterinary medical management to reduce capture-associated mortality, provide medical care to promote health and increase survival, and to conduct biomedical research to further the understanding of disease, nutrition, and reproductive physiology continued as an integral part of the Florida panther (Felis concolor coryi) recovery. Since veterinary involvement began in 1983, 159 immobilizations involving 58 individuals have been accomplished with one mortality (0.63%) in 1983, possibly capture-related. This fiscal year resulted in re-collaring 9 panthers and the capture and radio-instrumentation of 2 newly captured panthers. In addition, 8 kittens were hand-caught, examined, and released at 3 den sites. A total of 22 kittens have been hand-caught during this four-year period. A range of 18 to 23 individual panthers have been monitored by telemetry during this 4-year period. Presently, 18 panthers (8 males, 10 females) are being monitored. The panther population estimate is 30 to 50 adults. Serologic evidence indicates that they were exposed to or were infected with several potentially pathogenic agents: feline calicivirus, feline panleukopenia virus, feline rhinotracheitis virus, feline enteric coronavirus/feline infectious peritonitis, feline immunodeficiency virus/puma lentivirus. However, panthers were serologically negative for Brucella sp., Toxoplasma gondii, feline leukemia virus, and pseudorabies virus. Twenty-one deaths were documented during this 4-year period. In fiscal year 93/94, 71% of the 7 deaths were due to road kills, 14% to intraspecific mortality, and 14% to bacterial infection. Panthers were positive for 2 trematodes, 2 cestodes, 6 nematodes, 1 acanthocephalan, and 1 protozoan. No major changes in endoparasite loads were found compared to previous studies of the Florida panther (Forrester et al. 1985). Two studies were initiated this fiscal year, one on vitamin A and one on estradiol levels in panthers. No vitamin A deficiency was found, although,

vitamin A levels were correlated with several variables, including age and prey base. Apparently high estrogen levels in male panthers were suggestive of exposure to environmental estrogenic chemicals. There were no attempts to breed panthers in captivity during this period. One captive adult (#200) was euthanized this year due to a severe neurological disorder, leaving a total of 9 panthers currently in captivity.

The endangered Florida panther (*Felis concolor coryi*) is estimated to number less than 50 adult individuals and currently is protected under both state and federal endangered species statutes. This remnant population of the subspecies that once occupied the entire southeastern United States is now isolated in the remote cypress swamps and hardwood hammocks of southern Florida, primarily in the Big Cypress Swamp and Everglades ecosystems (Belden 1986). Human population growth in southern Florida with the concomitant loss of suitable panther habitat has been and continues to be the major threat to the continued survival of this rare mammal. A detailed review of the life history and status of the panther is presented in the United States Fish and Wildlife Service recovery plan (USFWS 1987). The critically small population size of the Florida panther makes the subspecies particularly vulnerable to demographic catastrophes. Chance events such as periodic road-kills, illegal hunting, or loss of prey species can be devastating to very small populations. In addition, disease outbreaks caused by adventitious agents are a constant threat to small populations, especially when those populations become relatively inbred over time (O'Brien and Evermann 1988). Decreased genetic diversity, increased neonatal mortality, abnormal reproductive traits, and lowered disease resistance often accompany such inbreeding in mammals (O'Brien et al. 1983 and 1985). There is reason to believe that the Florida panther is presently experiencing many of these problems (Roelke and Glass 1992; Seal et al. 1992; Dunbar 1993).

Preliminary research on the diseases and parasites of wild panthers was initiated in 1978. This was expanded in 1983 through a collaborative effort between the College of Veterinary Medicine at the University of Florida and the Florida Game and Fresh Water Fish Commission (GFC), which supported a 3-year veterinary residency position in Wildlife Medicine from 1983 to 1986 funded by the GFC. The continuation of biomedical research since 1 July 1986 was facilitated by the creation of a full-time wildlife veterinarian position with the GFC in 1986.

When the veterinary position was originally initiated in 1983, one of the primary objectives was to provide high quality medical care and insure safe handling during the capture and immobilization of free ranging and captive panthers. Since that time, the intensity and scope of the project's objectives have expanded to encompass many facets of medical management and biomedical research, including infectious diseases and parasites, mortality, pathology, environmental contaminants, reproductive physiology, and genetics. Many data have been generated dealing with these topics and are presented here, the breadth of which is unparalleled in most free-ranging carnivore studies.

The primary objective of this study is to document and elucidate the various biomedical factors that are contributing to the extinction of the Florida panther. Through the present multi-disciplinary approach, it is hoped this objective will be met. Furthermore, management strategies will be developed that will promote the recovery of the Florida panther.

Continuation of this project should reduce capture-related mortalities, increase survival rates for individual panthers requiring veterinary medical attention, and promote an understanding of the role that disease, parasites, nutrition, and genetic diversity play in the population dynamics of the Florida panther. This document also serves as the Annual Performance Report for work conducted under the contract entitled "Studies on Certain Health and Reproductive Parameters of the Florida Panther", D. J. Forrester, principal investigator. This yearly contract, covering 1 July 1993 through 30 June 1994, was between the GFC and the Department of Infectious Diseases, College of Veterinary Medicine, University of Florida, Gainesville, FL. I wish to thank the many individuals who contributed their time and effort to the Florida Panther Biomedical Investigation over the past four years. First of all I wish to recognize Dr. Melody Roelke and Dr. Carolyn Glass for their work in collecting much of the data for this report. I would like to thank field biologists David S. Maehr, Walter McCown, E. Darrell Land, Jayde Roof, Deborah K. Jansen, and Oron L. Bass Jr. for their efforts in location, capture, and

handling of panthers and Roy and Rowdy McBride, houndsmen, without whose expertise and efforts this project would be nearly impossible to accomplish. I thank Mr. Buck Thackeray of the Big Cypress National Preserve for his assistance in planning, organization, and field assistance. I also acknowledge Todd Logan, Ken Edwards, Larry Richardson, and Jim Krakowski of the U.S. Fish and Wildlife Service and Mike Petty and Mike Owens of Fakahatchee Strand State Preserve for their willing assistance in the field. I express my appreciation to veterinary assistants Mark Cunningham, Randy Roth, Pauline Nol, Marnie Lamm, Nicola Keeling, Renee Carleton, Lauren Ritchey, Chris Beal, Adam Birkenheuer, Andrena J. Anderson, and Don Coyner for their hard work and support given to the panther project throughout the last several years. I wish to recognize the scientific collaborators who have made many aspects of this project possible. They are Dr. Stephen J. O'Brien and Jan Martenson of the National Cancer Institute, Drs. David E. Wildt, Mark A. Barone, JoGayle Howard, and Onie Byers of the National Zoological Park, Dr. Robert Olmstead at the National Institute of Health, Drs. Don Shultz and Chuck Facemire of the U.S. Fish and Wildlife Service, Dr. Beth Callan of the University of Pennsylvania, Dr. Jim Evermann of Washington State University, Dr. Fred Scott of Cornell University, Laurie Wilkins of the Florida Museum of Natural History, and, finally, Drs. Donald Forrester, Ellis Greiner, Stephen Sundlof, Paul L. Nicoletti, Paul Gibbs, and Jack Gaskin of the University of Florida. I wish to thank Grace McLaughlin for her assistance with parasite collection and data analysis. For their laboratory and technical assistance, I acknowledge Mary Eichelburger, Eric Brown, Allison McKiernan, and Cordell Geisinger. Many thanks to veterinarians, Drs. Lee Young, Jeurgen Schumacher, Nancy Lung, Luisito Pablo and medical surgeon, Dr. Edward Staples of the University of Florida. Also thanks to Drs. Janet Stover and Scott Citino of White Oak Plantation, Dr. David Murphy of Lowry Park Zoo, Dr. Douglas Paige of Jacksonville Zoo, and Naples veterinarian Dr. John Lanier for their medical contributions to both wild and captive Florida panthers.

## METHODS

Radio telemetry from aircraft and ground tracking with dogs were used to locate radio-instrumented panthers. Non-radio-collared panthers were also located using dogs. Panther kittens were located by visual search of an area where a suspected parturient female had been repeatedly located. Environmental factors, as well as health and level of stress of each panther, were evaluated at each capture site to determine potential risk of immobilization and capture. If risk was acceptable, a compressed air Vario<sup>®</sup> dart rifle (Telinject USA, Inc.) was used to inject immobilizing drugs. Ketamine hydrochloride (Ketamine<sup>®</sup>), 8-9 mg/Kg, and Telazol<sup>®</sup>, 2-3 mg/Kg were used, in combination, as initial immobilizing agents.

Tranquilized panthers were removed from the tree by hand or allowed to fall into a net and cushioned "crash bag" as described by McCown et al. (1990). Panthers were monitored for vital signs and depth of anesthesia. During the last fiscal year, arterial blood oxygen saturation was measured using a portable pulse oximeter (Nellcor, Inc., Harward, California). Additional immobilizing drugs were administered as needed to maintain appropriate anesthesia enabling sample collection. An intravenous catheter was installed in the cephalic or saphenous vein, and isotonic fluids were administered to maintain adequate blood volume and pressure. Each panther was examined and, after stabilization, was radio-instrumented.

Additionally, body measurements and biological samples including blood, urine, skin biopsy, feces, hair, saliva, viral and bacterial culture swabs, and ectoparasites were collected as previously described (Roelke 1990). Samples were collected predominantly between January and April during 1990 through 1994.

Some panthers were administered prophylactic treatment of iron dextran and vitamin B-complex injectables following initial examination and collection of biological samples. However, this treatment was not conducted during the last 2 years. Each animal received vaccinations for antibody protection

against the viral agents feline panleukopenia, feline calicivirus, feline viral rhinotracheitis (Fel-O-Vax<sup>®</sup>, Fort Dodge), and rabies (Imrab<sup>®</sup>, Pitman Moore), all killed virus vaccines. Additionally, injectable anthelmintic drugs praziquantel (Droncit<sup>®</sup>, Haver) and ivermectin (Ivomec<sup>®</sup>, Merck & Co.) were administered to reduce parasitic infection.

Serum, collected in blood clot tubes, was analyzed for chemistry profiles and antibodies to the following disease agents: Brucella spp., Toxoplasma gondii, feline calicivirus (FCV), feline enteric corona virus/feline infectious peritonitis virus (FECV/FIP), feline immunodeficiency virus/puma lentivirus (FIV/PLV), feline panleukopenia virus (FPV), feline viral rhinotracheitis (FVR), pseudorabies virus (PRV), encephalomyocarditis virus, and feline syncytial forming virus (FeSFV). Feline calicivirus, FPV, and FVRV values were reported for samples obtained prior to vaccination with the killed preparation of these 3 viral vaccines.

Whole blood in EDTA was analyzed for complete blood count (CBC) and smears were prepared on glass slides for microscopic evaluation for intracellular parasites.

Surgeries were performed (in FY 92-93) on 4 panthers, 3 captive and 1 free-ranging. Cardiac surgery was performed by a medical team from Shands Hospital, University of Florida, Gainesville, on #205, a 2-year-old female panther diagnosed with atrial septal defect and tricuspid valve dysplasia. An orchiopexy was performed by veterinary surgeons at the Veterinary Medical Teaching Hospital (VMTH), University of Florida, on #207, a one-year-old male panther diagnosed as bilateral cryptorchid. A lung lobectomy was performed by VMTH surgeons on #204, a captive 2-year-old female diagnosed with chronic bacterial pneumonia due to Pseudomonas aeruginosa. A reproductive evaluation was performed on #38, an approximately 6-year-old female with a 3-year history of reproductive failure. Radiographic and ultrasound evaluations as well as celiotomy and uterine biopsy were performed by veterinary surgeons at VMTH.

Skin biopsies were taken as described by

Roelke (1990) from Florida panthers for genetic analysis using mitochondrial DNA and nuclear markers at the Laboratory of Viral Carcinogenesis, National Cancer Institute (Frederic, Maryland, USA) according to techniques described by O'Brien et al. (1990). Hair and/or whole blood were collected from free-ranging panthers captured during routine immobilizations and analyzed for mercury. Liver, kidney, muscle, brain, hair, and blood were collected at necropsy from dead panthers. Tissue samples were also collected from raccoons for mercury analysis. All samples were stored at either -10°C or -75°C until submitted for analysis. The reference laboratory used to analyze most panther tissue was the Patuxent Analytical Control Facility (U.S. Fish and Wildlife Service) in Laurel, Maryland. Some samples were analyzed at the GFC Fisheries Research Laboratory, Eustis, Florida. At the Patuxent Laboratory, tissue sample homogenates were digested under reflux in sulfuric and nitric acids (Monk 1961), and total mercury concentrations (the combined amount of organic and inorganic) were determined by cold vapor atomic absorption spectrophotometry (Hatch and Ott 1968) using a Spectro Products mercury analyzer equipped with a Varian VGA-76 vapor generation accessory. Tissue samples from raccoons were analyzed at the University of Florida, Gainesville, using similar techniques.

Semen was collected from male panthers by electro-ejaculation and stored by cryopreservation using techniques as described by Roelke (1990).

Dead *F. c. coryi* were subjected to complete post mortem examination by board-certified pathologists at VMTH, University of Florida. One panther was necropsied at the University of Georgia, Athens, Georgia, by pathologists of the Southeastern Wildlife Disease Cooperative Study. Analysis included gross and microscopic examination of tissues, serology, bacteriology, parasitology, and radiography. When specimens were fresh, attempts were made to collect and preserve living spermatozoa, ova, and skin. Hides and skeletal remains were deposited in the Florida Museum of Natural History, Gainesville, Florida.

## **RESULTS AND DISCUSSION**

### **FREE-RANGING PANTHERS**

#### **Field Capture and Radio Instrumentation**

The field capture season usually extended from January through March of each year, although some captures occurred as late as June. Captures during July through December were usually discouraged due to adverse weather conditions (hot temperatures and high water levels).

Eighteen to 23 individual panthers have been monitored by radio-telemetry each year during the 4-year period. Presently, 18 panthers (8 males and 10 females) are being monitored by radio-telemetry in southern Florida. With the exception of 1 adult male (#16), which occasionally inhabits the Everglades National Park (ENP), all radio-collared panthers presently inhabit lands north of Tamiami Trail (State Road 41), mostly (approximately 80%) within the Big Cypress National Preserve and Florida Panther National Wildlife Refuge. The population estimate for Florida panthers remains at 30 to 50 adults. Identification numbers and pertinent data on each animal presently being monitored can be found in Table 2.

Since veterinary involvement began in 1983, 159 immobilizations, involving 58 individual panthers, have been accomplished with one mortality in 1983 (0.63%), possibly capture related. In this four-year period, 74 immobilizations were accomplished, including the capture and radio-instrumentation of 23 first-time-captured panthers. This last fiscal year, 9 panthers were captured and re-collared, and, 2 panthers were first-time captured, for a total of 11 panthers handled. The 2 new panthers were a juvenile female (#55) and a pregnant adult female (#56).

For the first time, in FY 91-92, panther kittens were hand-caught and examined. To date, a total of 22 kittens have been hand-caught, 10 of which were placed in the captive breeding program during FY 91-92 and FY 92-93. There were 8 kittens hand-caught, examined, and released at 3 den sites this fiscal year. Data pertaining to kittens captured from April 1992 through May 1994 are presented in Table 1. Kittens were generally found to be in good health; however, elevated white blood cell counts, including elevated lymphocytes and eosinophils, indicated viral infections and high parasite loads, respectively, for some kittens. One kitten (#208) developed mange infestation due to Notoedus cati.

No significant medical problems due to immobilization or capture were encountered during this 4-year period. Most panthers were apparently in good health upon capture.

### Panther Mortality

Forty-one panther deaths have been documented since 1986; 21 occurred during this 4-year period, including 7 this fiscal year. Figure 1 depicts causes of mortality for free-ranging panthers >6 mo. of age from 1978 through 1994. In this fiscal year, 71% of the 7 deaths were due to road kills, 14% to intraspecific aggression, and 14% to bacterial infection. These figures approximate those recorded for the past 16 years. One panther (#47), whose death this fiscal year was originally thought to be due to intraspecific aggression, actually died from complications due to a congenital heart defect (cardiac atrial septal defect) exacerbated by the stress of intraspecific aggression. Necropsies were performed on each dead panther. Some necropsies were incomplete due to decomposition of the carcass. Tissue samples were stored at the GFC Wildlife Research Laboratory, Gainesville. Panther pelts and skeletons were deposited at the Florida Museum of Natural History, Gainesville. Some organ weights from dead panthers are shown in Table 3.

### Physical Condition and Body Weights

Two genotypes of Florida panthers are recognized: "authentic panthers", having historic Florida panther ancestry, and those with evidence of South American puma ancestry, designated as "Piper". The physical condition, body weight, and health of these 2 genotypes are sometimes compared.

All panthers examined over this 4-year period were generally in good health at time of capture. Some had rough and dry hair coats, and most vaginal papillomas observed at earlier captures had receded during the previous 2 years (#9 and #38 have minor vaginal hyperplasia). No oral lesions except cysts of Sarcocystis sp. found in the tongue of most panthers were noted. Body weights of authentic panthers captured from 1983 through 1994 are depicted by sex and capture location

(north of Alligator Alley versus south) in Figure 2. Mean body weights of both male (56.97 Kg) and female (39.42 Kg) panthers living north of the Alley are significantly greater than males (52.30 Kg) and females (34.47 Kg) living south of the Alley. This is possibly due to better nutrition, i.e., higher deer densities found north of the Alley as compared to south. Mean body weights by sex and age of authentic panthers captured from 1983 through 1994 are depicted in Figure 3.

#### Hematological and Serum Chemistry Values

Serum chemistries and complete blood counts revealed no significant abnormal values except for levels of enzymes associated with stress due to immobilization and capture.

Hematological data for all panthers captured from 1983 through 1994 is presently being analyzed and will not be reported until the FY 94/95 annual report.

Hematological values, before and after anthelmintic treatment, for kitten #208, which was naturally infected with the hookworm Ancylostoma pluriidentatum, are depicted in Table 4. For comparison, apparently normal hematological values from other felids are also shown in Table 4. Infection with A. pleuridentatum at a very early age (<2 mo.) apparently causes anemia.

#### Sero-Epidemiology

Serologic evidence collected from un-vaccinated Florida panthers captured March 1991 through February 1994, indicates that they were exposed to or were infected with several potentially pathogenic agents (Table 5): feline calicivirus (FCV), 31%; feline panleukopenia virus (FPV), 23%; feline enteric coronavirus/feline infectious peritonitis (FECV/FIP), 46%; feline immunodeficiency virus/puma lentivirus (FIV/PLV), 9% (1994 data); and feline syncytia-forming virus (FeSFV), 59% (1987-1992 data). However, they were serologically negative for Brucella sp., Toxoplasma gondii, feline leukemia virus, and pseudorabies virus (PRV).

Some panthers had been previously positive ( $\leq 8.3\%$  of those tested) for Toxoplasma gondii, depending on which laboratory conducted the tests. Panthers previously tested for titers to encephalomyocarditis virus were negative. The major change that can be found over the 4-year period is the increase in number of panthers that were serologically positive for FVR, 0% in FY 90-91, 91-92, and 92-93, but 38% in FY 92-93 and 93-94.

The reason for the increase in positive titers to FRV is unknown. No clinical signs have been observed that can be attributed to this disease. Monitoring efforts will continue during the next study period.

Feline Panleukopenia Virus.--Feline panleukopenia, also known as viral enteritis or feline distemper, is caused by the extremely environmentally resistant FPV. Feline panleukopenia is a highly contagious, devastating, viral disease causing mortality chiefly in young kittens, especially accompanying stress or co-infection with other agents or parasites (Scott 1990).

Neonatal kittens were first handled in 1992. This afforded an opportunity to examine levels of maternally derived antibody in the kittens, and, in the case of 1 kitten brought into captivity, the declining titers could be tracked over time. Additionally, 3 captive born cougars (F. c.) were also serially sampled to determine the rate of loss of maternal antibody. All neonatal kittens (less than 4 weeks of age) tested demonstrated passive transfer of maternal antibodies (>1:10) adequate to provide protection from FPV in the early weeks of life. The exact level of maternal antibody at the time of birth was not known for these litters, however, like the domestic cat, antibody levels in all serially bled cougar kittens declined over time.

At present, we do not have enough data to determine the half-life for panleukopenia antibodies in panther kittens. After maternal antibodies subside, kittens become vulnerable to infection with this virus. This may be a very critical time for survival of wild panther kittens. The number of early mortalities that occur in the wild panther population due to FPV is unknown but should be seriously examined. Van Rensburg et al. (1987) describes a significant negative impact of FPV on an island population of domestic cats. The primary effects following the introduction of FPV were lowered fecundity, a dramatic decrease in the population density, and adverse changes in the age structure. Despite an 82% decline in the population over a 5-yr period (from 3,409 down to 615 cats), no clinically affected animals were documented. Further, the authors believe that there was an annual epizootic of FPV among the susceptible young kittens at the time the maternal antibody waned. Perhaps wild, unvaccinated panther kittens are likewise succumbing to FPV.

A 64% decline in the prevalence of FPV has occurred in non-vaccinated non-neonates since the 1981-90 period. The level was 10% in FY 90-91 and 0% in FY 91-92. But, only 3 non-vaccinated panthers were captured in FY 92-93 and FY 93-94; all of which were positive for FPV. One cat was a neonate, possibly with maternal antibodies, 1 was a juvenile, and 1 was a breeding female. Therefore, sample size precludes a comparison of prevalence of titers between 1990 through 1994 and earlier reports.

Feline Calicivirus.--The prevalence of FCV antibodies (31% for 1991-94) is fairly comparable to levels observed in the past (38% for 1981-90). In 1991, FCV was cultured from a wild-caught kitten (#204) with oral and nasal lesions and from her asymptomatic mother (#31). FCV is thought to be endemic in the south Florida panther population. However, viral isolations for FCV and FVR were attempted on all suspicious lesions noted on panthers in the past and no virus was recovered. Like FPV, passive transfer of FCV antibodies was observed in the aforementioned 3 panther neonates.

Feline Immunodeficiency Virus/Puma lente virus.--Serologic evidence of FIV/PLV infection was detected in 14 of 38 (37%) panthers examined during 1978-1991 (qualitative assays, no titers determined) (Roelke et al. 1993). Six of 19 (31.6%) were positive in 1992 (Roelke 1992), and only 1 of 11 (9%) (Table 5) was positive in 1994. Of the 6 panthers positive in 1992, 1 remained positive, 3 were equivocal, and 2 were not re-tested in 1994.

FIV can cause severe immunosuppression and disease in the domestic cat. At this point, it is not clear what the true significance of the infection is to Florida panthers. In order to understand more about the pathogenicity and routes of transmission of FIV among panthers, a collaborative research effort was initiated with Dr. Robert Olmstead of the National Institutes of Health. Efforts have resulted in the isolation of 4 different viral strains of a FIV-like agent from the panther. Olmstead's DNA sequencing of this agent indicates that the panther virus isolates are similar to each other but are distinctly different from the previously described domestic cat FIV isolates. This new virus has been named puma lente virus (PLV), and analysis of the evolutionary distance between it and FIV suggest that it may have been associated with panthers and cougars for thousands of years.

Until it is proven otherwise, the presence of PLV must be viewed in light of what is known for domestic cats; the incubation period is long (years) and ultimately results in non-specific immunosuppression and death (Pedersen et al. 1988, Yamamoto et al. 1989). Thus, in order to limit transmission of PLV to seronegative captive panthers and other felid species, current management policy requires that seropositive animals be handled separately from seronegative animals when they

come into captivity. Transmission of this virus does not appear to occur readily. Several kittens born to seropositive mothers came into captivity (at 6 months of age) and remained negative over the following year. The only indication of maternally transmitted infection occurred in 2 litters where older kittens (>1 yr) were still with their mother and had contracted the identical virus as their mother by 16 and 22 months of age. Even with an estimated 25-30% of the wild population infected with PLV, the advantage of a low rate of maternal transmission may make it possible to create a PLV-free captive population by judiciously removing kittens from seropositive dams by 6 months of age.

Feline Enteric Coronavirus/Feline Infectious Peritonitis.--The prevalence of FECV/FIP was 46% of 13 panthers examined (IFA test) from 1991 through 1994. Since the antibody may be cross-reacting to other coronaviruses of canids (canine coronavirus) and swine (transmissible gastroenteritis virus), the presence of antibody titers typically found in the Florida panther may indicate exposure by way of food ingestion rather than natural infection with a feline coronavirus. The impact of this disease on the Florida panther population is unknown.

Feline Syncytia-forming Virus.--Approximately 59% (N=54) of all the Florida panthers that have been tested from 1987-1992 were found to be infected with FeSFV (Table 6). This virus is quite common in healthy as well as sick domestic cats (Gaskin and Gillespie 1973) and has been reported in conjunction with diseases such as FIP (Scott 1971), chronic progressive polyarthritis (Pedersen et al. 1980), and respiratory infections (Ellis 1985). However, a pathogenic role has yet to be established.

At this time the recognized importance of this virus to the Florida panther is that it destroys the fibroblast tissue cultures of infected individuals. These primary skin cultures are important for growing cellular material for DNA isolation used in genetic studies. Once the skin biopsy material is away from the host's virus-neutralizing antibodies, the virus is able to grow unchecked, which results in the destruction of the desired cells and the inability to maintain the cell cultures. This destruction of the cultures is quite costly in lost time, effort, and transportation costs and necessitates repetition of the surgical procedure to obtain another sample, which is often not possible with free-ranging animals.

Pseudorabies Virus.--Although no antibody titers against PRV have been found in Florida panthers (N=>30), it is believed that once a panther is infected with this virus, death is immediate, giving no chance for an immune response. The death of 1 panther (#29) in 1992 was attributed to acute infection with the PRV (Glass et al. 1994). PRV was isolated in cell culture from the brain tissue of this 3.5-year-old male Florida panther (#29). The virus was not isolated from other tissue collected at necropsy. Based upon a nested polymerase chain reaction, the virus was determined to have the classical wild-type virulent genotype, glycoprotein I<sup>+</sup>(gI<sup>+</sup>) and thymidine kinase<sup>+</sup>(TK<sup>+</sup>) (Glass et al. 1994).

The impact of pseudorabies on the panther population is unknown. However, since few un-explained deaths are found in radio-collared panthers, death due to pseudorabies appears to be minimal. However, the potential for catastrophic mortality due to pseudorabies exists. Since feral swine (Sus scrofa) carry the pseudorabies virus, and feral swine are a major prey for the panther, the potential for infection is great. But it is not known what factors are involved in allowing expression of the pseudorabies virus by feral swine. If environmental stressors are involved and these conditions exist, then viral shedding by swine could dramatically increase, causing large numbers of panther mortalities. Until such time that viral shedding and disease transmission is understood, there is cause for concern for the Florida panther.

## Parasites

Results of the parasite study are shown in Table 7. Fresh fecal samples were collected from 20 free-ranging panthers during this period. Eggs of endoparasites, primarily of the genera Alaria sp., Spirometra sp., Ancylostoma sp., were found in 85%, 60%, and 85%, respectively, of panthers examined. Nine panthers necropsied this period were examined for endoparasites. Ancylostoma pluridentatum was the most prevalent parasite (100%) of the panthers examined. Considering parasites and eggs of parasites, panthers were positive for 2 trematodes, 2 cestodes, 6 nematodes, 1 acanthocephalan, and 1 protozoan. No significant changes were noted in species, prevalence, or intensity of parasites found as compared to previous studies (Forrester et al. 1985). However, mean intensity of A. pluridentatum increased from 254 worms to 1003, and maximum numbers in any single panther increased from 744 to 2700 worms.

Ancylostoma pluridentatum appears to cause emaciation and anemia in young Florida panther kittens <2 months of age. Clinical signs attributed to natural infection with A. pluridentatum in a wild-born, captive panther kitten (#208) were poor body condition, lethargy, and below normal red blood cell numbers, hemoglobin concentration, and packed cell volume, and elevated eosinophil numbers. We suspect that kittens <2 months of age infected with even low numbers of A. pluridentatum may show signs of anemia suggestive of hookworm disease (Dunbar et al. In Press). Table 4 depicts the hematological values of kitten #208 before and after anthelmintic treatment for A. pluridentatum and a comparison of blood values to apparently normal felids. We recommend treating young panthers infected with A. pluridentatum with pyrantel pamoate (20 mg/Kg) per os and ivermectin (0.20 mg/Kg) per os. No clinical signs, even with heavy infections with A. pluridentatum, have been observed in adult Florida panthers, but treatment is advised.

Although collection and identification of ectoparasites ceased in 1992, much data from previous years were analyzed and summarized by Dr. Ellis Greiner of the University of Florida. The tables and figures concerning ticks on panthers in this report were constructed by Dr. Greiner.

Five species of ticks were collected from Florida panthers from 1984 through 1991: Ixodes scapularis, I. affinis, Dermacentor variabilis, Amblyoma americanum, and A. maculatum. The geographical distribution of these ticks in Florida is found in Figure 4. Tick prevalence on panthers by year is found in Figure 5, tick prevalence on panthers by year in Collier County is in Figure 6, and tick intensity on live panthers surveyed 1984 through 1991 is in Figure 7. Ixodes scapularis is the most prevalent tick found on Florida panthers in most years (75%) during 1984-1991, and I. affinis is consistently the least prevalent.

Three panthers, including 1 kitten (#208), examined within the last 2 years have experienced moderate to severe alopecia. The kitten was diagnosed with infestation of the mange mite, Notoedres cati. Due to similar clinical signs and response to treatment, we believe the 2 adult panthers were infested with the same parasite. Clinical signs resolved with multiple treatments of ivermectin given per os to the kitten and intramuscularly to the adults. The mite is possibly carried by bobcats (Felis rufus) in Florida.

Florida panthers continue to receive anthelmintic treatments during each capture, using praziquantel (Droncit, Haver) and ivermectin (Ivomec, Merk & Co.). However, some panthers are only captured every 2 years or greater; therefore, re-infection with endoparasites allows only minimal control of parasite infections.

## Mercury In The Free Ranging Florida Panther

Mercury contamination of largemouth bass (Micropterus salmoides) and alligators (Alligator mississippiensis) in various watersheds throughout the state of Florida has recently been identified as a human health issue (Delany et al. 1988, Hord et al. 1990). The entire Everglades watershed in southern Florida has been closed to alligator hunting, and health advisories have been issued to curtail the consumption of bass due to elevated mercury residues in the flesh of these species.

Mercury is accumulated and concentrated in the aquatic food chain, and the highest levels occur in the longer lived species at the upper trophic levels (Clarkson and Marsh 1982, Eisler 1987). The Florida panther is a top terrestrial mammalian carnivore in the southern Florida ecosystem. White-tailed deer (Odocoileus virginianus) and feral hogs are the preferred prey, but, in some areas, panthers also consume small mammals, e.g., raccoon (Procyon lotor), armadillo (Dasypus novemcinctus), and rabbit (Sylvilagus sp.), as a significant part of their diet (Maehr et al. 1990, Roelke et al. 1986).

We first became aware of mercury contamination in the Florida panther when a 3 to 4-year-old adult, radio-instrumented female (#27) died in the ENP in the summer of 1989. After a thorough pathologic examination culminating in a contaminants survey, the most probable cause of death was determined to be mercury toxicosis (USFWS 1989, Roelke 1990). The liver of this panther contained 110 parts per million (ppm, wet wt.) of mercury. For comparison, death due to mercury toxicosis was reported in feral domestic cats at Minamata Bay, Japan, with liver concentrations of 37-145 ppm (Takeuchi et al. 1977).

Distribution of Mercury in Panther Tissues.--As a result of the concern raised by the death of Florida panther #27, mercury concentrations were determined in archived and freshly collected tissue samples from 52 free-ranging panthers collected opportunistically between 1978 and 1991. The pattern of mercury distribution by geographical location for all panther tissues is very similar. Data from blood and hair is more extensive than for other internal body organs, therefore, these two tissues were used to discuss the geographic distribution of mercury. Mean levels of mercury in blood and hair were highest in panthers from the eastern portion of the range, particularly Shark River Slough (hair=55.532 ppm and blood=1.986 ppm), and lowest among those living to the north of Alligator Alley (NA) (hair=1.77 ppm and blood=0.089 ppm) (significantly different at  $p<0.1$ ). Panthers from Long Pine Key in Everglades National Park (ENP), Fakahatchee Strand State Preserve (FSSP), and Raccoon Point (eastern Big Cypress National Preserve - BCNP) had hair and blood mercury levels between these two extremes. Panthers from NA had significantly lower hair and blood mercury than did panthers from other areas ( $p<0.01$ ).

In the early part of this study (1983-1986), panthers living in the FSSP were generally underweight and anemic, had poor reproductive success, and consumed primarily raccoons and armadillos (Roelke 1986). At the time, the poor condition was considered to be primarily a nutritional problem. This study suggests that those panthers were also contaminated with elevated levels of mercury. The poor physical condition of the panthers and a very low deer density in the FSSP led to

the initiation of management actions in the fall of 1987 designed to increase ungulate density and availability to panthers. These actions included implementation of fire as a habitat management tool; creation of experimental food-plots, salt-licks, and feeders for deer; closure of the area to public hunting; and enhancement of law enforcement efforts to curtail illegal killing of deer.

There has been a decline in mercury levels in panthers in FSSP coincidental with the above actions. There was a significant difference ( $p < 0.001$ ) in mean ( $\bar{x} \pm 1$  S.E.) blood mercury values between the FSSP and NA prior to 1987 (FSSP=0.785 (1.17) and NA=0.058 (1.64)); however, the means (FSSP=0.141 (1.39) and NA=0.098 (1.16) after 1987 were not significantly different. Furthermore, there was no significant difference in either blood or hair values between the two time periods within the NA, while a significant difference ( $p < 0.005$ ) in the level of mercury in both tissues did exist within the FSSP. These data suggest that deer and hog abundance and availability is a major factor influencing diet composition and subsequent levels of mercury in panthers in the FSSP. It has now been determined that all panthers living in the FSSP during the early 1980's had higher mercury levels than the individuals living there since.

Source of Mercury for the Panther.--The most probable source of mercury contamination in panthers is via the food chain. The panthers north of Alligator Alley had the lowest levels of mercury in all tissues examined and fed primarily on white-tailed deer and feral hog. Although nothing is known about tissue mercury levels in the hog, mercury levels were less than 1.0 ppm in liver samples from approximately 100 southern Florida deer (Sundlof and Forrester, UF, pers. comm.). Panthers with the highest levels of tissue mercury were those which regularly consumed non-ungulates. These prey included raccoons, armadillos, rabbits, and alligators. Panther #27 fed only on small prey during the 15 months that she was monitored prior to her death. Of her 12 kills recovered, all were raccoons (O. Bass, ENP, pers. comm.). Other panthers with elevated mercury levels are known to have fed less frequently on these non-ungulate prey, yet had quite high levels. This is probably due to the level of mercury in the respective prey item; even the infrequent consumption of heavily contaminated prey could result in high levels of mercury in the panther.

Questions about mercury levels in prey species led to an examination of raccoons and other carnivores in the southern Florida ecosystem. As in the panther, the highest tissue mercury levels occurred in areas associated with the Shark River Slough drainage.

Significant levels of mercury were found in the raccoon, otter, and alligator but not in the bobcat (except for 1 animal from Shark River Slough in 1991). The bobcat and panther live in the same habitat, drink the same water, groom themselves in a similar fashion yet have distinctly different food habits (the bobcat consumes primarily cotton rats and marsh rabbits while panthers will consume aquatic web carnivores) (Maehr and Brady 1986, Maehr et al. 1990, Roelke et al. 1986). If the panther's mercury contamination was due to a direct air or water borne source, then bobcats should be as contaminated as the panther. That they are not implicates the panther's food source as the primary route of exposure.

Mercury levels found in the muscle of raccoons from the greater Shark River Slough environs were well above 1.0 ppm (range 1.01 ppm to 1.80 ppm) and were significantly higher ( $P < 0.05$ ) than in raccoons from other areas examined. Human health advisories recommend that when flesh is contaminated with mercury at the 1.0 ppm level or greater it should not be

eaten more than once a month and that consumption should be stopped at the 1.5 ppm level. Obviously, panthers in the Shark River Slough area, feeding on raccoons daily or less frequently over long periods of time, may develop considerable body burdens of mercury.

The significance of mercury and the potential long term untoward effects in certain sectors of the wild population should not be minimized. Increased mortality and lowered reproductive success due to chronic exposure to mercury is probably contributing to the lower than expected population densities of panthers in portions of their range and is likely hastening the extinction of this endangered mammal.

#### Major Conclusions From Mercury Report.--

As noted above, an intensive retrospective study of mercury levels in archived tissue samples was conducted following the discovery of mercury as a significant contaminant in the death of Florida panther #27. This study was completed in March 1992 (Roelke et al. 1992), and the most important conclusions and recommendations of that report are summarized below.

1. Mercury contamination of Florida panthers has been documented, with the highest levels observed in animals examined in the southern Everglades portion of their range and lowest levels observed in animals inhabiting the more upland, pine flatwood, and mixed hardwood hammock habitats in the northwest portion of their range.
2. Mercury appears to bio-accumulate through aquatic, carnivorous links in the panther food web; the primary dietary source of mercury for panthers appears to be raccoons.
3. Mercury contamination in raccoons is documented to occur in a distributional pattern similar to that of Florida panthers with highest body burdens observed from the southern Everglades region.
4. Elevated body burdens of mercury have been documented in Florida panthers whose diets appeared to comprise a higher relative occurrence of raccoons and/or included raccoons from areas likely to be more highly contaminated with mercury.
5. Mercury contamination may be contributing to lower reproductive performance of panthers in those areas where elevated body burdens of mercury have been documented.
6. Chronic exposure of Florida panthers to mercury appears to be compromising their relative health and possibly productivity, especially in Everglades portions of their ranges; mercury must be considered a threat to the continuing existence of this already endangered taxon.

7. Management practices that encourage greater availability of ungulate prey (i.e., white-tailed deer) may result in reduced dietary exposure to mercury of certain panthers.
8. Although various management actions may be taken to reduce exposure of certain panthers to mercury (i.e., prey management, land management, removal of individual panthers from contaminated areas) the ultimate need is to understand the environmental processes responsible for the availability of mercury in the areas and aquatic food web affecting panthers.

Mercury Analysis (1991-1994).--Table 8 depicts mercury levels in whole blood (ppm, wet weight) over time (1987-1992) for panthers captured in FY 91-92. In most instances, mercury levels have continued to decline. Florida panther #16 has variable mercury blood levels since he may, at times, reside in the ENP, where mercury levels in raccoons have been reported as high. Table 9 depicts mercury levels in hair (ppm, dry weight) of panthers during the same time period. These results are variable. Results for samples collected for mercury analysis in 1993-94 are pending.

In general, mercury levels have declined over the past few years for most panthers. However, no panthers presently inhabit the ENP, where mercury levels were found to be high in raccoons. Also, deer densities have increased in most areas of panther habitat, thus reducing the amount of mercury contaminated raccoons in the panther's diet. Studies on mercury levels in raccoons are being conducted by the U.S. Fish and Wildlife Service. Results are pending.

#### Nutrition

Some basic nutritional data was collected over the past 4 years, but was limited in nature and scope. Table 10 depicts some serum levels of selected minerals from free-ranging panthers. Most values appeared to be within normal ranges.

A study was initiated this fiscal year to determine the serum and liver concentrations of vitamin A (retinol and retinyl esters) in free-ranging panthers. This study was conducted to determine if hypovitaminosis A was occurring in panthers since this disease has been documented to cause congenital defects in other felids, such as cardiac atrial septal defects, low sperm volume, abnormal sperm, rough hair coat, low reproductive performance, and epithelial hyperplasia as are found in Florida panthers.

From November 1984 through March 1994, 54 blood samples were collected from 23 male and 22 female free-ranging (N=36) and captive (N=9) Florida panthers captured or immobilized for either radio-instrumentation or physical examination. Free-ranging panthers were captured on 1 or more occasions in southern Florida in the ENP; BCNP, including Bear Island (BI), Corn Dance (CD), and Stair Step (SS) units, and recently acquired public lands (APL) north of Interstate Highway 75 (I-75); Florida Panther National Wildlife Refuge (FPNWR); FSSP; Corbett Wildlife Management Area (CWMA); Seminole Indian Reservation (SIR); and privately owned lands (PL) in Collier and Hendry Counties. These areas were grouped into regions 1 (BI, FPNWR, SIR, APL, CWMA, and FSSP after 1988) and 2 (ENP, CD, SS, and FSSP before 1989) for comparison based on prey selection.

All captive panthers were wild-born and either hand-caught as kittens (N=3) or captured and immobilized as juveniles (N=3) or adults (N=3). Captive panthers were held in pens at three separate locations in Florida. Captive panther kittens less than four weeks of age were fed a milk replacer (KMR<sup>®</sup>, Pet Ag, Incorporated, Hampshire, Illinois) and weaned on a mixture of milk replacer and a moist feline diet (Nebraska Feline Brand<sup>®</sup>, Central Nebraska Packing, Incorporated, North Platte, Nebraska). Juvenile and adult panthers were fed the moist feline diet and, occasionally, portions of white-tailed deer carcasses. Duration of captivity prior to collection of samples used in this study ranged from 2 weeks to 8 years.

Blood was collected from both free-ranging and captive panthers by venipuncture of the cephalic, saphenous (medial and lateral), or jugular veins in serum separator tubes and allowed to clot. Blood was centrifuged at 2000 rpm for 10 to 15 minutes, and the serum was frozen at  $\leq -10\text{C}$  until analyzed. For comparison, some panthers were sampled as both free-ranging and captive.

Liver was collected at necropsy from each of 22 fresh or thawed panther carcasses and frozen at  $\leq -10\text{C}$  until analyzed. Necropsied panthers (17 male and 5 female) include those killed by vehicles (N=9) or other panthers (N=3), or that died due to cardiac defects (N=2), acute infectious diseases (N=2), mercury toxicosis (N=1), or unknown causes (N=5). All liver samples collected in this study were obtained from animals believed to be free of chronic or specific diseases that may have significantly affected vitamin A levels. Some panthers were sampled as live animals (serum analysis) and again at necropsy (liver analysis). For comparison to other free-ranging *F. concolor* subspecies in different habitats, livers were collected at necropsy from 2 juvenile and 1 adult free-ranging cougars (*F. c. stanleyana*) from Texas and 3 adult females (*F. c. oregonensis*) collected from hunter-kills from Washington. One cougar from Texas died of unknown causes during transport to Florida. Two died from traumatic injuries while free-ranging in northern Florida. These animals were being used in a study concerning possible Florida panther re-introductions. Livers collected from Texas cougars and Washington cougars were handled and stored similar to those from Florida panthers.

Serum and liver samples were analyzed for retinol and retinyl esters (vitamin A) at the Animal Health Diagnostic Laboratory, Michigan State University, East Lansing, Michigan, by normal phase isochromatic high speed liquid chromatography as described by Stowe (1982) and Dennison and Kirk (1979).

Age of panthers was estimated by tooth wear and facial, body, and pelage characteristics (Shaw 1979) or was determined from known birth dates. Ages ranged from 2 weeks to > 12 years.

Panther ages were grouped into seven classes for comparison: nursing kitten (0 to < 4 weeks), weaned kitten (4 wk to < 6 mo), juvenile (6 to < 18 mo), subadult (18 to < 24 mo), young adult (2 to 4 yr), mature adult (4 to < 8 yr), and older adult ( $\geq$  8 yr). Age classes were also grouped into pre-reproductive (4 wk to 24 mo) and reproductive ages ( $\geq$  24 mo).

Sample collection dates were grouped into 2 time periods, before and after January 1, 1989, to coincide with changes in ungulate prey densities.

Genotype of panthers was based on analysis of mitochondrial DNA and nuclear markers (O'Brien et al. 1990). Two genotypes were delineated and used for comparison: "authentic" Florida panthers and those with evidence of South American puma ancestry, designated as "Piper".

All values were log transformed prior to statistical analyses. All computations were performed using the SAS system (SAS Institute, Inc. 1990).

Retinol, retinyl esters, and total vitamin A levels in serum and liver from free-ranging Florida panthers were examined using analysis of variance (ANOVA) for effects of sex (male, female), age (juveniles, adult), capture location (regions 1 and 2), year of capture (1988 or before and after 1988), genotype (authentic or Piper), and prey selection (ungulate, non-ungulate).

Analysis of Variance also was used to make comparisons of vitamin A serum concentration between free-ranging panthers and captive panthers. The ANOVA model contained terms for status (captive or free-ranging) and as many factors that were found to significantly affect serum vitamin A concentrations in free-ranging panthers, and interactions of status with those factors as could be supported by the data. The free-ranging panther data included in the analysis was from only those panthers that were comparable to the captive panthers based on the previous ANOVA.

Comparisons of retinol, retinyl esters, and total vitamin A liver concentrations between free-ranging Florida panthers and captive panthers and between free-ranging Florida panthers and cougars were performed using ANOVA as described previously, with the exception that if the model contained only terms for status, then a *t*-test was performed. Overall mean values for serum retinol and total vitamin A from free-ranging Florida panthers were  $756.5 \pm 34.6$  SE pmol/ml (N=47) and  $1606.7 \pm 140.2$  SE pmol/ml (N=47), respectively. Mean values were found to have depended on sex (Figure 8), genotype (Figure 9), year of capture (Figure 10), and primary prey selection (Figure 11). Mean serum values for free-ranging female panthers were significantly higher (retinol,  $P=0.040$ ; vitamin A,  $P=0.0717$ ) than for males. Mean serum values for free-ranging Piper genotypes were significantly higher (retinol,  $P=0.027$ ; vitamin A,  $P=0.014$ ) than for authentic genotypes. Mean serum values for free-ranging panthers captured during 1984 through 1988 were significantly lower (retinol;  $P=0.011$ ; vitamin A,  $P=0.009$ ) than values for panthers captured during 1989 through 1994. Finally, free-ranging panthers primarily preying on wild ungulates had significantly higher (retinol,  $P=0.047$ ; vitamin A,  $P=0.033$ ) mean values compared to those primarily preying on non-ungulate prey. There were no significant differences ( $P>0.05$ ) in mean values for serum retinol or total vitamin A between free-ranging panthers captured from regions 1 and 2 or among various age classes. However, values from nursing kittens were not compared statistically to other age classes. Their values were very low and were dependent upon vitamin A levels in the milk of the dam.

Mean values of retinyl esters in the serum of free-ranging panthers ( $850.2 \pm 137.6$  SE) (N=47) were found to depend only on year of capture. Significantly lower ( $P=0.004$ ) values were found for those captured during 1984 through 1988 than for those captured during 1989 through 1994. However, values for nursing kittens were not compared statistically to other age classes for reasons previously mentioned, but values were extremely high.

Mean values for retinol, retinyl esters, and total vitamin A in the liver of free-ranging panthers were found to have depended only on age class (Figure 12). Adult panthers had significantly higher (retinol,  $P=0.013$ ; retinyl esters,  $P<0.0001$ ; vitamin A,  $P<0.0001$ ) values compared to juveniles. Differences in liver values for sex genotype, date of capture, prey selection, and region were not significant ( $P>0.05$ ).

Mean serum retinol values for captive panthers were significantly higher ( $P=0.029$ ) than for free-ranging panthers captured during 1984 through 1988. No other significant differences in mean serum retinol, retinyl esters, total vitamin A, or any vitamin A liver values were found among the various categories between captive and free-ranging panthers.

Mean liver values for retinyl esters and total vitamin A for cougars (from Texas and Washington) were significantly higher ( $P<0.05$ ) compared to mean values for juvenile free-ranging Florida panthers. However, no significant ( $P>0.05$ ) differences in liver values for retinol, retinyl esters, or total vitamin A were found between western cougars and free-ranging adult Florida panthers.

Although no vitamin A deficiency was found in Florida panthers, only very limited normal values data were available for comparison. However, the data is interesting since significantly lower vitamin A levels were found in young panthers less than 2 years of age and panthers preying on non-ungulates. Therefore, the potential for vitamin deficiency exists in panthers living in habitats with low deer density. Also, if a deficiency should occur, young panthers, dependent upon the dam for food, would succumb to the effects of hypovitaminosis before adult panthers, resulting in low recruitment to the population rather than adult mortality. Therefore, the maintenance of adequate deer and feral hog densities rather than more prey biomass (including non-ungulates) is again suggested as essential to maintaining a viable panther population.

Nutritional data from major prey species, as well as from the panthers, is important to understanding the health of Florida panthers. Nutritional studies will continue and possibly be expanded during the next study period.

### Hormone Analysis

Thyroxine.--Thyroid hormone ( $T_4$ ) affects the rate of metabolism, growth, and development of animals. Hyperthyroidism has been reported in the Florida panther (Roelke 1990). The major clinical signs of hyperthyroidism in domestic cats are weight loss, polyphagia, hyperactivity, polyuria, polydipsia (excessive thirst), hyperdefecation with marked steatorrhea (excess fat in the feces), heat intolerance, panting, and muscle tremors. Mild to moderate cardiomegaly (enlargement of the heart) may develop, and there is tachycardia, ventricular hypertrophy, and increased voltage of R waves. Cardiovascular disease may produce a hypertrophic congestive heart failure with plural effusion.

Normal  $T_4$  values in domestic cats range from 1.4 - 4.0 ug/dl. Hyperthyroid cats average 8.0 - 24.0 ug/dl. Clinical pathology in hyperthyroid cats causes an increased packed cell volume (PCV) in 45 percent of the cases, eosinopenia in 40 percent of the cases and erythrocytosis in 20 percent of the cases.

Mean ( $\pm$ SE) serum  $T_4$  values for free-ranging Florida panthers (N-41) by age class, captured 1983 through 1994 are shown in Figure 13.  $T_4$  values ranged from 1.17 to 7.42 ug/dl. The only panther that was close to the level of hyperthyroidism was #08, an old female, with values ranging from 2.61 ug/dl to 7.42 ug/dl over a 4 year time period. The second highest  $T_4$  value was from #29, a young male, with a value of 5.05 ug/dl. Although thyroxine ( $T_4$ ) values appeared to be higher in younger (<1.5 yr) and older (>8 yr) age classes, no apparent abnormal values indicating a hyperthyroid condition was found to exist in 41 panthers examined over an 11 year period. However,  $T_4$  values will continue to be monitored in Florida panthers.

Progesterone.--Serum progesterone levels in felids is variable depending upon the stage of the reproductive cycle. Correlation of pregnancy or pseudopregnancy with serum progesterone levels has been documented in domestic cats, pumas, lions, and other felids. We evaluated serum progesterone levels in 21 Florida panthers with known reproductive stage. Known pregnant female progesterone levels ranged from 5.2 ng/ml to 28.8 ng/ml ( $\bar{x}$ =15.8 ng/ml) compared to non-pregnant females which ranged from 0.7 ng/ml to 2.5 ng/ml. One female panther examined by laparoscopy was found to be non-pregnant but showed clinical signs of pregnancy (pseudopregnant) and had a progesterone level of 12.5 ng/ml.

We believe that pregnancy (or pseudopregnancy) can be determined by evaluation of serum progesterone values. Panthers having progesterone values exceeding 5 ng/ml can be classified as pregnant or pseudopregnant. Further refinement of the data will continue during the next study segment.

Estrogen/Testosterone.--There appears to be evidence that the panther population is displaying an increasing number of multiple morphologic and physiologic abnormalities that are likely a consequence of recent close inbreeding. However, the cause of these abnormalities is based on published reports relative to consequences of inbreeding in other species. Published data also exist that support causes other than genetic that may lead to similar abnormalities in other species. We evaluated one other potential cause of some of these abnormalities.

The Florida panther is likely exposed through the food chain to a variety of pesticides and other compounds, including polychlorinated biphenyls and polychlorinated dibenzo-p-dioxins known to disrupt the endocrine system. Exposure to these chemicals, some of which cause estrogen-like signs in animals, has resulted not only in thyroid disorders but also in deformed spermatozoa, decreased sperm count, and cryptorchidism in other species. In this initial study, we investigated the possibility that exposure to estrogenic pollutants as evidenced by skewed estradiol/testosterone (ET) ratios, was the cause of cryptorchidism in male panthers.

We examined sera from 24 panthers for estradiol and testosterone levels and compared these values to those of normal and cryptorchid panthers. We found no significant difference ( $F_{2, 21} = 1.13$ ) between mean estradiol levels (228 pg/ml  $\pm$  48 SE) of normal male panthers (N=5) and levels (267 pg/ml  $\pm$  32 SE) of cryptorchid males (N=14) (Table 11). However, we did find unusually high estradiol levels (545-670 pg/ml) in three males and skewed ET ratios in some males and females.

In conclusion, it is possible that some panthers had been exposed to estrogenic chemicals, however, we could not demonstrate any correlation between such exposure and cryptorchidism. Future studies are planned to determine normal estradiol levels and ET ratios in male and female panthers, possible pathways and degree of exposure to estrogenic compounds, and impacts, if any, of exposure.

#### Medical Management

Florida panther #205 underwent surgery in October 1992 to correct a cardiac anomaly and later died as a result of complications due to the anomaly and surgery. Florida panther #207 underwent surgery (orchiopexy) in August 1992 for bilateral cryptorchidism and, when evaluated in November 1993 was found to be sterile. Florida panther #204 was diagnosed with bacterial infection of the lung caused by Pseudomonas aeruginosa and underwent surgery (lobectomy) in August 1992 to remove a lung lobe. This panther is doing well in captivity at the present time. Florida panther #38 has not produced kittens, even though she was observed in the company of a fertile male panther (#42). She was captured and

temporarily placed in captivity in May 1993 for evaluation. Abdominal radiographs, ultrasound, vaginoscopy, and celiotomy with visual exam and palpation of the reproductive tract and biopsy and histological examination of the uterus revealed no abnormalities. It is believed this cat may have reproductive hormone abnormalities. After evaluation, she was returned to the wild.

Upon capture this fiscal year, #19 was found to have a front leg and foot infection. She was treated at the capture site and released. I believe she is doing well at the present time. #51 was treated twice for mange infection this fiscal year. He has not been re-evaluated since the last treatment. #09 was found to be very thin at last capture this year, probably due to old age. No treatments were administered.

#### Population Genetics

Piper stock panthers are those having some South American puma ancestry as compared to "authentic" panthers which are considered to be of historic Florida panther ancestry. Most Piper panthers inhabited the Everglades National Park compared to authentic panthers which resided in areas north of Tamiami Trail (State Road 41). However, the panther population in ENP became non-existent in 1991. Currently there are only 4 Piper panthers known to exist (2 males, #16 and #42, and 2 females, #23 and #55) and inhabit the Raccoon Point unit in the Big Cypress National Preserve.

The authentic panther population continues to produce animals with congenital abnormalities, probably due to close inbreeding and genetic depression. For example, approximately 50% of all free-ranging panthers (67% in authentic panthers, excluding Piper stock animals) are cryptorchid. Cardiac atrial septal defects have been found in 4 panthers since the beginning of the recovery project. Dorsal neck and mid-dorsal thoracic cowlicks and kinked tails are found in most authentic panthers but in few Piper animals. A high percentage of abnormal sperm, low sperm motility, and low sperm volume are present in most male panthers.

Pedigrees of wild-born panthers, living and dead, are shown in Figure 14.

#### Population Status

The Florida panther population estimate remains at 30 to 50 adults. Table 2 gives information on the current status of the 18 radio-collared free-ranging panthers.

#### Reproductive And Genetic Research

Male Reproductive Traits.--During this period, a total of 13 male panthers were electro-ejaculated, and semen from 9 was cryopreserved. Seminal traits from these males were very similar and included low volume, low spermatozoa concentration, and poor sperm motility (Table 12) (Barone et al. 1991).

To appreciate how much usable sperm we have frozen from each of our males, the number of "AI units" is calculated. One AI unit is the estimated number of post-thaw, motile sperm needed for one artificial insemination procedure ( $5 \times 10^6$  motile sperm per ml). Table 12 lists the individual semen characteristics and quantities of frozen sperm from all males currently represented in the sperm bank. Even with the best quality ejaculates we have procured from male #16, there is

only enough sperm for 5 AI breeding attempts from him, and successful impregnation would probably not be greater than 50% (based on domestic cat research). A minimum of 6 AI units per male is recommended (David Wildt, National Zoom, pers. comm.), but all together, we now have only 13.7 AI units total from 11 males.

In addition to the seminal defects described above, the majority (67%) of the living authentic panther males have a testicular descent abnormality (cryptorchidism) usually affecting only one testicle. Two young males (#47 and #207) were found upon initial capture to be bilaterally cryptorchid. However, #47 had one testicle descend at a later time, so #207 is presently the only bilaterally cryptorchid male panther. Cryptorchidism has been documented in the domestic cat and various wild felid species but at very low frequencies. Our studies with E. concolor have documented only 2 other cryptorchid males in over 100 captive and wild non-coryi cougars examined. This anomaly can affect either testicle or both, and those retained are generally atrophied and sterile due to the high body temperature.

Female Reproduction.--The Everglades population of Florida panthers became non-existent in August 1991 with the death of the last known female panther. Thirty days of intensive hunting effort during the spring, 1992, capture season and some effort in 1993 yielded no evidence of uncollared panthers in the area. Only one male (#16), now living primarily in the southern BCNP, occasionally visits the ENP.

Unlike in the ENP, the reproductive demographics of the eastern BCNP has changed positively during this study period. Because of the physical absence of any males in the area, panther #42 was originally targeted for translocation to the eastern BCNP, but, in January, 1992, he made the move on his own, emigrating into the area inhabited by females #23 and #38. This young male, born in the ENP, came from the south, crossing State Road 41 to establish a new home range overlapping that of both of these females. During the two-year period prior to this, no males had been documented in that area, and the 2 females bore no young. However, #23 has produced at least 2 litters of kittens. The latest kitten (#55) has left her mother and may soon be breeding. #23 may have recently denned but telemetry data indicates she may have lost this litter. Two of her kittens (#207 and #210) were placed in the captive breeding program and are presently 2 years of age and doing well. Unfortunately, despite regular encounters between #42 and #38, no kittens have been documented. This female (#38) is presently considered to be sterile.

Also, panther #09, the resident female in the FSSP, has not produced another litter since the removal of 2 kittens in February, 1991, and is considered a non-breeding female due to old age. #32 has produced no kittens since May, 1992, #36 has not produced kittens since 1991, #56 produced 2 kittens in 1994, #48 produced 3 kittens in 1993, and #19 produced 3 kittens in 1994.

Of the 11 radio-collared females, 2 (#9 and #11) are old and possibly non-producers, 1 (#38) is sterile, 1 (#55) is presently too young to breed, 4 (#19, #23, #32, #36) are prime age breeders but 2 (#32 and #36) of which have low reproduction rates, and 3 (#48, #52, #56) are young productive breeders. Therefore, of 11 known panther females, only 5 are considered good reproductive animals. In this light, the viability of the Florida panther population must be considered extremely poor.

Assisted Reproductive Technology Research.--In addition to intensive efforts to monitor reproductive output of free ranging panthers, artificial reproductive technologies for application to the Florida panther recovery effort were investigated. This effort involved three major areas of study: 1) establishing guidelines for handling, storage, and cryopreservation of domestic cat and panther semen for artificial insemination and *in vitro* fertilization (IVF), including the study of *in vivo* sperm transport and the viability of frozen-thawed versus fresh sperm, 2) development of an atraumatic laparoscopic artificial insemination technique in domestic cats and in cougars, and 3) delineation of the timing and necessary doses of reproductive hormones to stimulate ovulation for oocyte harvest or insemination.

The progress in developing a successful semen handling and AI technique in domestic cats was rapid (Barone, Nat. Zoo., pers. comm.). Since 1990 over 100 domestic kittens were produced by AI with approximately a 50% success rate. Adaptation of these successful domestic cat techniques to the cougar commenced in August 1991. During the past, private cougar owners who were willing to participate in the AI project were identified. Fertility evaluations of available males were made to identify good quality sperm donors. AI procedures were performed in females following exogenous hormonal stimulation (using pregnant mare serum and human chorionic gonadotrophin). Previously selected sperm donors were used for these inseminations. All together, 28 cougars were immobilized 95 times. Twenty-one of these events involved insemination of females, and 1 successful pregnancy has been achieved thus far with a live kitten born at Octagon Wildlife Sanctuary on February 10, 1992. One AI of a Florida panther was attempted in June, 1992, but was unsuccessful.

Development of the techniques of *in vitro* fertilization was pursued along with the AI project. Oocytes from Florida panther #205 were collected in June, 1992, because of the concern that her progressive heart condition would limit her reproductive life span. Fifteen oocytes were incubated with semen from a Florida panther, and 1 embryo resulted. This embryo was transferred into a recipient cougar on 9 June 1992.

Successful development of AI techniques may allow primary use of fresh Florida panther semen in a variety of both captive and wild situations. Combining the techniques of assisted reproductive technology with natural breeding may become the most effective means for rapidly increasing the numbers of panthers in captivity and in the wild.

## CAPTIVE BREEDING PROJECT

### Initiation Of The Captive Breeding Project

Available Florida panther reproductive and demographic data were analyzed by the Captive Breeding Specialists Group of the International Union for the Conservation of Nature and a Viability Analysis and Species Survival Plan was produced (Seal and Lacy 1989). Seal and Lacy reported that the wild panther population was in decline at a rate of 6 - 10% per year, with a loss of genetic diversity of 3 - 7% per generation. Further, they predicted that, without intervention in the form of a captive breeding program, the subspecies would be extinct within 25-40 years due to genetic and demographic factors.

There are numerous morphologic indicators to suggest that the Florida panther has already experienced a notable degree of inbreeding and concomitant genetic loss. These include "kinked-tail" and mid-thoracic cowlick traits (Belden 1986,

Wilkins 1990), very low semen quality with an extremely high percentage of abnormal sperm (Barone et al. 1991), high frequency of cryptorchids, and congenital heart defects.

All of the above stress the importance of moving ahead quickly and aggressively with captive breeding efforts. The primary goals of this project are to secure, in captivity, genetic representation of all wild founder individuals, to stabilize the loss of genetic diversity due to inbreeding, and to increase the number of individuals as a hedge against catastrophes. We have not progressed very well with these tasks. In the 4 years that have lapsed since the decision to proceed with the program, 12 of 28 (43%) identified wild founders have died. Of these 12 dead founders, only 2 (16.6%) have genetic representation in captivity. The projected success and long range survival of the panther via the captive breeding program were based on the premise that we could maintain 90% of the existing genetic diversity found in the wild for 100 years (Seal and Lacy 1989). To date, this has not been achieved.

### Selection Of Zoological Institutions

Four different zoological institutions have received endangered species permits from the U.S. Fish and Wildlife Service to participate in the captive breeding effort: White Oak Plantation, Yulee, FL; Miami Metro Zoo, Miami, FL; Lowry Park Zoo, Tampa, FL; and Jacksonville Zoo, Jacksonville, FL. However, the Miami Metro Zoo facility was damaged by hurricane Andrew in 1992 and cannot participate in the captive panther program at this time.

As of 30 June 1994, 9 Florida panthers (5 males and 4 females; 6 authentic and 3 Piper stock) are held in captivity for the captive breeding program. However, 1 of the 5 males is sterile. One female (#21) is an 8 year old and soon may be too old. She also is positive for FIV. The status and location of the 9 captive panthers are shown in Table 13.

There is limited or no available space for housing additional panthers should the breeding program become a reality. Therefore, other suitable institutions, capable of housing endangered species such as the panther, should be contacted and their support solicited.

## **CONCLUSIONS**

From the biomedical perspective, the Florida panther is advancing rapidly toward extinction. If areas south of state road 41, can be considered suitable panther habitat, then the population is still not utilizing all available areas. Also, only 2 panthers presently inhabit the FSSP. It seems apparent that the panther population is attempting to expand its range only minimally. Recent evidence suggests that breeding age females are not producing at their potential in spite of increasing deer densities in most areas. The increase in prevalence of congenital abnormalities, such as lethal cardiac defects, suggest an increase in the deleterious effects of inbreeding and genetic depression. Chronic low level mercury toxicosis and possible exposure to environmental contaminants, including estrogenic chemicals, may be lowering reproductive potential. The apparent evidence of the harmful effects of the exotic hookworm, *A. pluriidentatum*, on young kittens may be causing morbidity and mortality, thereby further reducing reproduction rates. High mortality of panthers due to collisions with vehicles on state highways and county roads continue to reduce recruitment and population numbers. Continued loss of

suitable habitat on private lands presently utilized by panthers, is a major threat to the panther population. Without immediate and aggressive intervention, the Florida panther will undoubtedly reach extinction.

## RECOMMENDATIONS

- A. Aggressively manage for optimum deer densities within panther habitat in order to maintain an adequate and long term prey base.
- B. Investigate and proceed with the introduction of non-coryi animals (genetic restoration) into existing panther habitat in southern Florida to overcome the observed deleterious effects of inbreeding. However, the potential risks of this project must be evaluated. Introduced subspecies must be assessed for possible health risks to the panther population. Quarantine and complete biomedical testing should be performed to reduce any risks of diseases and parasites carried by the introduced animals that may be harmful to panthers. Also, an evaluation must be performed that will assess the degree of success of the genetic restoration project. F<sub>1</sub> generations should be evaluated and monitored for degree and prevalence of congenital abnormalities, immune system status, and disease and parasite resistance as compared to authentic panthers.
- C. Support the installation of highway underpasses to reduce highway mortality.
- D. Continue to investigate the effects, if any, of exposure to environmental contaminants, including estrogenic chemicals.
- E. Utilize the captive panthers to produce both authentic and authentic X non-coryi offspring to be used for further releases in new available habitats.
- F. Continue to monitor health, vaccinate, and treat, as needed, all free-ranging panthers at least every 2 years.
- G. Continue to locate and examine all available panther kittens at an early age, preferably 2 weeks of age, to assess health of kittens and reproductive success of females.

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Table 1. Data pertaining to Florida panther kittens handled or captured at den site through 30 June 1994.

Date	Kitten #	Age (days)	Sex	Weight (lbs.)	Location	Genotype	Dam	Sire <sup>1</sup>	Status
4/7/92	K1	14-21	M	2.75	ACQ	A	FP#40	FP#26	Unk
4/7/92	K2	14-21	M	2.56	ACQ	A	FP#40	FP#26	FP#54
6/6/92	FP#208	9-12	F	1.25	FPNWR	A	FP#32	FP#12	Captive
8/20/92	FP#209	8-10	F	1.56	CDU	P	FP#23	FP#42	Captive
8/20/92	FP#210	8-10	M	1.75	CDU	P	FP#23	FP#42	Captive
6/18/93	K3	20-21	F	4.11	ACQ	A	FP#40	FP#26	Unk
6/18/93	K4	20-21	F	3.81	ACQ	A	FP#40	FP#26	Unk
6/18/93	K5	20-21	M	4.11	ACQ	A	FP#40	FP#26	Unk
10/30/93	K6	7-8	M	1.38	BI	A	FP#48	FP#12 <sup>2</sup>	Unk
10/30/93	K7	7-8	F	1.61	BI	A	FP#48	FP#12 <sup>2</sup>	Unk
10/30/93	K8	7-8	F	1.38	BI	A	FP#48	FP#12 <sup>2</sup>	Unk
4/21/94	K9	14	M	1.63	ACQ	A	FP#56	Unk	Unk
4/21/94	K10	14	F	2.11	ACQ	A	FP#56	Unk	Unk
4/21/94	K11	14	M	2.06	ACQ	A	FP#56	Unk	Unk
5/17/94	K12	10-14	F	2.11	FPNWR	A	FP#19	FP#51 or FP#12 <sup>2</sup>	Unk
5/17/94	K13	10-14	F	2.00	FPNWR	A	FP#19	FP#51 or FP#12 <sup>2</sup>	Unk

<sup>1</sup>Inferred from radio-telemetry data.

<sup>2</sup>Father of dam.

Table 2. Status of Living Free-Ranging Florida Panthers as of 30 June 1994.

FP #	Sex	Dob	Age	Genetics	Date	Location	Wt. (lbs.)	Crypt.	Spec. Prob.	Antibody Titers
9	F	80-82	12-14	A	1/21/94	FSSP	75	-	thin, dehydrated	FPL+
11	F	80-82	12-14	A	5/9/92	BI	88	-	collar not working	FPL+ FVR+ FCV+
12	M	81-82	12-13	A	1/4/93	BI	122	Y		FPL+ FCV+
16	M	6/85- 12/85	8.5	P	2/5/94	BCNP	121	N		FPL+ FIV+ FCV+
19	F	86	8	A	2/21/94	FPNWR	90	-		FPL+ FCV+
23	F	8/86	8	P	6/2/93	CDU	61	-		FPL+ FCV+
26	M	83-84	10-11	A	1/18/94	BI	129	Y		FPL+ FVR+ FCV+ FIV+
32	F	87	7	A	2/8/93	PL	74	-	Notoedres	FPL+ FCV+ FIP+
36	F	85-88	6-9	A	2/22/94	SIR	83	-		FPL+ FCV+
38	F	85-88	6-9	A	5/18/93	CDU	90	-	non-reproductive	FPL+ FCV+
40	F	87-88	5-6	A	1/24/94	ACQ	70	-	lost collar	FPL+
42	M	5/89	5	P	5/3/93	CDU	110	N		FPL+ FCV+
45	M	10/90	4	A	2/12/93	ACQ	134	Y		FPL+ FIP+
46	M	89-90	4-5	A	1/26/94	PL	125	Y		FPL+ FCV+
48	F	10/91	3	A	1/5/93	BI	68	-		FIP+
49	F	90	4	A	2/25/92	ACQ	72	-	collar not working	No serology done
51	M	89-90	4-5	A	1/19/94	FPNWR	116	N	Notoedres	FPL+
52	F	11/91	3.5	A	1/6/93	CI	68	-		FPL+ FIP+
54	M	3/92	2	A	1/17/94	FPNWR	108	N		FPL+ FCV+

FP #	Sex	Dob	Age	Genetics	Date	Location	Wt. (lbs.)	Crypt.	Spec. Prob.	Antibody Titers
55	F	1/93	1.5	P	1/25/94	CDI	58	-		FPL+
56	M	92	2	A	2/3/94	ACQ	68	-		FPL+

Table 3. Organ weights (in grams) for Florida panthers necropsied through 30 June 1994.

FP#	Sex	Age	Wt. (kg)	Liver	Heart	Kidney	Lungs	Spleen
07	M	10y	53.5	-	240	R 63 L 71	270	100
08	F	14y	31.8	840	200	-	635	200
20	M	5y	49.9	935	330	R 80 L 85	820	105
27	F	4y	25.0	360.5	97.1	-	-	26.1
29	M	3.5y	50.0	-	235	-	-	-
31	F	13y	42.6	-	230	-	-	-
34	M	5y	58.9	-	-	R 94.7 L 105.6	-	85
50	M	1.5y	61.2	630	280	R 100 L 100	-	150
N92-742	F	6 mo	11.8	335	69	R 34 L 33.7	-	50
CC29-94	M	8 mo	20.0	475	88 <sup>1</sup> 99 <sup>2</sup>	R 52 L 52	258	45

<sup>1</sup>without pericardial fat

<sup>2</sup>with pericardial fat

Table 4. Hematological values for a Florida panther kitten (FP 208), before and after anthelmintic treatment (on Day 11) for infection with *Ancylostoma pluriidentatum*. Values for another clinically normal panther kitten (FP 54) and felids of other types are included for comparison.

Blood value	FP#208			FP 54	Adult captive cougars <sup>b</sup>	Wild caught bobcats <sup>c</sup>
	Pre-Treatment Day 1 <sup>a</sup>	Post-Treatment Day 9	Treatment Day 23			
Red blood cells (X10 <sup>6</sup> /ul)	5.54	5.04	6.52	6.74	7.9±1.1 <sup>d</sup> (6.0-9.7) <sup>e</sup>	7.11±0.48 <sup>d</sup>
Hemoglobin (g/dl)	13.4	10.6	12.1	12.7	13.8±2.1 (10.5-17.8)	12.28±0.59
Packed cell volume (%)	32.8	27.8	34.8	31.3	38±6 (27-48)	36.47±2.24
Mean corpuscular volume (fl)	59.5	56.0	58.1	46.0	49±2 (46-54)	53.68±2.83
Mean corpuscular hemoglobin (pg)	24.1	21.2	18.6	18.8	17.6±0.8 (15.6-19.0)	-
Mean corpuscular hemoglobin concentration (%)	45.4	38.1	31.9	40.6	35.8±1.4 (32.0-38.2)	-
Eosinophils (% total leucocytes)	4	24	6	5	(0-6)	-
Eosinophils (number/ $\mu$ l)	284	1656	880	230	100±100 (0-400)	278±392

<sup>a</sup>Number of days in captivity

<sup>b</sup>From Hawkey and Hart (1986)

<sup>c</sup>From Heidt et al. (1988)

<sup>d</sup>Standard deviation

Table 6. (continued).  
<sup>e</sup>Range

Table 5. Seroprevalence of infectious disease agents in free-ranging Florida panthers, 1 March 1991 - 22 February 1994<sup>1</sup>.

	Number of Panthers		
	Examined	Positive	% Positive
Bacteria			
<u>Brucella</u> spp.	27	0	0%
Protozoa			
<u>Toxoplasma gondii</u>	5	0	0%
Viruses			
Feline calicivirus			
not vaccinated	13	4	31%
vaccinated	24	16	67%
Feline enteric coronavirus/ Feline infectious peritonitis			
KELA <sup>2</sup>	18	0	0%
IFA <sup>3</sup>	13	6	46%
Feline immunodeficiency virus/ puma lentivirus	11 <sup>4</sup>	1 <sup>4</sup>	9%
Feline leukemia virus	17	0	0%
Feline parvovirus (panleukopenia virus)			
not vaccinated	13	3	23%
vaccinated	24	24	100%
Feline rhinotracheitis virus (herpesvirus)			
not vaccinated	13	5	38%
vaccinated	24	7	29%
Pseudorabies virus	6	0	0%

<sup>1</sup>Table presents data from the latest capture date for individual panthers.

<sup>2</sup>KELA, kinetics enzyme - linked immunosorbent assay

<sup>3</sup>IFA, indirect immunofluorescence assay

<sup>4</sup>Data only from panthers captured in 1994.

Table 6. Syncytia forming virus antibodies in Florida panthers and the presence of tissue culture lysis, 1987-92.

ID#	Sex	Location <sup>B</sup>	Date	Age Class <sup>C</sup>	Tests <sup>A</sup>		Fibroblast Tissue Culture Lysis
					IFA	AGID	
#07	M	I-S.FS	01-26-85	6	+	nd <sup>D</sup>	
#08	F	I-S.FS	01-13-86	6	-	-	Growth
		I-S.FS	04-13-87	6	-	-	Growth
		C-WOP	06-01-88	6	-	nd	
#09	F	I-S.FS	01-08-87	5	+	+	Lysis
#10	M	I-S.FS	05-02-86	2	+	nd	
#11	F	I-BCNP	01-21-86	5	+	nd	Growth
		I-BCNP	01-02-90	6	+	nd	Lysis
		I-BI	05-09-92	6	pe	nd	
#12	M	I-N.FS	01-14-91	6	+	nd	na <sup>E</sup>
#13	M	I-BI/PR	01-27-86	5	+	nd	
#14	F	II-ENP	12-07-86	5	-	-	Lysis
		II-ENP	04-11-88	5	+	nd	Growth
		II-ENP	02-22-90	6	-	nd	
#15	F	II-ENP	12-13-86	5	-	-	Growth
		II-ENP	06-16-87	5	-	nd	
#16	M	II-ENP	01-12-87	2	-	-	Growth
		II-ENP	02-04-91	5	+	nd	na
		II-SS	01-23-92	5	pe	nd	
#17	M	I-PR	01-20-87	6	+	+	Lysis
		I-PR	01-26-89	6	+	nd	
#18	F	I-PR	01-22-87	6	+	+	Lysis
		I-PR	01-23-89	6	+	nd	
#19	F	I-BI	02-09-87	2	-	-	Lysis
		I-BI/PR	11-09-87	3	-	nd	Growth

Table 6. (continued).

ID#	Sex	Location <sup>B</sup>	Date	Age Class <sup>C</sup>	Tests <sup>A</sup>		Fibroblast Tissue Culture Lysis
					IFA	AGID	
		I-N.FS	02-16-90	4	+	nd	na
		I-FPNWR	01-06-92	5	pe	nd	

Table 6. (continued).

#20	M	I-PR	03-10-87	4	+	+	Lysis
		CAP/MMZ	07-06-87	4	+	nd	
#21	F	II-ENP	03-16-87	2	-	-	Lysis
		II-ENP	05-23-89	4	-	nd	
		C-WOP	06-09-92	5	pe	nd	
#22	F	II-ENP	03-18-87	2	-	-	Growth
			02-11-91	4	-	-	Growth

Table 7. Prevalence and intensity of parasites from Florida panthers (*Felis concolor coryi*)<sup>a</sup>, July 1990-June 1994.

Parasite	Parasites		Parasite eggs from feces			
	<u>No. panthers</u>		<u>No. panthers</u>		<u>Intensity<sup>b</sup></u>	
	Examined	Positive	Examined	Positive	Mean	Range
Trematodes						
<i>Alaria marciana</i>	9	6 (67%)	20	17 (85%)	1452	66-4,100
<i>Heterobilharzia americana</i>	9	1 (11%)	20	-	-	-
Cestodes						
<i>Spirometra mansonioides</i>	9	6 (67%)	20	12 (60%)	13565	2-69,600
<i>Taenia omissa</i>	9	4 (44%)	20	1 (5%)	-	-
Nematodes						
<i>Ancylostoma pluridentatum</i>	9	9 (100%)	20	17 (85%)	1127 <sup>e</sup>	-
<i>Ancylostoma caninum</i>	9	1 (11%)	20	-	-	-
<i>Capillaria aerophila</i>	9	-	20	2 (10%)	-	-
<i>Dirofilaria striata</i> <sup>c</sup>	8	6 (75%)	-	-	-	-
<i>Molineus barbatus</i>	9	1 (11%)	20	-	-	-
<i>Stongyloides sp.</i>	9	3 (33%)	20	3 (15%)	-	-
<i>Toxocara cati</i> & <i>T. leonina</i>	9	1 (11%)	20	6 (30%)	-	-
Protozoans						
<i>Cytauxzoon felis</i> <sup>d</sup>	33	8 (24%)	-	-	-	-

<sup>a</sup>Only data from panthers greater than 5 months of age is included.

<sup>b</sup>Eggs per gram of feces.

<sup>c</sup>Intensity: number of microfilaria per ml X=101,000 range=17,000-221,000.

<sup>d</sup>Reported positive if the individual panther tested positive at any time during the study period.

<sup>e</sup>Calculated from 8 panthers examined.

Table 8. Mercury concentration in whole blood (ppm, wet weight) over time for panthers captured in FY 1991-92.

ID#	1987	1988	1989	1990	1991	1992
#16 M	0.180	1.80		3.10	0.21	0.533
#19 F			0.100	0.11		<0.047
#26 M		0.069		0.053		<0.047
#28 M		0.320			0.024	<0.047
#36 F				0.035		<0.048
#38 F				0.650		0.067
#40 F				0.039		0.154
#44 M					0.172	0.147
#45 M					0.185	0.067
#46 M						0.147
#47 M						<0.047
#48 F						<0.047
#49 F						0.199
#50 M						<0.046
#51 M						0.055

Table 9. Mercury concentration in hair (ppm, wet weight) over time for panthers captured in FY 1991-92.

ID#	1987	1988	1989	1990	1991	1992
#16 M	4.00	82.00		90.00	67.00	31.80
#19 F	3.00		6.90	21.00		1.75
#26 M		1.20		0.82		1.33
#28 M		8.40			0.55	0.52
#36 F				0.77		1.04
#38 F				47.0		3.47
#40 F				0.90		9.90
#44 M					2.84	9.52
#45 M						2.21
#46 M						1.11
#47 M						6.81
#48 F						0.68
#49 F						1.11
#50 M						1.01
#51 M						6.25

Table 10. Levels (PPM) of selected trace minerals in liver, kidney, and serum from individual Florida panthers (FP).

Mineral	Free-Ranging FP					Captive FP			
	FP #29 5/27/92 <sup>A</sup>		FP #13			FP #206			FP #205
	liver	kidney	12/16/87 liver	2/27/86 kidney	serum	5/3/92 liver	1/2/92 kidney	serum	6/8/92 serum
B	<1.49	<2.07	<2.68	<3.64	<1.00	<3.13	<4.64	<1.00	<1.11
Co	<0.149	<0.207	<0.268	<0.364	<0.100	<0.313	<0.464	<0.100	<0.111
Mo	1.43	0.474	1.55	<0.727	<0.200	1.54	1.23	<0.200	<0.222
Sb	<1.49	<2.07	<2.68	<3.64	nd	<3.13	<4.64	nd	nd
Hg	1.28	0.770	2.2	1.8	nd	<1.5	1.8	nd	nd
Ba	<0.075	<0.103	<0.134	<0.182	0.069	<0.313	0.426	<0.100	<0.111
As	<0.746	<1.03	<1.34	<1.82	nd	<1.56	<2.32	nd	nd
Pb	<0.746	<1.03	<1.34	<1.82	nd	<1.56	<2.32	nd	nd
V	<0.075	<0.103	<0.134	<0.182	nd	<0.156	<0.232	nd	nd
Mg	523	536	596	587	22.3	502	533	22.0	24.8
Zn	101	81.9	279	83.8	1.27	107	91.9	1.31	1.11
Cr	<0.299	<0.413	<0.536	<0.727	nd	<0.626	<0.929	nd	nd
Se	3.79	10.4	2.55	7.09	nd	2.18	8.20	nd	nd
Cu	48.1	15.1	49.5	14.2	0.508	45.3	15.3	0.533	0.575
Mn	9.43	3.65	13.2	4.06	<0.050	7.68	3.47	<0.050	<0.056
Al	<1.49	<2.07	4.01	<3.64	<1.00	<3.13	<4.64	<1.00	<1.11
Cd	0.348	3.30	0.406	3.23	nd	<0.313	3.50	nd	nd
Tl	<3.73	<5.17	<6.70	<9.09	nd	<7.82	<11.6	nd	nd

Mineral	Free-Ranging FP					Captive FP			
	FP #29 5/27/92 <sup>A</sup>		FP #13			FP #206			FP #205
	liver	kidney	liver	kidney	serum	liver	kidney	serum	6/8/92 serum
Ni	<0.149	<0.207	<0.268	<0.364	nd	<0.313	<0.464	nd	nd

<sup>A</sup>Date sample collected

Table 11. Mean hormone levels ( $\bar{x} \pm$  S.E.) and estradiol/testosterone (ET) ratios for normal male, cryptorchid male, and female Florida panthers. Estradiol (E) and testosterone (T) levels are expressed in pg/ml. Ratios were derived from mean hormone levels.

Sex/Status	<i>n</i>	E	T	Ratio
Male/Normal	5	228 $\pm$ 48	414 $\pm$ 68	0.55
Male/Cryptorchid	14	267 $\pm$ 32	515 $\pm$ 70	0.52
Female	5	381 $\pm$ 79	187 $\pm$ 32	2.04**

\* $p < 0.05$ ;

\*\* $p < 0.001$

Table 12. Reproduction evaluation of stored sperm from Florida panthers.

ID#	Date	Age	Breeder Status	Reproductive evaluation (Series I-III) <sup>A</sup>						Sperm Bank <sup>A</sup>	
				# Testicles <sup>B</sup>	Motility <sup>C</sup>	Status <sup>D</sup>	Concentration <sup>E</sup>	Volume	TMS/Ejac <sup>F</sup>	# Pellets	AI units <sup>G,F</sup>
# 12 <sub>a</sub>	01-28-86	4-5 yr	Presumed	1	70%	2.5	10.8 x 10 <sup>6</sup>	0.77 ml	5.8 x 10 <sup>6</sup>	0	0
		6-7 yr	med	1	40%	3.0	10.5 x 10 <sup>6</sup>	2.20 ml	10 <sup>6</sup>	169	0.92
	01-31-88	9-10 yr	Presumed	1	50%	2.5	19.0 x 10 <sup>6</sup>	1.58 ml	9.2 x 10 <sup>6</sup>	51	1.50
		11-12 yr	med	1	40%	3.0	12.0 x 10 <sup>6</sup>	1.61 ml	10 <sup>6</sup>	95	0.77
	01-14-91		Presumed						15.0 x 10 <sup>6</sup>		
01-04-93		med						7.7 x 10 <sup>6</sup>			
# 13 <sub>d</sub>	12-15-87	6-8 yr	Suspect	2	nd	nd	nd	nd	nd	22 <sup>H</sup>	nd
# 16 <sub>a</sub>	02-02-88	28-34 mo	Unknown <sup>I</sup>	2	40%	3.5	2.0 x 10 <sup>6</sup>	0.53 ml	0.4 x 10 <sup>6</sup>	20	0.04
				2	-	-	0	0.22 ml	10 <sup>6</sup>	0 <sup>J</sup>	0
	02-21-90	4.5-5 yr	Presumed	2	70%	3.0	23.0 x 10 <sup>6</sup>	2.10 ml	0	83	3.38
		5.5-6 yr	med	2	60%	3.0	12.0 x 10 <sup>6</sup>	2.30 ml	33.8 x 10 <sup>6</sup>	122	1.66
	02-04-91	6.5-7 yr	Presumed						10 <sup>6</sup>		
01-23-92		med						16.6 x 10 <sup>6</sup>			
# 26 <sub>a</sub>	02-10-90	6-7 yr	Unknown <sup>K</sup>	1	50%	2.5	1.0 x 10 <sup>6</sup>	1.14 ml	0.6 x 10 <sup>6</sup>	1 <sup>L</sup>	0.06
		8-9 yr	wn <sup>K</sup>	1	50%	2.5	6.0 x 10 <sup>6</sup>	2.01 ml	10 <sup>6</sup>	64	0.60
	01-22-92		Presumed						6.0 x 10 <sup>6</sup>		

Table 12 (continued).

ID#	Date	Age	Breeder Status	Reproductive evaluation (Series I-III) <sup>A</sup>						Sperm Bank <sup>A</sup>	
				# Testicles <sup>B</sup>	Motility <sup>C</sup>	Status <sup>D</sup>	Concentration <sup>E</sup>	Volume	TMS/Ejac <sup>F</sup>	# Pellets	AI units <sup>G,F</sup>
# 28 <sub>d</sub>	03-11-89	20-26 mo	Unkno	1	10%	2.0	0.5 x 10 <sup>6</sup>	0.30 ml	0.02 x 10 <sup>6</sup>	0 <sup>J</sup>	0
			wn	1	30%	3.0	20.0 x 10 <sup>6</sup>	1.31 ml	10 <sup>6</sup>	8 <sup>L</sup>	0.79
	01-07-91	3.5-4 yr 6-7 yr	Unkno	1	40%	2.5	8.0 x 10 <sup>6</sup>	3.40 ml	7.9 x 10 <sup>6</sup>	170	1.09
			wn						10.9 x 10 <sup>6</sup>		
# 29 <sub>d</sub>	02-12-90	1.5 yr 2.5 yr	Unkno	1	30%	2.5	3.0 x 10 <sup>6</sup>	0.22 ml	0.2 x 10 <sup>6</sup>	0 <sup>J</sup>	0
			wn	1	30%	3.0	56.4 x 10 <sup>6</sup>	1.30 ml	10 <sup>6</sup>	50	2.20
# 33 <sub>d</sub>	03-05-89	3-4 yr	Suspe						21.9 x 10 <sup>6</sup>		
			ct	2	30% <sup>M</sup>	nd	1.0 x 10 <sup>6</sup>	0.93 ml	0.3 x 10 <sup>6</sup>	17	0.03
# 34 <sub>d</sub>	01-11-91	22 mo	Unkno	1	0%	0	4.0 x 10 <sup>6</sup>	2.77 ml	0	0 <sup>J</sup>	0
			wn								
# 39 <sub>d</sub>	02-19-90	3-4 yr	Unkno	2	50%	2.5	13.0 x 10 <sup>6</sup>	1.80 ml	11.7 x 10 <sup>6</sup>	3 <sup>L</sup>	1.17
			wn								
# 42 <sub>a</sub>	02-05-91	21 mo	Unkno	2	70%	3.0	2.0 x 10 <sup>6</sup>	1.30 ml	1.8 x 10 <sup>6</sup>	37	0.18
			wn								
# 43 <sub>d</sub>	01-05-91	16-17 mo	Unkno	1	-	-	0	0.21 ml	0	0 <sup>J</sup>	0
			wn								

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Table 12 (continued).

ID#	Date	Age	Breeder Status	Reproductive evaluation (Series I-III) <sup>A</sup>						Sperm Bank <sup>A</sup>	
				# Testicles <sup>B</sup>	Motility <sup>C</sup>	Status <sup>D</sup>	Concentration <sup>E</sup>	Volume	TMS/Ejac <sup>F</sup>	# Pellets	AI units <sup>G,F</sup>
# 46 <sub>a</sub>	01-31-92	2-3 yr	Unknown	1	38%	2.5	1.8 x 10 <sup>6</sup>	0.65 ml	0.43 x 10 <sup>6</sup>	33	0.04
#200 <sub>d</sub>	04-18-85	4-6 yr	Unknown	2	85%	3.0	23.0 x 10 <sup>6</sup>	1.27 ml	24.8 x 10 <sup>6</sup>	0 <sup>J</sup>	0
		5-7 yr	wn	2	na	na	na	na	10 <sup>6</sup>	many <sup>O</sup>	na
	04-29-86	7-9 yr	Unknown	2	45%	3.0	4.0 x 10 <sup>6</sup>	1.42 ml	na	0 <sup>J</sup>	0
		9-10 yr	wn	2	60%	3.0	na	na	2.6 x 10 <sup>6</sup>	many	na
	06-02-88	10-11 yr	Unknown	2	80%	3.0	1.0 x 10 <sup>6</sup>	2.60 ml	10 <sup>6</sup>	0 <sup>J</sup>	0
			wn	2	70%	3.5	8.1 x 10 <sup>6</sup>	4.46 ml	na	na	na
	10-04-89	12-13 yr	Unknown						2.1 x 10 <sup>6</sup>		
01-17-90		Unknown						25.2 x 10 <sup>6</sup>			
09-16-92		Unknown									
#202 <sub>a</sub>	06-08-92	2 yr	Unknown	2	60%	3.5	11.0 x 10 <sup>6</sup>	1.12 ml	7.4 x 10 <sup>6</sup>	0	0
N89-64 <sub>d</sub>	01-26-89	3 yr	Unknown	1	nd	nd	nd	nd	nd	12 <sup>H</sup>	nd

<sup>A</sup>Semen evaluation is based on sperm collected using a standard ejaculation protocol of 3 series of 10 stimulations each (Series I-III). Additional series stimulation is sometimes used to increase the amount of semen available for freezing. Therefore, AI units will include all semen frozen for Series I-V<sup>+</sup>. <sup>B</sup>Number of descended testicles; <sup>C</sup>Motility = percent motile sperm; <sup>D</sup>Status-quality of progressive sperm

Table 12 (continued).

movement, 1 (low) - 5 (high). <sup>E</sup>Number of sperm per ml of ejaculate; <sup>F</sup>TMS/Ejac = total motile sperm per ejaculate; <sup>G</sup>AI units - one AI unit is the number of motile sperm ( $5 \times 10^6$ ) necessary for one artificial insemination (AI) attempt, therefore, the number of units per male is calculated from the motility, concentration, and volume of the original ejaculate with an estimated  $\geq 50\%$  loss of viable sperm due to freezing; <sup>H</sup>Collected post-mortem; <sup>I</sup>Sired a litter approximately 1 week after this data; <sup>J</sup>Sperm quality was too low to allow for freezing; <sup>K</sup>Sired a litter in August 1990; <sup>L</sup>Sperm concentrated by centrifugation prior to freezing; <sup>M</sup>Estimated motility; <sup>N</sup>Also known as "Big Guy or PCO 10; <sup>O</sup>Poor sperm quality; <sup>P</sup>(Concentration) (Volume) (Motility) = Total Motile Sperm (TMS) per Ejaculate: (TMS/Ejac) (50% Freezer Loss) = AI Units  
( $5 \times 10^6$ ) Motile Sperm

na - data not available

nd - not determined

a = alive as of 6/30/94

d = dead as of 6/30/94

Table 13. Status and location of captive Florida panthers as of 30 June 1994.

ID#	Sex	Age (years)	Location	Status
FP21	F	8.0	White Oak Plantation	FiV Positive. Healthy upon annual exam
FP201	M	4.0	White Oak Plantation	Unilateral cryptorchid. Grade I/VI heart murmur upon annual exam.
FP202	M	4.0	White Oak Plantation	Grade III/VI heart murmur, otherwise healthy upon annual exam.
FP203	M	4.0	White Oak Plantation	Small left testicle. Healthy upon annual exam.
FP204	F	4.0	White Oak Plantation	Lobectomy performed in 1992 due to bacterial pyelitis. VMTH evaluated after surgery and doing well. III.VI heart murmur, otherwise healthy upon annual exam.
FP207	M	3.0	Lowry Park	Bilateral cryptorchid. Orchiopexy performed in 1993. Electroejaculated twice in 1993 and found to be sterile. Transferred to Lowry park. Testicular biopsy performed in 1993 and is sterile.
FP208	F	2.0	Lowry Park	Had mange ( <i>Notoedres cati</i> ) as kitten in 1992. Healthy upon annual exam.
FP209	F	2.0	Jacksonville Zoo	Grade I rear leg lameness due to injury at Jacksonville Zoo in captivity 08-20-92. Sick twice in 1993 (nonspecific). Healthy upon annual exam.
FP210	M	2.0	Jacksonville Zoo	Placed into captivity 08-20-92. Healthy upon annual exam. Separated 10.93 from sibling FP209 due to age.

## QUESTIONS:

*MR. KEN ALVAREZ:* Two questions about pseudorabies. If it represents a large potential threat, and we don't know much about it, how do we go about learning? And I understood the Department of Agriculture is trying to develop an oral vaccine for pseudorabies; is that correct? And does that have any application?

*DR. DUNBAR:* They are trying to develop A vaccine for pseudorabies. They have also got one for rabies. Do not get those two confused. I am not sure what the Department of Agriculture has to do with it in this country. But European countries have basically been working on those kinds of vaccines. The vaccines that are available today kill panthers. And I would assume that since this has been developed for feral swine and domestic swine, it is probably the same kind of vaccine, just in a different carrier state. It will probably kill panthers, too. I do not know what form it is coming in. It is probably a modified live virus.

*UNIDENTIFIED PERSON:* (Inaudible.)

*DR. DUNBAR:* Yes, it is being developed for hogs.

*UNIDENTIFIED PERSON:* (Inaudible.)

*DR. DUNBAR:* For the hogs, it probably does. We do not think we can eliminate pseudorabies with a vaccination probably anymore than we think we can eliminate rabies in the United States with vaccine programs they have. But

we can somewhat control it. And I think that is what we are after. I still think, in looking at that data, that it is still a long ways down the road. Even the vaccines they have today for domestic swine are not really effective. And they certainly are not effective in feral swine. This is an easy delivery system. But the product

itself I think is inferior.

*MS. LAURIE WILKINS:* I'm sure you covered this, or it was covered some time today, but please help me put this in prospective. You mentioned that the animals north of Alligator Alley were heavier. And that has been a situation that has existed over time. And in the past it was related to prey base, and then suggested that there were a number of other factors that were also related to prey base, to Vitamin A deficiency. So are you saying then that there is still a prey base problem south of the Alley? And are the animals south of the Alley also in poor physical condition, in addition to being underweight?

*DR. DUNBAR:* I can not find any particular problems with the cats south versus north of Alligator Alley, other than the body weights. And I'm not sure what that means. Maybe it is prey related and maybe it is not. So I guess that is about all I can say about it.

*MS. WILKINS:* I was just wondering if the change in the prey base, because of change in management of the habitat, might be on a decline, and we are just seeing it at another point in time in whether or not you have any prospective on whether there are more deer there now than there were in the past?

*DR. DUNBAR:* Yes, there seems to be in certain areas, and I think Fakahatchee Strand is an example of that, and probably Raccoon Point, too. We seem to have increased deer

densities in those areas when compared to earlier years. Just like Fakahatchee Strand, we do not have any additional cats than we had then. And we apparently have a higher deer density. So why aren't those cats moving to Fakahatchee Strand because prey is there? I don't know. And I think we have talked about these habitat behavior problems and everything else, and they probably know something we don't know.

