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ABSTRACT--Veterinary medical management has improved the safety of Florida panther (*Felis concolor coryi*) immobilizations. Since veterinary involvement began in January of 1983, 108 immobilizations involving 48 individuals have been accomplished with one possible capture mortality. This year a total of 118 hunt-days resulted in the capture and radio-instrumentation of 8 new juveniles and the re-collaring of 11 other panthers. Presently, 21 free-ranging panthers are radio-collared and are being monitored. Five panther deaths have been documented this past year: 2 (40%) intraspecific aggression, 1 (20%) cardiac anomaly, 1 (20%) road-kill, and 1 (20%) of unknown causes.

Six kittens were taken into captivity to initiate the captive breeding program. Twenty-seven founders were identified as potential contributors to the captive breeding program. Within the past 2 years 13 of these (48%) have already died, and only 2 of these have offspring in captivity. Five of the remaining living founders are now represented in captivity by 3 kittens. Serologic evidence from 19 different Florida panthers indicates that they were exposed to or are infected with several potentially pathogenic agents: feline panleukopenia virus (FPV) (10%), feline calicivirus (FCV) (30%), feline enteric corona virus/feline infectious peritonitis virus (16%), feline immunodeficiency virus (16%), and *Toxoplasma gondii* (8.0%). FCV was isolated from a mother and offspring with oral lesions. All were negative for feline leukemia virus and feline viral rhinotracheitis virus.

Mercury was identified as a significant contaminant in free-ranging panthers, raccoons, and otters but not in bobcats. Those animals with particularly high levels were found in the Shark River Slough of the Everglades National Park and adjacent wetlands. Panthers with elevated levels of mercury occur where they consume non-ungulate prey as part or all of their diet (raccoon is probably the primary source of mercury). Mercury levels in panthers living in the Fakahatchee Strand State Preserve (FSSP) have dropped significantly ($P < 0.01$) since the fall of 1987 when land management actions were initiated to enhance deer density. Females with elevated mercury had poorer reproductive success than those with low mercury levels.

Seven male panthers were electroejaculated, and semen from 5 was frozen for future artificial breeding research. Compared to other pumas, all male panthers have semen that is of low concentration, low volume, poor sperm motility, and with >90% abnormal sperm forms. Seventy-one percent of all living males are cryptorchids, only intergrade males have 2 testicles.

Heart murmurs were detected in all eight kittens born in 1990 and in 4 of 12 living adults. Congenital heart defects contributed to the death of 2 panthers and is seriously compromising a third. The abnormal male reproductive traits coupled with an increase in detectable heart murmurs raise a serious concern for the reproductive potential and genetic health of this subspecies.

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INTRODUCTION

The endangered Florida panther (*Felis concolor coryi*), declared the state mammal of Florida in 1982, is estimated to number less than fifty individuals (Hines et al. 1987) 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 is 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 already experiencing many of these problems (Roelke 1990).

Objective:

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. Further, management strategies are to be developed that will promote the recovery of the Florida panther. These actions involve the provision of high quality medical care during the capture and immobilization of free ranging and captive panthers and the evaluation of animal health with respect to selected parasites and diseases, mortality and pathology, environmental contaminants, reproductive physiology, and genetics.

Cooperative research:

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 1990 through 30 June 1991, was between the Florida Game and Fresh Water Fish Commission (GFC) and the Department of Infectious Diseases, College of Veterinary Medicine, University of Florida, Gainesville, Fl.

Acknowledgements:

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METHODS AND MATERIALS

Field Capture Technique:

A primary objective of veterinary involvement on the capture team was to insure safe handling and proper medical management of panthers during the capture effort. The decision to immobilize a treed panther was made after accessing its health, physical condition, and level of stress. Also considered were the ambient temperature, humidity, site conditions, and other factors that might result in an unacceptable risk. For details about special capture equipment, darts, and drugs used to immobilize panthers, refer to Roelke, 1990.

Following immobilization, each panther was given a thorough physical examination which included an evaluation of its health, physical condition, and reproductive status. Additionally, body measurements and biological samples were taken as previously described (Roelke 1990).

Clinical Pathology Analysis:

Samples of blood, urine, and feces were submitted for laboratory analyses which included complete blood count (CBC), serum chemistry profile, urinalysis, and fecal culture. Three different clinical laboratories were utilized during the past year depending on their proximity to our field location. These included Diagnostic Services Inc. in Naples, FL; James Archer Smith Hospital Laboratory in Homestead, FL; and the Veterinary Medical Teaching Hospital Laboratory (VMTH) in Gainesville, FL. When possible duplicate whole blood samples were shipped to the VMTH to validate the CBC results that were being obtained from the human hospital laboratories. In these cases the VMTH was used as the veterinary standard.

Post Mortem Analysis:

Five *F. c. coryi* specimens were examined during the past year. When decomposition was not advanced, the carcasses were subjected to a complete post mortem examination by pathologists at the College of Veterinary Medicine, University of Florida. A complete exam included gross and microscopic examination of tissues, serology, bacteriology, parasitology, toxicology, and radiography. When the carcass was very fresh, attempts were made to collect and preserve living gametes (oocytes and spermatozoa) and skin. Hides and skeletal remains were deposited in the Florida Museum of Natural History in Gainesville, FL.

Sero-survey of Infectious Disease Agents:

Serum samples collected from immobilized panthers were assayed for exposure to various infectious disease agents. A summary of the disease agents, serologic test methods, source and type of antigens used, number of samples tested, positive antibody thresholds, and references for the detailed test procedures are presented in Table 1.

Mercury Studies:

Tissue samples from 52 (29 male and 23 female) free-ranging Florida panthers were collected opportunistically between 1978 and 1991. Hair and whole blood from living panthers were sampled during routine immobilizations. Liver, kidney, muscle, brain, hair, and blood were collected at necropsy from dead panthers. Muscle and liver tissues were collected from four to seven raccoons (*Procyon lotor*) captured in 1990-1991 at each of nine sites (n=48) within representative panther habitat. Liver tissue was taken from 23 road- and hunter-killed bobcats (*Felis rufus*) (collected mainly in 1984-85) and 20 road-killed otters (*Lutra canadensis*)

(collected in 1984-1985 and 1989-1991). Muscle samples from alligators were collected from road-kills ($n=4$) and surgical biopsies from live-captured animals ($n=5$). All samples were stored at either -10°C or -75°C until submitted for analysis. The reference laboratory used to analyze panther tissue was the Patuxent Analytical Control Facility (U.S. Fish and Wildlife Service) in Laurel, Maryland. Tissue sample homogenates were digested under reflex in sulfuric and nitric acids (Monk 1961). Total mercury concentrations (the combined amount of organic and inorganic mercury) 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. Muscle and liver tissue samples from other carnivores were analyzed at the University of Florida, Gainesville, Florida, using similar techniques.

Genetic Analysis:

Blood and tissue samples for genetic analysis were obtained from 23 Florida panthers immobilized and/or necropsied in FY 1990-91. The blood (from living animals) and tissue samples (from dead animals) were processed and frozen for later analysis while the skin biopsies for fibroblast tissue culture were sent directly to the genetics laboratory at the National Cancer Institute.

Blood and tissue samples were collected from 17 wild-born cougars from British Columbia and southeastern Alaska (*F. c. vancouverensis*, *oregonensis*, and *missoulensis*) for inclusion in the Florida panther/puma population genetics research tissue bank.

Data Analysis:

All tissue mercury results in this paper are reported in ppm of mercury on a wet weight basis with the exception of hair which is presented as dry weight. The data for mercury concentrations in tissue were log-transformed prior to data analysis. Therefore, all reported means are geometric means rather than arithmetic. When comparisons involved more than two means, one-way analysis of variance (ANOVA) and the Student-Newman-Kuels multiple range test were used to detect differences; otherwise, differences between means were detected using Student's "t" test or the Paired-sign test as appropriate.

RESULTS AND DISCUSSION

MEDICAL MANAGEMENT OF FLORIDA PANTHERS

Field Capture of Free-Living Panthers:

The winter field capture season of 1991 extended from January 3 to May 10 and involved a total of 118 hunt-days by the field capture teams of the Florida Game and Fresh Water Fish Commission (GFC) and the National Park Service (Everglades National Park [ENP] and Big Cypress National Preserve [BCNP]). This hunting effort resulted in the capture of 8 uncollared panthers and the recapture of 11 collared panthers for a total of 19 field immobilizations (Table 2). All of the uncollared animals were the juvenile offspring of previously collared females living in the northwestern portion of the study area: Fakahatchee Strand State Preserve (FSSP), Florida Panther National Wildlife Refuge (FPNWR), Bear Island (BI) Unit of the BCNP, and adjacent private ranches. One female (#22) previously radio-instrumented but lost due to a malfunctioning collar, was recaptured in the ENP. Only two other uncollared animals were documented to exist in the Big Cypress Swamp study area. These were the recently dispersed offspring of a radio-collared female (#38).

Six of the 8 juveniles captured were removed from the wild to participate in the captive breeding program: The three female and three male kittens were transported to White Oak Plantation in Yulee, Florida.

As of 31 July 1991, 27 Florida panthers were part of our radio-telemetry and captive breeding projects. Eight animals were in captivity at White Oak Plantation, and 19 radio-instrumented, free-ranging panthers were being monitored; 15 in southwestern Florida, 2 in ENP, and 2 in eastern BCNP (Table 2).

Physical condition, body weights, and hematology of wild Florida panthers:

The physical condition, body weight, reproductive status, and selected hematology values (hemoglobin and packed cell volume) for male and female panthers examined in FY 1990-91 are presented in Tables 3 and 4, respectively. Data from these same animals captured previously are presented for comparison of respective parameters over time.

FLORIDA PANTHER LONGEVITY AND MORTALITY

Radio-Collaring of Panthers:

The 25 free-ranging, collared panthers still alive in 1990-91 have been captured and immobilized a total of 73 times (2.91 captures per panther) and have been followed for a total of 853 months, or an average of 32.8 months per panther (range 2 to 90 months) (Table 5). The average life post-collaring has increased from the 21.9 months for the 1981 - 1990 period (Roelke, 1990). This apparent increase is probably due to the addition of only 2 new uncollared animals this year compared to a mean of 7.5 new panthers for the previous 4 years.

Panther Mortality:

Five panther deaths were documented in FY 1990-91; 4 occurred among radio-collared animals, and 1 was the uncollared offspring of an instrumented female (#11). The causes of mortality for the 5 panthers are listed in Tables 5 and 6. Two of the 5 deaths were due to intraspecific aggression (40%), 2 were hit by cars and killed (40%), and 1 died of unknown causes (20%).

One of the road-killed panthers had severe heart disease which probably contributed to her death. This 9-month-old panther kitten was dying of congestive heart failure secondary to a congenital atrial septal defect when hit by a car. This is the second panther documented to have this congenital anomaly (see captive breeding section).

SERO-EPIDEMIOLOGY OF FLORIDA PANTHERS

Serum samples obtained from 19 wild Florida panthers collected in FY 1990-91 were screened serologically for 11 infectious disease agents known to be important to felidae and/or reported previously as endemic to other wildlife species in Florida. A summary of the prevalence and maximum titers are presented in Table 7, and the results from the individual panthers examined are presented in Table 8.

All samples tested were negative for feline viral rhinotracheitis virus antibodies and feline leukemia virus antigen. A variable number of panthers were positive for *Toxoplasma gondii* (0 or 8.3% depending on the lab), feline calicivirus (FCV) (30%), feline enteric corona virus/infectious peritonitis virus (FECV/FIPV) (15.8%), feline immunodeficiency virus (FIV) (15.8%), and feline panleukopenia virus (FPV) (10%). Results for *Brucella sp.*, pseudorabies virus, rabies virus, and feline syncytia forming virus are still pending.

Feline Panleukopenia Virus:

A marked decline in the prevalence of FPV positive animals was noted this year, 10% v.s. 64% (1981-90). This is probably due to the predominance of juveniles (80%) in the tested sample (only unvaccinated animals are tested for this disease). This is the first year we have had the opportunity to sample more than 2 kittens in a given year.

Eight kittens, 6 to 8 months old, were tested this spring, and none had detectable antibody levels for FPV (titers < 1:10). This finding substantiates our early hypothesis that kittens, less than 1 year of age, are the ones most at risk for succumbing to FPV (Roelke, 1990). Their mothers had antibody titers of 1:5,000 to >1:10,000 when last sampled (sampling ranged from 1 year prior to conception to 45 days after conception). Further, the mothers received booster immunizations for FPV at that time, and, therefore, should have had high titers when their kittens were born and first suckled. Even with high initial titers, all the passive antibody would be expected to be gone by 6 to 8 months rendering the kittens vulnerable to FPV. All the kittens were vaccinated against FPV when captured. Four of the kittens in captivity were retested 60 days after immunization, and all had developed titers (1:10 to 1:5,000). It is possible that without immunization, exposure to the virus in the wild would have resulted in a significant number of deaths.

Feline Calicivirus:

The prevalence of feline calicivirus (FCV) antibodies found this year was similar to what has been observed in past years (30% for 1990-91 verses 38% for 1981-90), but, for the first time, this year FCV was cultured from a wild-caught kitten (#204♀) with oral and nasal lesions. Calicivirus was isolated from the nasal passages, pharynx, and rectum of this cat. The lesions were mild and self-limiting and healed without any evidence of more serious disease (this kitten came into captivity so it could be monitored). The litter mate of this kitten (#201, also in captivity), captured one week prior to his sibling, did not exhibit any lesions nor could any virus be isolated from him. The mother (#31) was captured 2 weeks after #204, and we were able to culture calicivirus from her pharynx, but not from the nasal passages or rectum. She had lesions on the roof of her mouth and gum line, and she had the highest FCV titer recorded this year (>1:256), probably resulting from her recent encounter with the virus. Unlike the kittens, she had previously been immunized (on 01-12-89), and,

possibly, this prior exposure contributed to her pronounced antibody response not seen in the kittens (kittens 0 to 60 days post exposure: #201♂ = 1:2 to 1:12; #204♀ = 1:6 to 1:12) (see Table 8).

Feline Immunodeficiency Virus (FIV):

Serologic evidence of FIV infection was detected in 3 of 19 (15.8%) panthers examined this past year. Two of these panthers sero-converted since their last exam. Panther #42 is the offspring of sero-positive parents, #14♀ and #16♂. He was negative when tested at 10 months of age and was positive by 21 months, probably due to chronic exposure to his mother. Had the sero-positive father been responsible for the transmission of the virus we would have expected the conversion to have occurred earlier in his life. Panther #28 also sero-converted this past year. This male was negative when initially tested at 18 months of age and was strongly positive this spring at 3.5-4 years. During the 48-month interim, this male traveled widely and came in contact with at least 1 sero-positive female (#18). When and how he became exposed to FIV is not known.

Within the free-ranging Florida panther population there are animals with differing genetic backgrounds, those descending from historic *F. c. coryi* and those that are also carrying nuclear and mitochondrial genes common to Central or South America (O'Brien et al. 1990). There is a significant difference in the prevalence of FIV between the two different genetic stocks of Florida panthers. The incidence of FIV in those animals (>1 year of age) carrying non-North American genes (primarily the ENP population) is significantly higher ($z = 2.185$, $P = 0.0289$) than in those of pure *F. c. coryi* decent, 63.6% versus 25.9%. This may indicate that the virus originated in Latin America and was imported. Other alternatives exist. The virus occurs within families (mothers and offspring) and it has been determined that the ENP population can be traced back to a limited number of females (perhaps 1), therefore, it is plausible to suggest that the higher incidence of FIV within the ENP population is merely reflecting the familial/maternal connection between all those panthers. A less likely hypothesis is that environmental and ecological conditions in the ENP are conducive to viral survival.

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. Our efforts have resulted in the isolation of 4 different viral strains of FIV from the panther. His preliminary results indicate that the strains of panther virus are similar to each other, but are distinctly different from the previously

described domestic cat FIV isolates and that the virus has probably been associated with panthers for thousands of years (Olmstead, NIH, pers. comm.).

At this point, the significance of FIV to panthers is not understood. But until it is proven otherwise, the presence of the virus 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 to seronegative captive animals, current management decisions dictate that seropositive animals should be handled separately when they must come into captivity or not be taken into captivity at all. Thirty-eight percent (8 of 21) of the living wild adult panthers are known to be infected with FIV; consequently, our current lack of understanding of the natural history and pathogenicity of this virus may hinder our captive breeding efforts as we endeavor to bring genetic representation of all wild individuals in to captivity.

Feline Enteric Coronavirus/Feline Infectious Peritonitis Virus:

Panthers testing positive for FECV/FIPV by indirect immunofluorescence assay this year exhibited low titers (1:25) as in the past (Roelke, 1990). This sensitive assay can detect small quantities of the coronavirus group-specific antibody. Since the antibody may be cross-reacting to other coronaviruses of canids (canine coronavirus) and swine (transmissible gastroenteritis virus), the presence of low antibody titers may indicate exposure by way of food ingestion rather than natural infection with a feline coronavirus. At present, the finding of FECV/FIPV-positive animals is thought to be inconsequential.

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 the hunting of alligators, and health advisories have been issued to curtail the consumption of bass due to the elevated mercury residue 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 hog (*Sus scrofa*) are the preferred prey, but, in some areas, panthers also consume small mammals, e.g., raccoon, armadillo (*Dasypus novemcinctus*), and rabbit (*Sylvilagus sp.*), as a significant part of their diet (Maehr et al. 1990, Roelke et al. 1986). Additionally, two adult male panthers in the ENP have been documented to consume alligators (O. Bass, ENP, pers. comm.).

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 #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 (Table 9). Data from blood and hair are more extensive than for other internal body organs, therefore, these two tissues will be 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 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 ENP), Fakahatchee Strand State Preserve (FSSP), and Raccoon Point in eastern BCNP had hair and blood mercury levels between these two extremes. Panthers from NA had significantly lower hair and blood mercury than did panthers found in any all other areas ($p < 0.01$). Figures 1 and 2 demonstrate these relationships.

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 (Table 9).

The poor physical condition of the panthers and a very low deer density in the FSSP led to the initiation of management actions designed to increase ungulate density and availability to panthers in the fall of 1987. 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 precipitous drop in mercury levels in panthers in FSSP coincidental with the above actions. Figure 3 displays the mean mercury concentration in panther blood from FSSP and NA before and after the fall of 1987. There was a significant difference ($p < 0.001$) in mean blood mercury values between the FSSP and NA prior to 1987 (FSSP = 0.75 and NA = 0.08), however, the means after 1987 were not significantly different (FSSP = 0.12 and NA = 0.15). Further, 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.

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. Figure 4 displays the individual hair and blood mercury values for FSSP panthers and highlights the one female (#09) present throughout the entire period. Since 1987, #09 has gained 13 percent in body weight (10 lbs), has been documented to kill deer (as opposed to primarily raccoons previously), and has experienced a 77.2 percent drop in blood mercury levels (1985 = 0.630 ppm, 1987 = 0.598 ppm, 1988 = 0.363 ppm, and 1990 = 0.140 ppm). Additionally, #09's two surviving 1990 offspring had considerably lower blood mercury values than did her only other surviving offspring born in 1985 (0.140 ppm and 0.170 ppm versus 0.749 ppm, respectively).

Reproductive success:

Mercury passes through the placenta and is concentrated in the fetus at levels equal to or higher than those of the mother (Khera 1974). Further, the nervous tissue of the fetus is far more sensitive to the effects of mercury than is the adult nervous tissue. Even low mercury levels in the dam have resulted in profound neuronal disarray in the offspring when the exposure occurred during critical early stages of development (T.

Clarkson, UNY at Rochester, pers. comm.). The disruption of normal fetal development has been documented to cause abortions, stillbirths, congenital anomalies, and behavioral changes resulting in early neonatal death (Khera, 1974).

Our data indicate that mercury contamination may be affecting reproductive success in the female Florida panther in conjunction with otherwise generally poor nutrition. Figure 5 displays the average number of surviving young (>6 mos.) per female-year grouped by the mother's blood mercury level at the approximate time of pregnancy. Females with blood mercury values >0.5 ppm had significantly fewer ($p < 0.01$) surviving offspring (mean = 0.167 kittens/female-year) than females with blood mercury values <0.25 ppm (mean = 1.46 kittens/female-year).

While generally poor nutrition would otherwise explain lower reproductive success in females it must be considered that contamination may complicate the effect of poor nutrition on reproduction and in certain cases may be a primary factor responsible for lower reproductive success. In the absence of an adequate ungulate prey base such as deer and hogs, the panther will take less desirable prey such as raccoons, which may have low nutritional value as well as elevated mercury concentrations. Females living in the FSSP, which demonstrated high mercury levels and poor reproductive performance, also were typically in poor physical condition suggesting nutritional deprivation (Roelke 1986). As causes of poor reproductive success, the effects of mercury contamination in conjunction with poor nutrition are further confounded in that sub-optimal nutrition may mobilize mercury stored in the muscle tissue.

However, 3 females in the ENP and eastern BCNP, which were in good physical condition, also had high blood mercury values (i.e., #27♀ = 1.7 ppm, #14♀ = 1.25 ppm, #23 = 0.75 ppm) and experienced reproductive failures and/or death with neonatal dependent kittens. This would suggest that, at least in these cases, sub-clinical mercury toxicosis may have been a primary factor.

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 us to examine raccoons and other carnivores in the southern Florida ecosystem. Table 2 shows mercury concentrations in all non-panther carnivore tissue examined to date arranged by geographical location. As in the panther, the highest tissue mercury levels in other species 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 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 panthers mercury contamination was due to a direct air or water born 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 (Fig. 6). Human health advisories recommend that when flesh is contaminated with mercury at the 1.0 ppm level or greater it should be eaten more than once a month and that consumption should not be stopped at the 1.5 ppm level. Obviously, panthers in the Shark River Slough area, feeding on raccoons daily or less frequently but over long periods of time, may develop considerable body burdens of mercury.

The significance of mercury and the potential long-term 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 large portions of their range and is likely hastening the extinction of this endangered mammal.

REPRODUCTIVE AND GENETIC RESEARCH

Male Reproductive Traits:

Seven males were electroejaculated in FY 1990-91 to obtain semen for evaluation and cryopreservation. The youngest male was 16-17 months old and had no sperm. The next 2 youngest males were 22 months old. One of these males (#42) produced semen with sperm that had good motility (70%) but with a very low concentration of spermatozoa (2.0×10^6). The other young male (#34) had sperm but they were all dead (Table 11). These data suggest that the threshold for semen production occurs between 18 and 21 months of age.

Seminal traits from males with spermatozoa were very similar to those observed previously and included low volume, low spermatozoa concentration ($x = 20.5 \times 10^6/\text{ml}$), and poor sperm motility (Table 11). Semen from 5 of the males was of sufficient quality to be frozen and added to the Florida panther semen bank. There is now semen of modest quality from 7 males in the sperm bank. Semen from several other males has been collected and frozen, but due to immaturity, infertility, or post mortem collection, prospects for its utility is dubious.

Florida panther semen is extremely fragile, and the sperm do not survive the standard sperm handling and freezing protocols very well. This aspect of male Florida panther reproduction needs attention if we hope to be able to utilize banked genetic material in artificial breeding strategies.

To appreciate how many usable sperm we have frozen on each of our males, the motility, volume, and concentration must be factored together to calculate the total motile sperm per ejaculate. If we assume a conservative 50% loss of viable sperm during freezing (approximate value for western cougars) (M. Barone, NZP, unpublished data) and that 5×10^6 motile sperm are required per artificial insemination (AI) attempt, then the number of frozen AI units can be calculated for each male. Table 11 demonstrates that even with the best quality ejaculate we have procured (#16), there is only enough sperm for 3 AI breeding attempts, and successful impregnation would probably not be greater than 50% (based on domestic cat research). Obviously, we should

continue to bank sperm on all males whenever possible to increase the chances of preserving each male's genetic material in the event that he should die before procreating in the wild.

In addition to the seminal defects described above, the majority (10 of 14 = 71%) of living males are cryptorchid, having only one descended testicle. This highly unusual trait, which has not been documented in any other wild cougar, is probably the result of inbreeding. This anomaly can affect either the right or left testicle, and those retained are generally atrophied and sterile due to the high body temperature. Circulating testosterone concentrations are lower ($P < 0.05$) in the cryptorchid compared to the non-cryptorchid Florida panthers (0.27 ± 0.11 ng/ml versus 0.61 ± 0.11 ng/ml) which in turn were no different from other puma populations (0.81 ± 0.14 ng/ml, $P > 0.05$) (Barone et al. In press).

The only males in the population with two descended testicles are those panthers considered to be intergrades. In addition to having twice the testosterone levels and functional testicular mass, the semen from these males with genes common to Central or South America (O'Brien et al. 1990) had significantly higher ($P < 0.05$) sperm motility ($61.0 \pm 5.6\%$ versus $25.6 \pm 7.1\%$) and progressive motility (3.3 ± 0.1 versus 1.7 ± 0.3) than do the pure *F. c. coryi* males (Barone et al.). Sperm morphology was not different between the two groups, both had 92.2 - 94.3% abnormal spermatozoa with 40 - 43.4% acrosomal defects. These two traits were significantly higher in the Florida panther males than in any other group of wild or captive cougar yet examined.

The higher seminal and hormonal quality and the lower incidence of cryptorchidism in the hybrids demonstrate the positive effects that the infusion of new genetic material can have on a highly inbred group such as the Florida panther.

Female Reproduction:

The major female reproductive problems in the eastern BCNP and ENP are demographic. As of August 1, 1991, there are no documented female panthers living in ENP. This is due in part to the recent loss of the 2 adult females (#14 & #22). However, the situation was compounded by the lack of a breeding male from December 1986 until the spring of 1988. This allowed 1 of the 2 proven breeding females (#14) to remain barren while her son grew to sexual maturity (born in 1985/86). During the 2-year interim that #14 went unbred she could have raised a litter following the dispersal of her 1986 kittens. This gap in the reproductive output of the ENP population, coupled with the loss of #15♀ (plus her litter) and #27♀ in 1988, seriously crippled that

population's chances to recruit females into the population. The demographic situation was further compromised when the only surviving proven breeding female in ENP (#14) between 1988 and 1991 mated with her son, thus potentially jeopardizing the fitness and survivability of any offspring.

A very similar situation existed in the eastern BCNP (Raccoon Point and Jet Port areas). A mature female (#38) was captured there in February 1990 and a younger female (#23) immigrated into the area from ENP in the early summer of 1990. These are now the only females on the public lands south of Alligator Alley and east of SR 29 and are the only ones poised to recolonize that portion of the BCNP and the ENP. However, the area has been without any documented male activity since before January 1989.

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). They 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 abnormal sperm count (Roelke et al. 1986, Barone et al. In press), the cryptorchidism, 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 two years that have lapsed since the decision to proceed with the program, 13 of 27 (48%) identified wild founders have died (Fig. 7). Of these 13 animals, only 2 (15.4%) have genetic representation in captivity. Therefore, if we can actually

get offspring from all of the 14 remaining founders, we will have representation from only 59.3% of the individuals which were potentially available at the time this project was approved for implementation. The projected success and long range survival of the panther via the captive breeding program was 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). However, due to the delays imposed by a lawsuit to halt the captive breeding program (Fund for Animals and Holly Jensen verses the U.S. Fish and Wildlife Service), we are probably already well below that 90% level.

Selection of zoological institutions:

Four different zoological institutions have been identified as participants 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. Before these facilities can receive Florida panthers, they must be permitted by U.S. Fish and Wildlife Service and participate in an investigation of the health and disease profile of their felid collection. As an integral part of the profile, a questionnaire and felid inventory was created (Appendix I). These documents were aimed at insuring that Florida panthers would not be placed at risk by being exposed to potentially lethal disease agents or unhealthy environments. Conversely, the captive felid populations would not be unduly exposed to diseases unique to the wild Florida panther. At this time only White Oak Plantation has completed the entire process.

Proposed selection of animals for captive breeding project:

At the beginning of the 1991 capture season, an assessment of the living wild population was made to determine which animals might be appropriate candidates for inclusion in the captive breeding program. Adults and litters were ranked based on the importance of their lineage and the adult's age and previous reproductive history. Six females were identified which probably had litters in the summer and fall of 1990 (D. Maehr, GFC, pers. comm.). Table 12 lists the proposed kitten selection as it existed in mid-January, 1991. From this array, we planned to remove six kittens.

A list of potential adults for inclusion in the captive breeding project was also compiled (Table 13). Specific recommendations were made about each adult with respect to taking offspring as well as future contingency plans for removing that adult should it be deemed necessary. As of January 17, 1991, the consensus

of the Florida Panther Interagency Committee was that no adults needed to be taken from the wild for contribution to the captive breeding population. Several of the adults were targeted for future removal if their current litters did not survive or their reproductive output did not improve.

Founder representation of panthers removed from the wild:

Six panther kittens (3 males & 3 females), 6 to 8 months old, were brought into captivity in the spring of 1991. All captures went smoothly and the kittens were transported directly to White Oak Plantation without incident. Table 14 lists the outcome of each originally identified litter and gives the number, sex, age, and original priority of each kitten removed from the wild.

Table 15 lists the 8 captive panthers that were in the captive breeding population as of June 30, 1991. Their age, founder representation, genetic history, and deleterious traits are tabulated. Complete genetic representation (at the 99% level) of a given wild founder can be obtained by securing 7 or more of their offspring in captivity. This is based on the premise that an offspring receives 50% of its genetic make up from each parent. Therefore, if a founder were to be represented in captivity by only 1 offspring, then only 50% of that adult's genes would be in captivity. With each new offspring procured, a decreasing increment of genetic representation is added, e.g., 1st = 50%, 2nd = 25%, 3rd = 12.5%, 4th = 6.25%, etc. The proportional genetic representation of the 10 founders in our current captive population is as follows: 25% = #11♀; 50% = #14♀, #26♂, #40♀, ENP♂; 75% = #09♀, #31♀, #37♂; 93.75% = #12♂; and 100% = PCO 10♂.

This year's procurement of kittens represents a good start toward the ultimate goal of having complete genetic representation from all wild founders in the captive breeding program. Removing 7 or more kittens per founder would be ideal, however, initially it would be prudent to limit the number to insure that the ultimate survival of the wild population is not adversely effected. The number of kittens a given female contributes can be adjusted once female recycling and productivity following kitten removal has been assessed. At present, securing 3 to 4 offspring per founder should be possible over the next few years and would represent 87.5% to 93.8% of a given founder. However, these minimal numbers do not allow for individuals which fail to breed in captivity or should die before reaching sexual maturity.

Health status of captive kittens:*Growth rate in captivity*

All the kittens rapidly adapted to captivity; some were eating within 24 hours of their arrival. None of the animals exhibited the exaggerated escape behavior, nor did they damage their teeth or claws as have other wild panthers placed in more unnatural settings. The average daily weight gain (ADG) of the captive kittens was comparable to similarly aged wild kittens; $\text{mean} \pm \text{S.E. ADG} = 0.168 \pm 0.029$ lbs. for captive versus 0.160 ± 0.036 lbs. for wild (Table 16). However, the hemoglobin and packed cell volume changes over time were markedly different between the two groups (Table 16). The hemoglobin values for the captive kittens increased by 2.3 ± 0.69 gms/dl within the first 60 days of captivity while the wild kittens dropped by -1.02 ± 1.28 gms/dl over a 4 month period. These discrepancies can be explained by at least two possible factors that result in blood loss, intestinal parasites and biting arthropods. Although all the kittens received identical initial anthelmintic treatments when they were first captured, the captive ones had no opportunity to become reinfected with hookworms whereas the wild ones did. Also, the wild kittens had to contend with numerous mosquitos and ticks that did not afflict their captive cohorts.

Cardiac anomalies

The most serious problem that has been identified in kittens this year is congenital heart defects. The daughter of #11, killed by a car, was determined at necropsy to have had a congenital atrial septal defect. This kitten was dying of congestive heart failure secondary to a congenital atrial septal defect at the time. This was the second panther known to die with this type of cardiac lesion.

Also, it was determined that every panther kitten included in the captive breeding program ($n = 6$) plus the 2 left in the wild had heart murmurs, varying in intensity from 1^+ (mild) to 6^+ (severe). Panther kitten #205's murmur was so severe it could be felt through the chest wall. A cardiac evaluation of this kitten indicates that the tricuspid valve (the heart valve between the right atria and right ventricle) is congenitally deformed. Thus, blood is forced backwards into the right atria when the heart ventricles contract which has resulted in an overloading of the right side of her heart causing subsequent enlargement and dilatation. The anomaly in this kitten is very severe and the prognosis for long term survival is poor. She is currently in good health but, cardiologists predict that within 6 months to 2 years, she will experience cardiac failure and die. It should be

noted that she is the result of a consanguineous mating, sired by male #12. He also sired the kitten which had the lethal atrial septal defect this spring, and further, only 1 of 6 of his other offspring has an audibly normal heart.

The source of the murmurs in the other kittens has not yet been resolved. Definitive causes for most cardiac deformities generally have not been determined, but because of the documented inbreeding, lowered genetic diversity, and the high incidence of these anomalies in the panther population (not found in other puma's), a genetic basis is suspected.

CONCLUSIONS

From the biomedical perspective, the Florida panther is not faring well and is still sliding toward extinction despite efforts to stop the process. The major areas of concern are:

1) Stochastic demographic events-

- the panther is functionally extinct on all the public land south of Alligator Alley and east of S.R. 29 (3 female and 2 male panthers on > 1,200,000[±] acres with no opportunity to mate), whereas there was a breeding population in the ENP less than 5 years ago;

2) Degradation of genetic quality-

- within the last 5 years a rapid rise in the proportion of males that have only one descended testicle has occurred (now comprising 71% of all living males);
- a pattern of lethal congenital cardiac anomalies is suddenly evident this year, two panthers have died and another is predicted to die before she reaches sexual maturity, heart murmurs have been detected in all kittens born in 1990 (n = 8) and 30% of adults examined in 1991;

3) Serious environmental contamination with mercury-

- mercury is responsible for the death of 1 female panther, is strongly implicated in the death of 2 others in the Everglades National Park, and is correlated with lowered kitten survival and reproductive failure.

Actions to alleviate the above problems must be initiated or accelerated if these spiraling problems are to be turned around before it is too late. Actions to be considered are:

- A) translocate males to facilitate breeding in the eastern BCNP and FSSP;
- B) proceed with the captive breeding program as planned;

- C) maximize preservation of gametes to help preserve current genetic diversity;
- D) pursue assisted reproductive technologies that will contribute to points B and C;
- E) thoroughly study the incidence of mercury in the ENP, east Everglades, and southern BCNP and continue to study the dynamics of mercury in the ecosystem;
- F) support the possibility of introducing surrogate, non-endangered cougars, into ENP to elucidate the effects of mercury on panther/cougar physiology;
- G) secure habitat, especially on private lands north of Alligator Alley.

Unless panther density on public lands can be markedly improved by rectifying or mitigating demographic and contaminate problems and by halting the loss of genetic diversity, the future of the wild Florida panther is bleak.

LITERATURE CITED

- Barlough, J.E., R.H. Jacobson, D.R. Downing, K.L. Marcella, T.J. Lynch, and F.W. Scott. 1983. Evaluation of a computer-assisted, kinetics-based, enzyme-linked, immunosorbent assay for detection of coronavirus antibodies in cats. *J. Clin. Micro.* 17:202-217.
- Barone, M.A., J.G. Howard, M.E. Roelke, and D.E. Wildt. The unique ejaculate and hormonal characteristics of the Florida panther. *Proc. Therio.* In press.
- Barr, M.C., P.P. Calle, M.E. Roelke, and F.W. Scott. 1989. Feline immunodeficiency virus infection in nondomestic felids. *J. Zoo and Wildl. Med.* 20:265-272.
- Belden, R. C., 1986. Florida panther recovery plan implementation- a 1983 progress report. Pages 159-172 in S. D. Miller and D. D. Evertt, eds. *Cats of the World: Biology, Conservation and Management.* Proc. 2nd International Cat Symposium. Caesar Kleberg Wildlife Research Institute. Kingsville, Texas.
- Clarkson, T.W. and D.O. Marsh. 1982. Mercury toxicity in man. Pages 549-568 in A.S. Prasad (ed.). *Clinical, biochemical, and nutritional aspects of trace elements*, Vol. 6. Alan R. Liss, Inc., New York.
- Delany, M.R., J.U. Bell, and S.F. Sundlof. 1988. Concentrations of contaminants in muscle of American alligator in Florida. *J. Wildl. Dis.* 24:62-66.
- Eisler, R. 1987. Mercury hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish Wild. Serv. Biol. Rep. 85(1.10). 90pp.
- Evermann, J.F., M.E. Roelke, and M.B. Briggs. 1986. Clinical and diagnostic features of feline coronavirus infections of cheetahs. *Fel. Prac.* 26:21-30.
- Gaskin, J.M., and J.H. Gillespie. 1973. Detection of feline syncytia-forming virus carrier state with a microimmunodiffusion test. *Am. J. Res.* 34:245-247.
- Hatch, W.R., and W. L. Ott. 1968. Determination of submicrogram quantities of mercury by atomic absorption spectrophotometry. *Anal. Chem.* 40:2085-2087.
- Hines, T. C., R. C. Belden, and M. E. Roelke. 1987. An overview of panther research and recovery program. Pages 140-147 in R. R. Odom, K. A. Riddleberger, and J. C. Ozier, eds. *Proc. of the Third Symposium on Southeastern Nongame Wildlife.* Georgia Department of Natural Resources, Atlanta, Georgia.

- Jacobson, R.H., D.R. Downing, and T.J. Lynch. 1982. Computer-assisted enzyme immunoassays and simplified immunofluorescence assays: Applications for the diagnostic laboratory and the veterinary clinic. J. Amer. Vet. Med. Assoc. 181:1166-1168.
- Khera, K.S. 1974. Teratogenic effects of methylmercury in the cat: Note on the use of this species as a model for teratogeneticity studies. Teratology 8:293-304.
- Maehr, D.S. and J.R. Brady. 1986. Food habits of bobcats in Florida. J. Mamm. 67(1):133-138.
- Maehr, D.S., R.C. Belden, E.D. Land, and L. Wilkins. 1990. Food habits of panthers in southwestern Florida. J. Wildl. Manage. 54(3):420-423.
- Mia A.S., D.E. Kahn, M.M. Tierney, and J.E. Post. 1981. A microenzyme linked immunosorbent assay test for detection of FeLV in cats. Comp. for Immunol., Micro., and Infect. Dis. 4:111-117.
- Monk, H.E. 1961. Recommended methods of analysis of pesticide residues in food stuffs. Report by the Joint Mercury Residue Panel. Anal. Chem. 36:608-614.
- O'Brien, S.J., and J. F. Evermann. 1988. Interactive Influence of Infectious disease and genetic diversity in natural populations. Trends in Ecological Evolution 3:254-259.
- _____, M. E. Roelke, L. Marker, A. Newman, C. A. Winkler, D. Meltzer, L. Colby, J. F. Evermann, M. Bush, and D. E. Wildt. 1985. Genetic basis for species vulnerability in the cheetah. Science 227:1, 428-1, 434.
- _____, _____, N. Nuki, K. Richards, W. Johnson, W.L. Franklin, A. Anderson, O.L. Bass, R.C. Belden, and J.S. Martenson. 1990. Genetic introgression with the Florida panther (Felis concolor coryi). Nat. Geo. Res. In press.
- _____, D. E. Wildt, D. Goldman, C. R. Merrill, and M. Bush. 1983. The cheetah is depauperate in genetic variation. Science 221:459-462.
- Pedersen, N.C., E.W. Ho, M.L. Brown, and J.K. Yamamoto. 1988. Isolation of a T-lymphotrophic virus from domestic cats with an immunodeficiency-like syndrome. Science 235:790-793.
- Pirtle, E., M.E. Roelke, and J.R. Brady. 1986. Antibodies against pseudorabies virus in the serum of a Florida black bear cub. J. Am. Vet. Med. Assoc. 189:1164.
- Reagan, K.J., W.H. Wunner, T.J. Wiktor, and H. Koprowski. 1983. Antiidiotypic antibodies induce neutralizing antibodies to rabies virus glycoprotein. J. Virol. 48:660

- Roelke, M.E. 1990. Florida Panther Biomedical Investigation: health and reproduction. Annual performance report, Endangered Species Project E-1 II-E-6, Study No. 7506. Florida Game and Fresh Water Fish Commission. 176pp.
- _____, E. R. Jacobson, G. V. Kollias, and D. J. Forrester. 1986. Medical management and biomedical findings on the Florida panther (Felis concolor coryi). Annual performance report, Endangered Species Project E-1-10 7506. Florida Game and Fresh Water Fish Commission. 65pp.
- Scott, F.W., C.K. Cziza, and J.H. Gillespie. 1970. Maternally derived immunity to feline panleukopenia. J. Am. Vet. Med. Assoc. 156:439-453.
- _____, C.K. Cziza, and J.H. Gillespie. 1970a. Feline viruses. V. Serum-neutralization test for feline panleukopenia. The Cornell Veterinarian 60: 183-191.
- Seal, U.S., and R.C. Lacy. 1989. Florida panther (Felis concolor coryi) viability analysis and species survival plan. United States Fish and Wildlife Service Cooperative Agreement #14-16-0004-90-902, 208pp.
- Takeuchi, T., F.M. D'Itri, P.V. Fischer, C.S. Annett, and M. Okabe. 1977. The outbreak of Minamata disease (methly mercury poisoning) in cats on northwestern Ontario reserves. Environ. Res. 13:215-228.
- United States Fish and Wildlife Service. 1987. Florida panther (Felis concolor coryi) recovery plan. Prepared by the Florida panther interagency committee for the U.S. Fish and Wildlife Service, Atlanta, Georgia. 75pp.
- United States Fish and Wildlife Service. 1989. Status report on mercury contamination in Florida panthers. Prepared by the Florida panther Interagency Committee for the U.S. Fish and Wildlife Service, Atlanta, Georgia. 44pp.
- Wilkins, L. 1990. The Florida panther (Felis concolor coryi): A morphological investigation of the subspecies with a comparison to other North American and South American cougars. Report to the Florida Game and Fresh Water Fish Commission, Tallahassee, Fl. 66pp.
- Yamamoto, J.K., H. Hansen, E.W. Ho, T.Y. Morishita, O. Takiko, T.R. Sawa, R.M. Nakamura, and N.C. Pedersen. 1989. Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission. J. Am. Vet. Med. Assoc. 194:213-220.

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Appendix I. Florida Panther Captive Management and Health Protocol & Feline Inventory and Questionnaire.

Table 1. Serologic tests, antigens, and references used to determine antibody titers for selected pathogenic agents in wild Florida panthers, FY 1990-91.

Agent (antigen)	No. of samples	Test	Positive threshold	Reference
BACTERIA				
<u>Brucella</u> spp. (<u>Brucella abortus</u>)	19	Plate agglutination	≥ 1:25	USDA, no date
PROTOZOA				
<u>Toxoplasma gondii</u>	19	KELA ^{AB}	≥ 1:48	Jacobson et al. (1982)
	12	IHA ^{CD}		
VIRUSES				
Feline calicivirus (FP 255)	10	Plaque reduction	> 1:4	Scott (1977)
Feline enteric corona-virus/feline peritonitis virus	6	KELA ^{EB}	≥ 1:9	Barlough et al. (1983)
	19	IFA ^{FD}	≤ 1:25	Evermann et al. (1986)
Feline immunodeficiency virus	19	ELISA ^{GH}	Qual. ^I	Barr et al. (1989)
Feline leukemia virus	19	ELISA ^{GI}	Qual.	Mia et al. (1981)
Feline parvovirus	10	Serum neutralization	≥ 1:50	Scott et al. (1970a)
Feline viral rhino-tracheitis virus (FVR-Goldstein)	10	Plaque reduction	≥ 1:2	Scott (1977)
Pseudorabies virus	19	Latex agglutination ^K	> 1:4	Pirtle et al. (1986)
Rabies virus	19	Serum neutralization	≥ 6 IU	Reagan et al. (1983)
Syncicial forming virus	19	IFA	Qual.	Gaskin and Gillespie (1973)
	19	AGID ^L	Qual.	

^AKELA: kinetics enzyme-linked immunosorbant assay. ^BPerformed at Cornell Univ. Ithaca, NY. ^CIHA: indirect hemagglutination. ^D Performed at Wash. State Univ. Pullman, WA. ^EThese KELA results are considered to be "IFA-equivalent" titers. ^FIFA: indirect immunofluorescence assay. ^GELISA: enzyme-linked immunosorbant assay ^HPetech FTLV Antibody test, 100 Fore St., Portland, MA 04101, USA. ^IQualitative tests, no titers determined. ^JFeline Leukemia Virus test kits from VIRACHEKTM-FeLV by Symbiotics Corporation and LEUKASSAY[®] II by Pitman-Moore; ^KPseudorabies Virus antibody test kit-Latex agglutination from Viral Antigens, INC.; ^LAgar gel immunodiffusion.

Table 2. Summary of significant dates and events: Florida panthers FY 1990-1991.

ID# & SEX	CAPTURE			EVENT • LOCATION • COMMENT
	DATE	EST AGE	AREA ^A	
#09♀	07-...-90	8-10 yr	I-FSSP	Gave birth (sired by #37♂).
	02-22-91	9-11 yr	I-FSSP	Treed; Captured kitten #202♂ (sired by #37♂).
	02-25-91	9-11 yr	I-FSSP	Treed; Captured kitten #203♂ (sired by #37♂).
#11♀	05-...-91	9-10 yr	I-N.FS/PR	Gave birth in May 1990 (sired by #12♂). Only known offspring (PCO 192♀) was hit-by-car and necropsy showed that she had a atrial septal defect in her heart.
#12♂	01-14-91	10-11 yr	I-N.FS	Captured and immobilized for collar change on FPNWR, 1 mi. NW of Rock Island Gate. Electroejaculated and semen frozen by Dr. Mark Barone of the Natl. Zoo. Cat was in good condition, no sign of peculiar skin disease noted 1/89. Sired litters w/ #19♀, #11♀, and #31♀ in 1990.
	03-11-91	10-11 yr	I-N.FS	Illegally treed by renegade dogs.
#14♀	02-...-91	10 yr	II-ENP	Probably gave birth in Feb. 1991 (sired by #16♂, her son), kittens never documented.
	06-20-91	10 yr	II-ENP	Died of probable kidney failure with elevated mercury levels.
#16♂	02-04-91	5.5-6 yr	II-ENP	Captured and immobilized for collar change on W. side of Shark River Slough, S. of SR 41 (offspring of #14♀, sib of #21♀). Electroejaculated and semen frozen by Dr. Mark Barone. (Sired litter with #14♀ and 23♀, neither survived).
#17♂	07-20-90	11-13 yr	I-N.FS	Died of unknown causes on Noble's Lease, 4 mi. N. of Alligator Alley, 5 mi. E. of North Grade. Possibly sired a litter with #36♀.
#18♀	10-03-91	11-13 yr	I-N.FS	Died due to intraspecific aggression, probably from #28♂, on Gallegar Ranch, 1 mi. W. of McDaniel's fenceline.
#19♀	10-...-90	4.5 yr	I-N.FS	Gave birth (sired by #12♂, her father)(#19 is offspring of #11♀ and #12♂).
	02-05-91	5 yr	I-N.FS	Chased and treed 3 times on FPNWR in unsuccessful attempt to catch kitten(s).
	05-03-91	5 yr	I-N.FS	Tracked in attempt to locate kitten(s). Kitten #205♀ (sired by #12♂, sib #45♂) was found and captured without treeing #19♀.
	05-08-91	5 yr	I-N.FS	Treed; Captured #45♂ (sired by #12♂, sib #205♂).
#21♀	02-01-91	5-5.5 yr	C-WOP	Immobilized; artificially inseminated with semen from PCO 10♂, no offspring produced. Has been living in isolation due to FIV infection (offspring of #14♀, sib of #16♂).
#22♀	02-11-91	4.5-5.5 yr	II-ENP	Captured and immobilized to change dysfunctional radio-collar (offspring of #15♀, sib of #23♀). Cat had been "lost" between 9/89 and 2/90 despite extensive hunting efforts.

Table 2. (continued)

Capture Summary FY 90/91 p. 2

ID# & SEX	CAPTURE			EVENT • LOCATION • COMMENT
	DATE	EST AGE	AREA ^A	
	07-19-91	5-6 yr	II-ENP	Died of chronic emaciation (34 lbs, down from 70 lbs) with terminal cellulitis; both of unknown origin.
#23♀	07-...-90	3.5 yr	I-RP	Gave birth (sired by #16♂), lost litter at approx. 1 month (offspring of #15♀, sib of #22♀).
	02-08-91	4.5 yr	I-RP	Treed; capture aborted, cat too high in tree.
	05-10-91	4.5 yr	I-RP	Captured, immobilized, and changed collar. Treed immediately, but jumped and ran before being darted. Bayed and darted on the ground in a palm thicket. Cat was in good condition with a thick coat and good muscling, but had pale mucous membranes. A hog kill was found nearby. She continues to regularly associate with #38♀ without any males in the area.
#26♂	01-25-91	7-8 yr	I-PR	Cat was lying on his side in an open field and failed to move when buzzed by airplane. He moved off to the NE when approached and exhibited no further aberrant behavior (sired litters with #36♀ and 40♀).
	01-27-91	7-8 yr	I-PR	Treed; hunting for uncollared cats.
#28♂	01-07-91	4 yr	I-PR	Captured, immobilized, and radio-collar changed on Collier Ent. Land near Tony Strand. Electroejaculated and semen frozen by Dr. Mark Barone. Cat had lacerations on ear, multiple scrapes, scars, swelling and crepitation in right front foot. Antibiotics were administered. Cat tested FIV(+).
#29♂	01-08-91	4 yr	I-PR	Attempt to capture aborted. Cat moved onto private property. (Offspring of #11♀ and either #20♂ or #25♂).
	01-09-91	2.5 yr	I-PR	Captured (in PM), immobilized and radio-collar changed in the Nine section. Collar enterprise. Semen collected and frozen by Dr. Mark Barone. Cat was in excellent condition and had a cut over the right eye and inside of the lip that were healing well.
#31♀	08-...-90	9-11 yr	I-N.FS	Gave birth (sired by #12♂).
	02-19-91	10-12 yr	I-N.FS	Treed in Catherine's Island in unsuccessful attempt to capture kitten(s).
	02-21-91	10-12 yr	I-N.FS	Tracked and/or treed; Captured kitten #201♂ (sired by #12♂).
	02-27-91	10-12 yr	I-N.FS	Tracked and/or treed; Captured #204♀ (sired by #12♂).
	03-14-91	10-12 yr	I-N.FS	Captured, immobilized and radio collar changed on FPNWR. Cat had scrapes on elbow region of legs and lesions on the roof of her mouth, but was otherwise in excellent condition.
#32♀	01-11-91	4-5 yr	I-NFS	Captured (in AM), immobilized, and radio-collar changed in FPNWR. Possibly pregnant (sire #12♂), no kittens ever documented.

Table 2. (continued)

Capture Summary FY 90/91 p. 3

ID# & SEX	CAPTURE			EVENT • LOCATION • COMMENT
	DATE	EST AGE	AREA ^A	
#34♂	01-11-91	22 mo	I-PR	Captured (in PM), immobilized, and radio-collar changed on Seminole Indian Reservation Game Ranch (offspring of #31♀). Electroejaculation was performed by Dr. Mark Barone. Semen was not of sufficient quality to freeze.
#36♀	03-...-90	3-5 yr	I-PR	Gave birth (sired by #26♂ or #17♂).
	03-01-91	4-6 yr	I-PR	Treed on Seminole Indian Reservation Game Ranch in unsuccessful attempt to find kittens. Apparently they (n=2) had just recently dispersed. Recent hog kill found nearby.
#37♂	11-26-90	4.5 yr	I-FSSP	Hit-by-car and killed on SR 29 near Sunniland.
#38♀		4-6 yr	I-RP	Not immobilized this year. Continues to regularly associate with #23♀ without any males in the area.
#40♀	10-...-90	2.0-2.5 yr	I-PR	Gave birth (sired by #26♂).
	03-04-91	2.5-3 yr	I-PR	Treed on Noble's Lease in unsuccessful attempt to locate kittens. No kittens found, but tracks seen.
	04-29-91	2.5-3 yr	I-PR	Treed; hunting for kittens.
	04-30-91	2.5-3 yr	I-PR	Treed; captured #44♂ (offspring of #40♀ and #25♂).
	05-06-91	2.5-3 yr	I-PR	Tracked on Baker's Lease; captured #206♀ (sib of #44♂).
#41♀	09-26-90	2.0-3 yr	I-PR	Died from intraspecific aggression (probably with #28♂) at the W. end of Seminole Indian Reservation Game Ranch, 2 mi. N. of the Southern boundary.
#42♂	02-05-91	22.5 mo	II-ENP	Captured, immobilized and radio-collar changed N. of Chikika State Pk. Cat is no longer with mother and is in good condition. Electroejaculated and semen frozen by Dr. Mark Barone. (Offspring of #14♀ and back crossed to son #16♂).
#43♂	01-09-91	16-17 mo	I-PR	Captured (in AM), immobilized, and radio-collar changed on Noble's Lease. Electroejaculation was performed by Dr. Mark Barone. Semen was not of sufficient volume or quality to freeze (offspring of #19♀ backcrossed to her father #12♂).
#44♂	04-30-91	6 mo	I-BR/PR	Captured, immobilized, and radio-instrumented (offspring of #26♀ and #40♂). Cat was living on Noble's and Baker's Leases, but was captured in NE Bear Island. Abbreviated work-up was done to minimize time away from mother and they were together and 5 miles away the next day.
	05-06-91	6 mo	I-PR	Treed hunting for sibling(s); Captured #206♂.
#45♂	05-08-91	6 mo	I-N.FS	Captured, immobilized and radio-instrumented in SW FPNWR (offspring of #19♀ backcrossed to her father #12♂, sib of #205♀). Cat was in good condition and recovered in tent due to presence of standing water.

Table 2. (continued)

Capture Summary FY 90/91 p. 4

ID# & SEX	CAPTURE			EVENT • LOCATION • COMMENT
	DATE	EST AGE	AREA ^A	
#201♂	02-20-91	7 mo	I-N.FS	Captured and immobilized on Pacific Fruit Co. land (offspring of #31♀ and #12♂, sib of #204♀). Kitten was lighter weight than expected and had a rough hair coat. Removed from wild and transported to WOP for captive breeding program.
	04-23-91	9 mo	C-WOP	Immobilized for 60 day post quarantine exam and cardiac evaluation.
#202♂	02-23-91	7 mo	I-FSSP	Captured and immobilized in FSSP (offspring of #09♀ and #37♂, sib of #203♂). Kitten was in good condition. Removed from wild and transported to WOP for captive breeding program.
	04-23-91	9 mo	C-WOP	Immobilized for 60 days post quarantine exam and cardiac evaluation.
#203♂	02-25-91	7 mo	I-FSSP	Captured and immobilized in a cypress swamp (offspring of #09♀ and #37♂, sib of 202♂). Left testicle partially retained. Removed from wild and transported to WOP for captive breeding program.
	04-23-91	9 mo	C-WOP	Immobilized for 60 days post quarantine exam and cardiac evaluation.
#204♀	02-27-91	7 mo	I-N.FS	Captured and immobilized on Pacific Fruit Co. land (offspring of #31♀ and #12♂, sib of #201♂). Lesions on lip and tongue. Removed from wild and transported to WOP for captive breeding program.
	04-23-91	9 mo	C-WOP	Immobilized for 60 days post quarantine exam and cardiac evaluation.
#205♀	05-03-91	6 mo	I-N.FS	Captured and immobilized on FPNWR (offspring of #19♀ backcrossed to her father 12♂, sib of #45♂). Removed from wild and transported to WOP for captive breeding. Pronounced cardiac murmur detected at the time.
	07-02-91	9 mo	C-WOP	Immobilized for 60 days post-quarantine exam. Transported to VMTH for complete cardiac examination by Drs. King and Abbott. Diagnosed as having severe tricuspid valve dysplasia. This will probably be fatal. Returned to WOP.
#206♀	05-06-91	6 mo	I-PR	Captured and immobilized on NW Baker's Lease (Offspring of #40♀ and 26♂, sib of #44♂). Hog kill nearby. Removed from wild and transported to WOP for captive breeding.
	07-02-91	9 mo	C-WOP	Immobilized for 60 days post quarantine exam and cardiac evaluation.
PCO10♂	02-01-91	9-11 yr	C-WOP	Immobilized; collected sperm for AI of #21♀.

^ARefer to legend of Table 8 for location information.

Table 3. Physical parameters and reproductive status of male Florida panthers: FY 1990-91.

Location	ID#	Date	Age	Body WT ^A	Phys Cond ^B	HGB/PCV ^C	Repro ^D
Private Ranches East of Immokalee Hendry County	#17	20Jan87	8-10 yr	142	I	14.8/45.1	?/2/?
		26Jan89	10-12 yr	154	I	11.8/36.5	-/2/?
		20Jul90 ^E	11-13 yr	na ^E	na	na	/S
N. Fakahatchee Strand, Florida Panther National Wildlife Refuge, Bear Island Unit BCNP, and adjacent Private Ranches Collier and SW Hendry counties	#12	28Jan86	4-5 yr	122	I	14.3/41.0	+/1/Y
		31Jan88	6-7 yr	124	II	12.0/34.8	+/1/Y
		15Jan89	7-8 yr	120	II-	12.7/40.3	+/1/Y
		14Jan91	9-10 yr	110	I	12.5/39.6	+/1/Y
	#28	29Nov88 ^F	18-24 mo	93	I	11.1/33.8	?/1/?
		07Jan91	3.5-4 yr	120	II	13.5/41.4	+/1/?
	#29	03Jan89	6 mo	44	II	13.5/43.7	?/1/I
		01May89	11.5 mo	76	II	12.7/38.8	?/1/I
		12Feb90	20 mo	101	I	12.4/39.0	+/1/?
		09Jan91	2.5 yr	121	I	10.9/34.5	+/1/S
	#34	08Jan90	10 mo	62	I	11.4/36.5	?/1/I
		03May90	14 mo	68	II	11.8/35.7	?/1/I
		11Jan91	22 mo	110	I	11.3/35.4	+/1/?
	#43	01May90	10 mo	54	II	11.6/35.4	?/1/I
		05Jan91	16-17 mo	86	II	8.6/28.0	+/1/I
	#44	30Apr91	6 mo	33	II-	10.8/34.2	?/1/I
	#45	08May91	6 mo	38	II	11.6/37.1	?/1/I
	#201	20Feb91	7 mo	34	III	8.1/25.0	?/1/I
	#37	30Jan90	3-4 yr	102	II	10.7/35.3	+/2/Y
		26Nov90 ^E	4-5 yr		II	na	+/2/Y
	#202	23Feb91	7 mo	47	I	10.8/32.8	?/2/I
	#203	25Feb91	7 mo	45	II	9.8/31.4	?/1/I
Everglades National Park Dade Co.	#16	12Jan87	16-22 mo	84	II ⁺	13.1/38.0	+/2/?
		02Feb88	28-34 mo	86	III	12.0/33.9	+/2/?
		21Feb90	4.5-5 yr	104	II	9.7/29.9	-/2/Y
		04Feb91	5.5-6 yr	98	II	11.2/33.7	+/2/Y
	#42	06Mar90	10 mo	60	II	11.5/35.8	?/2/?
		05Feb91	21 mo	87	I	12.7/38.4	+/2/?

^APounds (lbs.); ^BPhysical condition score: I-Excellent, II-Good, III-Poor, IV-Grave; ^CHemoglobin (gms/100ml) PCV = packed cell volume; ^DReproductive status; results of semen evaluation (+ = fertile, - = sterile, ? = unknown)/number of descended testicles/documentated reproduction (Y = known sire, S = suspected sire, ? = unknown, I = immature). ^EDeath date; parameters not available (na) if carcass is decomposed. ^FOriginally hit-by-car near Ft. Myers airport.

Table 4. Physical parameters and reproductive status of female Florida panthers: 1990-91.

Location	ID#	Date	Age	Body WT ^A	Phys Cond ^B	HGB/PCV ^C	Repro ^D
Private Ranches East of Immokalee Hendry County	#18	22Jan87	8-10 yr	100	I	12.7/48.1	?/-/NPG
		23Jan89	10-12 yr	85	I	13.8/41.2	0/-/NPG
		30Oct90 ^E	11-13 yr	na ^E	na	na	0/-/na
N. Fakahatchee Strand, Florida Panther National Wildlife Refuge, Bear Island Unit BCNP, and adjacent Private Ranches Collier and SW Hendry counties	#31	12Jan89	8-10 yr	85	II	12.6/38.5	?/-/PG
		14Mar91	10-12 yr	92	I	12.7/39.9	2/7/NPG
	#32	03Feb89	24-30 mo	79	II	10.7/36.8	?/-/PG
		11Jan91	4-4.5 yr	76	II	11.1/33.5	0/-/SPG ^{1,4,5}
	#41	28Feb90	1.5-2 yr	61	I	14.1/45.7	?/-/NPG
		26Sep90 ^E	2-2.5 yr	na	na	na	0/-/na
	#204	27Feb91	7 mo	37	II	9.8/29.8	0/-/I
	#205	03May91	6 mo	31.5	II	9.9/37.1	0/-/I
Eastern Big Cypress National Preserve (BCNP), Collier Co.	#23	18Mar87 ^F	6-8 mo	31	II	12.6/36.2	0/-/I
		19Feb90 ^F	3.5 yr	55	II	10.0/31.9	0/-/SPG ^{1,4,5}
		10May91	4.5 yr	63	II	8.6/33.2	0/-/NPG ^G
Everglades National Park, Dade Co.	#22	18Mar87	6-8 mo	33	II	15.7/43.3	0/-/I
		07Apr87	7-9 mo	36	II	11.8/34.4	0/-/I
		15Jun87	9-11 mo	43	II	9.8/30.0	0/-/I
		24Mar88	16-18 mo	60	II	9.8/30.1	0/-/NPG
		11Feb91	4.5 yr	70	II	10.9/33.9	0/-/NPG

^APounds (lbs.); ^BPhysical condition score: I-Excellent, II-Good, III-Poor, IV-Grave; ^CHemoglobin (gms/100ml) PCV = packed cell volume; ^DReproductive status; number of kitten documented within previous 12 months/age of kittens in months on capture date/pregnancy status of female at capture (PG - pregnant, NPG - not pregnant, SPG - suspect pregnant, PPG - probably recently pregnant but litter was lost, I - immature). Diagnosis of pregnancy status based on ¹association with a male and subsequent ²denning behaviour, ³palpation of fetuses, ⁴nipple &/or mammary development, &/or ⁵elevated progesterone; ^EDeath date; parameters not available (na) if carcass is decomposed; ^FCaptured in Everglades National Park on these dates; ^GNo male in area to breed with.

Table 5. Documented panther longevity, injury and mortality for panthers alive in FY 1990-91.

ID# & Sex	Capture Date		Field ^A Captures	Months Collared		Age ^C	Date	Dead or Injured Panthers Cause/Location ^D
	Initial	Latest		Total	Recent ^B			
#09♀	01-26-85	01-08-87	2	24	RFW ^{E-1}	5-7 yr	01-08-87	Treated for gunshot wound & osteomyelitis of rear foot, Cco Returned to wild 1/87
	01-31-87	03-14-90	4	90	15	9-11 yr	* Alive *	
#11♀	01-21-86	01-02-90	3	65	18	9-11 yr	* Alive *	
#12♂	01-28-86	01-14-91	4	65	6	9-10 yr	* Alive *	
#14♀	12-06-86	02-22-90	3	54	16	9-10 yr	* Alive *	(Died 7/2/91 - suspect renal disease plus mercury contamination, ENP, Dco)
#16♂	01-12-87	02-04-91	4	53	5	5.5-6 yr	* Alive *	
#17♂	01-20-87	01-26-89	2	42	18	11-13 yr	07-20-90	Unknown- Bnz & Hr ^F , Cco
#18♀	01-22-87	01-23-89	2	44	20	11-13 yr	10-30-90	Intrasp aggression, Hco
#19♀	02-09-87	02-16-90	4	53	16	5 yr	* Alive *	Radio-collar malfunctioned, "lost" cat between 9/89 and 2/90
#21♀	03-16-87	07-23-88	2	18	RFW ^{E-2}	2.5-3 yr	07-23-88	Hit-by-car, Palm Dr., SE Homestead, Dco. Alive in captivity at WOP.
#22♀	03-18-87	02-11-91	5	51	5	5 yr	* Alive *	(Died 7/19/91 - cause of death is undetermined at this time)
#23♀	03-18-87	06-22-88	5	15 ^G	RFW ^{E-3}	21-22 mo	06-22-88	Stranded on island, removed from wild
	02-22-89	05-10-91	7	43 ^G	2	5 yr	* Alive *	Returned to wild, ENP
#26♂	03-01-88	02-10-90	2	40	16	7-8 yr	* Alive *	
#28♂	11-29-88	11-29-88	1	0	RFW ^{E-4}	18-24 mo	11-29-88	Hit-by-car, Ft. Myers airport, Lco
	03-15-89	01-07-91	2	28	6	4-4.5 yr	* Alive *	Returned to wild
#29♂	01-03-89	01-09-91	4	30	6	3 yr	* Alive *	
#31♀	01-12-89	03-14-91	2	30	3	10-12 yr	* Alive *	

Table 5. (continued)

Mortality and longevity p.2

ID# & Sex	Capture Date		Field ^A Captures	Months Collared		Age ^C	Date	Dead or Injured Panthers Cause/Location ^D
	Initial	Latest		Total	Recent ^B			
#32♀	02-03-89	01-11-91	2	29	6	4-4.5 yr	* Alive *	
#34♂	01-08-90	01-11-91	3	17	6	28 mo	* Alive *	
#36♀	01-27-90	-----	1	17	17	4-6 yr	* Alive *	
#37♂	01-30-90	-----	1	10	10	4-5 yr	11-26-90	Hit-by-car, SR 29 N. of SR 84, SunL, Cco.
#38♀	02-08-90	-----	1	17	17	4-6 yr	* Alive *	
#40♀	02-26-90	-----	1	16	16	3-3.5 yr	* Alive *	
#41♀	02-28-90	-----	1	7	7	2-2.5 yr	09-26-90	Intrasp aggression, Hco.
#42♂	03-06-90	02-05-91	2	16	4	26 mo	* Alive *	
#43♂	05-01-90	01-05-91	2	14	5	24 mo	* Alive *	
#44♂	04-30-91	-----	1	2	2	6 mo	* Alive *	
#45♂	05-08-91	-----	1	2	2	6 mo	* Alive *	
#201♂	02-20-91	-----	1	0	RFW ^{E-5}	7 mo	02-20-91	Captive breeding program
#202♂	02-23-91	-----	1	0	RFW ^{E-5}	7 mo	02-23-91	Captive breeding program
#203♂	02-25-91	-----	1	0	RFW ^{E-5}	7 mo	02-25-91	Captive breeding program
#204♀	02-27-91	-----	1	0	RFW ^{E-5}	7 mo	02-27-91	Captive breeding program
#205♀	05-03-91	-----	1	0	RFW ^{E-5}	6 mo	05-03-91	Captive breeding program
#206♀	05-06-91	-----	1	0	RFW ^{E-5}	6 mo	05-06-91	Captive breeding program
PCO♂ 10	11-02-84	-----	1	0	RFW ^{E-6}	3-5 yr	11-02-84	Hit-by-car, Ochopee SR41, Cco. Alive in captivity at WOP.
PCO♀ 192	02-04-91 ^H	-----	0	0	0	9 mo	02-04-91	Cardiac failure (atrial septal defect) & then hit-by-car, SR 29 at "Wooden Bridge" SunL, Cco.

^AField captures = total number of times panther was captured and immobilized in the wild (field).

^BMonths collared: As of June 30, 1991 the "Total" months since initial collaring and the number on months since the most "Recent" capture.

^CAge at death or injury and removed from the wild or if still alive and free-ranging, estimate age on June 30, 1990.

^DLocation: County-Collier (Cco), Dade (Dco), Hendry (Hco), Lee (Lco); ENP-Everglades Nat. Park; SunL-Sunniland; WOP- White Oak Plantation.

^ERFW = Removed from wild:

¹Illegally shot. Fractured metatarsal, osteomyelitis, removed from wild for one month. Treated at Miami Metro Zoo Hospital. Returned to wild.

²hit-by-car, removed to fix fractured femur, still in captivity.

³stranded on island surrounded by water in Shark River Slough, ENP.

⁴uncollared panther, hit-by-car, rehabilitated and released.

⁵chosen for captive breeding program, now at White Oak Plantation.

⁶uncollared panther, hit-by-car, rehabilitated, still in captivity.

^FBnz & Hr - skeleton was reduced to bones and hair.

^G#23 was in captivity from 6-22-87 until 5-14-88 (11 months) then returned to the wild and was removed 1 month later. She was then held in captivity for 8 more months before released on 02-22-89.

^HThis kitten was the unradio-collared offspring of FP#09 F.

Table 6. Causes of documented panther mortality, FY 1990-91.

Category of Death/Injury	Collared		Uncollared		Total	
	No.	(Percent)	No.	(Percent)	No.	(Percent)
Road killed/Injured	1	(25.0)	(1) ^A		1	(20.0)
Intraspecific Aggression	2	(50.0)	0	0	2	(40.0)
Congenital Heart Defect	0		1 ^A	(100.0)	1	(20.0)
Unknown cause ^B	1	(25.0)	0		4	(20.0)
TOTAL	4		1		5	

^AThis 9 month-old kitten of FP#11 was dieing of cardiac failure due to an atrial septal defect when she was hit by a car and killed. She would have died shortly had the car not intervened.

^BSkeletal and hair remains only were recovered on these animals, cause of death could not be determined.

Table 7. Prevalences and reciprocal titers for antibodies to selected pathogens in individual wild Florida panthers, FY 1990-91.

Agent ^A	Number of panthers			Reciprocal titer	
	Examined ^B	Positive	Percent	Minimum	Maximum
BACTERIA					
<u>Brucella</u> spp.	19 ^C			<25	
PROTOZOA					
<u>Toxoplasma gondii</u>	19 ^D	0	0	<48	<48
	12 ^E	1	8.3	<64	64
VIRUSES					
Feline calicivirus ^B	10 ^F	3	30.0	<2	>256
Feline enteric coronavirus/ Feline infectious peritonitis (coronavirus)	6 ^D 19 ^G	0 3	0 15.8	<9 <5	25
Feline immunodeficiency virus	19	3	15.8	Qual. ^H	
Feline leukemia virus	19	0	0	Qual.	
Feline parvovirus ^B (panleukopenia virus)	10 ^F	1	10.0	<10	50
Feline rhinotracheitis virus ^B (herpesvirus)	10 ^F	0	0	<2	
Pseudorabies virus	19 ^C				
Rabies virus ^B	19 ^C			≤0.2 IU	
Feline syncytia forming virus	19 ^C			Qual.	

^ARefer to Table 1 for details of specific test used. ^BResults are presented for samples collected from panthers prior to vaccination; ^CResults are pending on these samples; ^DKELA results, Cornell University, Ithaca, New York. ^EIHA results; ^FEight of these 10 were less than 1 year of age. ^GIFA results, Washington State University, Pullman, Washington. ^HQualitative assays, no titers determined.

Table 8. Seroprevalence of selected disease agents in Florida panthers, captured in FY 1990-91.

ID#	SEX	LOCATION ^B	DATE	TESTS ^A									
				AGE CLASS ^C	FPL	FCV	TOX	FVR	FELV	FIP	FIV	BRUC	PRV
# 12	M	I-BI/FS	01-28-86	5	500	<2	—	<2	—	—/—	—	nd ^D	nd
		I-BI/FS	01-31-88	5	100 ^V	6 ^V	26	<2 ^V	—	—	—	—	—
		I-N.FS	01-15-89	6	500 ^V	6 ^V	21	3 ^V	—	—/—	—	nd	nd
		I-N.FS	01-14-91	6	5,000 ^V	3 ^V	10	16 ^V	—	—/ <25	—	pe ^B	pe
# 16	M	II-ENP	01-12-87	2	<10	48	6	<2	—	—/+	+	—	—
		II-ENP	02-02-88	4	<10	240	12	<2	—	—/—	+	—	—
		II-ENP	02-21-90	4	<10	8	<48	<2	—	/+	+	—	—
		II-ENP	02-04-91	5	50 ^V	48 ^V	6	<2 ^V	nd	/ <25	+	pe	pe
# 21	F	II-ENP	03-16-87	2	<10	2	15	<2	—	—/—	+	—	—
		II-HST	07-23-88	4	<10	<2	<10	<2	—	—	—	—	—
		C/GFC	10-19-88	4	nd	nd	nd	nd	nd	nd	—	nd	nd
		C/GFC	12-29-88	4	nd	nd	nd	nd	nd	/—	nd	nd	nd
		C/GFC	01-28-89	4	nd	nd	nd	nd	nd	nd	—	nd	nd
		C/GFC	02-24-89	4	nd	nd	nd	nd	nd	nd	—	nd	nd
		C/GFC	05-23-89	4	>10,000 ^V	24 ^V	<10	16 ^V	—	—	—	nd	nd
		C/WOP	01-17-91		nd	nd	nd	nd	nd	nd	+	nd	nd
# 22	F	II-ENP	03-18-87	2	<10	<2	4	<2	—	—/—	—	nd	nd
		II-ENP	04-09-87	2	nd	nd	nd	nd	nd	/—	nd	—	—
		II-ENP	03-24-88	3	<10	32	5	<2	—	—	—	nd	nd
		II-ENP	02-11-91	4	<10	<2	4	<2	nd	/ <25	—	pe	pe
# 23	F	II-ENP	03-18-87	2	<10	<2	3	<2	—	—	nd	nd	nd
		II-ENP	04-09-87	2	nd	nd	nd	nd	nd	nd	nd	—	—
		II-ENP	06-01-87	2	<10	2	3	<2	—	—	—	nd	nd
		II-ENP	06-22-87	2	nd	nd	nd	nd	nd	nd	—	nd	nd
		II-ENP	05-12-88	3	nd	nd	nd	nd	nd	/—	nd	nd	nd
		C/GFC	10-21-88	4	nd	nd	nd	nd	nd	/—	—	nd	nd
		C/GFC	11-05-88	4	nd	nd	nd	nd	nd	nd	—	nd	nd
		II-ENP	02-19-90	4	10,000 ^V	48 ^V	<48	3 ^V	—	/—	—	—	—
		I-RP	05-10-91	5	5,000 ^V	128 ^V	15	6 ^V	—	/ <25	—	pe	pe
# 28	M	I-CORK	11-29-88	2	<10	<2	nd	<2	nd	nd	nd	nd	nd
		C/GFC	12-30-88	2	nd	nd	nd	nd	nd	/—	—	—	—
		C/GFC	02-24-89	3	nd	nd	nd	nd	nd	/—	nd	nd	nd
		C/GFC	03-02-89	3	10,000 ^V	<2 ^V	<10	<2 ^V	—	—	nd	nd	nd
		I-PR	01-07-91	4	48 ^V	2 ^V	2	2 ^V	—	—/ <25	+	pe	pe
# 29	M	I-BI/PR	01-03-89	1	<10	<2	47	<2	—	—/—	—	nd	nd
		I-BI/PR	05-01-89	2	50 ^V	2 ^V	58	<2 ^V	—	—/—	—	—	—
		I-SIR	02-12-90	3	10,000 ^V	6 ^V	<48	<2 ^V	—	/—	—	—	—
		I-PR	01-09-91	4	500 ^V	32 ^V	5	5 ^V	nd	—/ <25	—	pe	pe

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ID#	SEX	LOCATION ^B	DATE	AGE CLASS ^C	TESTS ^A									PRV
					FPL	FCV	TOX	FVR	FELV	FIP	FIV	BRUC		
# 31	F	I-N.FS	01-12-89	6	5,000	<2	<10	<2	-	-/-	-	-	-	
		I-N.FS	03-14-91	6	1,000 ^V	>256 ^V	23	<2 ^V	nd	/ <25	-	pe	pe	
# 32	F	I-N.FS	02-03-89	4	<10	<2	<10	<2	-	-/-	-	-	-	
		I-N.FS	01-11-91	5	50 ^V	<2 ^V	5	3 ^V	-	- / <25	-	pe	pe	
# 34	M	I-CAI	01-08-90	2	<10	<2	<48	<2	-	/-	-	-	-	
		I-PR	01-11-91	3	500 ^V	3 ^V	15	12 ^V	-	- / <25	-	pe	pe	
# 42	M	II-ENP	03-06-90	2	>10	<2	<48	<2	-	/-	-	-	-	
		II-EE	02-05-91	3	50	<2	14	<2	nd	/ <25	+	pe	pe	
# 43	M	I-N.FS	05-01-90	2	nd	nd	nd	nd	-	nd	nd	nd	nd	
		I-PR	01-09-91	2	100 ^V	<2 ^V	7	<2 ^V	nd	- / <25	-	pe	pe	
# 44	M	I-N.FS	04-31-91	1	<10	<2	8	<2	-	/ <25	-	pe	pe	
# 45	M	I-N.FS	05-08-91	1	<10	48	1	<2	-	/ <25	-	pe	pe	
# 201	M	I-N.FS	02-20-91	2	<10	<2	2	<2	-	/ 25	-	pe	pe	
		C/WOP	04-23-91	2	5,000 ^V	12 ^V	nd	<2 ^V	-	/ <25	-	pe	pe	
# 202	M	I-S.FS	02-23-91	2	<10	<2	5	<2	-	/ <25	-	pe	pe	
		C/WOP	04-23-91	2	500 ^V	4 ^V	nd	<2 ^V	-	/ <25	-	pe	pe	
# 203	M	I-S.FS	02-25-91	2	<10	<2	1	<2	-	/ <25	-	pe	pe	
		C/WOP	04-23-91	2	10 ^V	6 ^V	nd	<2 ^V	-	/ <25	-	pe	pe	
# 204	F	I-N.FS	02-27-91	2	<10	6	nd	<2	-	/ 25	-	pe	pe	
		C/WOP	04-23-91	2	100 ^V	12 ^V	-	<2 ^V	-	/ 25	-	pe	pe	
# 205	F	I-N.FS	05-03-91	1	<10	8	3	<2	-	/ 25	-	pe	pe	
		C/WOP	07-02-91	2	pe	pe	nd	pe	pe	pe	pe	pe	pe	
# 206	F	I-BI/PR	05-06-91	1	<10	<2	13	<2	-	/ <25	-	pe	pe	
		C/WOP	07-02-91	2	pe	pe	nd	pe	pe	pe	pe	pe	pe	
PCO 10	M	I-MO	11-02-84	4	<20	40	-	5	-	-	-	-	-	
		C/WOP	11-20-84	4	nd	nd	nd	nd	nd	/-	nd	nd	nd	
		C/WOP	06-13-87	5	nd	nd	nd	nd	nd	nd	-	nd	nd	
		C/WOP	06-02-88	5	5,000 ^V	12 ^V	<10	8 ^V	-	-/-	-	nd	nd	
		C/WOP	01-17-91		nd	nd	nd	nd	nd	nd	-	nd	nd	

Table 8. (continued)

LEGEND: SEROPREVALENCE TABLE

^aSEROLOGICAL TESTS:

BRUC - brucellosis, +/- = plate agglutination test screens serum for antibodies against bacteria Brucella spp., titer = tube agglutination; FCV - feline calicivirus, SN antibody > 1:4 titer considered positive; FELV - feline leukemia virus, ELISA test detects presence of virus; FIP - feline infectious peritonitis - KELA test determines antibody to coronaviruses (+ or -) at Cornell /IFA titer determined at WADDL, $\geq$ 1:25 positive; FIV - feline immunodeficiency virus, ELISA test detects presence of antibodies; FPL - feline panleukopenia virus, serum neutralizing antibody titer, test detects and cross reacts with all parvoviruses, titers $\geq$ 1:50 are considered positive; FVR - feline viral rhinotracheitis, SN antibody titer $\geq$ 1:2 considered positive; TOX - toxoplasmosis, caused by protozoan Toxoplasma gondii, KELA antibody titer > 1:48 considered positive; PRV - pseudo-rabies virus, latex agglutination antibody titer > 1:4 considered positive.

^bLOCATION OF PANTHER- (major population followed by locality)

MAJOR POPULATIONS: I- Southwestern Florida, Big Cypress Swamp & Fakahatchee Strand and surrounding ecosystem including privately owned land; II- Southeastern Florida, Everglades National Park; C- captivity.

LOCALITY OF RANGE: ACQ - Acquisition lands (BCNP) east of BI and north of SR 84; BI - Bear Island Unit of Big Cypress National Preserve; CAI - Catherine Island, SW of Immokalee, north and south of Hwy 856 and west of Hwy 29; CORK - Corkscrew Swamp Sanctuary and Ft. Myers area; EE- East Everglades; ENP - Everglades National Park; FS- Fakahatchee Strand both north and south of SR 84; GFC - Florida Game and Fresh Water Fish Commission, Wildlife Research Laboratory, Gainesville, FL.; HST - Homestead, Florida; MMZ - Miami Metro Zoo, Miami, FL.; MO - Southwestern Monument Unit, Big Cypress National Preserve; N.FS - Northern Fakahatchee Strand; PR - Private ranches east of Immokalee; RP - Raccoon Point, Eastern Big Cypress National Preserve; S.FS - Southern Fakahatchee Strand (Fakahatchee Strand State Preserve); SIR -Seminole Indian Reservation; SS -Stair Steps Unit of Big Cypress National Preserve; WOP - White Oak Plantation, Yulee, FL.

^cAGE CLASS: 1 - kitten (0-6 months); 2 - juvenile (7-18 months); 3 - subadult (19-24 months); 4 - young adult (2-4 years); 5 - mature adult (5-8 years); 6 - older adult (> 8 years).

^dnd- not determined

^epe- test results pending

^vCertain test results on this date may be influenced by vaccinations received on the previous immobilization date. Panthers were vaccinated with killed virus vaccine (Fort Dodge: FEL-O-VAX[®]) for FPL, FCV, and FVR.

Table 9. Mercury concentrations (ppm, wet wt.) in tissues from free-ranging Florida panthers from various locations, 1978 - 1991.

LOCATION	WHOLE BLOOD		HAIR		LIVER		KIDNEY		MUSCLE		BRAIN	
	n	Mean(S.E.) ^A	n	Mean(S.E.)	n	Mean(S.E.)	n	Mean(S.E.)	n	Mean(S.E.)	n	Mean(S.E.)
North of Alley	42	0.095(1.15) ^B	43	1.66(1.23) ^B	10	1.15(1.24)	7	0.407(1.24)	6	0.084(1.27)	3	0.052(1.43)
Fakahatchee St.	12	0.384(1.35) ^{CD}	7	7.18(1.62) ^C	2	20.2 (1.08)	4	6.35(1.18)	4	0.974(1.26)	2	1.08(1.32)
Raccoon Point	2	0.620(1.26)	2	42.3 (1.63)	1	15.0	nd ^E		nd		nd	
Shark Slough	7	0.794(1.59) ^D	8	56.4 (1.24) ^D	2	40.6 (2.02)	2	2.23(2.62)	2	4.44 (1.04)	2	3.18 (1.62)
Long Pine Key	14	0.232(1.21) ^C	8	10.9 (1.35) ^C	3	18.1	1	4.20	1	0.290	2	0.200
Corbett WMA		nd		nd	1	12.1	1	5.20	1	0.240	1	0.240

^AThe mean is the geometric mean determined from log-transformed data and the standard error (S.E.) should therefore be applied as x or ÷ of the mean.

^{B,C,D} Within columns, means with different superscripts differ ($P < 0.05$). Means without superscript notations were not compared statistically due to small sample size.

^End - not determined.

Table 10. Mercury concentrations (ppm, wet wt.) in tissues from various southern Florida carnivores, 1984 - 1991.

LOCATION	RACCOON				BOBCAT		OTTER		ALLIGATOR	
	MUSCLE		LIVER		LIVER		LIVER		MUSCLE	
Northwest to Southeast (approximate)	n	Mean(S.E.) ^A	n	Mean(S.E.)	n	Mean(S.E.)	n	Mean(S.E.)	n	Mean(S.E.)
North of Alley (Bear Island)	7	0.32(1.33) ^B	8	1.51(1.27) ^B	8 ^E	0.10(1.79)	3 [†]	0.19(1.77)	1 [†]	0.34
Fakahatchee Strand St. Preserve	6	0.47(1.21) ^{BC}	6	5.46(1.35) ^C	5	0.13(1.88)		nd ^F		nd
Collier Seminole SP (far west SR 41)		nd		nd		nd	2 [†]	1.01(1.64)		nd
Carnestown (west SR 41)		nd		nd		nd	6 [†]	3.07(1.22)	1 [†]	0.38
Raccoon Point	5	0.48(1.27) ^{BC}	5	13.4 (1.29) ^{CD}	7	0.16(1.95)		nd		nd
Oasis (east SR 41)		nd		nd		nd	8 [†]	2.98(1.26)	3 [†]	0.32(1.17)
Loop Road	6	1.43(1.30) ^D	6	15.4 (1.23) ^{CD}		nd		nd		nd
Water Conservation Area 3A	5	1.41(1.41) ^D	5	15.8 (1.14) ^{CD}		nd		nd		nd
Shark Slough	5	1.80(1.24) ^D	5	24.0 (1.15) ^D	1 [†]	5.08	1 ^{†G}	6.78	5	2.96(1.20)
Long Pine Key	5	1.22(1.24) ^{CD}	5	17.5 (1.33) ^D	1	0.08		nd		nd
Taylor Slough	4	1.01(1.68) ^{CD}	4	4.88(1.87) ^C		nd		nd		nd
Flamingo	6	0.22(1.24) ^B	6	1.35(1.10) ^B		nd		nd		nd
Card Sound Road		nd		nd	1 [†]	0.22		nd		nd

^AThe mean is the geometric mean determined from log-transformed data and the standard error (S.E.) should therefore be applied as x or ÷ of the mean; ^{B,C,D}Within columns, means with different superscripts differ ($P < 0.05$). Means without superscript notations were not compared statistically due to small sample size; ^ETwo of these samples were collected in the Florida Panther National Wildlife Refuge (0.99 & 0.08 ppm); ^Fnd - not determined; ^GCollected from Chikika State Park in the eastern Everglades; [†]Road-killed animals.

Table 11. Fertility assessment and contribution to frozen sperm bank for male panthers captured in FY 1990-91.

ID#	Date	Age	Breeder Status	Reproductive evaluation					Sperm Bank	
				# Testicles ^A	Motility ^B	Status ^C	Concentration ^D	Volume	# Pellets	AI units ^E
# 12	01-31-88	6-7 yr	Presumed	1	40%	3.0	10.5 x 10 ⁶	0.77 ml	169	1.92
	01-14-91	9-10 yr	Presumed	1	50%	2.5	19.0 x 10 ⁶	1.58 ml	51	1.15
# 16	02-02-88	28-34 mo	Unknown ^F	2	40%	3.5	3.5 x 10 ⁶	0.53 ml	20	0.07
	02-04-91	5.5-6 yr	Presumed	2	70%	3.0	23.0 x 10 ⁶	2.10 ml	83	3.38
# 26	02-10-90	6-7 yr	Unknown ^G	1	40%	3.0	1.0 x 10 ⁶	1.14 ml	1 ^H	0.05
# 28	01-07-91	3.5-4 yr	Unknown	1	30%	3.0	20.0 x 10 ⁶	1.31 ml	8 ^H	0.79
# 29	10-09-91	2.5 yr	Suspect	1	30%	3.0	56.4 x 10 ⁶	1.30 ml	50	2.20
# 34	01-11-91	22 mo	Unknown	1	0%	0	4.0 x 10 ⁶	2.77 ml	0 ^I	—
# 42	02-05-91	21 mo	Unknown	2	70%	3.0	2.0 x 10 ⁶	1.30 ml	37	0.16
# 43	01-05-91	16-17 mo	Unknown	1	—	—	0	0.21 ml	0 ^I	—

^ANumber of descended testicles; ^BMotility = percent motile sperm; ^CStatus- quality of progressive sperm movement, 1 (low) - 5 (high).

^DNumber of sperm per ml of ejaculate; ^EAI units - one AI unit is the number of motile sperm (5 x 10⁶) 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 ≥ 50% loss of viable sperm due to freezing; ^FSired a litter approximately 1 week after this date; ^GSired a litter in August 1990; ^HSperm concentrated by centrifugation prior to freezing; ^ISperm quality was too low to allow for freezing.

Table 12. Proposed selection of panther kittens for captive breeding project: January 17, 1991.

FEMALE		KITTEN		Location ^A	Priority ^B	Recommendation ^C
ID#	Age	Age	Sire			
KITTENS BORN IN 1990						
#31	10-12y	5.5mo	#12	I-N.FS/PR	1 ⁺	Pull ALL (up to 2) Dam (<i>D</i>) is old and both sire (<i>S</i>) and <i>D</i> are founders (<i>F</i>).
#9	8-10y	6.5mo	#37	I-FSSP	1 ⁺	Pull ALL (up to 2) <i>S</i> is dead and both parents are Fs, <i>D</i> has history of poor survivorship of young. <i>S</i> is FIV positive- test kittens.
#36	4-6y	9.5mo	#26 or #17	I-PR	2 ⁺	Pull ALL or PART (Depending on number of #31 and #09 kittens). Both <i>S</i> 's are unrepresented Fs and one of the possible <i>S</i> 's is dead (#17). <i>D</i> is FIV positive- test kittens.
#11	8-10y	8.5mo	#12	I-BI/PR	2 ⁺	Pull PART <i>D</i> is an old F, but she does have three living collared offspring (<i>F</i> ₁ = #19F & #29 and <i>F</i> ₂ = #43M).
#40	2.5-3y	3.0mo	#26	I-PR	2 ⁺	Pull PART <i>D</i> is young <i>F</i> (possibly daughter of #36) <i>S</i> is a F. <i>D</i> and <i>S</i> are FIV positive- test kittens.
#19	4.5y	2.5mo	#12	I-N.FS	4 ⁺	Select these only if other kittens are unavailable. Living mother (#11) is <i>F</i> and this litter is a back cross with the <i>D</i> 's father.
POSSIBLE 1991 SPRING LITTERS						
#32	3.5-4y	----	#12	I-N.FS	2 ⁺	Litter possibly due in March. <i>D</i> is a young <i>F</i> (possible daughter of #31) and <i>S</i> is F.
#14	8-9y	----	#16	II-ENP	3 ⁺	<i>D</i> is a <i>F</i> and her <i>F</i> , #21F is in captivity. <i>S</i> of Litter would be #14's son (#16), due in March.

^ALocation: see Table . (I-Big Cypress population, II-Everglades population); ^BPriority in order of preference 1⁺ = top choice, 4⁺ = lowest choice; ^CRational used to determine priority of selection, *D* = dam; *S* = sire *F* = founder.

Table 13. Potential adult panthers for inclusion in captive breeding program: January 17, 1991.

ID#	Age	Location ^A	Priority ^B	Status ^C	Recommendation
FEMALES					
#31	10-12y	I-N.FS/PR	1 ⁺	FOUNDER	Old female, if 1990 kittens are gone, PULL ALL 1991/92 kittens- if none produced consider pulling female in 1992 (son is #34M)
#09	8-10y	I-FSSP	1 ⁺	FOUNDER	Old female, if 1990 kittens are gone, PULL any 1991/92 kittens- if none produced consider pulling female in 1992
#23	4.5y	II-ENP	1-2 ⁺	FOUNDER	Young/mature female- one litter died-PULL ALL or PART of next litter. Consider pulling female if no breeding male is in area by 1992. This female has spent 19 months in captivity and could easily adapt again.
#38	4-6y	I-E.BCNP	1-2 ⁺	FOUNDER	Young/mature female- cycles regularly, but no male is in area. Consider PULLING only if no male is in area by 1992.
#11	8-10y	I-BI/PR	2 ⁺	FOUNDER	Older female- Proven breeder. Consider pulling only if no F ₁ (preferred) or F ₂ 's are available
#32	3.5-4y	I-N.FS	2 ⁺	FOUNDER	Young female-give a couple of years (possibly #31's F ₁). However, she has lost at least one litter and regularly associates with male without producing young.
#36	4-6y	I-PR	2 ⁺	FOUNDER	Young/mature female- give a couple of years before would consider pulling female.
#40	2.5-3y	I-PR	2 ⁺	FOUNDER	Young female-give a couple of years (possibly #36's F ₁)
#14	8-9y	II-ENP	3 ⁺	FOUNDER	Her F ₁ , #21F is in captivity. Would not consider pulling female.
#19	4.5y	I-N.FS	4 ⁺	NON-FOUNDER	Young female (she is #11's F ₁)

Table 1

Adults for captive breeding p.2

ID#	Age	Location ^A	Priority ^B	Status ^C	Recommendation
<u>MALES</u>					
#12	8-10y	I-N.FS/PR	1 ⁺	FOUNDER	Old male, get 1990 or 1991 kittens. Would consider pull only if no F ₁ 's or F ₂ 's are available.
#26	7-8y	I-PR/BI	1 ⁺	FOUNDER	Mature, get 1990 or 1991 kittens, consider pulling in 1992 in no kittens survive.
#29	2.5y	I-PR/BI	2 ⁺	FOUNDER	Young/mature- Only offspring of dead #20M. Give more time for breeding before considering pulling.
#28	3.5-4y	I-PR/BI	2 ⁺	FOUNDER	Young/mature- give more time for breeding.
#16	5-5.5y	II-ENP	3 ⁺	1/2 FOUNDER	Sibling #21F is in captivity. Unknown father is dead. Back crossed son (#42) is alive.
#34	22mo	I-PR/SIR	4 ⁺	NON-FOUNDER	Young - would pull only if mother (#31) dies before her F ₁ 's can be obtained and this male does not breed.
#42	26mo	II-ENP	4 ⁺	NON-FOUNDER	Young - back crossed son of #14F bred with her son #16M (Aunt/1/2 sibling #21F is in captivity).
#43	17mo	I-PR/SIR	4 ⁺	NON-FOUNDER	Young - back crossed son of #19F bred with her father #12M.

^ALocation: see Table . (I-Big Cypress population, II-Everglades population); ^BPriority in order of preference 1⁺ = top choice, 4⁺ = lowest choice.

Table 14. Outcome of 1990 litters and kittens captured in 1991 for captive breeding project.

Female ID#	Number and Sex [^] of kittens		Age when captured	Priority [^]	Comments
	In litter	Now in captivity			
#31♀	1:1:0	1:1:0 (#201♂, #204♀)	7.0 mo	1 ⁺	#201♂ captured 02-20-91. #204♀ captured 02-27-91.
#09♀	2:0:0	2:0:0 (#202♂, #203♂)	8.0 mo	1 ⁺	#202♂ captured 02-23-91. #203♂ captured 02-25-91.
#36♀	0:0:2	0:0:0		2 ⁺	Litter dispersed before capture could be attempted. Delay was due to litigation.
#11♀	0:1:0	0:0:0		2 ⁺	Only one kitten documented and this was hit by a car and killed. She was suffering from cardiac failure due to atrial septal defect at the time.
#40♀	1:1:0	0:1:0 (#206♀)	6.0 mo	2 ⁺	#206♀ captured 05-06-91. Radio-collared male kitten (#44♂) remained in the wild.
#19♀	1:1:0	0:1:0 (#205♀)	6.0 mo	1 ⁺	#205♀ captured 05-03-91. Radio-collared male kitten (45♂) remained in the wild.

[^]Sex 0:0:0 = males:females:unknown; [^]Original priority in order of preference established at beginning of capture season (1⁺ = top choice, 4⁺ = lowest choice).

Table 15. Florida panther founder representation in captivity, June 30, 1991.

ID# & Sex	Age	Founders Represented		Genetic stock ^A	Deliterious traits ^B	
		Dam	Sire		# Testicles	Heart Murmurs
PCO 10♂	9-10 yr	? ½	? ½	Hybrid	2	0
#21♀	5.5 yr	#14 ½	ENP ½	Hybrid	na	0
#201♂	11 mo	#31 ½	#12 ½	Pure	1	1 ⁺
#202♂	12 mo	#09 ½	#37 ½	Hybrid	2	3 ⁺
#203♀	12 mo	#09 ½	#37 ½	Hybrid	1	1 ⁺
#204♀	11 mo	#31 ½	#12 ½	Pure	na	3 ⁺
#205♀	7 mo	#11 ¼	#12 ¾	Pure	na	6 ⁺
#206♀	7 mo	#40 ½	#26 ½	Pure	na	1 ⁺

^ADetermined by mitochondrial DNA and APRT analysis (O'Brien et al., 1990).

^BNumber of descended testicles; heart murmur grades- 0 (not detected), 1⁺ (mild) to 6⁺ (severe).

Table 16. Comparison of growth rates of wild and captive kittens less than 12 months of age.

Location	ID#	Date	Age	Body WT ^A	Phys Cond ^B	Wt. Gain per Day ^C	HGB/PCV ^D
N. Fakahatchee Strand, Florida Panther National Wildlife Refuge, Bear Island Unit BCNP, and adjacent Private Ranches Collier and SW Hendry counties	#29	03Jan89	6 mo	44	II		13.5/43.7
		01May89	11.5 mo	76	II	0.271	12.7/38.8
	#30	06Jan89	9-10 mo	55	II		12.0/32.8
		02May89	13-14 mo	75	II	0.172	11.6/35.1
	#34	08Jan90	10 mo	62	I		11.4/36.5
		03May90	14 mo	68	II	0.052	11.8/35.7
Fakahatchee Strand State Preserve	#10	13Jan86	5-7 mo	34	III		11.3/30.0
		02May86	9-11 mo	55	II	0.192	12.9/35.0
Everglades National Park Dade Co.	#22	18Mar87	6-8 mo	33	II		15.7/43.3
		07Apr87	7-9 mo	36	II-	0.150	11.8/34.4
		15Jun87	9-11 mo	43	II	0.100	9.8/30.0
						0.112 ^E	
Captivity White Oak Plantation	#201	20Feb91	7 mo	34	III		8.1/25.0
		23Apr91	9 mo	52	II	0.290	12.6/38.5
	#202	23Feb91	8 mo	47	I		10.8/32.8
		23Apr91	10 mo	59	I	0.200	10.5/31.7
	#203	25Feb91	8 mo	45	II		9.8/31.4
		23Apr91	10 mo	50	II	0.088	11.7/35.2
	#204	27Feb91	7 mo	37	II		9.8/29.8
		23Apr91	9 mo	44	II	0.127	13.8/42.0
	#205	03May91	6 mo	31.5	II		9.9/37.1
		02Jul91	8 mo	40	II	0.142	12.3/39.5
	#206	06May91	6 mo	33	II-		10.2/33.4
		02Jul91	8 mo	42	II	0.158	10.9/33.2

^APounds (lbs.); ^BPhysical condition score: I-Excellent, II-Good, III-Poor, IV-Grave; ^CAverage weight (lbs.) gain per day, calculated between the two recorded dates, mean $\pm$ S.E. is 0.160 ± 0.036 for wild and 0.168 ± 0.029 for captive kittens; ^DHemoglobin (gms/dl) PCV = packed cell volume, mean $\pm$ S.E. for the change in hemoglobin over time was -1.02 ± 1.28 for wild and 2.3 ± 0.69 for captive kittens; ^ECalculated for entire period March-June.

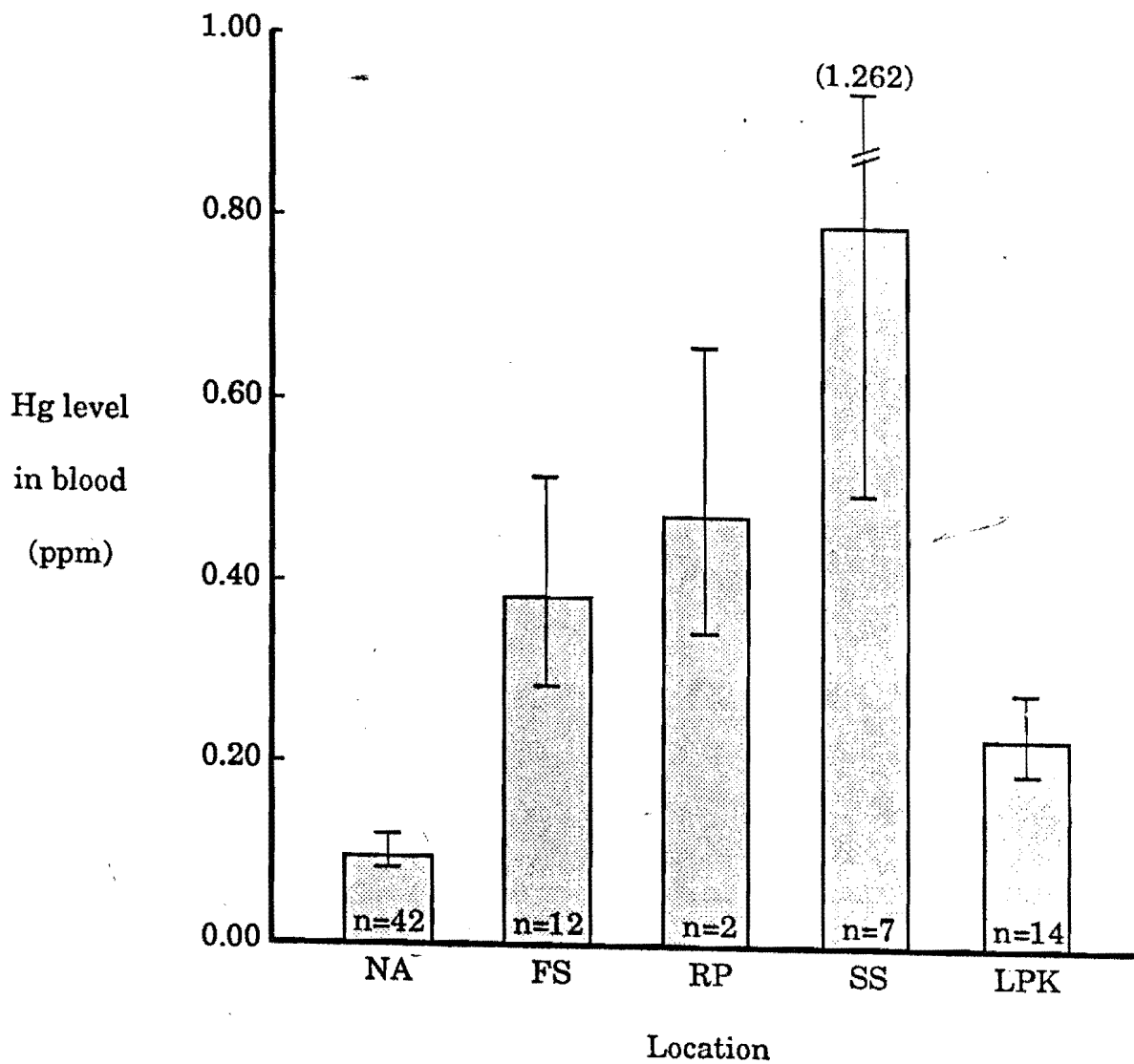


Figure 1. Mercury (Hg) concentrations (geometric mean $\pm$ S.E.) in whole blood of panthers by capture area: north of Alligator Alley (NA); Fakahatchee Strand State Preserve (FS); Raccoon Point (RP); Shark Slough (SS); Long Pine Key (LPK).
n = number of samples.

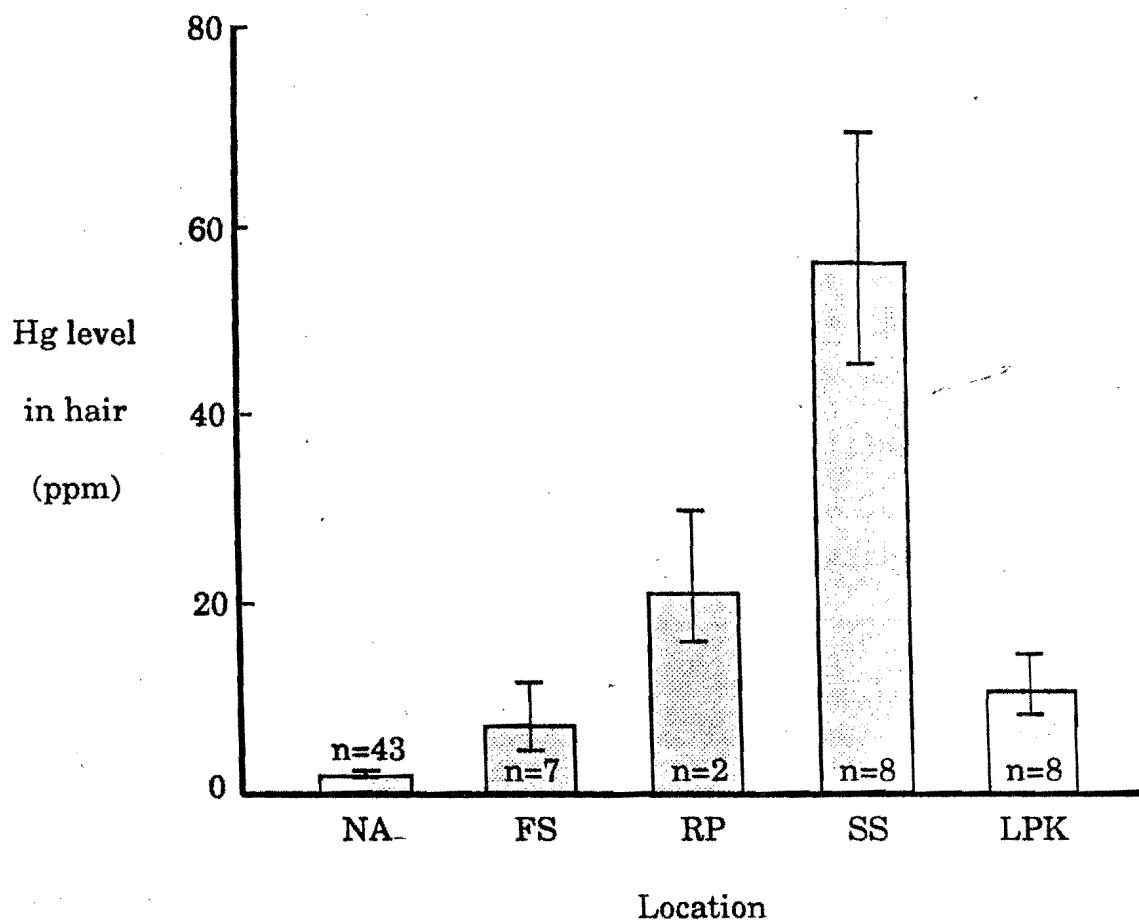


Figure 2. Mercury (Hg) concentrations (geometric mean $\pm$ S.E.) in hair from Florida panthers by capture area: north of Alligator Alley (NA); Fakahatchee Strand State Preserve (FS); Raccoon Point (RP); Shark Slough (SS); and Long Pine Key (LPK). n = number of samples.

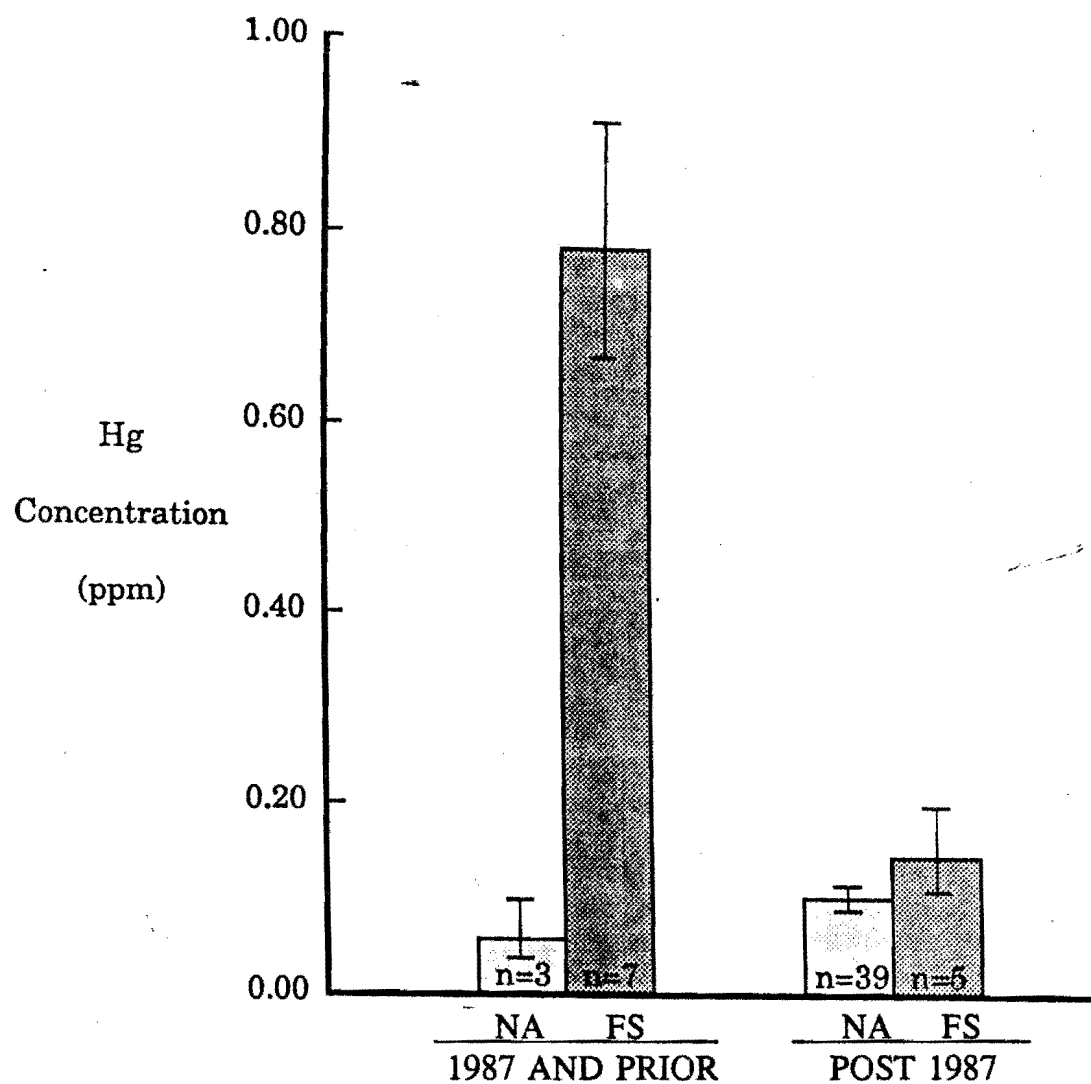


Figure 3. Comparison of blood mercury (Hg) concentration (geometric mean $\pm$ S.E.) in panthers from the Fakatachee Strand State Preserve (FS) & north of Alligator Alley (NA) before and after the fall of 1987. n = number of blood samples.

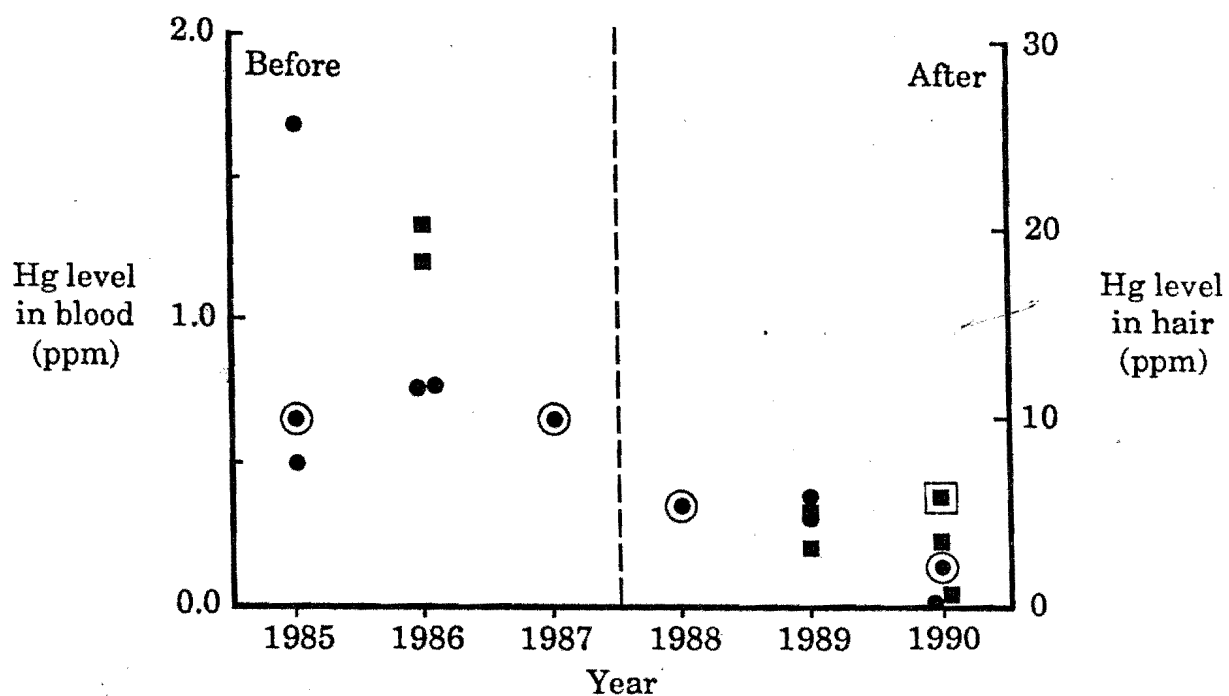


Figure 4. Mercury (Hg) concentrations (geometric mean $\pm$ S.E.) in blood {●} and hair {■} of panthers in the Fakahatchee Strand State Preserve before and after the fall of 1987. Samples ⊙ and ⊠ are from #09♀, the only panther in this area throughout the entire study period.
 n = number of blood samples.

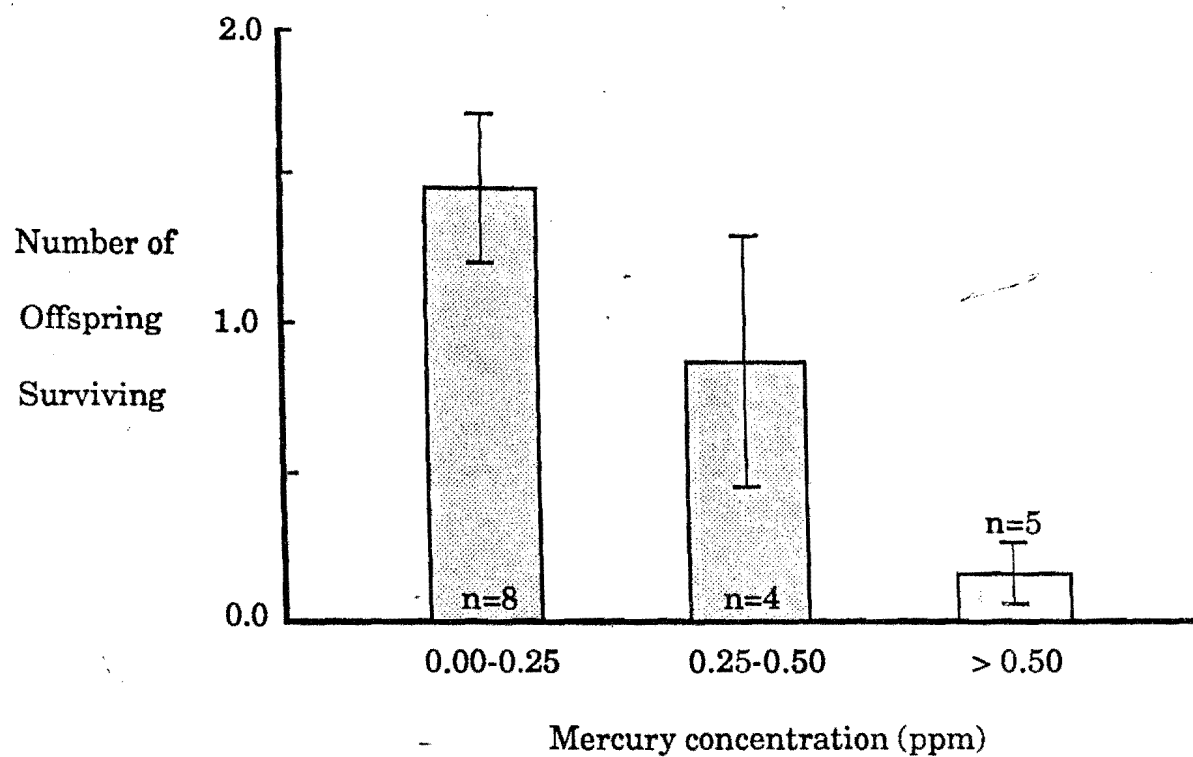


Figure 5. Mean number of offspring ($\pm$ S.E.) surviving to age of 6 months per female-year by whole blood mercury concentration in female parent ($n=17$) during pregnancy.

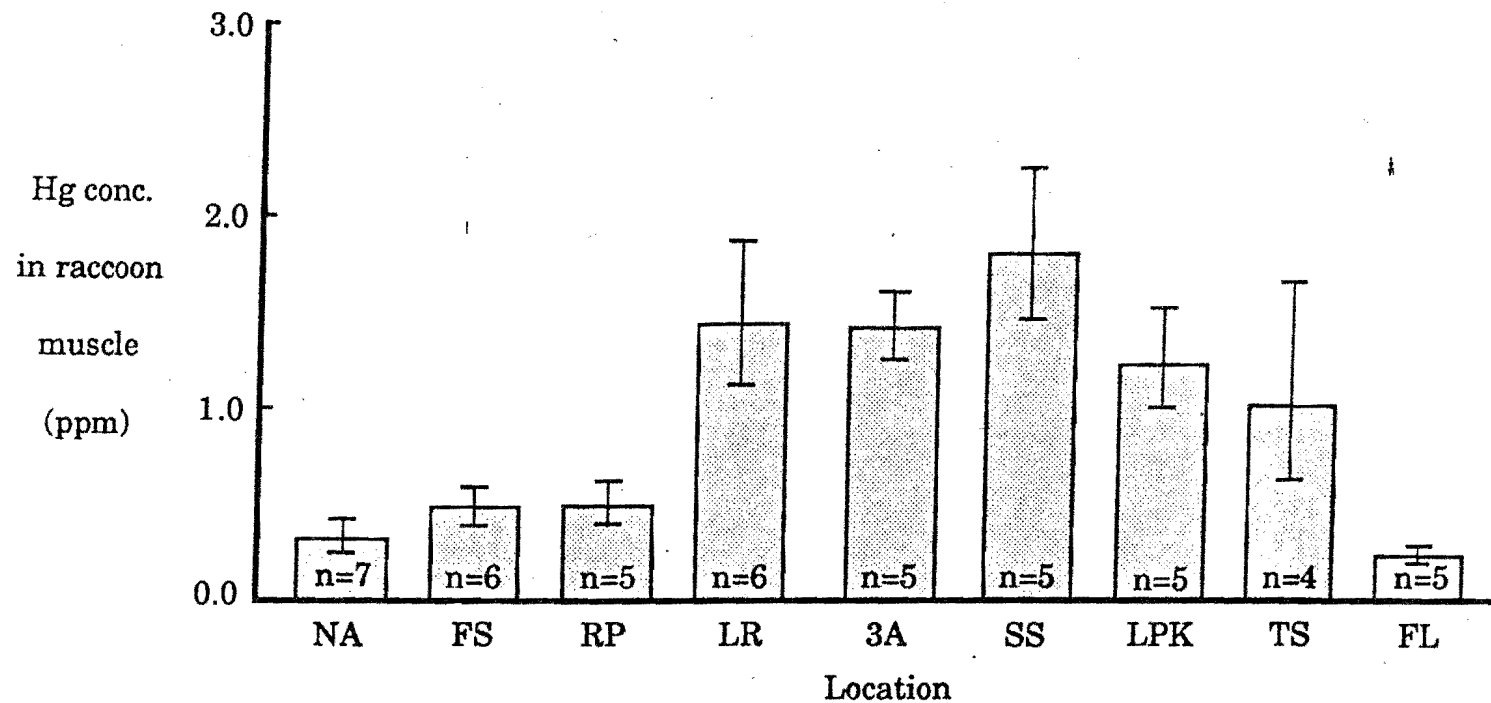
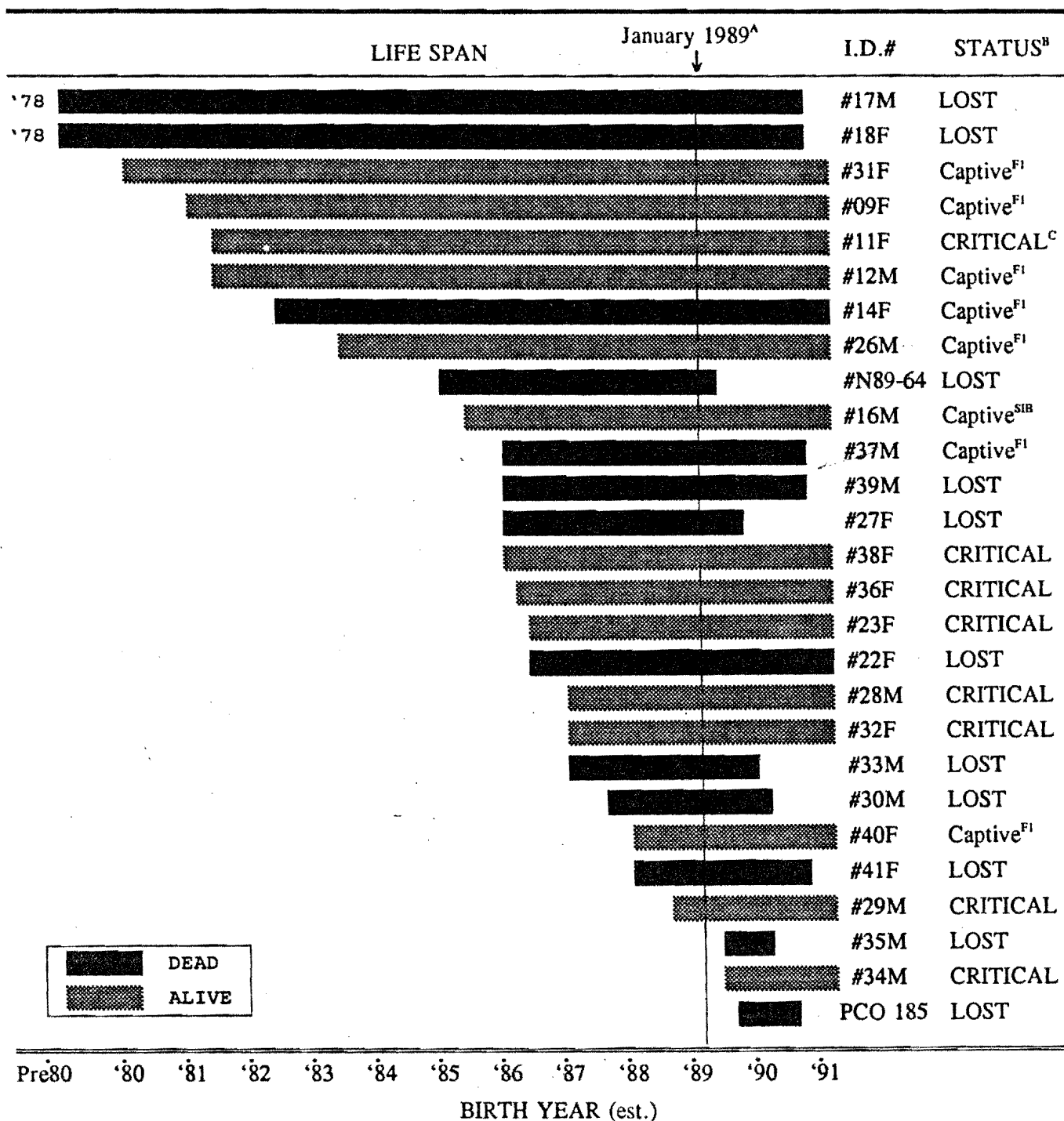


Figure 6. Mercury concentration (geometric mean $\pm$ S.E.) in muscle from raccoons by capture area: north of Alligator Alley (NA), Fakahatchee Strand State Preserve (FS), Raccoon Point (RP), Loop Road (LR), Water Conservation Area 3A (3A), Shark Slough (SS), Long Pine Key (LPK), Taylor Slough (TS) and Flamingo Key (FL).

Figure 7. Founders identified for captive breeding population: January 1, 1989 to July 30, 1991.



^aPlanning for a captive breeding program and identification of possible founders started January 17, 1989.

^bLost - animal died with no known surviving decedents; Captive - F¹ or sibling (Sib) genetic representation is in captivity; Critical - no genetic representation in captivity.

^cA back crossed F² of this female is currently in captivity, however, the animal is not expected to reach breeding age due to serious heart defect.

APPENDIX I

FLORIDA PANTHER CAPTIVE MANAGEMENT AND HEALTH PROTOCOL

I. INSTITUTION EVALUATION

A. Facilities wishing to obtain Florida panthers

1. Questionnaire and felid inventory is to be completed by zoo veterinarians and curators to cover the following topics:
 - a. Summary of current inventory and activity with felids in past 2 yrs;
 - b. Check list indicating results of survey of infectious diseases and summary of results;
 - c. Nursery and hand-rearing techniques used at facility;
 - d. Isolation and quarantine protocol;
 - e. Vaccination history and results of serologic tests and parasite examinations;
 - f. Past and present health problems in felids at facility;
 - g. Husbandry of cougar/puma/panther or Felis species with similar management needs (diet, sanitation, and description of enclosure).
2. Infectious disease and parasite evaluation.

In order to protect the captive zoo stock from infection from wild panthers and vice versa, all felids in the receiving facility will be tested for the following diseases agents before Florida panthers can be received. An initial survey of all resident felids is to be completed on samples collected no longer than 1 year prior to receiving panthers. If following completion of the survey, the arrival of panthers should be delayed for any reason then testing of facility felids should continue following the "on line facilities guidelines" (see I.B. below). Facilities with animals testing positive for any of the following agents will be evaluated by the Florida panther captive management committee on a case-by-case basis in determining the suitability of placement of panthers with that facility and/or special precautions that will be taken to prevent transmission of disease to or from the wild panthers.

a. MANDATORY TESTING (Must use designated laboratory)

(1) *Feline infectious peritonitis virus/feline enteric corona virus (FIPV/FECV)*

Send serum to:

Dr. Jim Evermann
Wash. Animal Disease Diagnostic Laboratory
Bustad Hall, Rm. 155-N
Washington State University
Pullman, Washington 99164 (509) 335-9696

(2) *Feline immunodeficiency virus (FIV)*

Submit fresh heparinized whole blood (preferable) or serum for CITE test and "Western Blot" analysis to:

Dr. Stephen O'Brien/ Jan Martenson
FCRF-NCI-PRI, Lab of Viral Carcinogenesis
Building 560, Room 11-10
Frederick, Maryland 21701
(301) 846-1299

- (3) *Feline leukemia virus (FELV)*
Elisa and/or IFA performed at a reliable lab of the facility's choice.
- (4) *Cytauxzoan felis and Haemobartonella*
Submit fresh EDTA whole blood or methanol fixed blood smears to:

Dr. John Harvey
Clinical Pathology
Veterinary Medical Teaching Hospital
Build. 215, 16th Ave.
Univ. of Florida
Gainesville, Fl. 32610 (904) 392-4382

b. Desirable **OPTIONAL** testing

- (1) Viral diseases prevented by vaccination. Testing should be mandatory if facility does not have an active vaccination program.

Feline panleukopenia virus (FPLV)
Feline calicivirus (FCV)
Feline viral rhinotracheitis virus (FVRV)

Submit serum to either:

Dr. Jim Evermann (see FIP above)

OR

Dr. Fred Scott (Preferred lab)
Cornell Feline Health Center
College of Veterinary Medicine
Cornell University
Ithaca, N.Y. 14853 (607) 253-3414

- (2) *Encephalomyocarditis virus (EMCV)*

Although this virus has caused sporadic deaths to various species of non-domestic animals in the southeastern U.S. (particularly in Florida from Tampa and south), none of the documented cases have involved exotic felids. Currently, EMCV is not considered to be a serious threat to felids. There is anecdotal information indicating that 26 African lions died following consumption of flesh from infected rhinoceros and/or elephant who died of EMCV, but this was never confirmed. If a particular facility has a history of animal loss to this virus, then testing of felids and other select zoo animals (esp. wild rodents) should be MANDATORY. Otherwise, the testing of felids should be optional, but

encouraged, in order to learn more about the presence and prevalence of this virus.

Submit serum to:

Dr. Jack Gaskin
Dept. of Infectious Diseases
University of Florida
College of Veterinary Medicine
Gainesville, Florida 32610 (904) 392-1841

(3) *Toxoplasma gondii*

This parasite causes sporadic disease problems in felids. It appears that many captive and wild non-domestic felids are infected with the organism (depending on the laboratory running the test), but differences between facilities have been observed. Therefore testing for this agent would be important to establish the prevalence rate for a particular facility and to learn more about the persistence and cycling of this agent in captive felids.

Submit serum for IgG, IgM, and antigen in batches to:

Dr. Michael Lappin
Dept. of Clinical Sciences
College of Vet. Medicine
Colorado State Univ.
Ft. Collins, CO 80523 (303) 491-1274

c. Testing of Feral cats

Representative feral cats on the premises should be captured and tested for the above MANDATORY and OPTIONAL diseases as described above.

B. "On line facilities" with resident Florida panthers

1. Institute questionnaire and felid inventory will be updated every 2 years.
2. Testing of newly acquired felids:

All incoming felids will be screened for the MANDATORY tests as described above (I.A.2.a.) preferably prior to shipment to the recipient facility or within one week of arrival and at the end of the 30-60 day quarantine. EMCV and other OPTIONAL tests should be included as necessary as described in I.A.2.b. The only exception to this will be incoming wild Florida panthers which will be bled as they are removed from the wild and will be held in strict quarantine until the initial test results are known (see Sec. II below).

3. Testing of resident non-domestic and domestic felids:

All resident felids (including those born on the premises) shall be tested at least once every 2 years for the MANDATORY and OPTIONAL tests as described above (I.A.2.a.& b.)

4. Test positive animals:

The Florida panther propagation group and Florida Game and Fish Commission's (GFC) veterinarian should be consulted if any of the animal's have positive test results. Recommendations will be made on a case-by-case basis depending on the agent and the magnitude of the potential problem.

II. FIELD EVALUATION AND SAMPLING AT CAPTURE

Standard Field Protocol

The following procedures and analyses will be performed on incoming Florida panthers by the GFC veterinarian. Some of the specific treatments may be modified depending on the age of the animal.

- A. Physical exam including weight and measurements.
- B. Complete blood count (CBC) and Serum Chemistries
- C. Serology (Same labs as in I.A.2.a. & b.). Results for agents with * will be available within 48 hr of receipt by laboratory. The remainder will be completed within 30 days.
 1. FIP*
 2. FIV*
 3. FeLV*
 4. Toxoplasma*
 5. EMCV
 6. FPLV
 7. FCV
 8. FVRV
 9. Brucella (Dr. P. Nicolitti, Univ. Fl, Gainesville, FL)
 10. Pseudorabies (Dr. P. Gibbs, Univ. Fl, Gainesville, FL)
- D. Virology - Viral isolation culture of nasal, pharyngeal, and rectal swabs.
- E. Parasitology
 1. Fecal O + P (floatation, McMaster, and sedimentation)
 2. Microfilaria exam
 3. Occult heartworm exam
 4. Cytauxzoan and Haemobartonella exam
- F. Ectoparasite Removal - thorough manual removal of all ticks (specimens are preserved).
- G. Fecal bacterial culture for enteric pathogens (*Salmonella sp.*, *Campylobacter sp.*, etc.)
- H. Other routine samples possibly taken- saliva, skin biopsies, hair, vaginal smears and biopsies, urine, and blood for mercury analysis.
- I. Treatments: This schedule may be modified if kittens are very young.
 1. Vaccination - Fel-O-Vax® 3cc divided IM (FPLV, FCV, FVRV killed), Imrab® 1cc IM (rabies virus killed)
 2. Ivermectin - SubQ (200ug/Kg)

3. Praziquantel - SubQ or IM (5mg/kg)
4. Iron dextran - 1-3 cc IM
5. B-vitamins - 3-5 cc SQ
6. IV and SQ lactated ringers - 1-3 liters

III. QUARANTINE/ACCLIMATION PERIOD AT RECEIVING INSTITUTION

A. Quarantine period: 0 - 30 days post capture

1. Close observation should be made to note appetite, attitude, behavior, and condition. Physical or "routine" changes should be made to alleviate stress or observed aberrant behavior as needed (e.g. visual barriers, blockade exhibit, white noise, etc.).
2. Whole, natural food items should be available to entice cats to eat (preferably venison and rabbit).
3. Fecal Exams (parasite and microbiologic)- depending on which agents were present when panther was removed from the wild, it may be desirable to repeat tests at least every 2 weeks and treat accordingly. Collect and SAVE ALL FECES defecated, label with date and time collected, refrigerate or freeze as directed by Dr. Roelke. Do not unnecessarily disturb the panther, collect as you can, OK if the feces are a day or two old. These are important samples and will be used for food habits, parasite, mercury excretion, and fecal steroid studies.
4. Remedial therapy may be necessary to treat dehydration, weight loss, etc.
5. If handling of the panther is required due to self-trauma, illness, injury, stress or anorexia, the panther should be immobilized, tranquilized, or physically restrained as the staff veterinarian deems necessary for treatment. The GFC panther veterinarian should be contacted for consultation prior to the action (preferred), but if this is not possible, they should be notified within 24 hours.
6. Preferred immobilization drugs (and dosages) for wild panthers include ketamine hydrochloride (approx. 8-12 mg/kg IM), Telazol® (6.0-7.0 mg/kg IM), or a combination of ketamine and Telazol® (K= 8-12 mg/kg + T=1.5 mg/kg). Additional ketamine, diazepam, and/or medazolan can be added as needed to stabilize (diazepam or medazolan 1-5 mgs IV slowly! or IM). Doses for stressed, sick, or anorectic animals should be scaled down according to their condition. Atropine or glycopyrolate can be used if desired. After induction, anesthesia of the panther can be maintained by either additional injectable drugs or gas anesthesia as desired.

B. Post-quarantine exam, at 30-60 days post capture:

STANDARD VETERINARY EXAMINATION PROTOCOL

1. Routine procedures to be followed every time the panther is handled

- a. Complete Physical Examination including body weight, measurements, dental exam and prophylaxis (with dentist if necessary).
 - b. Collect Samples for the following procedures
 - (1) Whole blood in EDTA:
 - (a) CBC with reticulocyte count
 - (b) Blood parasite screen (particularly *Cytauxzoan* and microfilaria)
 - (2) Serum:
 - (a) Serum chemistry panel including electrolytes and CPK
 - (b) Submit serum for serologic testing for all the agents listed in II.C. above (at post quarantine exam and at required intervals).
 - (c) **ALWAYS BANK SERUM** - Freeze 5-10 cc minimum for the "Florida Panther Serum and Tissue Bank" at the Wildlife Research Lab (GFC) in Gainesville and a minimum of 5cc for your own serum bank.
 - (d) Save and freeze the blood clot.
 - (3) Heparinized whole blood:
 - (a) Genetic and FIV studies - ship to Dr. S. O'Brien at NCI (I.A.2.a.(2)). Submit 10-60cc depending on the size and condition of the panther.
 - (b) Mercury analysis - 10cc frozen whole for later analysis.
 - (4) Hair (1.0+ gm desired) and whiskers (3/side). Pluck and freeze sample, also save any shaved hair.
 - (5) Feces for enteric pathogen analysis
 - (a) Fecal ova and parasite exam (submit to Univ. of Florida - contact Roelke for appropriate address).
 - (b) Bacterial stool culture (*Salmonella*, *Campylobacter*, etc.)
 - (6) Urine - Urinalysis (S.G., dipstick, sediment evaluation, ALWAYS freeze supernatant remainder). On healthy young animals catch urine opportunistically if voided during anesthesia or manually express bladder. If you suspect urinary problems or if the animal is an older adult obtain a urine sample by catheterization or cystocentesis.
 - (7) Ectoparasites - remove and preserve all specimens.
 - (8) Viral isolation swabs - rectal, pharyngeal, and nasal (freeze $\leq -75^{\circ}$).
 - (9) Dental impressions
 - (10) Other samples or procedures as needed or requested.
 - c. Treatments
 - (1) Booster vaccinations - Fel-O-Vax and Imrab (rabies)(see IV.D.)
 - (2) Routine de-worming as necessary (see IV.E.)
 - (3) Other medications as clinical presentation indicates
2. Non-routine and special procedures
- a. Skin biopsies for genetic studies - send fresh to NCI (I.A.2.a.(2)).
 - b. Radiographs of chest, skull/teeth, hips and shoulders, and growth plates of long bones (DV and LAT views). The initial set of films will be used to evaluate previous trauma or exposure to lead, heart silhouette, and epiphyseal status. Radiographs of long bones are desirable on subsequent immobilizations to document epiphyseal closure. Other radiographs indicated only if necessary for medical evaluation.

- c. Cardiac evaluation - include EKG and ultrasound if possible. Utilize more detailed procedures if initial examination indicates it is warranted.

IV. LONG TERM HEALTH MAINTENANCE

A. Documentation of enteric parasites

1. Fresh fecal samples should be collected at day 0, 1 month, 2 months, and 6 months for complete parasite analysis and submitted for analysis: Contact M. Roelke for appropriate address.
2. Routine fecal O + P should be performed at least monthly after quarantine with treatment as necessary until panther is cleared of parasites. Then test at least every 6 months thereafter.

B. Subsequent health monitoring of individual cats

1. Cats will be immobilized and evaluated as per the Standard Veterinary Examination Protocol (sec. III.A.6.).
2. Cats are to be immobilized and evaluated at 6 months and 12 months post-capture of the first year and at least every 12 months thereafter. Samples as outline above (III.B.1.) should be obtained whenever the panther is handled.

C. Rigid health screening of other exotic felids entering collection:

1. Evaluation of past medical history before entry.
2. Proper quarantine procedures followed.
3. Screened per I.A.2. Infectious Disease Protocol before and after quarantine.

D. Vaccination Protocol

1. Vaccines - **USE ONLY KILLED VACCINES ON FLORIDA PANTHERS**
 - a. Fel-O-Vax PCT 1-3 cc IM (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa).
 - b. Rabies (killed) - Imrab 1cc IM (Rhone Merieux, Inc., Athens, Georgia).
 - c. Others may be considered at a future date as deemed necessary (i.e., FeLV, FIP, Lepto, etc.).
2. Vaccination Schedules

COLLECT AND SAVE SERUM EACH TIME CAT IS VACCINATED

so that response to vaccination can be evaluated.

a. FEL-O-Vax

- (1) Kittens < 16 weeks old - give at least two 1cc IM doses of vaccine.
 - (a) Born to vaccinated queens and/or queens with adequate FPLV titers: Isolate kitten and begin vaccinations at 6 weeks and repeat every 2-3 weeks until at least 16 weeks of age.
 - (b) Born to non-vaccinated queens and/or queens with low or absent FPLV titers: Isolate kitten and begin vaccinations immediately and repeat every 2-3 weeks until at least 16 weeks of age
- (2) After initial vaccination schedule, kittens should be vaccinated at 6 and 12 months and then annually.
- (3) Juveniles (> 16 weeks), subadults, and mature panthers - give 2-3cc IM.
 - (a) Vaccinate at capture, at 30 day quarantine exam, at 6 and 12 month exams, and then annually.

b. Rabies

(1) All kittens

Vaccinate at 16 weeks, 20 weeks, 12 months, and then annually

(2) Juveniles (> 16 weeks), subadults, and mature panthers

Vaccinate at capture, at 30 day quarantine exam, at 12 months, and then annually

E. Anthelmintic Protocol

1. Anthelmintics (Suggested - will depend on type of parasite)

a. Pyrantel Pamoate - 12mg/lb PO

b. Ivermectin - 200ug/kg SQ

c. Praziquantel - 5.0mg/kg IM or SQ

d. Other safe, accepted anthelmintics as required

2. Anthelmintic Schedule

a. Kittens (< 16 weeks)

(1) de-worm for nematodes every 2 weeks until 16 weeks of age

(2) After 16 weeks, de-worm as required by fecal examination

b. Juveniles (> 16 weeks), subadults, and mature panthers

(1) de-worm at capture, at 30 day quarantine exam, and then as required by fecal examination

V. MEDICAL PROBLEMS

A. Involving exotic cat collection (excluding Fl. panthers)

Any outbreak of infectious disease should be immediately reported to the Commission veterinarian and the Fl. panther captive management committee so that measures can be taken to protect the Florida panthers.

B. Involving the Florida panthers

1. Minor problems can be treated on site at the respective facility.

2. Major illness and or surgical treatment. Please contact GFC's panther veterinarian for consultation. If condition warrants it, the panther should be transported to the College of Veterinary Medicine for work-up and treatment.

VI. MORTALITY AND NECROPSY PROTOCOL

All panthers are to be necropsied by the Dept. of Pathology, College of Veterinary Medicine following a standard Florida panther protocol (available from GFC). Chill carcass as soon as possible and contact appropriate people as listed in the necropsy protocol (primarily Dr. Melody Roelke (904) 336-2230, Dr. Lois Roth (904) 392-1221, and Laurie Wilkins (904) 392-6467 or 1271). If extenuating circumstances will not allow expedient transport and necropsy at Gainesville, carefully follow the details of the protocol (SAVE ALL TISSUE AND FLUIDS). Please supply a detailed clinical record and description of illness and cause of death (if known).

VII. MANAGEMENT PROTOCOL

- A. The management of Florida panthers and facility requirements will vary based on the age of the animal and the planned long-term strategy for the individual.

1. Age of panther

- a. Kittens (neonatal) - animals taken from dens, probably still nursing, will generally be less than 6 weeks of age.
- b. Juvenile kitten 4 - 12 months, still dependent on mother.
- c. Young adult 1-3 years - dispersing and independent from parent, generally has not yet established territory.
- d. Mature adult - 3 years or older, sexually mature.

2. Long-term management strategy

- a. Category I animal maintained in captivity for life.
- b. Category II animal has potential of future release.

B. Specific requirements by age class

1. Kitten Age Class

a. Physical environment:

(1) During isolation period

- (a) Complete isolation of panther litters from other felids and panthers up to 16 weeks of age or at least 4 weeks post entry if kittens are older.
- (b) Kittens should be maintained in ambient temperature range of 85-90°F until approximately four (4) weeks of age at which time temperatures can be decreased depending on health status.

(2) After initial isolation period

- (a) Kittens may be maintained in other nursery facilities that contain other species, however, they are to remain separated from other animals during the daily management routine.
- (b) The nursery is not to include convalescing animals.

b. Nursery staff:

- (1) During isolation period there will be no contact with other zoo felines.
- (2) If caretaker has domestic cats -
 - (a) Cats are to be screened under infectious disease protocol (above) and results provided to staff veterinarian for documentation.
 - (b) Any caretakers with sero-positive cats will be reviewed on a case-by-case bases with staff veterinarian, curator and GFC veterinarian.

c. Nursery Protocol:

- (1) Wash hands before and after handling with betadine solution. Wear clean smock (only used with kitten in nursery).
- (2) Check body temperature daily. Normal range is 99-101°F.

- (3) Record weight daily under consistent circumstances.
- (4) Kitten enclosure is to be cleaned and disinfected daily or more, depending on the circumstances.
- (5) Sterilize bottles and nipples in boiling water for 10 minutes before every feeding.
- (6) Kittens should be stimulated to defecate or urinate after every feeding.
- (7) Vaccination and anthelmintic protocol to be followed as described above (see IV.D.& E.)

d. Diet

- (1) Kittens less than 8 weeks of age should be fed Kitten Milk Replacer (KMR) by Borden.
 - (a) KMR should be fed based on label formula at a rate of 25-40% of body weight per day in divided feedings.
 - (b) Kittens should be fed every 2-3 hours for first 3 to 4 weeks of age. After this time the frequency may be reduced as the kitten's condition allows.
 - (c) Young kittens should be fed by a bottle and positioned properly during nursing so as not to inhale formula. Older kittens can be fed their milk from a dish.
 - (d) At 3 to 4 weeks of age, meats can be offered to kittens in addition to formula, i.e., feline diet, baby food, etc.
- (2) Kittens 8 weeks of age and older
 - (a) Weaning from a milk diet can be started at this age
 - (b) Kitten is to be weaned onto a commercial balanced meat diet.

e. Post isolation

After initial isolation period or 16 weeks of age, kittens may be maintained as per other age classes.

2. Remaining Age Classes

a. Facility requirements

Must be in compliance with FGFWFG regulations.

- (1) Management house - category I & II panthers
 - (a) Minimum 200 sq. ft. concrete pad, roofed.
 - (b) Seclusion stall to facilitate isolation, immobilization, and crating, not less than 25% of pad square footage, with adequate ventilation.
 - (c) Automatic water desirable.
 - (d) Den box - 4'L x 3'H x 2.5'W
 - (e) Any chain link should be lined with snow fencing on the interior.
- (2) Outside enclosure
 - (a) Minimum square footage
 - i) Category I panther, 1600 sq. ft.
 - ii) Category II panther, 3200 sq. ft.
 - (b) Barrier material should be minimum 9 gauge chain link.
 - i) Covered facility - minimum 8' height.

- ii) Open facility - minimum 10' height with 3' turnback.
- iii) Concrete footer or wire, 12" deep.
- iv) Recommended hot wire at 9' height on inside of barrier.
- v) Barrier to be lined with wood snow fencing for initial introduction of cats to facility.
- (c) Furnishings
 - i) Emphasis on natural vegetation to provide animal privacy.
 - ii) Automatic waterer desirable.
 - iii) Provide scratch logs and natural climbing material.
- (d) Facility should allow for separation of animals and ability to transfer from one compound to another.

b. Diets

- (1) Category I - convert to commercial diet as soon as possible.
- (2) Category II - convert to commercial diet as soon as possible.
 - (a) Periodic feeding of whole food (carcass) once a week, provided food is screened for health consideration, e.g., brown or grey rabbits.
 - (b) Conversion to live food only when scheduled for release with adequate time -- 4-5 months in advance.

C. Shipment/movement of panthers between institutions

- 1. Transportation is to be made by participating institutions or if necessary by commercial airlines. No commercial animal dealers or movers are to be used.

FELINE INVENTORY AND QUESTIONNAIRE

FACILITY SUMMARY:

FLORIDA PANTHER RECOVERY PROJECT (1/1/91)

Page 1-A

Zoo or Facility's Name: Address (include Phone & FAX No.): Today's date:	Researcher's &/or Contact's Name: Address (include Phone & FAX No.):
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I. Please list the species of felids currently held at your facility:

SPECIES NAME		Number/Sex (0.0.0)	Activity within last 24 months			
Common	Scientific		Acquired	Births	Transferred	Deaths & causes

II. In the past 5 years, has your facility had any major problems (disease outbreaks, barbiturate intoxication, etc.) that resulted in severe illness or death of any of your felines? Please describe.

III. Do any of the animals in your facility have chronic or recurrent disease problems that might be transmissible to Florida panthers?

FACILITY SUMMARY: (continued)

Page 2-A

Zoo or Facility's Name:

IV. Do you routinely sanitize darts & surgical instruments (including dental instruments) between animals? Please describe protocol.

V. Give test results for the following disease agents. Indicate the number of individuals testing positive or negative in the appropriate column. Are these results from samples collected within the last 12 or 24 months? _____ Have samples from all your felids been included? _____ (Follow the Florida Panther Health Protocol for acceptable laboratory and testing protocol).

Summary of test results Agent	Non-Domestic			Feral Domestic cats		
	POS	NEG	List positive titers/Test Date	POS	NEG	Titers/Date
MANDATORY TESTS:						
Feline infectious peritonitis (FIP)						
Feline immunodeficiency virus (FIV)						
Feline leukemia virus (FeLV)						
<u>Cytauxzoan felis</u>						
<u>Haemobartonella felis</u>						
OPTIONAL TESTS:						
Feline panleukopenia virus (FPLV)						
Feline calicivirus (FCV)						
Encephalomyocarditis virus (EMCV)						
Feline viral rhinotracheitis virus (FVRV)						
<u>Toxoplasma gondii</u>						
Other:						

VI. Please describe your nursery and hand rearing procedures (use back of sheet if needed, also attach any written protocols you use). Describe your quarantine and isolation protocol (include description of facility).

FELINE COLLECTION: (Include data for all felids held during the past 2 years; living & dead)

Page 3-A

Zoo or Facility's Name:										
SPECIES NAME		ID# &/or Name		Date Acquired	BIRTH (Date)			RELATIVES IN ZOO (ID#/sex)		IF WILD
Common	Scientific ^A		Sex		Captive	Wild	Age	Parents ^B	Offspring	Capture location ^C
1.										
2.										
3.										
4.										
5.										

^AInclude subspecies if known

^BFor genetic studies, please include pedigree and capture location information for parents, this is very important if they were wild caught

^CIndicate capture location on a map

GENERAL HUSBANDRY INFORMATION FOR FELIS SPECIES: (OK to use down arrows for repeat information)

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Zoo or Facilities' Name:		Researcher's &/or Contact's Name:	
ID# or Name from Pg 3	DIET & SUPPLEMENTATION Include: Type/brand/vitamins amount fed per day/frequency/fast days live prey?	SANITATION PROTOCOL & SCHEDULE Give interval of fecal collection/cleaning/ disinfection (type used)	ENCLOSURE DESCRIPTION Give size, general materials used, flooring, & vegetation, distance from other cats
1.			
2.			
3.			
4.			
5.			

Zoo or Facility's Name:												
ID# or NAME from Pg 3	VACCINATION HISTORY ^{1,3} (Dates)					SEROLOGIC TESTS ^{2,3} (Dates : Results)					PARASITES	
	FPLV	FCV	FVRV	FelV	RABIES	FIP	FELV	FIV	TOX	Other	Species Observed	Treatment (Dates/drugs)
1.												
2.												
3.												
4.												
5.												

Code above dates appropriately (include data for past 2-3 yrs if available):

¹Indicate Brand Name/manufacturer of vaccine used and Type (killed, modified-live, etc.)

A
B
C

²Indicate Laboratory used and

D
E
F
G

type of test performed (ELISA, FA, serum neutralization, etc.)

³Pathogens: FPLV- feline panleukopenia virus; FCV- feline calicivirus; FVRV- feline viral rhinotracheitis virus; FelV- feline leukemia virus; FIV- feline immunodeficiency virus; TOXO- Toxoplasma gondii

MEDICAL HISTORY:

Page 5-A

Zoo or Facility's Name:						
ID# or NAME from Pg 3	ACUTE PROBLEMS Traumatic, toxicologic, infectious, parasitic, etc. Date (diagnosis, treatment, & outcome)		CHRONIC AND RECURRENT PROBLEMS Diarrhea, vomiting, respiratory, gingivitis, parasitic, etc. Date(s) (diagnosis, treatment, & outcome)		REPRODUCTIVE HISTORY Dates & comments: Matings/ Births/ Pregnancies	
	1.					
2.						
3.						
4.						
5.						