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Project Title: Florida Panther Research

Study Title: Florida Panther Biomedical Investigation

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Abstract--Veterinary medical assistance to reduce capture associated mortality, provide medical care, and conduct biomedical investigation to promote better understanding of disease and reproductive physiology continued as an integral part of endangered Florida panther (Felis concolor coryi) research and management. Twenty free-ranging Florida panthers inhabiting the Big Cypress Swamp and Everglades ecosystems of southern Florida were either immobilized and/or captured with no significant medical problems. Twenty-two, free-ranging, radio-collared adults are presently being monitored in southern Florida. Health status of each animal captured varied but was considered good for free-ranging panthers. Internal parasitism, especially by Ancylostoma pluriidentatum and congenital abnormalities affecting health and reproduction are two major factors that continue to compromise population viability. Extensive surgical intervention to treat congenital defects was performed, with mixed results, on 3 captive and one free-ranging panther. The captive-breeding program continued with addition of two wild-caught kittens for a total of 10 placed in captivity. Five mortalities were documented, four free-ranging and one captive panther. Six panther kittens were known born in the wild this period.

The endangered Florida panther (Felis concolor coryi) is estimated to number less than fifty adult individuals, and currently is protected under both state and federal endangered species statutes. This remnant population of the subspecies that once occupied the entire southeastern United States is now isolated in the remote cypress swamps and hardwood hammocks of southern Florida, primarily in the Big Cypress Swamp and Everglades ecosystems (Belden 1986). Human population growth in southern Florida with the concomitant loss of suitable panther habitat has been and continues to be the major threat to

the continued survival of this rare mammal. A detailed review of the life history and status of the panther is presented in the United States Fish and Wildlife Service recovery plan (USFWS 1987).

The critically small population size of the Florida panther makes the subspecies particularly vulnerable to demographic catastrophes. Chance events such as periodic road-kills, illegal hunting, or loss of prey species can be devastating to very small populations. In addition, disease outbreaks caused by adventitious agents are a constant threat to small populations, especially when those populations become relatively inbred over time (O'Brien and Evermann 1988). Decreased genetic diversity, increased neonatal mortality, abnormal reproductive traits, and lowered disease resistance often accompany such inbreeding in mammals (O'Brien et al. 1983 and 1985). There is reason to believe that the Florida panther is presently experiencing many of these problems (Roelke 1990, 1991; Roelke and Glass 1992; Seal et al. 1992).

Continuation of this project should reduce capture-related mortalities, increase survival rates for individual panthers requiring veterinary medical attention, and increase an understanding of the role disease, parasites, nutrition, and genetic diversity play in the population dynamics of the Florida panther.

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METHODS

Triangulation from aircraft and ground tracking with dogs were used to locate radio-instrumented panthers. Non-radio-collared panthers were also located using ground tracking dogs. Panther kittens were located by visual search of an area where a suspected parturient female had been repeatedly located.

Environmental factors, as well as health and level of stress of each panther, were evaluated at each capture site to determine potential risk of immobilization and capture. If risk was acceptable, a compressed air Vario[®] dart rifle (Telinject USA, Inc.) was used to inject immobilizing drugs. Ketamine hydrochloride (Ketamine[®]), 5-10 mg/Kg, and Telazol[®], 4-8 mg/Kg alone or in combination, were used as initial immobilizing agents.

Tranquilized panthers were removed from the tree by hand or allowed to fall into a net and cushioned "crash bag" as described by McCown et al. (1990). Panthers were monitored for vital signs and depth of anesthesia. Additional immobilizing drugs were administered as needed to maintain appropriate

anesthesia enabling sample collection. An intravenous catheter was installed in the cephalic or saphenous vein and isotonic fluids administered to maintain adequate blood volume and pressure. Each panther was examined and radio-instrumented after stabilization. Additionally, body measurements and biological samples including blood, urine, skin biopsy, feces, hair, saliva, viral and bacterial culture swabs, and ectoparasites were collected as previously described (Roelke 1990). Samples were collected predominantly between January and April, 1993.

Each panther was administered prophylactic treatment of iron dextran and vitamin B-complex injectables following initial examination and collection of biological samples. Each animal also received vaccinations for antibody protection against viral agents feline panleukopenia, feline calicivirus, and viral rhinotracheitis (Fel-O-Vax®, Fort Dodge), and rabies (Imrab®, Pitman Moore), all killed virus vaccines. Additionally, injectable anthelmintic drugs praziquantel (Droncit®, Haver) and ivermectin (Ivomec®, Merk & Co.) were administered to reduce parasite infestation.

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

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

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

Two male panthers, one captive (FP#200) and one free-ranging (FP#12) were electroejaculated as described by Roelke (1990), to obtain semen for evaluation and cryopreservation.

Skin biopsies were taken as described by Roelke (1990) from Florida panthers #50 and #208 for genetic analysis using mitochondrial DNA and nuclear markers at the Laboratory of Viral Carcinogenesis, National Cancer Institute (Frederic, Maryland, USA) according to techniques described by O'Brien et al. (1990).

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

RESULTS

Twenty free-ranging Florida panthers were captured in the Big Cypress Swamp/Everglades ecosystem in southern Florida (Table 1). One (FP#54) was captured twice for radio-collaring. No significant medical problems due to immobilization and capture were encountered. Two previously un-collared panthers (FP#53 and 54) were radio-instrumented and released, and 2 kittens (FP#209 and 210) were captured and placed in the captive breeding program (Table 2). Also, a litter of 3 kittens (not radio-collared or numbered, but ear tattooed) of FP#40 and one kitten from FP#23 were located and found in apparently good health. Presently 22 free-ranging radio-collared panthers are being monitored in southern Florida.

Wild-caught panthers were seropositive for FCV (14 tested, 5 positive, positive titer range = 1:8 - 1:192), FPV (16 tested, 12 positive, positive titer range = 1:100 - 1:10,000), and FIP/corona (16 tested, 9 positive, positive titer range 1:25 - 1:625). Serum chemistry and complete blood counts of wild-caught panthers revealed no significant abnormal values except for levels of enzymes associated with stress due to immobilization and capture.

Fresh fecal samples were collected from 7 panthers captured in the wild. Endoparasites, primarily of the genera Alaria spp., Spirometra sp., and Ancylostoma spp. occurred in each sample. Ectoparasites found included 5 species of ticks, including Dermacentor variabilis, Ixodes scapularis, I. affinis, Amblyomma maculatum, and A. americanum, and fleas common to southern Florida. One free-ranging 6-year-old female panther (FP#32) was found with severe alopecia and a presumptive diagnosis of infestation with Notoedres cati was made. Diagnosis was based on a definitive diagnosis made in her captive kitten (FP#208) having similar clinical signs where Notoedres cati was isolated. Florida panther #32 was treated with injectable ivermectin and clinical signs resolved within 3 months. Florida panther #32 was the only panther of those captured this period found positive for Cytauxzoon felis by microscopic examination of RBC's, with no clinical signs associated with the parasite.

Although hair and blood samples were collected from panthers this period, analysis for mercury was not conducted. However, results from some panthers captured last period were available. Mercury levels in whole blood collected from 5 free-ranging panthers captured during the preceeding period were considered well within normal limits, ranging from <.049 PPM to .142 PPM. These panthers were either captured or found dead in southern Florida, all outside the Everglades ecosystem.

Six kittens, 2 in one litter (FP#209 and 210, 3 in another [dam = FP#40]) and one uncaptured kitten born to FP#23, were known born this period. Also, one litter of 2 kittens (FP#53 and N92742) born just prior to this period was

#208? monitored for health. One (#N92742) of these 8 known kittens died as a result of injuries sustained from collision with a vehicle on state road 29 (SR29), one (FP#53) was killed by another panther (intraspecific aggression), and 2 (FP#209 and 210) were captured and placed in the captive-breeding program. Florida panther #209, when captured, was very thin and emaciated. It is believed this kitten would have died if it had not received medical attention. Three kittens (not numbered) born to FP#40 and the observed but not captured kitten born to FP#23 were apparently healthy and doing well when last observed.

Florida panther #205 underwent surgery in an attempt to correct a cardiac anomaly and later died as a result of complications due to the anomaly and surgery. Florida panther #207 underwent surgery (orchiopey) for bilateral cryptorchidism and has not been evaluated for success of the operation. Florida panther #204 was diagnosed with bacterial infection caused by Pseudomonas aeruginosa of the lung and underwent surgery (lobectomy) to remove a lung lobe. This panther is doing well in captivity at the present time. Florida panther #38 did not produce kittens for three years in the wild, even though she was observed in company with a fertile male panther. She was captured and temporarily placed in captivity for evaluation. Abdominal radiographs, ultrasound, vaginoscopy, and celiotomy with visual exam and palpation of the reproductive tract and biopsy and histological examination of the uterus revealed no abnormalities. It is believed this cat may have reproductive hormone abnormalities. After evaluation, she was released into the wild.

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Results of skin biopsies taken from FP#50 and 208 have been incorporated into the data base for genetic profile of the Florida panther population.

The two kittens (FP#209 and 210) that were wild-caught and placed in captivity bring the total captive panther population to 10 (seven at White Oak Plantation, Yulee, Florida; 2 at Jacksonville Zoo, Jacksonville; and one at Lowry Park Zoo, Tampa) (Table 2). All are in good health at the present time. The artificial insemination procedure in captive panther #21, performed last period, was determined to be unsuccessful.

Two panthers (FP#12 and 200) were electro-ejaculated this period. Results from FP#12 are pending and FP#200 produced a fair volume of sperm with high percent (80%) motility.

Five mortalities, 4 free-ranging and one captive Florida panthers, were documented this period. One panther (N92742) died as a result of collision with an automobile on SR29, 3 (FP#28, 47, and 53) died as a result of injuries sustained from intraspecific aggression, and one captive (FP#205) died as a result of complications from surgery.

Post mortem examination was conducted on FP#205, FP#N92742, and FP#28. Cause of death for FP#205 was heart failure; FP#N92742 was trauma associated with collision with car. FP#28 was unknown cause of death due to tissue autolysis. Necropsies on panthers FP#47 and FP#53 are pending.

DISCUSSION

This study is proceeding on schedule.

Adequate annual recruitment into a population is essential to maintain or increase animal numbers. Population size and growth rate must attain some

optimum level to preclude extinction. The panther population in Florida is limited in size and ability to achieve adequate annual recruitment to increase numbers. Demographic, environmental, disease, and genetic factors continue to suppress population levels. Lack of, and continued loss of, suitable habitat, conflicts with an ever expanding human population, and low genetic diversity contribute to loss of population vigor. Internal parasitism, especially by Ancylostoma pluriidentatum, and congenital abnormalities, both affecting health and reproduction, continue to reduce population viability. Effects of low level, chronic mercury toxicosis on immune system function and reproduction and as a contributor to development of congenital defects needs further monitoring. Research into the rates and causes of kitten mortality coupled with aggressive medical management of wild kittens at a very early age may be essential to reverse the present population trend.

The Florida panther remains in jeopardy of extinction. Extinction could occur in 25-40 years (Seal and Lacy 1989) without management intervention. Even with aggressive management, factors previously mentioned that are beyond control may still render the subspecies extinct. However, we must continue our efforts to save this magnificent animal from extinction. To do otherwise, would certainly be unthinkable.

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Table 1. Summary of significant data collected from wild Florida panthers captured FY 92/93.

ID#	Sex	Age (Yr.)	~~last~~ Capture		Significant Data/Comments
			Date	Location ^a	
FP12	M	12	01-04-93	BCNP	Routine capture. Electro-ejaculated (results pending). Apparently healthy.
FP23	F	7	06-03-93	BCNP	Piper stock. Radio-collar signal weak. Re-collared. One kitten observed near capture site. Apparently healthy.
FP31	F	12	01-06-93	FPNWR	Routine capture. Still has heart murmur, otherwise healthy.
FP32	F	6	02-08-93	FPNWR	Hair loss possibly due to mange mite (<u>Notoedres cati</u>). Her kitten (FP208) in captivity at Lowry Park, was diagnosed with <u>Notoedres sp.</u> Treated with Ivermectin and mostly noraml upon last visual exam. Is late in having a litter.
FP34	M	4	02-19-93	Collier property	Routine capture. Unilateral cryptorchid. Very low sperm mobility, otherwise healthy.
FP38	F	5	05-28-93	BCNP	Not known to be reproducing. Captured and temporarily placed in captivity for repro. exam by VMTH. No diagnosis. Suspect hormone imbalance. Continuing investigation and monitoring. Released to wild.
FP42	M	4	05-04-93	BCNP	Piper stock. FiV pos. apparently healthy.
FP44	M	3	02-17-93	ENP	Unilateral cryptorchid. Killed by male panther 07-07-93.
FP45	M	3	02-12-93	South of Seminole Res.	Routine Capture. Healthy.
FP47	M	2	01-04-93	FPNWR	Routined capture. Died due to congestive heart failure associated with large atrial septal defect and stress of injuries associated with fight with male panther.

ID#	Sex	Age (Yr.)	Capture		Significant Data/Comments
			Date	Location ^a	
FP48	F	2	01-05-93	FPNWR	Routine capture. Healthy.
FP50	M	2	02-09-93	Alico	Routine capture. Healthy.
FP52	F	2	01-06-93	Catherine Is.	Routine capture. Healthy. Possibly has kittens.
FP53	M	1	02-10-93	BCNP	Killed by another male panther 02-20-93.
FP54	M	1	02-10-93 05-04-93	BCNP	Routine capture. Healthy.
FP209	F	1	08-20-92	BCNP	Captured and placed into captive breeding program at Jacksonville Zoo. Kitten of FP23.
FP210	M	1	08-20-92	BCNP	Captured and placed into captivity at Jacksonville Zoo. Kitten of FP23.

^aBCNP = Big Cypress National Preserve; FPNWR = Florida Panther National Wildlife Refuge; ENP = Everglades National Park.

Table 2. Status and location of captive Florida panthers.

ID#	Sex	Age - <i>at end of FY</i> (years)	Location	Status
FP21	F	7.5	White Oak Plantation	Piper stock. FiV positive. Healthy.
FP200	M	11-13	White Oak Plantation	Piper stock. FiV positive. Healthy. VMTH evaluated past femur fractures repair and prognosis good.
FP201	M	3	White Oak Plantation	Unilateral cryptorchid. Grade I/VI heart murmur. Healthy.
FP202	M	3	White Oak Plantation	Grade III/VI heart murmur. Healthy.
FP203	M	3	White Oak Plantation	Small left testicle. Healthy.
FP204	F	3	White Oak Plantation	Lobectomy performed due to bacterial pneumonia. VMTH evaluated after surgery and doing fine. Grade III/VI heart murmur, otherwise healthy.
FP205	M	2	White Oak Plantation	Had surgery for atrial septal defect. Died after surgery.
FP207	M	2	Lowry Park (transferred FY 93/94)	Bilateral cryptorchid. Orchiopexy performed. Electroejaculated after surgery and found to be sterile. Grade I/VI heart murmur.
FP208	F	1	Lowry Park	Had mange (<u>Notoerdes cati</u>) as kitten. Grade II/VI heart murmur. Healthy.
FP209	F	1	Jacksonville Zoo	Grade I rear leg lameness due to injury as kitten. Placed into captivity 08-20-93. 92
FP210	M	1	Jacksonville Zoo	Placed into captivity 08-20-93. Healthy.