

Analysis of scientific publications related to the Florida Panther

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INTRODUCTION

The Fish and Wildlife Service (FWS) recently appointed a new Florida panther recovery team to revise the panther recovery plan. To support this revision and to assist the recovery team in setting priorities, FWS and Florida Fish and Wildlife Conservation Commission (FWC) commissioned an independent critical review of literature related to ecology and management of the Florida panther. The four authors of this report were recruited to conduct the review.

The main objectives of this review are to identify strengths and weaknesses of existing data, previous analyses, and published interpretations of data and analyses related to the panther, and to recommend future analyses and research priorities. To support our review, we were asked to produce an annotated bibliography of Florida panther literature. We were asked to include all scientific publications, including unpublished reports, related to conservation and management of the Florida panther.

METHODS

The 4 members of the Scientific Review Team met each other in Naples, Florida on April 29 2002. At that meeting, we also met several personnel from FWC, US Fish and Wildlife Service, and National Park Service who are leading efforts on panther management and recovery. We learned about the history of management of the panther, and were provided with maps and copies of most of the literature in electronic format. None of the SRT members had prior knowledge of scientific controversies related to panther management. During the meeting we learned that there were disputes, but we were unaware of the nature of these disagreements.

The SRT classified the approximately 3000 pages of published and unpublished papers on the panther into 4 topical areas and assigned ourselves, in sub-teams of two persons, to review the literature in each subject area. About 20% of the papers straddled topical areas and were assigned to 2 or more sub-teams. Each of us read 1500-2000 pages of material in our 2 assigned subject areas by September 2002, at which time each pair of reviewers provided a list of 8-11 key papers in their topical area for the other SRT members to review. Thus each paper was read by at least 2 of the 4 members of the SRT, and a considerable portion was read by all members. During this phase, SRT members carefully avoided making comments to each other, and avoided comments from other interested parties, so that each reviewer could independently evaluate the literature on its merits.

During November 4-7 2002, the SRT again met in Naples, where for the first time we discussed our evaluations of the literature. Each of us approached the meeting with strong reactions to the literature, and concern that his opinion might not be shared by the rest of the SRT. In each subject area, however, there was a remarkable level of agreement about the strengths and weaknesses of the data and analyses. We also noted ambiguities in some of the papers, and started to develop a list of queries that we could send to particular authors to clarify these issues. On November 5, we enjoyed a half-day helicopter tour of panther habitat, courtesy of the National Park Service. During the last 2 days of the meeting, we continued our discussions, and made ourselves available for persons to express their opinions on scientific issues.

Either at or immediately after the November meeting, each SRT member shared their annotations and comments on each paper for collation into an annotated bibliography. We used ProCite ® software to organize reviewer notes on the content, and reviewer comments, into a bibliographic

database. During December 2002, we collated queries specific to each of several authors. We E-mailed queries to the respective authors, and invited each author to attend an SRT meeting on January 31 2003. Each author responded to their queries via phone or Email before the meeting, so that we could devote that meeting to matters of interpretation rather than matters of fact.

On 31 January 2003, the panther SRT met in Athens, Georgia. We separately interviewed each of the following persons: Sonny Bass, Chris Belden, Jane Comiskey, Mark Cunningham, John Kasbohm, Darrell Land, and Dave Maehr. Cunningham and Land participated by speaker phone from Florida; the others attended in person. After a discussion of scientific issues, we invited each interviewee to give us their perspective on any issues related to ecology and management of the panther.

During Spring 2003, the SRT drafted this report, organizing it along the same topical themes we had used to organize the literature. All SRT members participated in writing each section, and this report reflects our consensus view in early June 2003. FWC will send this draft out for review, and will use reviewer comments to revise the document in Fall 2003. At that time, we may add some general comments on the recovery program and reflections on the review process. We append to this report (a) a list of suggested reviewers, and (b) our annotated bibliography in the form of a ProCite database.

Acknowledgments. This review was funded by FWC and USFWS. We thank all the authors who responded candidly and helpfully to our requests for information; Dave Maehr and Darrell Land received a disproportionate share of queries and responded promptly and thoroughly. Scanning dozens of documents into pdf format and providing copies to the SRT was a Herculean task, and we thank Darrell Land for his speed and good humor in attending to it. We thank Deborah Jensen and John Donahue for arranging a helicopter tour of panther habitat, Roy McBride for a field tour, and other staff of NPS and FWC for attending to the many logistical details of our visits. Justin Waskiewicz entered and edited data in the bibliographic database.

HABITAT RELATIONS

During 1981-2002, a number of reports, popular articles, books or book chapters, and scientific papers, some with rigorous and some not so rigorous peer reviews, were written about habitat use and habitat requirements of the Florida panther (*Puma concolor coryi*), yet a clear consensus of panther-habitat relationships has not emerged. In fact, the issue of panther habitat requirements is quite controversial. The controversy hinges primarily on the spatial and temporal limitations of the data, and how habitat use data were collected, analyzed, and interpreted. In a series of papers (Maehr and Cox 1995, Maehr 1997a and b, Kerkhoff et al. 2000, Maehr et al. 2001), David Maehr and his colleagues drew conclusions about the relative importance of forests to panthers and the reluctance of panthers to stray far from forest cover. Some of their conclusions were criticized, most vigorously by Jane Comiskey, but also by other biologists and managers involved with Florida panther recovery, and even by some of Maehr's coauthors on the very papers from which the conclusions were drawn. Scientifically sanctioned (e.g., peer reviewed research) conclusions regarding Florida panther habitat requirements are critical because they affect land management decisions, particularly those decisions made in US Fish and Wildlife Service Section 7 consultations on land development and mitigation, where the "best available science" is the standard.

The Florida panther Science Review Team (SRT) recognized several issues, or areas of controversy, surrounding the scientific literature related to panther habitat requirements. These include, but are not limited to:

- The use of daytime telemetry locations to depict 24-hour habitat use patterns.
- Selective use of telemetry locations to analyze panther habitat use data.
- Use of individual locations rather than individual panthers as the sampling unit.
- Currency of habitat maps used in analysis of telemetry data.
- Telemetry error and use of a point-to-pixel approach to plot telemetry locations.
- The distance from forest cover panthers are likely to travel.
- Panther home range size relative to percent forest cover within the home range.
- Big Cypress National Preserve and Everglades National Park as panther habitat.
- Analytical techniques used to analyze telemetry data.
- The inadequacy of the peer-review process.
- Selection of reintroduction sites for establishing population(s) of panthers outside of south Florida.

The important questions seem to be “Are the data as presented reliable?” and “Should those responsible for recovery of the Florida panther use these data to make critical decisions that may affect Florida panther persistence?” The SRT’s response to these questions is detailed below. Because of the interrelatedness of the issues listed above, not all will be discussed individually.

About 75% of the habitat related publications reviewed by the SRT were authored or coauthored by David Maehr, thus most of the following discussion focuses on the work of Dr. Maehr. It is critically important to note that the SRT strived to present an objective opinion of the literature with regard to its scientific rigor.

One of the most critical papers regarding panther habitat requirements was Maehr and Cox (1995), which identified the importance of forest types to panthers (previously reported in Maehr et al. 1991), noted Florida panthers need large forested areas to meet daily requirements (mean = 20,816 ha), reported 96% of all locations occurred in or within 90 m of a preferred forest type, found an inverse relationship between male panther home range size and percent forest in the home range, and implied that Everglades National Park (ENP) and Big Cypress National Preserve (BCNP) contained marginal habitat and may be avoided by panthers. This publication is repeatedly cited in subsequent manuscripts, authored or co-authored by Maehr, as evidence of the above ideas. For instance, Maehr and Meegan (2001) cited this paper in developing criteria for evaluating panther habitat for land management decisions. In other examples, Maehr belittled ENP and BCNP as panther habitat (Maehr 1997b, Maehr et al. 2002), modeled and provided guidelines for assessing panther habitat (Maehr and Meegan 2001, Meegan and Maehr 2002, Maehr and Deason 2002), and concluded that Florida panthers are forest obligates, while reinforcing the idea that panthers are reluctant to cross non-forested habitat greater than 90 m in width (Maehr et al. 2001).

In response to publications by Maehr and colleagues, Comiskey et al. (2002) challenged the validity of some of the conclusions drawn from these publications, based in large part on the idea that daytime telemetry alone cannot completely describe Florida panther habitat requirements, particularly for an animal that is most active at night.

The SRT evaluated the scientific literature, spoke with several of the authors of the key habitat publications and reached the following conclusions:

Use of daytime telemetry locations to describe habitat use

Maehr et al. (1989, 1990) described nocturnal activities of 4 dams monitored 4,600 hours and 6 solitary panthers monitored for 130 hours. However, all other analyses are based on telemetry locations gathered between 6 and 11 AM from a fixed-wing aircraft. This time window was selected because of unfavorable flying conditions at other times of the day (D. Land, personal communication). Thus, all analyses of available telemetry data, regardless of author, are restricted to daytime telemetry locations. However, Florida panthers apparently are most active at night (Maehr et al. 1990), and daytime telemetry is insufficient to describe habitat use of animals that are active at night (Beyer and Haufler 1994, Dickson et al. in review). In the opinion of the SRT, the use of daytime telemetry data should be limited to describing daytime panther habitat use patterns. Extrapolating daytime telemetry locations to describe 24-hour habitat use by Florida panthers is unjustified, and conclusions based on this approach are unreliable. The SRT emphasizes these data are useful for describing daytime habitat use patterns, identifying daytime bedding sites, documenting seasonal movements and dispersal, and outlining home ranges. However, to interpret these data beyond their limits is scientifically untenable. Further, the SRT noted that many of the publications that pushed these data beyond their limits, (e.g., Maehr 1992, Maehr and Cox 1995, Kerkhoff et al. 2000), failed to mention that the data were collected in daylight hours only; whether purposeful or not, this is an inexcusable oversight by both the authors and reviewers of the publications.

Selective use of telemetry locations

Between 1981 and 2002, more than 55,000 radio locations on more than 100 Florida panthers were recorded, yet most publications have not made full use of the locations available at the time of data analysis. The SRT discovered several unexplained examples of data sub-setting or selective use of data. For example, Maehr et al. (1991) tracked 26 Florida panthers during 1985-90, but without explanation, used only 9 of the 26 panthers (7,025 locations) to describe cover type use by panthers. Maehr and Cox (1995) similarly failed to state they excluded 6,000 of 14,448 locations and 18 of 41 panthers from their analyses. Alternatively, Dees et al. (2001) excluded panthers and locations from their analyses, but fully explained the reasons for excluding data. While there may be legitimate reasons to exclude data from certain analyses, it is incumbent upon the author(s) to explicitly state which data were and were not used, and to fully explain the reasons for excluding data.

Locations versus panthers as the sampling unit

Although it was not always clear from the description in the methods section of some publications, the analyses in at least two publications (Maehr and Cox 1995, Kerkhoff et al. 2000) used locations rather than panthers as the sampling unit to determine habitat use of panthers. The SRT believes this is an incorrect approach because a single panther with an abnormally large number of locations could bias the analysis. For example, suppose that a researcher had 54,750 locations on a single animal, and 5 locations for each of another 50 animals. A pooled analysis would be nothing more than the habitat selection of a single animal, because use by the other 50 animals would be swamped by the one animal with many locations. Although panther data are not this skewed, simple pooling makes sense only under the patently

false assumption that animals with more locations are “better panthers” or “more representative panthers” than those with fewer locations.

In addition, inferential statistics from such a pseudo-replicated analysis are strongly biased toward “statistical significant” results (Hurlburt 1984). This problem is not remedied by having an equal number of locations per animal. The proper approach would be to calculate the habitat composition in the home range of each panther then average across panthers (Aebischer et al. 1993). The final answer may not change using this approach, but the analysis is defensible, whereas using locations (i.e., all locations from all panthers combined) as the sampling unit is indefensible.

Currency of habitat maps

Telemetry data have been collected for Florida panthers over a long time period (1981-2002), but there is no indication that vegetation maps used in habitat use analyses have been updated and ground-truthed to stay current with analyses of telemetry data. If researchers fail to update vegetation maps, they could reach erroneous conclusions about panther habitat use. For instance, what was a forest patch in 1985 may be an agricultural field in 1995. If this is not reflected on vegetation maps used in analyses of panther locations from 1995, then the wrong conclusions about habitat use may be drawn. The SRT has insufficient information to know to what degree this may be a problem, but recommends attention to this potential problem in future analyses.

Telemetry error

No matter how good the telemetry tracking system, and how qualified the persons operating the tracking equipment, location error occurs in all telemetry studies; this error must be accounted for when analyzing location data (White and Garrott 1990). Almost all telemetry data from radio-marked Florida panthers were collected using aerial telemetry. White and Garrott (1990) noted errors in excess of 0.5 km had been reported for aerial location estimates when the animal was not visually located. They further note that altitude above ground, air speed, location procedures, prevalence of landmarks, and investigator fatigue or discomfort affect aerial location accuracy.

Most papers that analyzed telemetry locations to determine habitat use and home range of Florida panthers noted that locations were gathered using aerial telemetry, and reported altitude and flight speed; however, only 3 publications estimated telemetry error. Belden et al. (1988) estimated error as 230 m and Janis and Clark (2002) estimated a mean error of 176 m with 95% of locations within 489 m. Dees et al. (2001) estimated telemetry error averaged 77 m with 95% of locations within 200 m. Maehr et al. (1991) **assumed** (without explanation) that telemetry error was 100 m; in retrospect, this probably was an underestimate. The most influential paper on habitat use (Maehr and Cox 1995) did not provide any estimate of telemetry error, and implicitly assumed error was 0 m, the most crucial example being when Maehr and Cox (1995) concluded from their analysis that “...96% of panther locations were within 90m of large forest patches.” Later, Maehr et al (2001) and Maehr and Deason (2002) cited Maehr and Cox (1995) incorrectly when they claimed that Maehr and Cox (1995) showed panthers were reluctant to cross non-forested areas greater than 90m. This is particularly disturbing because all the data upon which these conclusions were based were collected during daylight hours (never acknowledged by the authors) and may have no relevance to what panthers do at night when they are most active.

Unfortunately, the 90-m distance seems to have been adopted as one of the standards for computing mitigation for loss of panther habitat in land development projects. The SRT finds no empirical basis for the idea that panthers don't travel over 90 m from forest cover, and we recommend that use of this idea be discontinued immediately. Indeed, Maehr et al. (1992) reported that an adult male panther north of the Caloosahatchee, in an area of fragmented forest, regularly crossed areas much larger than 90 m within his home range. The conclusion that 96% of panther locations were within 90 m of forest cover is likely incorrect because Maehr and Cox (1995) used a point-to-pixel approach to plotting locations and did not allow for telemetry error. Extending this conclusion to justify the characterization of habitat patches farther than 90 m from forest cover as unlikely to be used by panthers is unsupportable and bad science.

Home range

Belden et al (1988), Maehr et al. (1991) and Comiskey et al. (2002) reported home range size for male and female Florida panthers, and all agreed that males (435-650 km²) and females (193-396 km²) required relatively large areas to meet their needs. The SRT believes that all these estimates are reliable. Estimates of home range size are probably more sensitive to the statistical algorithm used (e.g., minimum convex polygon, adaptive kernel, fixed kernel) than to location error or daytime bias in locations.

Importance of forests to panthers

Maehr (1992), Maehr and Cox (1995) and Kerkoff et al. (2000) concluded panther home range size was inversely related to the amount of forest cover within the home range. Using fractal analysis, Kerkoff et al. (2000) added that when the amount of forest cover within a home range drops below about 25%, "...the likelihood of intensive panther use declines dramatically", and because their study area contained only about 22% forest cover "...the region may be, from a panther-centered view, on the verge of collapse." Comiskey et al. (2002), on the other hand, also used fractal analysis, but failed to find a relationship between amount forest cover within a home range and home range size, and rejected the idea of a forest-centered view of panther habitat selection. Rather, they contended the panther is a habitat generalist that occupies all natural habitats available to them.

Kerkoff et al. (2000) used data collected in a 20,000 km² area of south Florida during 1981-93 (41 individuals; 12,783 locations), whereas Comiskey et al. (2002) used data collected in a 25,000 km² area of south Florida during 1981 through mid-2000 (102 individuals; 49,889 locations). Comiskey et al. (2002) also used a subset of data (1981-93, 18,118 locations, 52 panthers) in a fractal analysis for comparison with Kerkoff et al (2000).

The SRT found both these fractal analyses difficult to interpret and of little value. Comiskey et al. (2002) used GIS layers for vegetation that were not always concurrent with telemetry locations, and estimated home range using the Minimum Convex Polygon approach, which may include large areas of unused habitat in the home range. All the location data were diurnal locations, which should not be used to describe how panthers use habitat on a 24-hour basis. Despite these shortcomings, the SRT agreed Comiskey et al. (2002) made a credible case that the relationship between panther home range size and amount of forest within the home range is weak, and convincingly showed that panther used home ranges that had less than 25% forest.

Disregarding the fractal analyses, and the severely pseudo-replicated analyses that used the animal as the sampling unit, there is reasonable evidence forests are the most important habitat

for diurnal locations of panthers. For instance, Table 2 of Maehr and Cox (1995) presents percent composition of panther home ranges, and supports the importance of forests to panthers within the area north of Interstate 75 and west of State Route 29. Although the analysis for their Table 2 was not described in the methods section, these data appropriately use the animal as the sampling unit. Similarly, Table 4 in the draft 2002 Conservation Strategy (Florida Panther MERIT Subteam 2002), convincingly illustrates the importance of forests as preferred diurnal locations for panthers.

Other analyses attempt to demonstrate the importance of forest based on apparently straightforward frequency distributions of patch sizes used by panthers. For instance, 75% (Maehr and Cox 1995) to 81% (Maehr and Meegan 2001) of panther locations were within forests larger than 500 ha. However, despite their apparent simplicity, these statistics by themselves say nothing about the preference for large forests because they were not compared to the frequency distribution of forest patches *available* to panthers. The fact that 19% to 25% of panther locations occurred in patches smaller than 500 ha could reflect either aversion, indifference, or preference for small patches – depending on the availability of small forest patches to each radio-tagged panther. This is an important issue, because Maehr and Deeson (2002) use this 500 ha minimum patch size as the *single most important factor* in their Panther Habitat Evaluation Model.

The SRT strongly recommends the use of compositional analysis (Aebischer et al. 1993), or another statistically appropriate method, to compare the distributions of forest patch sizes available to panthers to those used by panthers. We also caution that such analyses should develop a frequency distribution of “available” patch sizes separately for each animal. An analysis using availability based on patch sizes pooled across animals would be misleading because it entails the assumption that a huge patch of forest (in Florida Panther NWR, for example) is “available” to every panther (a panther in the Everglades, for example). The analysis should also use locations buffered to reflect location error.

Big Cypress National Preserve and Everglades National Park as panther habitat

Maehr and Cox (1995) excluded (without mention in the publication) panthers and radio locations south of I-75 and east of SR 29 from analysis because “...we viewed the southeastern area of occupied range as fundamentally different and not typical of preferred habitat” (written response to SRT inquiry). Maehr et al. (2001) referred to public areas south of I-75 (i.e., BCNP and ENP) as population sinks for panthers because they do not meet the Panther Habitat Evaluation Model (PHEM) habitat requirements (model score is highly dependent on a 500-ha minimum size of forest patch – see above). Maehr et al. (2002) argued that 600,000 ha of National Park Service land in south Florida is marginal panther habitat because none of 27 subadult panthers captured during 1986-2000 began their dispersal to the southeast of their natal range, which was primarily in the core area north of I-75 and west of SR 29. They attribute lack of dispersal to the southeast to limited forest cover and low prey density. Earlier, Bass and Maehr (1991) concluded reproduction does not occur in unforested areas of ENP (a conclusion Bass no longer ascribes to; personal communication January 31, 2003). Comiskey et al.’s (2002) analyses did not support any of these conclusions, and reported that 1 male and 5 females occupied home ranges in ENP with < 15% forest cover.

The SRT recognizes that prior to genetic restoration in 1995, most of the panther population, including those that were radio-collared and monitored, were in what was considered the core

panther area north of I-75 and west of SR 29. Following, genetic restoration, panthers freely occupied habitats south of the core area. Thus, conclusions about the habitat value of ENP and BCNP depend partly on whether a study used data from periods when the parks were and were not occupied by panthers. The SRT is not persuaded by the arguments that the 2 federally managed areas are poor panther habitat. On the other hand the post-1995 population increase in these areas does not convince use that these areas will continue to support a dense panther population. Arguments over whether ENP and BCNP, or any other land outside the “core area,” are suitable panther habitat will be resolved as we observe panther survival and reproduction in these areas over the next few years.

Analysis of telemetry data

The SRT concludes that, to date, Florida panther telemetry data, overall, have not been properly analyzed. Fractal analyses (Kerkoff et al. 2000, Comiskey et al. 2002), point-to-pixel approach (Maehr and Cox 1995), minimum convex polygons (Belden et al. 1988, Maehr et al. 1991, Comiskey et al. 2002), spatial models (Meegan and Maehr 2002), and other techniques not clearly described (Maehr 1992) have provided some useful information, but each has been seriously flawed. The SRT recommends a more appropriate technique, such as compositional analysis (Aebischer et al. 1993, Florida Panther MERIT subteam 2002). Even this approach cannot address some important issues (e.g., nocturnal habitat use) until appropriate data are available.. Thus, the SRT beseeches Florida panther researchers to collect and analyze night-time telemetry data to provide a more complete picture of Florida panther habitat use.

The inadequacy of the peer-review process

As the SRT reviewed the Florida panther habitat literature it became obvious Maehr and Cox (1995) has been used to influence land management decisions involving Florida panthers, despite its use of daytime telemetry locations to describe 24-hour habitat use, assumption of zero location error, pseudo-replication, and failure to compare sizes of forest patches used by panthers with those available to them. Sadly, the peer review process failed to prevent publication of these errors. Particularly egregious conclusions of this paper (or conclusions later attributed to the paper by Maehr and colleagues) are that panthers are reluctant to traverse areas farther than 90 m from forest cover, that panthers require forest blocks >500 ha, and that panthers are forest obligates. These conclusions also form the basis of a habitat model (Meegan and Maehr 2002, Maehr and Deeson 2002) that might be used to evaluate and sanction development projects within panther range.

The peer-review process similarly failed to detect that later manuscripts inappropriately cited Maehr and Cox (1995) as supporting conclusions not stated therein – such as panthers being ‘reluctant to cross’ 90 m of non-forest – perhaps because reviewers assumed Maehr would not misinterpret his own work. These examples illustrate the failure of the peer review process to prevent publication of seriously flawed analyses and illustrate how the conclusions drawn from a flawed peer-reviewed paper can be accepted as fact by repeated citation and mis-citation. The SRT questions the reliability of subsequent publications that uncritically rely on Maehr and Cox (1995) and cautions those responsible for the management and recovery of the Florida panther that the peer-review process can fail, at times seriously. At least one SRT member reviewed one or more of the papers involved in this particular example, and we emphasize that reviewers and editors share responsibility for the failures reported here. We earnestly hope our review will not create any new errors or reinforce other existing errors by failing to notice them.

Selection of reintroduction sites for establishing panthers outside of south Florida.

Maehr et al. (2002a) suggested that natural dispersal of panthers, abetted by habitat restoration and perhaps by translocation of females, may allow panthers to re-establish a breeding subpopulation north of the Caloosahatchee River. The SRT concurs that this is a reasonable possibility, and would significantly enhance panther recovery. However, recovery and delisting of the panther requires 3 viable populations within historic range (US FWS 1995, 1999). Because such populations are unlikely to occur via natural recolonization, reintroductions will almost certainly be required. FWC conducted well-designed experiments that will assist in the mechanics of reintroduction (Belden and Hagedorn 1993, Belden and McCown 1996). The collation and analysis of data on panther dispersal (Maehr et al. 2002a) also will be useful in planning reintroduction efforts. However, the SRT was dismayed that little substantive work has been done on identifying reintroduction sites and preparing for the social and political challenges involved in such an effort.

Jordan (1994) provided a good start on this challenging issue. However, this document lacked several important elements, including: a discussion of the biological links between each criterion and expected success of a reintroduction, a weighting scheme for criteria, sensitivity analysis of different weighting schemes, site boundaries based on something more meaningful than county lines, a discussion of opportunities to use education and other tools to alter social factors impeding public acceptance, GIS analysis of landscape connectivity and potential for population expansion (the report acknowledges the importance of connectivity, but ignores it anyway), and a more sophisticated road analysis (the report treats a 6-lane freeway the same as a public dirt road). Perhaps more important, Tasks 2, 3, 4, and 5 of the report (page 10) had target dates between August 1994 and February 1996. As of early 2003, none of these tasks have been completed, and some may not have been started. The SRT's impression is that, as an agency, FWS does not have its heart in the reintroduction effort. This impression was reinforced by several persons interviewed during our review. The SRT is aware that University of Tennessee is conducting some analyses that may indicate significant progress on this issue. We strongly urge FWS to vigorously promote this process.

SUMMARY – HABITAT RELATIONS

Definition of habitat requirements of the Florida panther is one of the most contentious aspects of panther ecology. Most of the controversy revolves around conclusions drawn by David Maehr and colleagues regarding the relative importance of forest and forest cover to panthers. Maehr and colleagues concluded Florida panthers are forest obligates, they strongly prefer large blocks ($>500 \text{ km}^2$) of forest cover, they are reluctant to traverse areas greater than 90 m from forest cover, and they avoid areas lacking large forest tracts such as most of ENP and BCNP. Those with an opposing view argue the data are insufficient to draw these conclusions because of the diurnal bias in telemetry locations and selective use of data by Maehr and colleagues. The SRT recognizes the selective use of data can be legitimate, but affirms it is incumbent upon authors to divulge which data were deleted and why. The SRT agrees with the view that daytime telemetry is insufficient to describe a 24-hour pattern of habitat use by Florida panthers. Although we recognize the available data clearly point to the importance of forests and forest cover to panthers, we find no basis for the idea that panthers are reluctant to move greater than 90 m from forest cover, that panthers avoid forest patches smaller than 500 ha, or that BCNP and ENP do not provide useful habitat for panthers. Clearly, panthers use a variety of habitats and survive and reproduce in areas that do not meet the criteria of PHEM. In fact, we recommend that PHEM not

be used to make land management decisions regarding Florida panthers. On a positive note, we recognize the value of the available data in describing daytime habitat requirements, determining home range attributes, and describing dispersal patterns. To our surprise, we find that, to date, no one has completely and properly analyzed available telemetry data nor, until recently, attempted to consistently obtain nocturnal locations.

RECOMMENDATIONS – HABITAT RELATIONS

The SRT recommends the following regarding Florida panther habitat relations

1. Analyze existing telemetry location data using an appropriate technique such as compositional analysis (Aebischer 1993, Dickson and Beier 2002). Use buffers around each location based on empirical estimates of location error. Use panthers, not panther locations, as the sampling unit. Use GIS layers or habitat maps that were current at the time the data were collected. Conduct the analysis at 2 levels (Johnson 1980): First, compare the habitat within panther home ranges to the habitat available within the geographic range of the Florida panther. Second, and more important, compare locations within a home range to habitat available in that home range.
2. Some panthers have been equipped with GPS collars and nocturnal locations are now or soon will be available; we assume plans were made to achieve an adequate sample from panthers in different parts of their range at all times of the year. Analyze these data to get a complete picture of panther habitat use both within and outside the “core area.”
3. Vigorously pursue efforts to identify at least one appropriate site for reintroducing panthers and re-establishing population(s) of panthers outside of south Florida. The analysis must include justification of the criteria used, sensitivity analysis of weighting schemes, GIS analysis of landscape connectivity and potential for population expansion, and rigorous consideration of roads and other factors. Develop plans to use education and other tools to alter social factors that might impede public acceptance.
4. Develop habitat models, using the best available data and acceptable statistical approaches, which predict habitat occupancy as a function of landscape attributes. These approaches should: a) be based on the full range of empirical data and not exclude outlying observations, particularly those that do not adhere to conventional wisdom about habitat suitability; b) fully incorporate uncertainties in the data and statistical models, including telemetry location error and biases induced by non-representative sampling of activity patterns; c) predict at appropriate scales of spatial and temporal resolution; and d) explicitly incorporate alternative, plausible biological hypotheses as alternative statistical models. Evaluate these using information-theoretic approaches and incorporate AIC model weights or other measures of belief in these alternatives in predictive, decision-making models.
5. Sensitivity analysis should be conducted on any habitat model prior to using it to make decisions on management or land use. Each poorly estimated factor (such as panther aversion to roads) should be varied across the range of reasonable values for its impact on the map of predicted habitat, as well as priority assigned to various parcels. At a minimum, this would suggest future analyses to better estimate the important factors.
6. In the future, use radio telemetry to focus on specific unanswered research questions rather than to simply continue monitoring. In addition to issues mentioned above, the

SRT recommends using radio-telemetry to quantify nocturnal panther use of different sized patches of row crops and other types of agricultural land, areas at various distances from freeways, and areas at various distances from urban development. Such data are sorely needed for predictive models of habitat use in a changing landscape. Managers also need fine-scale data on dispersal movements with respect to potential barriers such as roads and the Caloosahatchee River. Focusing on the research question will dictate the location of tagging efforts, the ages of animals tagged, types of radio-tags deployed (GPS versus VHF), and types of analysis.

7. To be considered “best available science” a published paper must completely identify data used and data available, justify selective use of data, acknowledge the limitations of diurnal data, account for location error, and use the animal as the sampling unit. Most published papers on habitat use by panthers, including Maehr and Cox (1995) and papers relying on it, do not meet these criteria. These publications should not be used by management agencies to make decisions that affect the persistence of the Florida panther or to justify any action that may be detrimental to Florida panthers.
8. The debate about whether panthers are forest obligates is an unproductive argument that polarizes opinion, wastes time, and does nothing for the management and recovery of the Florida panther. The SRT recommends all stakeholders put aside personal agendas and work constructively to preserve the Florida panther.

PREY RELATIONSHIPS

Prey selection, prey abundance, and the effect of select variables (e.g., hunting, water levels, weather variables) on prey availability seem key issues as they relate to Florida panther persistence. Competition with other predators may also be a concern. While there is general agreement on which foods are important to Florida panthers, there is some disagreement on whether prey densities in some parts of panther range are sufficient to support a panther population, and uncertainty about the effects of hunting and habitat quality on the density and well being of the prey population. However, these issues do not appear contentious, and may reflect a general lack of sound data on prey abundance, distribution, and general health.

Schortemeyer et al. (1991) presented a history of events since the early 1900s that have affected Florida panthers and their prey. Prior to the 1980's, there was no intentional management on public lands to increase prey for panthers. During the 1980's, actions were taken to reduce access to public lands, reduce hunting pressure and harvest on white-tailed deer (*Odocoileus virginianus*) and wild hogs (*Sus scrofa*), reduce the use of hunting dogs, and protect does and fawns. These initial efforts were not data-based, but by the 1990's, data were available and used to refine prey management strategies. Schortemeyer et al. (1991) outlined the positive effects of specific management initiatives, and concluded: 1) efforts to control wild hogs and armadillos (*Dasypus novemcinctus*), which were introduced for reasons unrelated to panther management, could negatively affect panthers; 2) higher prey densities may be achieved by improving habitat conditions; and 3) recreational hunting does not adversely affect deer behavior or numbers. In addition, they recommended further research concerning predator, prey, and human interactions.

Florida panther food habits

The SRT found only 2 publications that described food habits of the Florida panther, but both appeared to be based on solid data. Maehr et al. (1990) described the food habits of Florida panthers in southwest Florida (Collier, Hendry, Lee, Glades, and Highland counties), after examining 270 scats collected during 1977-89, and 38 kill sites discovered during 1986-89. Wild hogs dominated in estimated biomass consumed in the northern part of the study area, while white-tailed deer dominated in the southern part of the study area. Dalrymple and Bass (1996) examined 113 kill sites from 9 radio-collared panthers, and 272 scats collected at kill sites and other random locations in ENP during 1984-91. They concluded white-tailed deer, which composed an estimated 78% biomass consumed, were the dominant prey species of Florida panthers. Marsh rabbits (*Sylvilagus palustris*) and raccoons (*Procyon lotor*) were secondary prey. In SRT discussions with state and federal biologist and land managers, the conventional wisdom was that white-tailed deer and wild hogs were the dominant prey species of Florida panthers, while rabbits, raccoons, armadillos, etc. were of secondary importance.

Both papers on food habits depended on examination of kill sites and scat samples. It apparently is not difficult to differentiate panther kills from kills of other potential predators (Shaw 1983). Johnson and Belden (1984) determined mountain lion scats and bobcat scats could be differentiated with a relatively high degree of confidence. While the SRT recognizes that items dominant in the diet of Florida panthers vary over panther range, we believe the literature describing Florida panther food habits is reliable. We also note the existence of an unpublished data set on Florida panther food habits in the BCNP (personal communication, Deborah Jansen, NPS). The SRT supports and encourages the analysis and publication of these data, which should make an important contribution to the understanding of panther food habits.

Impact of hunting

Hunting of deer and other game species is a form of competition with panthers for what may be a limited resource. Hunting also disturbs land (e.g., vehicle tracks and hunting camps) and wildlife, including panthers and deer. Doubtless both competition and disturbance have negative impacts on panthers. The real issue is whether these impacts are large enough to warrant management intervention. Several participants in a round table discussion of issues relative to Florida panther survival expressed concern over the impacts of recreational harvest of deer in panther range, and recommended more research on this issue (Branan 1986). In 1985, a panel was formed to make recommendations for deer management on Big Cypress National Preserve. They concluded legal buck hunting did not affect herd productivity or rate of increase, and suggested panther predation might focus on older does (Downing et al. 1986). The panel recommended reduced deer harvests and they encouraged managers to more carefully monitor deer and wild hog population trends. Steelman et al. (1999) reviewed regulation changes designed to reduce the harvest of deer and wild hogs on Big Cypress National Preserve and the impacts of those changes. In a 5-year study of radio-tagged deer on Bear Island, Land (1991, 1994) reported poached deer (2) nearly equaled the legal harvest (3), and that hunters took about as many deer (5) as panthers did (4), but bobcats killed even more deer (10). Land (1991) concluded, "Our data do not support contentions that hunting in Bear Island has adverse impacts upon the white-tailed deer population, and in particular, female deer. The deer herd provides a stable prey base for panthers and is not prone to wide fluctuations in survival patterns." Janis and Clark (2002) found panthers responded to human activity (i.e., hunting, ORV use), but from an energy intake/energy expenditure perspective suggested hunting did not have a negative impact on panthers. They also

found kill rates by female panthers were trivially higher in the hunted area than in the non-hunted control area before, during, and after the hunting season. They reported that they had insufficient statistical power to make inferences about how hunting affected kill rates by male panthers.

The SRT finds these studies of hunting impacts inconclusive. The raw number of kills reported by Land (1991) suggests hunters take about as many deer as panthers, a statistic that neither supports nor refutes the contention that deer hunting reduces panther fitness. Only Janis and Clark (2002) attempted to test whether hunting and harvest of deer affected fitness (predation rates) of Florida panthers, and their negative results are reasonably persuasive evidence that recreational hunting is benign. However, the study was not replicated (a single hunted area compared to a single non-hunted area), and thus site effects are confounded with the effects of hunting. Their results do not rule out the possibility that panther kill rates (and other measures of fitness) on Bear Island might increase if hunting were halted.

Despite the lack of strong evidence, the SRT is not inclined to recommend new research on this issue, largely because it does not seem important to managers, most of whom apparently feel recreational harvest of deer does not negatively affect deer availability for panthers. Management agencies are taking steps to reduce hunt-related disturbance on Bear Island by closing deer camps and rehabilitating vehicle tracks, and seem satisfied these measures are benefiting deer and panthers on Bear Island. No one interviewed by the SRT expressed concern about this subject.

Relationship among soil, hydrology, prey density, and panthers

The SRT found little to no quantitative data on prey densities. Belden and Hagedorn (1993) suggested, for translocations, a density of 1 deer/36 ha could support a population of 50-60 panthers, but we found no specific information on prey densities required to support existing populations. Responding to a query from the SRT, Darrell Land (FWC) noted “Spotlight surveys, track counts, and aerial surveys have all been used to monitor deer numbers, but their reliability as true estimators is very suspect.” Cramer and Portier (2001), who attempted to model panther movements in relation to some anthropomorphic and environmental variables, noted “The lack of specific deer density data on much of the study area makes this the weakest source of data.”

McCown et al. (1991) collected 39 and 43 deer, respectively, from the Eastern Monument Unit (EMU) and Bear Island Unit (BIU) of Big Cypress National Preserve (BCNP) and concluded deer health and productivity were reduced in EMU by poor forage quality. In a more inclusive study (more years, larger sample), McCown (1991 [also McCown 1994]), concluded deer and panthers in the cypress-dominated habitats of the Corn Dance Unit of BCNP and the Fakahatchee Strand State Preserve were “...smaller, leaner, less thrifty, less productive and less numerous...” than in the Bear Island Unit of BCNP. Dees et al. (2001) used 1,940 telemetry locations collected during 1989-98 to describe panther habitat use in relation to prescribed fires and suggested panthers were attracted to burned areas less than 1 year old because deer were attracted there by the improved quality of the habitat. Roelke (1990) posited a north-south cline in which prey density, panther body mass, panther reproduction, and various measures of panther health all declined from north to south. This apparently corresponded to a shift from a diet of feral hogs and deer in the north to a diet of deer, raccoons, and armadillos in the south, and Roelke (1990) hypothesized the small, more aquatic-based prey provided a vehicle for mercury poisoning of panthers. Fleming (1994) linked the quality of forage to soil quality, but presented

no data on the quality of either soils or forage. Maehr et al. (2002) analyzed dispersal movements of 27 panthers during 1986-2000 and argued lack of panther dispersal to the southeast was due to limited forest cover and low prey densities in the Everglades area. All of these studies suggest density of prey (mainly deer), and by implication, panther densities, are limited by vegetation and soil types, but they present remarkably little quantitative analysis of these relationships.

Land (1991) and Fleming (1994) found an inverse relationship between white-tailed deer fawn survival and water levels in BCNP and the Florida Everglades, respectively. Fawns had low survival when water levels were high, especially during the February fawning season, presumably because fawning areas were concentrated in areas easily exploited by predators. The 9-year study in the Everglades (Fleming 1994) suggested seasonal flooding was an important positive factor, with intermediate hydroperiods (3-6 months of flooding) correlated with higher deer numbers than shorter or longer hydroperiods.

Maehr and Lacy (2002) expanded this idea by arguing the demographic surge of panthers after genetic introgression was due to increased deer numbers, which were in turn due to several years of little flooding, and that deer and panther numbers can be expected to collapse during the next period of wet conditions. Their argument is supported only by a graph of deer numbers for one management unit, with no data suggesting a deer increase throughout panther range since 1995. Furthermore, the graph does not support their argument: 3 of the 6 high water events depicted in the graph were followed by deer increases, and most of the large year-to-year variation in deer numbers appears unrelated to hydrologic events. Roy McBride (personal communication) alleges the last several years (when both deer and panther numbers appear to have increased) have had longer than average hydroperiods and higher than average water levels. Thus, it is unclear to the SRT how recent changes in water levels may have affected deer populations and panther numbers.

Taken as a whole, these studies suggest important relationships among soil, vegetation, hydroperiod, prey density, and panther fitness. The SRT believes the time is ripe for a thorough analysis of these relationships, using some combination of existing data and new data. Detailed soil and vegetation maps probably exist in digital form, and some of the data sets used in previous papers (i.e., annual deer surveys in various game management units, data on panther on panther health across occupied range, hydrologic data), have been expanded by another decade of research. Other data sets (i.e., panther diets) apparently have not been augmented during the last decade. Nonetheless, existing data may allow a rigorous investigation of at least some of these ideas.

Competition with other carnivores

Black bears (*Ursus americanus*), bobcats (*Lynx rufus*), and alligators (*Alligator mississippiensis*) are potential competitors with Florida panthers for prey; however, the SRT did not find any publications addressing this subject. Maehr et al. (1989) reported only 1 of 12 wild hogs released in occupied panther home ranges was taken by panthers, while other predators took 4 of 12 hogs. Also, Land (1991) reported 10 of 26 radio-collared deer (46%) that died during the study were taken by bobcats; bobcats and panthers combined accounted for 64% of the mortality. These results suggest to the SRT that competition for prey may be an important issue, and is fertile ground for investigation.

SUMMARY – PREY RELATIONSHIPS

The Florida panther's main prey is white-tailed deer and wild hogs; however, there are few reports on prey densities. Data suggest recreational hunting has little effect on prey numbers and thus is not detrimental to panther persistence; hunting does not seem to be an issue of concern. Competition between panthers and other predators for what may be a limited resource has not been explored, but is fertile ground for further research. Prey densities have been linked to hunting, soil types, hydrology, vegetation, and other environmental factors. Literature describing these relationships is, for the most part, inconclusive, and worthy of research effort.

RECOMMENDATIONS – PREY RELATIONSHIPS

The SRT recommends:

1. Develop a plan for determining the relationship among hydrology, soils, vegetation, abundance of prey (especially deer and hogs), habitat use by panthers, and panther fitness (population density, body mass, survival rates, reproduction). The results could provide meaningful estimates of desired minimum levels of prey in reintroduction sites, suggest whether intensive management of prey is warranted in any parts of currently occupied habitat, and influence management of wetlands in which water levels are controlled. We suspect that some of these analyses could be conducted with existing data sets. If existing data are lacking or in some way defective, we caution that this analysis may require sampling extensively across space and/or time, and thus would require a major commitment of resources. FWC and FWS would need to weigh this against other important objectives, such as determining nocturnal habitat use, translocating panthers north of the Caloosahatchee, establishing a new population outside of south Florida, monitoring levels of genetic introgression, and investigating the correlation between fitness and introgression level.
2. Continue research in managing vegetation (control of exotics, use of fire) to enhance prey populations. This is not to suggest that management should focus on "farming prey". Rather, vegetation management should focus on broad ecosystem objectives, with prey enhancement among the benefits.
3. Assess the effect of competition by bobcats and other predators on the availability of deer and other prey for panthers.

GENETICS

There is no debate about the fact that the Florida panther had little genetic variation prior to the introduction of 8 female *P. c. stanleyana* from Texas into south Florida in March 1995 (Roelke et al. 1993, Maehr 1998, Culver et al. 2000). Furthermore, both proponents (Seal 1991) and opponents (Maehr 1998) of the introgression program agree that genetic restoration is insufficient for recovery of the panther, which cannot be considered recovered until it has a much larger geographic range, including range outside of south Florida. However, almost every other aspect of the role of genetics in conservation and management of the Florida panther is contentious.

Most management agencies and scientists believe that (a) the lack of genetic variation is a result of human overkill of panthers and their prey, and destroying panther habitat, with the most

severe impacts occurring since about 1890; and (b) by 1994, this anthropogenic impoverishment of the genome presented an urgent threat to survival of the population. David Maehr has led a vigorous challenge to these ideas. The counterargument starts with the idea that the Florida peninsula, which was narrower during the last glacial period, may have at least started the process of genetic divergence of south Florida panthers from other populations of *Puma concolor*, long before any human impacts (Maehr 1998, Wilkins 1994). A related idea is that the panther may have evolved some beneficial genetic adaptations, which should be conserved (Maehr 1998). Second, despite strong evidence of phenotypic abnormalities plausibly related to deleterious alleles (Roelke et al. 1993), critics assert that the panther population is demographically vigorous and that these abnormalities thus have little practical consequence at the population level (Maehr 1997b:89, Maehr and Caddick 1995).

The genetic restoration program also has come under fire. Critics have questioned whether the target of 20% genetic introgression is justified (Maehr 1998), and assert that management agencies use genetic restoration as an excuse not to confront critical habitat issues (Maehr 1998, Maehr and Lacy 2002). They also worry about overachieving the target level of 20% introgression, which might swamp *coryi* genetic material or cause outbreeding depression (Maehr and Lacy 2002).

Genetic variation of Florida panthers

In a study of 31 puma subspecies throughout North and South America, *Puma concolor coryi* had the lowest value for each of 6 measures of variation in microsatellite loci (Culver et al. 2000). For most measures, *P. c. coryi* manifests one half to one eighth the variation of other subspecies. Similarly, Roelke et al. (1993) reported that allozyme polymorphism at 41 loci was 4.9% for *P. c. coryi* compared to a range of 7.3% to 17.1% for 6 subspecies of western North American pumas, that average heterozygosity was 1.8% for *coryi* compared to 2.0% to 6.7% for the western subspecies, and that minisatellite variation (“DNA fingerprinting”) was 85% lower in *P. c. coryi* than in western pumas.

Prior to the 1995 introgression program, there apparently was an infusion of non-*coryi* genes via release of at least 7 animals, including at least 3 females, from “Piper stock” into the Everglades during 1957-1967 (O'Brien et al. 1990). The Piper stock was comprised of captive *Puma concolor*, apparently dominated by genes of South American pumas, but including at least one Florida panther founder. Panthers apparently were extirpated east of Shark River Slough about 1990 (Bass and Maehr 1991), removing the animals that had the largest representation of Piper genes. Although the level of genetic admixture between these hybrids and panthers west of Shark River Slough was remarkably low (O'Brien et al. 1990), some introgressed genes probably remain in the panther population.

According to Maehr (1998), “there is no debate about” the basic fact of low genetic variation in the Florida panther.

When did Florida panthers lose genetic variation?

The strong preponderance of evidence suggests that Florida panthers lost half or more of their genetic variation since the 1890-1920 period. Culver et al. (2000) found that samples from 4 “ancient” (i.e., museum specimen) Florida panthers contained 6 microsatellite alleles that were absent from the 6 modern *coryi* samples they analyzed. The decline in microsatellite genetic variability was evident in polymorphism (50% in 6 ancient specimens, 20% in 4 modern

samples), mean heterozygosity (42% declining to 5%), and mean number of alleles per locus (2.3 declining to 1.2). Similarly, the incidence of cowlicks (a trait unrelated to fitness, but which is probably genetically controlled) increased from about 33% (2 of 6) during 1896-1898 to over 94% in modern specimens from southwest Florida (Wilkins et al. 1997). The most dramatic evidence that the loss of genetic material was not abating prior to the genetic restoration program was that cryptorchidism (which is almost certainly under genetic control) increased four-fold during 1970-1992, a statistically significant increase despite small sample sizes (Roelke et al. 1993).

Culver et al. (2000) had small sample sizes for Florida panthers (4-6 ancient specimens in each analysis, 6 modern specimens). Because the focus of Culver et al. (2000) was the entire Western Hemisphere, they did not offer any statistical analysis of these temporal changes. Nonetheless, these point estimates of temporal change suggest a striking loss of genetic material in recent decades.

In response to an inquiry from the Scientific Review Team about the “ancient” samples, Melanie Culver (University of Arizona, personal communication to P. Beier, November 2002) stated: “The 6 turn of the century Florida panthers were mostly from the area where the type specimen was from, [namely] Sebastian, Fla. They were all from central, not southern Florida. The dates [of the “ancient” samples] are 1890-1922, except one specimen from 1965 from the Florida Museum of Natural History. In retrospect, 1965 is more likely to be post-bottleneck and should not have been included with the other [ancient] specimens. The heterozygosity should probably be recalculated without the 1965 specimen.... I don't know how many other voucher museum specimens could be located. I tried to get all I could find and collected 12, of which only 6 yielded usable microsatellite DNA. The Harvard Museum would not let me sample the 'type' specimen.”

In arguing that genetic divergence of Florida panthers may have started prior to modern human influences on the species, Wilkins (1994) and Maehr (1998) apparently were referring to panthers in south Florida, rather than the historic race *P. c. coryi*. The argument is that the peninsular nature of Florida (which was narrower until sea level receded to its current level about 8,000 years before present) may have started the process of differentiation before the influence of Euro-Americans (Wilkins 1994). This geographic semi-isolation (“one side short of an island” – Maehr 1998) could have started the process of genetic divergence via the processes of genetic drift and isolation by distance. Maehr (1997b:89) flatly asserted “mating between closely related individuals has always occurred more frequently in Florida than elsewhere in the species range.” This divergence could then have been accelerated by selection for “traits that permit [the panther] to survive in the harsh south Florida landscape” (Maehr 1998).

We do not find these arguments persuasive for several reasons. First, the genetic evidence suggests that most variability was lost since 1890 and that erosion was continuing until the early 1990's (Roelke et al. 1993, Culver et al. 2000). Second, analysis of museum skulls suggests that 19th century panthers in Florida resembled *P. c. coryi* in Louisiana more than they resembled contemporary *P. c. stanleyana* in Texas, suggesting considerable gene flow throughout the range of *P. c. coryi* through most of the 19th century (Wilkins et al. 1997). Third, pumas apparently recolonized North America from Central America starting about 10,000 years before present (Culver et al. 2000), and there would have been little time for panthers to have experienced restricted gene flow in the narrowed Florida peninsula of the last glacial period. Also, the Florida peninsula is tens of miles wide, and thus may not strongly resemble an island.

Finally, if genetic isolation of the Florida panther was a natural process driven by geography and abetted by local selection pressure, we would expect to see even stronger genetic effects on the pumas of Vancouver Island, which is an island rather than a peninsula, has only 35% of Florida's land area, and may be harsher than Florida in some respects (e.g., it lacks many species of small prey). But in fact, *P. c. vancouverensis* is 40% to 280% higher in each measure of microsatellite variation than *P. c. coryi* from Florida (Culver et al 2000). We conclude that there is no evidence that peninsularity caused genetic divergence prior to the isolation and reduction of the panther population by recent human activity.

Recommendations: The SRT recommends a statistical analysis of the likelihood that the temporal changes in microsatellite diversity reported by Culver et al. (2000) could be the result of chance. We suspect that, despite the sample of fewer than 12 animals, these differences will be statistically significant, regardless of whether the 1965 specimen is considered “ancient” or “modern.” However, until the analyses are done, skeptics can continue to deny these changes. Because no new sampling or laboratory procedures would be needed, the cost of this analysis would be negligible.

If the above analysis is statistically inconclusive, the SRT recommends additional analyses – using a larger sample of modern and pre-1960 samples – to address the temporal trends in genetic diversity. Tissue samples have been collected from many modern panthers uninfluenced by the recent introgression or Piper stock. We assume these tissues have been appropriately stored by FWC and would be available for analysis. It may be more difficult to increase the number of pre-1960 tissue samples, because Culver (personal communication, above) suggests that there may be only 12 museum specimens. However, another effort may extract usable DNA from the 6 museum specimens that did not yield DNA for Culver, and doubtless some persons possess hides from panthers killed circa 1900 that could also be sampled after careful screening for authenticity.

Evidence for local genetic adaptation

There is no evidence for genetic fixation of locally adaptive genes that help the Florida panther thrive in the south Florida landscape, or in the larger range of *P. c. coryi*. Furthermore, unless the selection pressure for a particular trait was very strong, it is unlikely that such genes would have become fixed in *P. c. concolor* during the brief time (less than 10,000 years) since pumas apparently recolonized North America (Culver 2000). However, demonstrating such adaptations is difficult, and the lack of evidence should not be taken as rebutting their existence. Locally-adaptive genes may exist. Because maintenance of genetic patterns at all scales is a basic goal of conservation biology (Noss and Cooperrider 1994), it is prudent to assume such genes do exist, and management should strive to conserve these genes in the population. We discuss the “threat of outbreeding depression” (Maehr 1998) below.

The impact of decreased genetic variability on panther fitness

Maehr (1997b:89) argued that the panther was adapted to a long history of inbreeding, and that “while features such as cowlicks, crooked tails, and cryptorchidism are probably manifestations of inbreeding, these traits do not appear to have disrupted the demography of the panther.” Maehr correctly makes a meaningful distinction between inbreeding (loss of genetic material due to random mating effects in small populations) and inbreeding depression (loss of fitness due to fixation or near-fixation of deleterious genetic variants). He is also correct that cowlicks and crooked tails are unlikely to reduce fitness of panthers. However, there is substantial evidence

that genetic impoverishment was having a negative impact on panther fitness prior to genetic restoration in 1995.

Roelke et al. (1993) discussed four abnormalities prevalent in the pre-introgression panther population that may be related to low genetic variability, namely cryptorchidism, low sperm quality, atrial septal defect, and opportunistic infections. Each of the four traits has been shown to be genetically determined in some species, and pedigree analysis (Roelke et al. 1993) suggests that cryptorchidism is related to the level of inbreeding in individual panthers.

Although all large felids have low sperm quality, motile sperm per ejaculate in the Florida panther is 18-38 times lower than in other puma subspecies (Roelke et al. 1993). Panther sperm had a 40% incidence of acrosomal abnormality, which renders sperm unable to fertilize the ovum. Prior to genetic restoration, panthers had a significantly greater frequency of malformed spermatozoa (94.3% per ejaculate) than any other puma subspecies (Roelke et al. 1993).

Cryptorchidism occurred in 56% of panther males examined 1978-1992, compared to 0 of 40 free-ranging western and South American pumas (Roelke et al. 1993). Cryptorchidism rose dramatically over time in the panther population, occurring in only 1 of 8 males born before 1980, compared to 11 of 17 males born after 1985, and 5 of 6 of males born 1989-1992 (Roelke et al. 1993). Two males captured in 1992 were bilaterally cryptorchid and thus sterile, and cryptorchidism was associated with documented matings of close relatives (Roelke et al. 1993: Figure 7).

Roelke et al. (1993) also reported that atrial septal defect or *patent foramen ovale* apparently caused deaths of 2 panthers (ages 2 and 5 years) and that a third panther failed to survive surgery to correct an atrial septal defect and a defective tricuspid valve. The link between this defect and genetics is less clear than for the reproductive abnormalities; environmental contaminants could also play a role. Roelke et al. (1993) did not report the rate of occurrence of heart defects in necropsied panthers, but did report that heart murmurs consistent with, but not diagnostic of, atrial septal defect occurred in 80% of Florida panthers compared to 4% of other pumas.

Finally, low genetic variability may increase susceptibility of pumas to infectious disease and parasites. Roelke et al. (1993) reported that the pathogen and parasite load was relatively high in Florida panthers. At least one agent (*Pseudomonas aeruginosa*, which caused 1 panther death) is unexpected except in hosts with disarmed immune systems. Mark Cunningham (telephone conversation with SRT, January 31 2003) also reported that fungal infections ("ringworm") were common in Florida panthers but absent in Texas-Florida hybrids, apparently due to compromised immune systems of Florida panthers.

In downplaying the impact of genetic impoverishment, Maehr (1997b, 1998) and Maehr and Caddick (1995) admit that most of these defects probably do have a genetic basis, but argue that they may have minor impact on *individual* fitness, and negligible impact on *population* fitness. Attempting to dismiss the evidence that genetic impoverishment reduces individual fitness, Maehr (1998) accused Roelke et al. (1993) of "a selective and biased approach." No such bias was evident to the SRT. Maehr's other attempts to trivialize genetic impacts of individual fitness are speculative ideas that run counter to common sense. For example, although it may take only one good sperm to fertilize an ovum, bilateral cryptorchidism reduces fitness of the victim to zero, and reduces the fitness of its neighbors to the extent that the sterile male competes with them for resources. Although opportunistic bacterial and fungal infections may be commensal rather than parasitic relationships, the burden of proof lies with proponents of this paradigm shift.

Similarly, common sense suggests that a heart defect that reduces oxygenation of the systemic blood supply probably has a negative impact on the animal.

It is somewhat more plausible to argue that genetic defects could have minimal impact at the *population* level. The argument requires that the panther population has sufficient demographic vigor to maintain itself near carrying capacity, and that demographic performance is not impaired due to the presence of genetically impoverished panthers. To support this argument, Maehr and Caddick (1995) estimated kitten survival rate at 84% to 87%. This estimate, described in 4 sentences within a 3-page editorial note that presented no underlying data, cannot be accepted as scientifically rigorous (see *Demography* section below). The second argument offered by Maehr and Caddick (1995) is a plot of the *number* of known births versus known deaths by year (their Figure 1). As discussed below (*Demography* section), these data say nothing about whether population growth rate is positive or negative.

The lack of any supporting evidence does not disprove Maehr's hypothesis that inbreeding has no impact on panther demography. However, conceptual chains of causation (from genetic impoverishment to genetic defects to individual fitness to population fitness) favors the alternative hypothesis that genetic impoverishment decreases population fitness. The SRT believes that this conceptual linkage, along with the documentation of defects linked to genetic impoverishment, justified the decision to embark on genetic restoration. If Maehr's hypothesis is incorrect and had been adopted, risk of extinction would have continued to increase. If Maehr's hypothesis is correct, genetic restoration, by causing loss of some local adaptations, will probably make a positive growth rate somewhat less positive. This disparity in the consequences of potential error further justifies the decision for introgression.

The only conclusive test of Maehr's hypothesis would have been to decide not to embark on genetic restoration, and then to carefully document population trend; this test was rendered moot by the release of Texas pumas into south Florida in March 1995. The next-best opportunity to test the alternative hypotheses is to compare demographic performance (survival and reproduction) of contemporaneous, sympatric Florida panthers and Florida-Texas hybrids. Careful collection and rigorous analysis of the appropriate data constitute the SRT's main recommendation on this issue.

Preliminary evidence supports the hypothesis that genetic impoverishment had reduced panther fitness and that genetic restoration is increasing fitness. In addition to dramatic decreases in incidence of abnormalities, kitten survival rate is 72% for hybrids compared to 52% for pure Florida panthers (Shindle et al. 2001), and opportunistic ringworm infections are nonexistent in hybrids but common in pure panthers. Another striking line of evidence for the superiority of hybrids was inadvertently provided by Maehr and Lacy (2002), whose Figure 2 purports to show changes in numbers of deer and panthers during the last 20 years. The figure shows a sizable irruption of deer in the late 1990's, with the hybrid panther population rapidly responding by approximately doubling in size. The figure also depicts a more prolonged and 3-fold larger increase in deer numbers during the late 1980's that pure Florida panthers failed to exploit, despite Maehr's consistent position that the panther population was demographically vigorous and showing all the signs of a population tracking its carrying capacity during those years.

Recommendations: The SRT strongly recommends that managers aggressively collect field data on vital rates (litter size, kitten survival, subadult survival, adult survival) and important phenotypic traits related to fitness (cryptorchidism, atrial septal defects, opportunistic infections,

sperm defects, body condition) of hybrid and pure Florida panthers during the few years that these animals can be reliably identified by pedigree analysis, and before the pure Florida panther blood line ceases to exist. These results should be analyzed promptly and published in a rigorous, peer-reviewed venue. By 2003 it is already impossible to know the pedigree of many panthers, so data already collected or soon to be collected will be the best opportunity to have a simultaneous comparison of the 2 genetic lines. The availability of 50%, 25% and 0% introgressed hybrids gives us a brief window of time in which to definitively determine how genetics influences individual traits and vital rates in an analysis not confounded by extraneous spatial or temporal factors. Even if reliable genetic markers are developed (as we recommend), within 10 years it is likely that few if any “pure-bred Florida panthers” will exist to serve as a strong comparison to hybrid animals.

We believe that this analysis will confirm the hypothesis that genetic impoverishment was compromising demographic performance of the panther population. We adamantly reject the idea that, in light of the undeniable fact that genetic introgression is a *fait accompli*, there is no point in collecting such data. For decades, many scientists have doubted whether genetics is relevant to *in situ* conservation (Soule and Mills 1998, Lande 1988, Caro and Laurenson 1994, Caughley 1994, Nash 2001). The first and only poster child for the role of genetics, the cheetah, has lost most of its credibility (Laurenson et al. 1995). A recent excellent analysis of butterfly extinction (Saccheri et al. 1998) showed that genetics, while important, was less important to extinction than other factors, and the lessons of this study are arguably of limited relevance to the species and landscapes typically managed for conservation. The story of decline and recovery of prairie chickens (Westemeier et al. 1998) is more persuasive, but is based on comparisons of populations before and after introgression, without measuring the genetic variability or the demographic performance of inbred and outbred individuals, or controlling for effects of place and time. The Florida panther story promises to be the most dramatic and conclusive example of the relevance of genetics to conservation – or it could be a striking example of the futility of genetic management. Future decisions of great importance to conservation deserve to be informed by the results of the panther introgression experiment. It would be derelict in the extreme to forgo the opportunity to rigorously address this issue.

Apart from any monitoring to compare panthers based on hybrid status, the SRT strongly recommends continued monitoring to document the incidence of traits that might indicate a resurgence of inbreeding depression. Increased incidence of traits such as cryptorchidism or congenital heart defects should promptly trigger consideration of additional introgression.

Many of the types of data we request have been collected since the report of Roelke et al. (1993), but have not been analyzed or published. Additional data should be collected. In evaluating atrial septal defects, we recommend supplementing evidence based on heart murmurs with definitive evidence using Doppler echocardiography. Samples of convenience should be discouraged in favor of statistically defensible sampling designs. For instance, necropsies of animals found dead may overestimate of the incidence of disease or heart defects; pathology screening and echocardiography of randomly-captured live animals is much more likely to yield unbiased estimates. Doubtless tissue samples have been and continue to be collected and stored that will allow researchers to quantify genotypes of individual animals. Such data should be used to determine the correlation between individual genetic impoverishment and phenotypic defects.

The introgression program

In its final Environmental Assessment of the planned introgression program, the US Fish and Wildlife Service (1994) assumed that high and increasing levels of sperm abnormality, cryptorchidism, congenital heart defects, and immune deficiencies were caused by increased prevalence of deleterious alleles, and cited these trends as indicating the need for a genetic restoration program. Relying on Seal (1992, 1994), the Service also reasoned that hybridization historically had occurred between *P. c. coryi* and parapatric subspecies, of which only *P. c. stanleyana* is extant, with Texas being the closest area known to have potential donors. Accordingly, animals from Texas populations of *P. c. stanleyana* were selected for release into the existing south Florida population. The decisions to use wild-caught females of about 2-3 years of age as gene donors, and to release them in vacant panther territories, were intended to minimize social disruption and the risk of animals leaving the release sites. Appropriate selection criteria and veterinary procedures were used to decrease the risk of introducing unwanted genes or diseases into the Florida population. The goal was to mimic historic gene flow, and not to replace or swamp the *P. c. coryi* gene pool.

In 1991 the US Fish and Wildlife Service (Jordan 1991) had decided against a genetic restoration program. At that time the Service argued that genetic restoration should not proceed until after experiments on captive animals to determine whether outcrossing would reduce abnormal traits and whether outbreeding depression would occur. Although these experiments had not been done, or even initiated, the need for experiments on captive animals was not mentioned in the 1994 Environmental Assessment. The intervening analyses by Seal (1992, 1994, both included as Enclosures to the 1994 Environmental Assessment) and the report of Roelke et al. (1993) apparently had persuaded managers that the situation was more urgent than previously thought. The 1994 environmental analysis did call for careful monitoring to prevent swamping of *coryi* genes, and glibly asserted that in such an emergency “all translocated individuals and their progeny could be removed at any time.” Even without the benefit of hindsight, in 1994 it should have been obvious that within one or two decades, Texas progeny would include most of the population, and removing them would be tantamount to extinction.

The murkiest aspect of the program is the target of 20% introgression of non-*coryi* genes to be achieved in one release of 8 females in 1995 followed by 1 female per generation thereafter. The SRT searched in vain for an early explanation for these numbers. USFWS (1994) cited a September 1994 workshop (Seal 1994), which cited an October 1992 workshop (Seal 1992) as the source of these numbers. Seal (1992), however, simply stated the 20% target without explanation and credited the basic idea to Seal (1991). The 1991 paper did not give a quantitative target, but concluded that “further analyses will be needed to determine the optimal amount and rate of genetic introgression.”

Phil Hedrick, who later (1995) published a justification for the 20% target, was not cited or listed as a participant for the workshops summarized by Seal (1991, 1992), nor was he mentioned in the Environmental Assessment for the genetic restoration program (US Fish and Wildlife Service 1994). The SRT finds it rather startling that the selection of the 20% target may not have been justified prior to its adoption. Hedrick was listed as a participant in the September 1994 workshop (Seal 1994), but by that time the 20% target was 2 years old.

Maehr (1998) asked “is [genetic introgression] appropriate at this time, and is the number (about 20% of the estimated population) of relocated animals necessary?” Although the SRT agrees that

the target may not have been explained in a timely fashion, Hedrick provided rigorous and persuasive arguments that a 20% level of introgression has < 20% risk of causing the loss of any locally-adapted allele, could eliminate highly deleterious alleles, and would greatly reduce (but probably not eliminate) moderately deleterious alleles (Hedrick 1995). The bulk of the effect would occur via the 20% introgression in the first generation, with smaller marginal returns from introgression of one individual per generation after the first generation. Hedrick concluded that gene flow after the first generation may not be needed to eliminate deleterious alleles, because selection will continue to do the work in the absence of continued introgression. However, assumptions about the effective population size of panthers strongly affect the expected outcomes.

Maehr (1998) attacked Hedrick's analysis, asserting that "the assumptions upon which Hedrick built his model were products of earlier workshops that based their conclusions on antiquated data," specifically referring to kitten survival rates. Maehr's criticism is unfounded, in that Hedrick's analysis made no assumptions about survival rates or any other field data on panthers (other than effective population size, for which appropriate caveats are given). To the contrary, Hedrick's analysis hinged entirely on numbers of introduced animals, strength of natural selection, level of dominance among alleles, and whether alleles are neutral, adaptive, or deleterious.

A pedigree analysis suggested that, as of December 1999, an introgression level of about 18-22% had been achieved (Land and Lacy 2000). Four of eight Texas pumas were still alive at that time, and five of the eight pumas had produced at least 36 descendants, of which 25 were probably still alive. The 5 reproducing females are equivalent to about 3 effective founders due to unequal representation of the Texas females in the population's gene pool.

Maehr and Lacy (2002) questioned Land and Lacy's analysis, pointing out that the true level of introgression may be much higher than 22%. The SRT agrees that this issue is not settled, in part because true effective population size, which contributes the denominator to any calculation based on pedigree analysis, is not known and is unlikely ever to be known with sufficient certainty to calculate the level of introgression.

Recommendations: The SRT strongly recommends the prompt development of genetic markers to monitor the level of introgression in the Florida panther population. The rapid expansion of the population since introgression, and the impossibility of calculating effective population size (which differs widely and unpredictably from census population size – Waples 2002) is rapidly making pedigree analysis useless as a measure of the degree of hybridization. If geneticists can identify genetic markers peculiar to the 5 Texas females that contributed genes to the Florida panther population, these markers would provide an efficient and accurate means of monitoring the trajectory of introgression. Seal (1994) made a similar recommendation, and suggested that mitochondrial DNA or microsatellites might provide ideal markers. Warren Johnson and colleagues (abstract titled *Preliminary results of Florida panther genetic analyses* for the May 2003 Mountain Lion Workshop in Wyoming) state that 175 samples (presumably individuals) have been genotyped at 23 microsatellite loci for the purposes of developing such markers, but the success of this effort was not described in the abstract.

Until clinical signs of inbreeding depression are again observed, or a better method of estimating genetic introgression is developed, we recommend consulting Hedrick, Roelke-Parker, or Culver

about whether to continue to introduce an additional Texas puma per generation, or whether the rate of future introgression should be reduced.

Certainly any future introductions should avoid the claim that “all translocated individuals and their progeny could be removed at any time.” We believe the 1995 restoration was justifiable, but it, and any future introgression, should be viewed as an irreversible experiment, with appropriate up-front analysis of likely impacts.

Risk of outbreeding depression

In its Environmental Assessment (US FWS 1994), the Fish and Wildlife Service considered outbreeding depression an implausible outcome of the restoration program, citing the fact that the program simply seeks to emulate natural gene flow between parapatric subspecies. The Service further noted that “outbreeding depression would be unprecedented for a cross between such closely related and recently diverged mammalian populations as the Florida and Texas *F. concolor*.” The SRT concurs with this assessment, which is buttressed by two lines of evidence that became available since 1994. First, a rigorous analysis (Hedrick 1995) suggests a low risk of outbreeding depression from a genetic restoration program of this magnitude. Second Culver et al. (2000) suggest that pumas recolonized North America only during the last 10,000 years, and that all putative North American subspecies probably should be subsumed into a single subspecies. Thus the divergence of these subspecies is probably far more recent than would have been guessed in 1994.

Nonetheless, the “threat of outbreeding depression” (Maehr 1998) should not be dismissed out of hand. Because maintenance of genetic patterns at all scales is a basic goal of conservation biology (Noss and Cooperrider 1994), it is prudent to assume locally adaptive genes do exist, and management should strive not to eliminate these genes from the population.

Recommendation: Until clinical signs of inbreeding depression are again observed, we recommend reconsidering the idea of introducing an additional Texas puma per generation. Because the genetic restoration program has been so successful, the marginal benefit of additional gene flow may no longer outweigh the marginal risk of outbreeding depression.

The SRT also recommends that researchers continue to monitor morphological traits such as pelage color or body mass that might indicate local adaptation. We acknowledge that these data will be difficult to interpret.

The importance of genetic management in the big picture of panther conservation

Maehr (1997b, 1998), and Maehr and Lacy (2002) imply that genetic restoration is diverting attention from more critical issues of conserving habitat for Florida panthers and expanding panther range beyond south Florida. For instance, Maehr and Lacy (2002) stated that “genetic restoration will have been only a stop-gap measure... unless the population can be increased to much larger size than can be supported in the present [occupied] habitat” and bemoan “the apparent abandonment or indefinite postponement of plans to reintroduce the panther to other parts of its former range.” Similarly, Maehr et al. (2001) asserted that the recovery program “now targets a single solution, genetic restoration”

The SRT concurs that habitat conservation, and establishment of panthers outside of south Florida, are the most important elements of panther recovery. However, we feel it is unhelpful to frame the problem as genetic restoration *versus* habitat conservation and expansion. We note that proponents of restoration explicitly made statements similar to Maehr’s at the outset of the

restoration program. For instance, Seal (1991) warned against “creation of a false sense of management accomplishment and a masking of underlying environmental difficulties” and explicitly listed “securing and enhancing the wild population” as the first priority for conservation – with genetic restoration third. Proponents of introgression, and all recovery documents for the panther, explicitly consider genetic restoration a necessary, but not sufficient, recovery action.

Our reading of the FWS plan to reintroduce panthers outside of south Florida (Jordan 1994) suggests that FWS has had little stomach for reintroduction. In addition to many important analyses lacking in that plan (discussed in *Habitat Relations* section, above), there has apparently been little progress on the important tasks listed on page 10 of that report. The SRT’s impression is that, as an agency, FWS has not had its heart in the reintroduction effort. This impression was reinforced by several persons interviewed during our review.

Although the SRT agrees with the importance of reintroduction and other non-genetic issues, and that the agencies have been less than vigorous in attacking these issues, we do not see that the agencies used introgression as an excuse to duck the hard issues. Reintroducing panthers outside of south Florida is a politically difficult issue that managers will tend to avoid, regardless of whether genetic restoration is on the table. Even if this accusation is true, we think it may be more productive to promote actions on the important non-genetic issues on their own merits.

SUMMARY – GENETICS

- Florida panthers lost half or more of their genetic variation since the 1890-1920 period. There is no strong evidence to suggest that the peninsular nature of Florida caused genetic divergence prior to the isolation and reduction of the panther population by recent human activity.
- Coinciding with this loss of genetic variation, panthers experienced increased incidence of cryptorchidism, acrosomal sperm defects, atrial septal defects, and possibly opportunistic infections. The rate of cryptorchidism increased over the 30 years prior to genetic introgression. All of these traits are plausibly related to increased incidence or fixation of deleterious alleles.
- Since introgression, rates of these defects have dramatically decreased, hybrids seem to have substantially higher kitten survival than pure Florida panthers, and the hybrid population seems to exhibit greater demographic vigor. Aggressive, focused data collection is needed to confirm or refute these suggested patterns, and to provide guidance for future use of genetic management in conservation.
- The levels of introgression achieved is likely to remove deleterious effects of inbreeding with little or no risk of outbreeding depression. The genetic restoration program seems to have been a success. It has been an undeniably helpful step, and probably a necessary condition, for panther recovery.
- Genetic restoration is *not sufficient* as a strategy to conserve the species. Both proponents and opponents of the introgression program agree that the panther cannot be considered recovered until it has a much larger geographic range, including range outside of south Florida.

- During the period of genetic introgression, there has been a lack of strong leadership in implementing the four main steps to reintroduction recommended by Jordan (1994, each with target dates between August 1994 and February 1996). However, this lack of political will may be unrelated to any diversion of attention due to introgression efforts. We do not think it is helpful to argue that FWS and FWC have used the introgression program as an excuse to ignore the pressing need to expand the geographic range of the panther.
- At the time FWS and FWC made the formal decision to embark on the introgression program, there was no justification for the target of 20% introgression. Hedrick (1995) provided strong justification after the fact.
- It is not clear whether the plan to continue (after the 1995 introgression via 8 female Texas pumas) to introduce one non-*coryi* individual per generation is needed. Because of the apparent success of the genetic restoration program, the marginal benefit of additional gene flow may no longer outweigh the marginal risk of outbreeding depression.
- Despite a preliminary estimate that the 20% target was achieved (Land and Lacy 2000), this estimate depends largely on an unknown denominator (total population size), and is based on the assumption that the fraction of the total population gene pool is the same as the fraction of effective population size comprised of Texas-equivalents.

RECOMMENDATIONS – GENETICS

- Managers should aggressively collect field data on vital rates (litter size, kitten survival, subadult survival, adult survival) and important phenotypic traits related to fitness (cryptorchidism, atrial septal defects, opportunistic infections, sperm defects, body condition) of hybrid and pure Florida panthers. These results should be analyzed promptly and published in a rigorous, peer-reviewed venue. Within a few years, it will be increasingly difficult to sort out the blood lines, and the data from 1995 to about 2004 will be the best opportunity to make a simultaneous comparison of the 2 genetic lines. Such comparisons are needed to assess the success of the introgression program.
- Thus the importance of the above recommendation transcends the Florida panther. The management of Florida panther genetics will probably become the textbook example of the practical impact of genetic management for wild populations. This story is more relevant to conservation than the butterfly example of Saccheri et al. (1998), and has the potential to be better documented than the prairie-chicken example of Westemeier et al. 1998) or the controversial cheetah story (Caro and Laurenson 1994, Laurenson et al. 1995). Conservation science desperately needs, and currently lacks, a well-documented, persuasive case study of the practical application of genetics in conservation of an endangered animal. This has the potential to be that example.
- Apart from any monitoring to compare panthers based on hybrid status, the SRT strongly recommends continued monitoring to document the incidence of traits that might indicate a resurgence of inbreeding depression. Increases in traits such as cryptorchidism or congenital heart defects should promptly trigger consideration of additional introgression.
- We need a rigorous estimate of the level of introgression achieved, including a range of plausible values, with an explicit discussion of the assumptions used in the calculations. Pedigree analysis is no longer useful as a measure of introgression, and should be replaced

with genetic markers, probably based on mitochondrial or microsatellite DNA. Such markers will open the door to sophisticated analyses of the correlation between panther fitness and the degree of hybridization of individual animals.

- The historical changes in microsatellite variation of *P. c. coryi*, using existing data collected by Culver et al. (2000), should be subject to statistical analysis to confirm the recentness of genetic erosion. In addition, it would be useful to conduct additional analyses, using a larger sample of modern and pre-1960 samples, to address the temporal trends in genetic diversity, and to serve as a baseline for comparison to the genetics of post-introgression panthers.

DEMOGRAPHY

Our discussion about panther demography is organized by the demographic parameters or relationships that are fundamental to understanding and predicting panther dynamics. We especially focus on those areas where information is critical to managers, in enabling the assessment of likely population outcomes under alternative recovery scenarios. We also discuss efforts to date directed toward viability modeling (PVA). For each parameter, factor, or approach we describe results (if any) that may be said to be “reliable:” that is, the data, analyses, models, or assumptions are capable of supporting inferences about panther demography. We describe situations where the information is not reliable, for instance because data were inappropriately collected or analyzed, or functional relationships or assumptions are not supported. We make recommendations for each for improving the reliability of demographic information, particularly as regards applications to conservation decision-making. Although the focus is demography, there is unavoidable overlap with the sections of this report that deal with genetics, habitat and prey relationships, and biomedical issues.

We have organized the references pertaining to demography according to the pertinent demographic parameter or functional relationship. These are summarized in relation to the study area, key citations, and a brief description of the methods used in Table 1. A more detailed commentary on each paper follows in the annotated bibliography. Many of these studies dealt with more than one demographic parameter or issue; indeed, in some cases demography was not the main focus of the paper. Because the actual number of studies (versus papers) is relatively small, we have included all studies that related (directly or indirectly) to demography. We discuss the relative strengths and weaknesses of inferences from each study for each demographic parameter.

Abundance and density

To our knowledge there are no statistically-derived estimates of panther abundance or density. This lack is perhaps unsurprising given the secretive nature of the species, which tends to render impracticable methods based on visual detection (e.g., direct counts, distance sampling), and the difficulty and expense of applying methods dependent on capture-recapture (Williams et al. 2002). In addition to these technical/ logistical difficulties, rigorous estimation is hampered by the fact that, to our knowledge, there has never been a systematic, range-wide effort to detect and estimate panther numbers. Range-wide estimates instead have depended on accumulating estimates from local studies, usually over non-overlapping time intervals, which by definition render such estimates suspect (Table 1). For example, “current verifiable population size” as reported in McBride (2000, 2001) consists of cumulative counts of radiocollared cats and

sightings of uncollared cats over the various study areas in South Florida. However, it is not known over what period these observations were gathered, or whether some of cats counted therein have meanwhile exited the population via mortality (a possibility acknowledged by McBride 2001).

Although the estimate of Maehr et al (1991a) of 46 panthers ($110/\text{km}^2$; Table 1), based on radiotelemetry observations, lacks a confidence interval or statistical underpinnings, it appears to be a reasonable approximation of panther density in the “core area” (as defined by Maehr et al. 1991a). However, this estimate cannot be extrapolated to South Florida without unsupportable assumptions (e.g., that densities are the same throughout as in the core area), and it certainly cannot be extrapolated to areas north of the Caloosahatchee.

It is remarkable that 22 years of intensive research has produced only one reasonably rigorous estimate of the density of the panther population in south Florida, namely that of Maehr et al. (1991). Nonetheless, the SRT does not believe that producing a rigorous estimate of population size should necessarily be a high priority. One argument for a rigorous estimate of N is that assessing the level of genetic introgression depends critically on N , which forms the denominator of estimates based on pedigree. With no estimate of N , critics of introgression can argue that introgression may be as high as 45% (Maehr and Lacy 2002). Because introgression is now a *fait accompli*, this need may be less pressing. Genetic markers may provide a direct measure of introgression that does not depend on population size (See section on genetics).

There are two reasons that an estimate of N may not be needed. First, seat-of-the pants estimates (minimum number known alive [MNKA], new animals encountered per 10 days of capture effort, expansion and contraction of occupied range) are sufficient to persuade most reasonable observers that N was probably ≤ 30 animals during 1930-1965, was stable or slowly fluctuating at 30 to 70 during 1980-1995, and has grown rapidly since 1995. Second, estimates of per-capita age-specific fecundity and survival rates may provide a useful index of population performance at much less expense than estimating N .

If reliable estimates of N or D are deemed important, these must be based on a rigorous sampling design, collection of appropriate field data, and valid statistical analyses (Williams et al. 2002). For panthers, logistical considerations probably rule out methods based on direct counts, distance estimation, or conventional mark-recapture, the latter because recaptures are relatively infrequent. However, mark-recapture methods employing camera “trapping”, analysis of DNA from hair samples, and other novel approaches could be used, together with conventional recaptures (physical and via radiotelemetry).

Population growth rates

Although as noted above reliable estimates of abundance are unavailable, the general trend in abundance appears to be fairly well established. It appears that the population was critically low during the period 1930-1965 (probably 30 or fewer individuals), stable or slowly fluctuating at 30 to 70 adults during 1980-1995, and has grown rapidly since 1995. However, in the absence of either quantitative estimates of relative abundance, or reliable projections from demographic estimates (below), more quantitative statements about rates of growth cannot be made. In particular, no reliable estimates of variability in λ are available; these estimates are essential components of PVA (see below).

No scientific paper should use graphical presentations of raw numbers of kittens produced compared to deaths of radio-tagged animals as in Shindle et al (2001: Fig 4) and Maehr and Caddick (1995: Fig. 1). The position of points in such a plot largely reflects researchers' ability to detect births and deaths, and these data say nothing about the birth and death *rates* needed to calculate whether population growth rate is positive or negative. Such figures were misinterpreted by Maehr and Caddick (1995) as indicating a "positive growth rate;" this is an inexcusable substitute for the appropriate analysis of population dynamics. Calculation of growth rate requires per capita vital rates, which are not depicted in figures like these.

It is important to note that the entire body of evidence for demographic vigor of Florida panthers prior to genetic restoration hinges on this misinterpretation of Figure 1 of Maehr and Caddick (1995), plus their assertion that kitten survival rate exceeded 80% (discussed below). All subsequent arguments (Maehr 1997, Maehr 1998, Maehr et al. 2001, Maehr and Lacy 2002) repeat and often overstate the two central claims of this paper, but offer no new supporting data or analysis. The most egregious overstatement occurs in a population modeling paper (Maehr et al. 2002) that cites Maehr and Caddick (1995) as the source of "better data" than used in previous models.

Population range and dispersal

Much basic information exists, much of it highly relevant to management, regarding the historical and current distribution of panthers, and patterns of (and barriers to) dispersal (e.g., Belden 1989, Maehr et al. 2002a). Panther dispersal tends to be frustrated due to existing natural barriers (oceans and other large water bodies) and anthropogenic barriers (freeways, urban areas, large expanses of row crops and improved pasture). Male dispersal is further frustrated by a lack of female panthers in areas north of the Caloosahatchee. Dispersing male panthers are able to find tenuous habitat linkages across the Caloosahatchee River, and dispersal to that region would doubtless be enhanced by habitat restoration. The study of panther dispersal (Maehr et al. 2002a) is one of a small handful of papers to provide meaningful insight into dispersal dynamics of *Puma concolor*.

There appears little doubt that panthers suffer from a highly constricted range relative to their historical distribution, and that significant natural and anthropogenic barriers exist to dispersal, range expansion, and ultimately, population growth. Further research should focus on assessing specific factors that constrain or enhance dispersal, and in quantifying the uncertainty in the relative importance of these factors, particularly those potentially under management control.

Reproductive rates

Although reliable per capita estimates of reproduction are generally not available for panthers, some aspects of panther recruitment are reasonably well documented. Litter sizes range from 1 - 4 kittens, with modal values of 1-2 (Shindle et al. 2001, Land 1994). However, published data appear to be inadequate to provide reliable estimates of variability that could be used to generate litter frequency distributions for use in PVA. Shindle et al. (2001: Appendix IV) listed over 75 panther litters. Although this list does not indicate how long after birth the litters were handled, there may be sufficient data in FWC files to estimate litter size at birth, and variation in this parameter. Alternatively, estimates from closely related populations (e.g., Texas panthers) should be used to supplement this relatively meager information.

It seems to be fairly well established (e.g., Maehr et al. 1989) that panthers can reproduce as early as 26 months. It also appears that density, habitat conditions, and other factors may raise or lower this age. However, because only a limited number of panthers have been monitored from birth to reproduction, there is no basis for estimating a frequency distribution for first reproduction ages, or for relating reproductive success to factors such as habitat. Because this parameter can be critical to population projection/ PVA, reliable information on the range in values, and factors responsible for variation in the parameter, seems critical. Again, in the absence of adequate data for panthers, a frequency distribution for first age at reproduction could be inferred from data on closely related cougars.

Although of limited quality, there are at least some data on litter size and age at first reproduction. In contrast, and despite decades of data collection, estimates are lacking on other vital rates such as percent of adult females breeding and inter-birth intervals. These rates are critically important in assessing population performance, and their absence precludes meaningful population projection and viability analysis. In addition, tables of “known births” (e.g., Maehr and Caddick 1995) are of little value in determining reproduction rates, without an accurate basis of reference (population of breeders).

The SRT recommends that existing data be used to estimate frequency distributions for litter sizes and ages and first reproduction, and to the extent possible other components of reproduction rates such as percent of females breeding and inter-birth interval. Suitable data to estimate these quantities may not exist; therefore, direct estimation of reproductive recruitment (e.g., via Jolly-Seber or other approaches [Williams et al. 2002]) should be considered. Where empirical estimates are impracticable, frequency distributions for these rates should be approximated from closely related populations (e.g., Texas cougars). Finally, reproduction and its components are intrinsically variable, and emphasis must be placed on estimates of this variability, and in separating statistical uncertainty from demographic and environmental variability in reproductive rates.

Survival rates

No existing paper has published, nor has any PVA used, scientifically defensible estimates of stage-specific survival rates for panthers, despite the fact that appropriate data exist from which to make such calculations (Maehr et al. 1991b, Land 1994, Shindle et al. 2001). The estimate of 80% kitten survival (Maehr and Caddick 1995) is especially indefensible for several reasons. First, this estimate is described in 4 sentences within a 3-page editorial note that presented no underlying data, and thus is not scientifically rigorous. Moreover, it is higher than the 82% estimate for Florida panthers of all ages, mostly adults (Maehr et al. 1991b). It would be unprecedented in studies of vertebrates for the survival rate of juveniles to equal or exceed adult survival rate. Finally, this estimate is contradicted by a more recent estimate of 52% for pure Florida panther kittens (Shindle et al. 2001, whose report included underlying data). Use of this estimate of kitten survival in PVA is unjustifiable.

Similarly the age distribution of animals at death (e.g., Shindle et al. 2001: Figure 7) reflects survival rates only under restrictive assumptions about population growth rate and stability of age distribution over time (Williams et al. 2002:337-342). Because such graphs tempt readers to naïve and probably incorrect estimates of survival rates, they should not be displayed in annual reports. Survival rates should be recalculated on basis of exposure days and actual age at the start of monitoring, for each sex-age class (Williams et al. 2002: 343ff).

The SRT strongly recommends that survival and other vital rates be properly estimated, using approaches that are not highly dependent on an accurate estimate of the baseline population. For example, radiotelemetry or mark-recapture estimates of survival, where the data are gathered under a rigorous sampling design, are far preferable to ratios based on crude mortality numbers divided by population estimates (Williams et al. 2002). Even if the mortality numbers are reliable (and they frequently are not), such estimates are highly sensitive to errors in the estimation of population size. In addition to these estimates, statistical comparisons, using data starting in 1995, should be made between the survival rates of progeny of released Texas pumas and pure Florida panthers.

Cause-specific mortality

Although a list of lethal agents has been accumulated, it is for the most part impossible to assess these in terms of their relative demographic importance. This is so because many of these agents were discovered by post-mortem analysis of a relative handful of panthers; in turn, many of these were discovered because of the nature of the mortality (e.g., road kill, Maehr et al. 1991b) or other non-random method of sampling. Because of these difficulties, currently available analyses cannot be used either to support or refute the importance of road mortality to panthers. Maehr et al. (1991b; N = 8 mortalities of radio-tagged panthers) and Shindle et al. (2001: Appendix VII; N = 59 mortalities of radio-tagged panthers) provide useful breakdowns on causes of mortality that are free of such bias, and suggest that vehicles cause 12% to 22% of panther deaths.

Even with radio-telemetry, the cause of death is often ambiguous because of rapid deterioration of specimens if not discovered immediately, leaving post-mortem examination capable of determining only those causes leaving obvious physical evidence. For example, 22% of the mortalities of radio-tagged panthers reported by Shindle et al. (2001: Appendix VII) were of “unknown” causes – identical to the percentage of road-kills. Nonetheless, data available through telemetry (Shindle et al. 2001, Taylor et al. 2002) are the best information on cause-specific, and appropriate analyses of these data should be a priority. Statistical analyses must properly deal with nuances of telemetry or other data structures (e.g., staggered entry, censoring, and multiple, competing mortality risks). These data should be reanalyzed on the basis of exposure-days to calculate annual cause-specific rates, which could then be used to analyze the population impact of various alternative scenarios.

The SRT strongly recommends that the radiotelemetry data be properly analyzed to obtain cause-specific mortality (and where possible seasonal changes in these rates, differences among age-sex classes, etc.). Conclusions about cause-specific mortality based on samples of convenience should be avoided because such samples are biased with respect to probability of detection. This analysis is especially important given increasing road kill during the current population expansion.

Carrying capacity

Carrying capacity is critically important to demography. Assumptions about carrying capacity have great impact on population projections and PVA (see below), and it is essential that estimates of carrying capacity are based on the best possible biological information. Although there likely are behavioral, physiological and other feedback mechanisms that limit panther population growth given infinite resources, most notions of carrying capacity for panther and other carnivores are linked to acres of habitat and biomass of prey. Thus, discussion of these topics in the “habitat” section of this report is highly relevant to demography. To select but one

example: differing assertions about the quality of habitats (some currently occupied by panthers and some not) potentially result in orders of magnitude difference in carrying capacity, both within the “core range” (Maehr et al. 1991a), as well as in portions of the former range into which panthers may either expand or be reintroduced.

Functional relationships

Little seems to be known about key functional relationships among demographic parameters, such as density dependence or inbreeding effects on demographic parameters. Assumptions about these relationships are in turn critically important in predicting demographic response. For instance, even assuming that there is a functional relationship between density and reproductive rates, the functional form and parameter values of that relationship are likely to be imperfectly known, if they are known at all. Thus, we could have a monotonically negative density relationship, or an Allee effect (i.e., changing direction at the low end of density); in either case there are many choices for the function’s shape. In turn, the results of population projections and PVA are highly sensitive to alternative functional forms (Runge and Johnson 2002).

Unfortunately, canned programs such as VORTEX, RAMAS, etc. tend to gloss over this issue, typically providing either default (assumed) function forms and parameter values, a limited range of alternative functions, or both.

The SRT recommends that priority be given to research in investigating, and where possible statistically estimating critical functional relationships in panther demography. These would include, at a minimum, estimating the effects of density and inbreeding on reproduction and survival rates. PVA and other modeling exercises must take into account uncertainty in these relationships; at the moment, the approach can only be characterized as “faith based.”

Population projection and viability modeling

At least three “population viability analyses” (PVA) have been conducted for panthers, although these have not been independent, being based on overlapping data and many assumptions in common. The only major, published attempt to date to synthesize the panther demographic data and project viability was the result of a workshop involving agency biologists and university consultants (Ellis et al. 1999, Maehr et al. 2002b). In the workshop, previous analyses were “updated” and new analyses were conducted based on a range of data and expert opinions among two agency biologists, two University ecologists, and a “population ecologist” who was one of the developers of the computer model used, VORTEX (Lacy et al. 1995). Based on input estimates and assumptions, a “consensus” model emerged that predicted high (virtually 100%) probability of persistence for 100 years, although some individual estimates and assumptions resulted in the opposite conclusion (100% probability of extinction by 100 yr). Leaving aside the unreasonable assumption of certainty about intrinsically uncertain events, parameter values used in these projections varied widely among experts, and in the degree to which the values were supported by data (survival rates seemed to be guesses, or if empirical, based on very small samples). Critical assumptions about functional form (e.g., density dependence, inbreeding effects) seem to have been based on model defaults, with little examination of credible alternatives. For instance, only the model developer initially invoked an assumption of inbreeding depression (using the model default for this), but this factor somehow emerged as important in the “consensus” model.

Modeling for conservation must always balance realism (dictating the incorporation of biological detail) with the need for models to be understandable, and capable of parameterization using

field data (Conroy et al. 1995, Williams et al. 2002). Modeling for panthers must focus on issues that are relevant to panther life history, incorporating data-based estimates of parameters where available, information from congeners or informed opinion when not, and in both cases fully exploring the implications of uncertainty in these relationships via sensitivity analyses and statistical modeling (Burnham and Anderson 2002, Williams et al. 2002). On the other hand, models should not require the user to quantify life history details that are unknown (or that cannot be addressed via sensitivity analysis). In particular, models that require a large number of arbitrary rules for behavior (as do some of the spatially explicit population models [Conroy et al. 1995]) or otherwise force a large number of arbitrary (and often critical) assumptions (as in VORTEX and RAMAS), should be avoided. Models should be built around a core of solid information about the species, which necessarily dictates models with relatively few parameters and simple structure. Again, however, uncertainty in model parameters and functional relationships *must* be taken into account via sensitivity analysis, regardless of the simplicity or complexity of the modeling approach.

Sensitivity analysis conducted by Maehr et al. (2002b) suggested that neither habitat trend nor continued genetic augmentation, nor interactions between them, influenced extinction risk. However, unfortunately they did not conduct sensitivity analysis for the biggest changes in their model compared to previous panther PVA, namely changing kitten survival from 0.50 to 0.80, changing carrying capacity K from 50 breeders to 70 breeders, and changing starting population size from 50 breeders to 60 breeders. Clearly some combination of these changes caused extinction risk to plummet from 100% to about 0%. Understanding the sensitivity to these changes is more important than the change in point estimate of extinction risk.

Although VORTEX and other canned procedures can reveal sensitivity to some parameter estimates, they are opaque on many other issues, especially those related to functional relationships. For instance, once the user is forced to select a mating system, the “polygynous” choice for panthers renders males demographically irrelevant. Clearly this is an unrealistic oversimplification, but its impacts cannot be explored, except by comparing outputs to results using the even less-realistic “monogamous” choice. Similarly, the user can choose only among 2 or 3 caricatures of density-dependent relationships, and cannot gain a nuanced appreciation of how these important functional relationships influence extinction risk. The SRT recommends that any future PVA models should be built from scratch and explicitly consider statistical uncertainty, variation (demographic, environmental) in parameters, and uncertainty in key functional relationships such as density-dependence and the effects of inbreeding.

The SRT also recommends that, to the extent possible, statistically valid estimates of reproduction rates, survival rates, and variation in these rates, be incorporated into future PVA. Clearly, this calls for a re-analysis of data along the lines discussed earlier, particularly to adequately estimate age- and sex- specific survival rates and per-capita reproduction rates. The SRT also recommends that sensitivity analyses, first in the context of a simple stage-projection matrix or a stochastic PVA be conducted to examine variation in λ in response to ranges of values for key demographic factors, particularly those subject to great uncertainty (e.g., kitten survival rates, assumed inter-birth intervals). Sensitivity analyses should be extended to allow comparison of alternative recovery actions. For instance, proposed actions (e.g., highway underpasses) have presumed impacts (reduction of deaths from collisions). What do models predict about the impacts of the anticipated reduction on λ ? Do differing assumption about the modeled growth influence these predictions? Such an approach might allow a more quantitative

assessment of the impact of highway mortality on panther. It should also allow managers to assess the relative value and cost-effectiveness of management actions to reduce highway mortalities, compared with other possible actions (e.g., providing habitat connectivity north of the Caloosahatchee, a re-introduction in Arkansas, or other possible actions) that compete for dollars and attention. Thus, sensitivity analyses should be used not only to predict overall extinction risk, but more importantly to analyze of alternative recovery actions, especially where these analyses may suggest a change in recommended management activities. Only after these basic sensitivity analyses are completely evaluated should more complex models be considered.

Ultimately, of course, model structure must incorporate all essential, plausible alternative hypotheses about demographic response, including age structure and density dependence (including Allee effects). To the extent possible all parameters and functional relationships should be based on appropriate analysis of field data that takes into account both random errors (due to sampling) as well as systematic biases induced by flaws in design or data collection. Where possible, data should be analyzed under alternative biological models, and measures of model uncertainty calculated for each (e.g., using AIC or Bayesian approaches [Burnham and Anderson 2002]). Where data are not available, ranges (or distributions) of parameter values should be based on studies from congeners or conspecifics. Population projections, viability analyses, and decisions based on these models, data, and assumptions must fully incorporate the range of uncertainty in each of these elements. Bayesian approaches, in which existing knowledge (including both statistical estimates and expert opinion) is incorporated in prior distributions, subject to updating as additional data become available, may be an ideal approach for integrating the various component of uncertainty.

The SRT strongly discourages “consensus” approaches to input values for population modeling. These lead to a false sense of reliability, particularly if “consensus” was reached by force of argument, rather than weight of evidence. Rather, the data and opinions should be used as prior estimates (appropriately weighted) in a Bayesian or other approach. In general, there is a serious need to take into account the various sources of uncertainty in these projections, among them uncertainty in the input values and assumptions. Management alternatives could then be evaluated considering all these sources of uncertainty. This approach could also be used to evaluate the potential value (to decision making) of reducing various sources of uncertainty, e.g., by further research or improved estimates.

Experimental releases and impact of reintroductions

The success of experimental releases of Texas cougars into north Florida (Belden and McCown 1996, Belden and Hagedorn 1993, Jordan 1991, 1994) strongly suggest that reintroduction of panthers to vacant parts of historic range is biologically feasible. In addition, deliberate genetic introgression from cougars into the extant panther population appears to have dramatically stimulated population growth and reduced the prevalence of cats with characteristic signs of inbreeding depression (Shindle et al. 2001).

It seems all but certain that the panther will not be recovered solely by better management of the existing population in south or south-central Florida. Recovery of the panther will require reintroduction of the species in north Florida, or in other states within the historic range of panthers, in addition to habitat conservation and other measures such as genetic introgression. Knowledge seems sufficient at this time to make at least provisional recommendations as to the nature of releases. Experience with experimental releases (Belden and Hagedorn 1993, Belden

and McCown 1996) suggests advantages and disadvantages of various combinations of the source (captive-reared versus wild) and age-sex composition of the release stock. For instance, captive-raised animals seem to establish home ranges more quickly than wild-caught animals, but also are more likely to be involved in encounters with humans. Thus, reintroduction schemes might need to balance the advantages of more rapid establishment, with the disadvantage of more negative encounters with humans. Proposed, minimum release number of 10 cats (likely more, depending on age-sex composition and source) seem reasonable, and take into account the likelihood of both natural and human-induced mortality, including some deliberate persecution. The optimal combination of wild vs. captive-reared releases seems unknown, although it is reasonable to conjecture that releases of wild females are more critical than are those of males. In addition, the optimal sequence of release is unknown, but intelligent predictions can be made under alternate scenarios (e.g., releases of wild-caught males followed by several females into each male range; versus release of several females followed a single, captive-reared male who would be removed once he had bred all the females; Belden and McCown 1996). While these “rules of thumb” may represent the best current information, it is important that the assumptions upon which these rules are based are subjected to testing. To that end, the SRT recommends that current information about the impacts of reintroduction and introgression, based on experimental releases to date, be summarized in the form of predictions (in conjunction with PVA; see above) as the basis for discriminating among alternative reintroduction schemes (numbers, composition, timing, location, etc.) When one or more alternative is selected, monitoring data should be collected so as to test these predictions, for example in an adaptive management framework (Walters 1986, Williams et al. 2002).

Finally, the SRT notes that south central Florida north of the Caloosahatchee River is part of the current geographic range of the panther, as evidenced by the regular occurrence of male panthers there. Thus re-locating panthers from South Florida into this area should not be considered reintroduction per se, but rather a within-range translocation. The distinction between “reintroduction” and “translocation” is of more than mere semantic importance, given the sensitivity of the reintroduction issue.

SUMMARY – DEMOGRAPHY

We review studies that have dealt with the assessment of demographic parameters for Florida panther. We find that some aspects of panther demography are reasonably well understood. General patterns of population growth and dispersal, and certain components of reproduction such as litter size and age at first reproduction, are fairly well known. Data are poor or do not exist for other components, such as interbirth interval and proportion of females reproducing. Data exist with which to estimate survival rates for kitten and adult panthers, but much of this data has not been properly analyzed, so that statistically defensible estimates of survival rates are not available. Likewise, much of the data on cause-specific mortality is inherently biased (e.g., based on incidental reports of highway mortality), but data exist (e.g., from radiotelemetry) that has yet to be properly analyzed based on exposure-days to yield annual rates of cause-specific mortality. Population modeling (e.g., PVA) has principally employed “black box” models such as VORTEX and RAMAS that require unrealistically detailed demographic information and unverifiable (but critical) assumptions. Application of these models has emphasized “consensus” approaches that tend to obfuscate important uncertainties in demography.

RECOMMENDATIONS – DEMOGRAPHY

The SRT makes the following specific recommendations regarding demography of Florida panthers:

1. Quantitatively assess factors that constrain or enhance dispersal. Quantify uncertainty in the importance of dispersal as a factor limiting panther populations.
2. Develop statistically valid estimates of per-capita reproduction rate and of critical components of recruitment, including estimated frequency of litter sizes, ages at first reproduction, inter birth interval, and proportion of females breeding.
3. Use available data to make statistical comparisons between the survival of released Texas cougars and pure Florida panthers.
4. Develop statistically valid estimates of age- and sex- specific survival rates, using methods such as Kaplan-Meier or Heisey-Fuller that take into account exposure days. Emphasis should be on estimates based on radiotelemetry or other mark-recapture approaches. Methods that rely on raw mortalities, age distribution, or other approaches that are heavily dependent on untested (and untenable) assumptions should be avoided.
5. Develop statistically valid estimates of cause-specific mortality rates, based on appropriate analyses of radiotelemetry data. Avoid the use of raw mortality data that are heavily biased toward detection of certain mortality events (e.g., car collisions).
6. Give priority to quantifying critical functional relationships in demography, notably density-dependence and inbreeding effects. The inevitable uncertainty in these relationships must be taken into account in PVA or other modeling.
7. Develop population models that reflect a) panther natural history and b) do not require levels of detail about panther demography that are unknown (and largely unknowable). Explicitly address uncertainty about parameter estimates via sensitivity analyses, simulations, and incorporation of uncertainty into decision-making models. Avoid the use of canned programs (e.g., VORTEX, RAMAS) that tend to obfuscate these sources of uncertainty. Use, to the extent possible, statistically valid estimates of reproduction rates, survival rates, and variations (distributions) in these rates.
8. Analyze data where possible under alternative biological/ sampling models and use modern information- and decision- theoretic methods to account for “model uncertainty” in predictive estimation and the application of models to conservation decision making.
9. Do not use “consensus” approaches for modeling that obfuscate uncertainty in parameters, assumptions, and functional forms.
10. Summarize current information about the impacts of reintroduction and introgression, based on experimental releases to date as predictions, testable under adaptive management (Walters 1986, Williams et al. 2002).

BIOMEDICAL ISSUES

Descriptions of the new scientific field of conservation medicine read like an overview of Florida panther biomedical program needs and concerns. For instance, Osterfeld et al. (2002; p.18) state,

“Conservation medicine is...devoted to understanding the interactions among (1) human-induced and natural changes in climate, habitat structure, and land use; (2) pathogens, parasites, and pollutants; (3) biodiversity and health within animal communities; and (4) health of humans.” Roelke (1990) listed similar emphases for the Florida panther recovery program. One of the most important aspects of conservation medicine, especially for species imperiled by human impacts, is monitoring through time (Munson and Karesh 2002). Important monitoring topics include biomagnification (or bioaccumulation) of pollutants in the environment, disease transmission and susceptibility, emerging and growing health issues as they relate to destruction and alteration of wild habitats, and the identification of sentinel species for tracking various health issues. Thus, biomedical research is an important field for wildlife conservation, particularly as it concerns Florida panthers. Consequently, Florida panther investigations can contribute to our understanding of this felid and its conservation, and contribute to wildlife conservation in general.

The SRT found less controversy about biomedical aspects of panther research than the other issues we examined. Nonetheless, biomedical data are potentially important for the survival of the subspecies and biomedical monitoring requires consistent data collection. Thus, we gave special focus on consistency and completeness of biomedical information collected over the course of Florida panther recovery efforts. Early phases of biomedical research on the Florida panther recovery program included innovative, pioneering research. We seek to assure biomedical aspects of the recovery program continue to supply meaningful data.

The SRT reviewed 21 peer-reviewed publications dealing specifically with biomedical topics, including reproductive characteristics, parasites, physical abnormalities, diseases, and toxins. In addition, we reviewed publications on related topics, such as genetics, artificial insemination, animal capture and handling, hormone disruptors, and population structure. Plus, we requested data forms, protocols, and some specific databases for review. Our objective was to identify areas of Florida panther biomedical investigations requiring increased attention.

Physical abnormalities

Several physiological abnormalities have been identified in Florida panthers. Many of these abnormalities have been implicated in mortalities and in reduced reproductive capabilities. Although in many cases the cause of these abnormalities cannot be proven in the wild, the SRT found that most researchers made reasonable inferences based on the preponderance of evidence.

Taylor et al. (2002) documented mortalities from an atrial septal heart defect, an aortic aneurysm, an esophageal tear, pleuritis, and a pyothorax, for a combined total of 5% of 73 panther mortalities. An additional 6 atrial septal heart defects were documented, 3 of which may have contributed to the deaths of the animals involved. Cunningham et al. (1999) reported 6 of 33 panthers necropsied between 1985 and 1998 displayed atrial septal defects. Taylor et al. (2002) believed the cardiovascular defects were congenital anomalies, and that they were probably associated with inbreeding. However, there are few comparative data from other *Puma concolor* populations to determine the level of threat these may play in Florida panther populations compared to other wild populations of pumas. Kinked tails and cowlicks have also been reported in Florida panthers and are likely related to inbreeding.

Abnormal characteristics related to reproductive physiology are especially important in endangered species and have received much attention in Florida panther investigations. Cryptorchidism, testicular volume, and semen characteristics have been examined and are of

particular concern due to direct effects on reproductive capabilities. As Roelke et al. (1993) point out, these may be related to inbreeding and low genetic variability. There is no direct evidence of this, however, it is a plausible explanation given the correlation of such defects with inbreeding in other species, plus the observed increase in the occurrence of some traits with time in the Florida panther population. Reduction of such traits with genetic introgression would provide additional support for inbreeding as the cause of these abnormalities.

Cryptorchidism occurred in 56% of males examined from 1978 to 1992, and was increasing in prevalence (Roelke et al. 1993; Figure 6) from 1970 to 1992. In addition, bilateral cryptorchidism has been documented (2 of 9 known living males in 1992).

Although sperm quality is generally low in large felids, motile sperm per ejaculate in the Florida panther is 18-38 times lower than in other *Puma concolor* subspecies (Roelke et al. 1993). The Florida panther also has a 40% incidence of acrosomal abnormality, which renders sperm deficient in fertilization potential. They also have a significantly greater frequency of malformed spermatozoa (94.3% per ejaculate) than other puma subspecies (Roelke et al. 1993).

Recommendations.

1. Careful monitoring of physiological abnormalities correlated with inbreeding and depletion of genetic variability. Continued monitoring with the same intensity and vigilance during the genetic restoration period of panther recovery efforts will not only be important for this subspecies, but have the potential of being an exceptional contribution to our understanding of small populations overall.
2. Increased collaboration and studies comparing physiological traits of Florida panthers with those in other populations of *Puma concolor*.

Disease

Disease has not been considered a major mortality factor in the Florida panther (Maehr et al. 1991, Taylor et al. 2002). Taylor et al. (2002) reported 2% of panther mortality was due to disease, based on postmortem examinations of 73 panthers from 1978-1999. However, a wide array of pathogens (i.e., bacterial, viral, and parasitic diseases) or antibodies to pathogens have been documented in the Florida panther population, including feline calicivirus (FCV), feline panleukopenia virus (FPV), feline immunodeficiency virus/puma lentivirus, feline enteric coronavirus/feline infectious peritonitis (FIP), rabies, pseudorabies, *Bartonella henselae*, and *Toxoplasma gondii* (Roelke 1990, Roelke et al. 1993, Glass et al. 1994, Rotstein et al 2000).

Roelke et al. (1993:36) indicated “disease agents could contribute to the extinction of this endangered subspecies.” There are 3 reasons to be concerned about disease, despite the apparently small documented impact of diseases on panthers. First, there are probably fewer than 100 non-juvenile panthers; thus, every mortality counts. Second, there is only one population. Although sloughs and highways are impediments, there is no absolute barrier that could prevent a disease from sweeping through the population. In this regard, no other management action would contribute more to conservation medicine of panthers than the establishment of a new population outside of south Florida. Finally, if immuno-responses have been compromised through the effects of inbreeding, the population may be more vulnerable. The genetic restoration program may be ameliorating this last risk.

Although the SRT found no fault with pathogen/parasite screening and analyses, we are concerned about the apparent lack of consistency in data collection over time, and the lack of

regular annual reports and assessments of the findings. Such monitoring would provide timely identification of increases in pathogen effects and allow for actions and further analyses to resolve such outbreaks. From the literature provided to the SRT, it is unclear if this type of monitoring is taking place. Although monitoring intervals have been recommended for basic blood parameters (Dunbar et al. 1997), we were unable to determine if the same had been stated for monitoring disease and parasite contact in the Florida panther. For example, the 1998-2002 annual reports clearly list the hematological values and the serum antibody titer values for FIV, but it is unclear whether those values are available each year between 1993 (Roelke et al. 1993) and 1998 (Land et al. 1999). We also note ongoing monitoring for some internal parasites such as helminthes (D. Land, personal communication) to determine correlations between parasite loads and environmental, physiological, or pedigree characteristics. Such study is of particular scientific and conservation value, especially considering the effects such parasites may have on panther health (c.f. Dunbar et al. 1994).

Recommendations

1. Continued screening for pathogens on a regular basis. Conduct regular reviews by a small panel of veterinary experts to evaluate data collected to date and suggest modifications to protocols and analyses.
2. Increased pursuit of samples from other populations of *Puma concolor* for comparison, or collaborators willing to use the same screening techniques for a comparative study.

Environmental toxins

There are a number of reasons for concern about contaminants and their potential effects on the survival of the Florida panther, not the least of which is the small population. Furthermore, part of the Florida panther population lives near the lower end of the Everglades hydrologic system, which is subject to pollution from urban, suburban, industrial and agricultural land uses. Finally, as a top predator, the panther may be subject to bioaccumulation of toxins.

Panther tissues have been screened for a variety of toxins, including potential endocrine disrupters. In the early 1990's, serious concerns were raised about one toxin, mercury, which Roelke (1990) considered "a significant contaminant." Roelke documented high levels of mercury in blood and hair samples, and implicated mercury toxicosis in 3 panther deaths. Taking a more skeptical view of the evidence, Taylor et al. (2002) affirmed no panther death has been documented as directly caused by mercury. Facemire et al. (1995) stated "...environmental contaminants [including mercury] may be a major factor contributing to reproductive impairment" and they made a compelling case for the role of toxicants in sub-lethal effects in the Florida panther population, especially effects on male reproductive capability.

Sileo et al. (1997) initiated a study of endocrine disruptive chemicals and their association with congenital anomalies of the Florida panther. However, the results were incomplete, and the issue apparently has not been pursued vigorously.

Certainly the boldest scenario involving mercury was the "north-south health cline" in panthers posited by Roelke (1990). Roelke found panthers north of Interstate 75, particularly on the private ranches east of Immokalee, the Florida Panther National Wildlife Refuge, Bear Island Unit of the Big Cypress National Preserve, and adjacent ranches were in excellent health and in better condition (body mass, reproduction) than those in the Everglades National Park or Fakahatchee Strand State Preserve. This cline was correlated with a southward decrease in the

abundance of deer and hogs, and a southward increase in panther consumption of raccoons and alligators. These latter species are relatively high in the aquatic food chain, and thus could accumulate toxins that find their way into ground water. Consistent with this, Roelke et al. (1991) found high levels of mercury in raccoon tissues, again higher in the south than in the north. (Apparently incinerators in the Miami metropolitan area could cause increased atmospheric mercury in the south.) Roelke et al. (1991) further stated individual female panthers with high mercury levels tended to have lower reproductive success.

This apparent cline was based on relatively few panthers, and it is difficult to disentangle the cause-and-effect relationships when so many factors (i.e., prey abundance, prey consumption, mercury, body mass, reproduction) vary along the same gradient. Nonetheless, Roelke and colleagues assembled several consistent lines of evidence and painted a persuasive case for this cline, and for the role of mercury in the cline. The SRT was therefore surprised to find no publications since 1993 that either confirm or refute the patterns suggested by Roelke, despite the fact another decade of tissues have been collected, and another decade of data on panther body mass and reproduction are available. Although some of the mercury assays have been reported (Taylor 1997), there have been no reports since 1997, and apparently no attempt to correlate mercury levels with panther fitness. Although Taylor (2002) asserted mercury 'studies are underway,' the recent report by Shindle et al. (2001) made no mention of it. D. Land and M. Cunningham (personal communications to SRT, 13 January 2003) indicated tissues from 1997 have been stored, and mercury assays will be completed on them. They also indicated mercury levels in panthers may have declined since 1991. Until existing data are analyzed, however, the SRT does not feel complacent about this issue.

The SRT believes that tissue samples and data now available would allow a fruitful re-examination of the association between mercury concentration levels and panther health and reproduction. Such analysis should consider all possible alternative explanations for any emergent patterns.

Recommendations.

1. Continued, regular collection and analysis of panther tissue and blood for the presence of environmental toxins and pollutants, including mercury and potential endocrine disruptors (Sileo et al. 1997).
2. Thorough analysis of all existing data on geographic clines in toxins and panther health and reproduction. Such analyses must also include correlations between tissue levels of toxins and the fitness of individual panthers, rather than simple geographic correlations.
3. If the previous analyses suggest mercury (or any other toxin) may still be a problem, research should identify the ultimate source of the mercury (toxins), and follow it through the food chain by sampling aquatic plants, invertebrates, and the vertebrate prey consumed by panthers.

Capture and handling

One area of some additional interest by the SRT is the use of telazol, medetomidine, or a combination of either with ketamine instead of straight ketamine. The SRT is aware of the general advantages of telazol and medetomidine over the use of ketamine for general anesthesia. However, we also note the advantages straight ketamine has over either of these other drugs when the primary capture method is by treeing the target animal with the use of hounds. Despite

the disadvantages of ketamine in many other situations, one important advantage of this drug is the retention of muscle tone, thus reducing the potential for the cat falling from the tree. We did not have and did not seek information about the percentage of cats that fell from trees during capture operations, but we also note the special arrangements for such occasions (i.e., the use of a “crash bag”) to cushion the falling cat. In addition, we note the reduced amount of tiletamine hydrochloride/zolazepam hydrochloride with ketamine in situations where cats are treed high in the tree (Taylor 1998), and the risk of orphaning kittens with extended anesthesia sometimes related to the use of ketamine.

We note kitten captures are not attempted until the age of one year or more. Given the importance of kitten survival information in population models, and the lack of a rigorous published estimate, collaring and marking kittens younger than one year of age may be warranted. However, this does present a set of additional risks that must be weighed against the potential gains in knowledge about this population.

Finally, we note that Taylor et al. (1998) and Sileo et al. (1997) mentioned loss of information due to incomplete record keeping and inadequate sample storage (i.e., autolysis), respectively. The SRT encourages increased diligence in collecting tissues, storing tissues and data forms, and backing up data.

Recommendations.

1. A review of drugs used for immobilization and capture along with related tradeoffs.
2. A review of kitten capture techniques and results in other field studies of *Puma concolor* and the potentials for investigations of the Florida panther.
3. Increased diligence in collecting tissues, storing tissues and data forms, and backing up data.

RECOMMENDATIONS – BIOMEDICAL ISSUES

1. FWC and FWS should conduct an immediate and thorough analysis of **existing** data on geographic clines in toxins, diseases, and panther health and reproduction. Such analyses must include correlations between tissue levels of toxins and the fitness of individual panthers, in addition to geographic correlations. The analyses should consider how the incidence of abnormalities in panthers is correlated with, or interacts with, genetic status (heterozygosity, degree of hybridization with Texas pumas). It appears considerable tissues and data have been warehoused for a decade. The marginal cost of the analyses would be small, and these analyses are sorely needed to guide future data collection.
2. As suggested by the results of the analyses recommended above, FWS should collect and analyze panther tissue and blood for the presence of environmental toxins and pollutants, including mercury and potential endocrine disruptors (Sileo et al. 1997). If mercury may still be a problem, research should identify the ultimate source of the mercury, and follow it through the food chain by sampling aquatic plants, invertebrates, and the vertebrate prey consumed by panthers. These results will be important not only for this subspecies but also for our understanding of how to conserve other small populations.

3. Panther researches should try to collaborate with puma researchers elsewhere to compare physiological traits, diseases, and contaminant loads of Florida panthers with those of other populations of *Puma concolor*.
4. A review of drugs used for immobilization and capture along with related tradeoffs.
5. A review of kitten capture techniques and results in other field studies of *Puma concolor* and the potentials for investigations of the Florida panther.
6. Increased diligence in collecting tissues, storing tissues and data forms, and backing up data.

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Table 1. Estimates of demographic parameters for Florida panthers.

Demographic parameter/ relationship	Component	Mean or Mode (range)	Study area	Period	References	Methods used/ comments
Abundance/ density		D= 1/ 110 km ² N= 46	S. Florida ("core area")	1985-1990	Maehr et al. (1991)	Estimate for "core area" based on radiotelemetry
		N=62	S. Florida	2000	McBride (2000)	Cumulative counts of radiocollared panthers
		N=78	S. Florida	2001	McBride (2001)	Cumulative counts of radiocollared panthers
Population growth		$\lambda > 1$	S. Florida	10 years (dates not specified)	Maehr and Caddick (1995)	Plots of "known births vs. known deaths"
		$\lambda > 1$	S. Florida	1981-2001	McBride (2000, 2001)	Capture rates, counts of newly-collared cats
Dispersal distance	Juvenile males	58.7 km (22-100)	S. Florida	1986-2000	Maehr et al. (1991a)	Radiotelemetry of juveniles
	Juvenile males	68.4 km	S. Florida	1986-2000	Maehr et al. (2002a)	Radiotelemetry of juveniles
	Juvenile females	20.3 km	S. Florida	1986-2000	Maehr et al. (2002a)	Radiotelemetry of juveniles
Home range size	All ages/ sexes	(53-1183 km ²)	S. Florida ("core area")	1985-1990	Maehr et al. (1991a)	Estimate for "core area" based on radiotelemetry
	Resident males	519	S. Florida ("core area")	1985-1990	Maehr et al. (1991a)	
	Resident females	103	S. Florida ("core area")	1985-1990	Maehr et al. (1991a)	

Reproduction	Litter sizes	1.8 (1 - 4)	SW Florida	1993-1994	Land (1994)	Litter counts of radio-marked females. Probably biased low.
		Mode =1 (1 - 3)	SW Florida	1995-2001	Shindle et al. (2001)	Radiotelemetry
	Age at first reproduction	10.5 - 2 yr	BCNP	1986-1987	Maehr et al. (1989)	Radio telemetry from 9-m to 26 months with litter (inferred that conceived < 20mo)
Survival	All ages	0.83 (0.69-1.00)	SW Florida	1987-1990	Maehr et al. (1991b)	Telemetry. Heisey-Fuller estimates (not stratified by age-sex)
		(Frequency distribution of ages at death—no direct survivorship estimates computed)	SW Florida	1995-2001	Shindle et al. (2001)	Ages at death. Evidently includes both radiocollared and found, uncollared cats. Concern here for discovery bias.
	Kitten	0.959	SW Florida	1993-1994	Land (1994)	Revisits to dens/ litter counts (0- 6 mo). Mixture of ages, biased.
		0.84	S. Florida	10 years (dates not specified)	Maehr and Caddick (1995)	Plots of "known births vs. known deaths"
	Birth to 6 mo	0.62	SW Florida	1995-2001	Shindle et al. (2001)	Revisits to dens/ litter counts (0- 6 mo). Mixture of ages, biased.

Cause-specific mortality	Juvenile	0.975	SW Florida	1995-2001	Shindle et al. (2001)	Telemetry; not adjusted for exposure days; includes Texas cougars. Not clear if this is an annual or a periodic rate.
	Juvenile	0.933	SW Florida	1993-1994	Land (1994)	Radiotelemetry (6 mo - 1 yr)
			SW Florida	1987-1990	Maehr et al. (1991b); Shindle et al. (2001: Appendix VII)	Telemetry; crude mortality estimates include non-radioed cats (so biased toward auto collisions)
	All ages	Rates not estimated	SW Florida	1988-1989	Maehr (1989)	Documentation of road kills of uncollared cats
		Listed percent of each source occurred, but rates not estimated	SW Florida	1995-2001	Shindle et al. (2001)	Radiotelemetry
Carrying capacity		Rates not estimated	South Florida	1978-1999	Taylor et al (2002)	Carcass examination of collared and uncollared cats
	All ages, sexes	Not directly estimated but could be inferred from home range estimates and habitat preference.	South Florida	1985-1990	Maehr et al. (1991a)	Radiotelemetry; Serious methodological questions about home range and habitat inferences

	All ages, sexes	Not directly estimated but could be inferred from home range estimates, "frustrated dispersals". Thought by Maehr to have been at carry capacity prior to Texas introductions.	South Florida	1986-2000	Maehr et al. (2002a)	Radiotelemetry; Serious methodological questions about home range and habitat inferences
Functional relationship	Density-dependent dispersal	Positive relationship to density	S. Florida	1986-2000	Maehr et al. (2002a)	Radiotelemetry of juveniles
	Genetic introgression	20%	SW Florida	1995-2001	Shindle et al. (2001)	Extrapolation based on apparent reduction in kinked tails and cowlicks
Viability analysis	Probability of persistence over 100yr	0 to 1 (mode =1) depending on assumptions	Florida	1999	Ellis et al (1999), Maehr et al (2002b)	VORTEX simulations.
Reintroduction	Reintroduction (likelihood of success)	No formal estimates; success "likely" depending on assumptions and protocols	Florida	1991-96	Jordan (1991, 1994), Belden and Hagedorn (1993), Belden and McCown (1996)	Review of candidate release sites; Experimental releases of cougars