Statement of
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Before the Committee on Resources
U.S. House of Representatives

Concerning
National Canada Lynx Survey

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Mr. Chairman and Members of the Committee:

Thank you for the opportunity to appear before you today to talk about the National Canada Lynx Survey. I am Kevin McKelvey and I am a research scientist working for the Rocky Mountain Research Station of the USDA Forest Service. I am the scientist with the responsibility of overseeing the National Lynx Survey effort, including design, analysis, reporting and results publication. Today, I would like to describe the background and objectives, survey methods, DNA analyses, and measures used to ensure quality and reliability associated with the National Lynx Survey.

Background

In 1994, the Rocky Mountain Research Station was charged with evaluating the current state of knowledge concerning forest carnivores, including the Canada lynx. Their published findings (Ruggiero et al. 1994) indicated that knowledge gaps concerning forest carnivores, and lynx in particular were huge. In 1998, with the proposed listing of the lynx under the Endangered Species Act, the potential consequences of this lack of knowledge became critical. The Rocky Mountain Research Station was charged with collating and evaluating all of the knowledge concerning lynx, their prey, competitive interactions, and ecological context.

As a part of this effort, in 1999, Dr. Keith Aubry, Yvette Ortega, and I finished an analysis of the historical records for lynx in the contiguous United States. However, these data are ambiguous concerning the current range of the species. To build an effective conservation strategy, we need to determine where extant populations of lynx are and where they are

not. The first step is to determine where there are lynx, secondly, to determine numbers and look for evidence of reproduction- that is, residency in an area - and finally, to determine patterns of habitat use and conservation needs (Figure 1). The National Lynx Survey was designed as the first step in this multi-stage process, with follow-up surveys in areas where lynx are detected serving as the beginning of the second step.

Dr. Leonard F. Ruggiero, Dr. John R. Squires, Gregory W. McDaniel and I at the Rocky Mountain Research Station developed and published the data collection methods used in the survey. Dr. L. Scott Mills, of the University of Montana, Kristine Pilgrim, Dr. Michael Schwartz, and I developed and published the DNA methods used to distinguish lynx from other species. The survey is based on peer reviewed and published research. The protocols included standards for training in field methods, standards for field data collection, and standards for the DNA analysis of hair samples to determine if the hair was from lynx or from another species. The National Lynx Survey is funded by and reports directly to the National Lynx Steering Team, an interagency oversight group headed by Kathy McAllister, Deputy Regional Forester for Region 1 of the USDA Forest Service. The National Lynx Survey has three primary leaders: James Claar, (Region 1, USDA Forest Service), Dr. L. Scott Mills, and me. I have general oversight and design of the entire survey effort. James Claar is responsible for coordinating with the field offices, distributing funds and materials, and training. Dr. Mills, Director of the Carnivore Conservation Genetics Laboratory, is responsible for the protocols associated with DNA analysis. This laboratory is jointly supported by the University of Montana, the Rocky Mountain Research Station, and Region 1 of the Forest Service. Because Dr. Mills is testifying at these hearings and will describe the DNA methods, I will limit my discussion of DNA protocols.

In order to be effective, we determined that the National Lynx Survey needed to have the following characteristics:

1) It had to produce unambiguous results. We didn't want to spend a lot of time doing extensive follow-ups in areas that contained no lynx.

2) It needed to cover large areas of land, and therefore needed to be compact and inexpensive. It was critical that the method not be so cumbersome that surveys would be largely confined to roaded areas.

3) It needed to be a method that worked in the summer. Winter methods cannot be applied in avalanche-prone or extensive roadless areas.

4) It needed to be effective enough that lynx populations can be reliably found. It is just as important to specify where lynx likely do not exist as to determine where they exist. These two understandings are required to define current distribution.

5) Because the survey was to be applied by a large number of people with various backgrounds, it had to be simple and straightforward, and not demand special skills. Field work had to be limited to data collection only.

These considerations led us to discount most of the current survey methods. The hair snagging method, however, used scent stations to collect hair and DNA analysis to determine species. It satisfied all the requirements for the survey. After we detected lynx using hair snagging, we could then employ more intensive methods, such as snow tracking, to verify the detections and gain additional information regarding lynx populations.

Survey Design

The goal of the National Lynx Survey is to detect lynx and help to define current range. It is a presence/absence survey. Therefore, the study has to be designed to detect lynx, if present, with high likelihood. If this goal is achieved, failure to detect lynx indicates their absence or extreme scarcity, allowing possible range delineation. We tested the probability of detection directly by implementing the survey in as many areas as possible where lynx are known to be present.

Detection testing in the contiguous United States is limited because we know of so few locations where lynx occur. In Northwest Montana, we know of approximately 20 lynx in the Clearwater drainage around Seeley Lake, Montana because our research group is conducting a large radio-telemetry study in the area. We know that lynx occur in the Okanogan National Forest in northwest Washington State, based on ongoing camera surveys. We know of a tiny group in Wyoming, probably no more than 5 individuals that exist in the northern portion of the Wyoming range. Lastly, we know that lynx exist in northern Maine. Additionally, there was evidence of lynx occurrence in Glacier National Park and in the Pioneer Range in Southwest Montana. We placed surveys in all these locations and have currently run them for at least one year.

While extensive, the surveys could not cover the entire historical range of the lynx. We therefore centered grids with transects on large contiguous areas of designated lynx habitat. Additionally, we specified that the survey be run in each location for 3 years. We took a number of measures to regularize methods and ensure consistency. We used common training with the same instructor across the survey, and we provided a “kit” for

each survey. The kit contained everything necessary to conduct the survey. Important components (hair snares, visual attractants, desiccant filled vials, lure etc.) were all produced at a central facility to ensure consistency. An extremely detailed field manual was also included in each kit.

Additionally, the field protocol was simple: people had to bait the lures as specified (we provided the measurement spoons), place the transects on a grid, set up each station as specified, collect hair 2 weeks later, place hair in the provided vials and the associated carpet pads in plastic bags (also provided), label the vials and bags and mail all vials and the associated pads to us. As long as there was sufficient supervisory control to assure that these steps were done properly, there is no reason that crews of variable make-up and skills could not successfully carry out the protocol.

DNA Analysis of Hair

Hair vials were shipped to the Missoula Lab in boxes or envelopes and were transferred unopened to our "hair lab," a facility on the University of Montana in a separate building from the lab in which we performed polymerase chain reaction (PCR) amplification.

Participants in the National Lynx Survey sent written reports to the Forest Service Regional Office in Missoula, or to the Missoula Lab. The written reports consisted of a set of maps showing the location of transects, vegetation forms, and a record of the stations from which hair had been collected. By matching information within the written reports with the vials and pads received at the Missoula Lab, we could detect any addition or deletion of samples that might have occurred. Additionally, we requested information concerning problems encountered in implementing the survey and ideas as to how the survey could be improved. These suggestions have led to a variety of minor changes in the field protocol.

The extracted DNA is then taken from the hair lab located on the University of Montana to the main laboratory located in the USDA Forest Service Forestry Sciences Laboratory, both in Missoula. Species identification methods were developed using extensive internal and external blind tests, as well as geographic range tests to confirm that the DNA differences used to separate species were consistent within the species and consistently different between species. Species identification of black bear and brown bear, coyote, wolf/dog, foxes, and mustelids, such as fisher, marten, or weasel is also performed. Additionally, other species are identified by sequencing the DNA and matching the derived base pair strings to data from Genbank, a database that serves as the primary international receptacle for DNA data. Positive and negative controls are included in every reaction. The positive control is a sample from a known organism of the target species. The

positive control demonstrates that if a sample from the target species is present we are able to detect it. The negative control is water, and is used to test for the presence of contaminants in the reagents. The results of all laboratory reactions, in the form of gel images, are incorporated into lab books along with the species identification and associated notes.

We consulted extensively with the Fish and Wildlife Service Forensic lab in Ashland, Oregon concerning how to best preserve the chain-of-evidence associated with forensic samples. Records of all of the gels we have run are kept in lab books, all of the extracted DNA samples are preserved in 20-below-zero freezers, and all hair samples are held in sealed, desiccant filled vials, in locked cabinets in our hair extraction lab. If there are issues associated with a specific sample, we can readily access the DNA analyses, extracted DNA, and the original hair sample.

Follow-up Surveys

We initiate follow-up surveys when we identify a lynx sample in an area where, prior to the survey, we did not know that lynx were present. Where access permits (and it has so far) we utilize an extremely intensive winter-long snow tracking protocol designed and tested by Dr. John Squires to find lynx in preparation for trapping and subsequent radio-tracking. This allows us to separate detections associated with pets, lone wanderers, fur farm escapees, and falsified or unexplained samples from lynx detections associated with populations of conservation interest. We are running two such surveys this winter in the Boise and Shoshone National Forests, the only heretofore unknown lynx locations associated with the National Lynx Survey to date.

Check-backs and Validation

There are 2 potential errors that can affect a survey. First, the survey could falsely identify lynx in areas where they do not exist. The second is that the survey could fail to detect lynx in areas in which they do exist (Table 1).

The first error, false positives, is primarily controlled by the rigor of the lab work. In this context, we demonstrated that the genetic assays we use for species identification are consistent across the ranges of all of the potential felids, and were diagnostic 100% of the time in rigorous double-blind tests. The extreme reliability of these assays is the primary strength of the method, and one of the primary reasons we chose DNA analysis.

Even though we have processed more than 1200 hair samples with sufficient DNA to amplify, we have only found 4 samples of lynx in areas where we were unaware of their presence prior to the survey. These occurred on the Boise and Shoshone National Forests.

We are engaging in follow-up surveys of the types mentioned earlier in both areas this winter. We believe that the use of well-tested DNA analyses, combined with intensive follow-up surveys virtually eliminates the possibility of false positive results.

The second error, failing to detect lynx when they are, in fact, present cannot be entirely eliminated, but can be controlled through thorough field methods. To reduce the chances of failing to detect lynx, the survey employs a large number of approaches (Table 1). However, the real test of any survey is determined by directly testing its efficacy in the field. That is why we have placed so much emphasis on placing survey grids in areas in which lynx presence is known or strongly suspected.

Lynx Detections Not Associated With Lynx Conservation

There are lynx detections that occur within the National Lynx Survey that are not of conservation concern. For instance, lynx are domesticated both as pets and in fur farms, and may wander off or escape. Additionally, even though we have protocols to keep the lynx detection stations out of sight from roads or trails, and to limit the knowledge of their locations, people can, and have, planted lynx hair within our survey. To separate these occurrences from actual lynx populations, we rely on follow-up surveys. In these surveys, we look for evidence of multiple lynx, family groupings (the young-of-the-year travel together with their mother), and the spatial extent of the track data. Additionally, because we collect hair from the snow along all lynx tracks encountered, we may be able to evaluate the population more directly. As an example, on one of our test grids we obtained 12 hair samples associated with lynx, and 7 of these samples were from different individual lynx. If lynx hair were planted in areas that contain no lynx, in our follow-up surveys we would not find tracks, lynx hairs associated with the tracks, or other evidence of lynx such as scat. We, therefore, believe that the overall integrity of the survey is robust and will detect the presence of escaped pets, or willful data manipulation.

Summary

In summary, Mr. Chairman, we believe we can verify the scientific authenticity of the National Lynx Survey based on the reasons I have cited: survey methods, DNA analyses, and measures used to ensure quality and reliability associated with the National Lynx Survey. We believe the integrity of the overall survey has been maintained. This concludes my statement; I would be happy to answer any questions you or members of the Committee might have.

Literature cited not included in the attached National Lynx Survey

Ruggiero, L. F., K. B. Aubry, S. W. Buskirk, L. J. Lyon and W. J. Zielinski. 1994. The scientific basic for conserving forest carnivores: American marten, fisher, lynx, and wolverine in the western United States. USDA Forest Service General Technical Report RM-234.

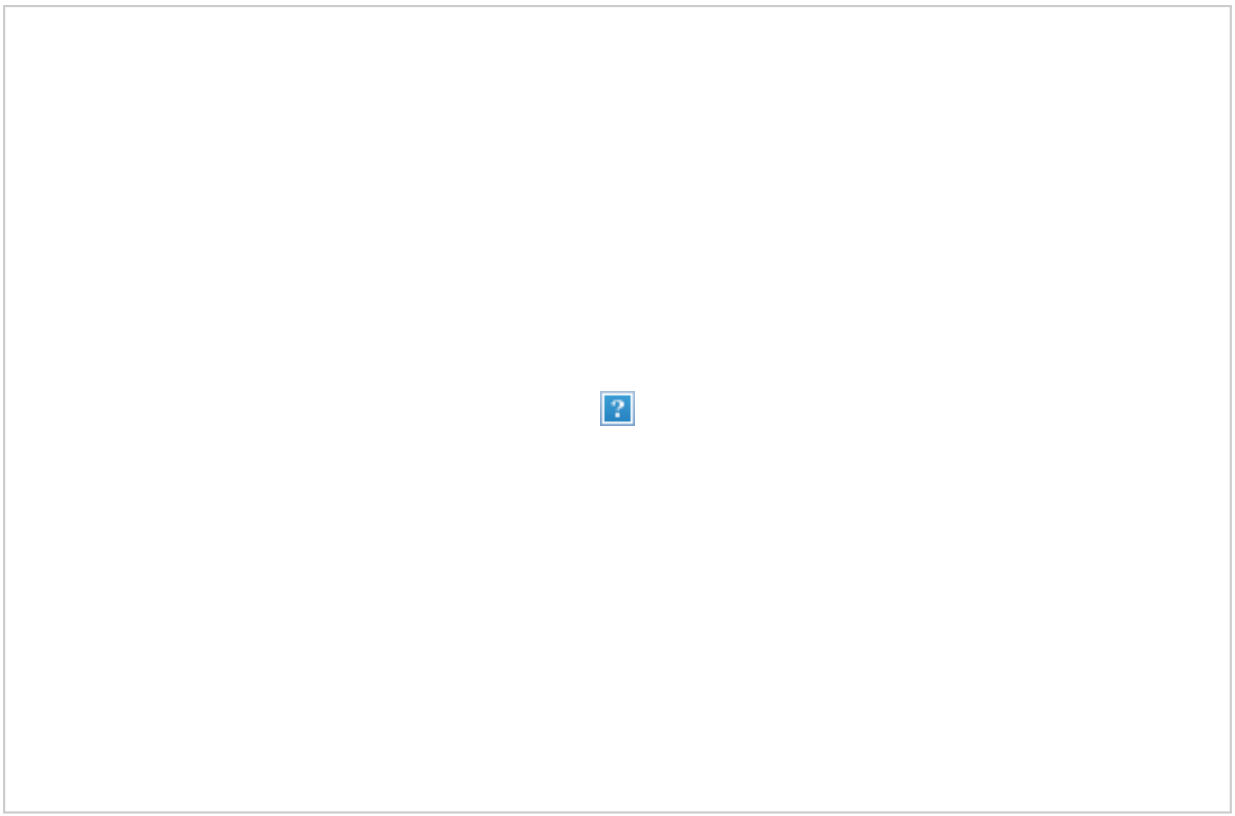

Figure 1. A schematic representation of the process for identifying areas where lynx can be studied, or conserved. The National Lynx Survey primarily answers the first question: are lynx present? From Aubry et al. (2000).

Avoiding false positive results	**Detecting lynx when present**
Geographic range tests of DNA methods Test results consistent Blind tests of DNA methods 100% success Quality controls in the lab Careful documentation of samples, reactions Positive and negative controls on each reaction Total separation between extraction and PCR Follow-up surveys for all lynx identifications outside of test grids	Use of a method that allows representative surveys of roadless areas. Testing the efficacy of the method In Kluane lynx detected on 45% of transects We use the best lure tested Saturation of the sample areas with 125 stations in 25 transects Conducting the survey for 3 years If protocol is not followed, the local survey doesn't count towards the 3 years Complete standardization of all materials and training used in the survey

	Geographic range tests of DNA methods Test results consistent Blind tests of DNA methods 100% success Multiple DNA extractions if PCR is unsuccessful About 80% amplification rate Positive controls on every reaction Running multiple test grids to directly evaluate survey efficacy

Table 1. Protocols in the National Lynx Survey designed to eliminate false positive results and to both increase and test the likelihood that the survey will detect lynx when present.

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