

OVERVIEW OF THE NATIONAL LYNX SURVEY

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Background

Survey objectives

In 1999, we finished a thorough analysis of the historical records for lynx in the contiguous United States (McKelvey et al. 2000a). Though the effort was exhaustive (See: McKelvey et al. 2000a, Appendix 8.1 for a complete list of the data sources), the data were equivocal both in terms of defining historical range, and even more so current range. The primary factors that lead to uncertainty concerning distribution were: 1) the inability to separate native populations from large numbers of lynx that periodically flushed out of Canada and 2) the cessation or extreme limitations placed on trapping lynx in most states during the 1980's. The first factor likely led to recent verified lynx across most of the historical range (ID, MN, NH, OR, UT, and WI all had verified lynx occurrences in the early 1990's). The second factor led to a decrease in the physical remains produced, and this could easily be confused with a recent decline in range. Because the historical data were so equivocal, it was clear that one step towards building an effective conservation strategy was to determine where extant populations of lynx are and where they are not. As outlined in Aubry et al. (2000; This Section. See particularly the section titled: A Research Framework), determining the location of researchable populations was a multi-step process. The first step was to determine where there were at least some lynx, secondly to determine numbers and look for evidence of reproduction, and lastly, to determine patterns of habitat use and conservation needs. The National Lynx Survey (NLS) was primarily designed to fulfill the first of these steps, with follow-up surveys in areas where lynx were detected serving as the beginning of the second step-determining numbers and reproduction (e.g. residency).

In order to be effective the NLS needs to have a number of properties:

1) It has to produce unambiguous results. In our collation of historical data, we uncovered hundreds of recent anecdotal accounts of lynx occurrence, most of which were probably incorrect. The last thing we needed was additional equivocal or ambiguous data. While the process outlined in Aubry et al. (2000; This Section) included follow-up surveys, and these could serve to verify weak data, these surveys are expensive and time consuming. Clearly we didn't want to spend a lot of time doing extensive follow-ups in areas that contained no lynx.

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

3) It needs to be a method that works in the summer. Winter methods cannot be applied in avalanche-prone or extensive roadless areas. Further, methods such as snow tracking, that require specific snow conditions, will be ineffective in areas where the necessary conditions are rare.

4) It needs 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 it will be applied by a large number of people with various backgrounds, it has to be simple and straightforward, and cannot demand special skills. This means that critical data analysis cannot occur in the field. Field work has to be limited to data collection.

These considerations lead us to discount most of the current survey methods. Snow tracking is inherently difficult, requiring special skills. It can only be done in the winter and, even with photographs or other backup documentation, most of the critical analysis is done in the field. Therefore snow tracking, while a very sensitive method for detecting rare organisms, requires highly trained crews and cannot be applied everywhere. Remote camera sets are bulky, expensive, prone to breakdown, and the data are often ambiguous. Scent stations have been used to attract bobcats (Sargeant et al. 1998, Diefenbach et al. 1994), generally using areas of smooth sand to collect track data, but sand is heavy and bulky, and the resultant data are tracks with associated uncertainties. We were, however, intrigued by an unpublished hair-snagging method being prototyped by John Weaver (Weaver 1997). This method used scent stations, but hair was collected rather than tracks and DNA analysis used to determine species. This method, if it worked, could satisfy all of the requirements associated with the NLS.

Testing the methods

There were major uncertainties that needed to be addressed before we could consider basing a national effort on hair snagging. First, was it effective? That is, when lynx were in an area, did they reliably rub and leave hair? The second question was: are there reliable diagnostic DNA tests that can felid separate species, and which will work reliably given the low quality of DNA associated with the hair samples we were likely to obtain? And finally, could we develop a protocol that would incorporate this detection method into an effective survey?

To test for efficacy, we needed an extensive area with many lynx. Within this area, we could test the limited question: Given that lynx encounter a scent station, do they rub and leave a hair sample? Because we didn't have an area that fit this description in the contiguous U.S., we performed this test in Kluane NP, Yukon (McDaniel et al. 2000; Section 2). We used many aspects of Weaver's (1997) detection method. The basic station included a rub-pad (a carpet pad with nails sticking through) baited with a scent lure and catnip. Above the carpet pad a pie plate was suspended as a visual lure (McKelvey et al. 1999; Section 3). This set is a modified version of sets used by trappers

to catch lynx, and we assumed that the basic design was sound. We therefore chose to test a variety of scent-lures, as it seemed to us that the lure would be critical to the efficacy both of attracting lynx, and for eliciting rubbing behavior. We therefore designed a cafeteria-style survey with transects containing 5 potential lures. All 5 lures were recommended to us by lynx researchers. John Weaver declined the opportunity to participate in the test (he was offered the lead), and refused to allow us to test his proprietary lure.

The Kluane results were encouraging. The best lure recorded lynx at 27% (21/78) of the stations (39% of the stations with lynx hair), and 45% of the 5-station transects contained a lynx hair sample (McDaniel et al. 2000; Section 2). Additionally, the lure that worked best for lynx had fewer non-target samples from coyotes and bears than did the other lures. We felt that the results from Kluane indicated that an effective presence/absence survey based on hair snaring could probably be constructed.

The second component was to determine whether reliable diagnostic DNA methods could be developed. To explore this, we first looked to published methods in the literature. Foran et al. (1997) described multi-species identification tests based on analysis of mitochondrial DNA (mtDNA). However, these tests proved unreliable for hair from felids, and we developed our own methods (Mills et al. 2000; Section 2). We tested these methods exhaustively, doing both internal and external double-blind tests, and obtaining geographically representative samples to assure that the tests were diagnostic for each species across its range. In addition to our published methods for separating felids (lynx, bobcat, cougar, and domesticated cat), we utilized similar published methods to identify canids (red and gray fox, and wolf/dog; Paxinos et al. 1997) and developed methods to identify bears (black and brown; K. L. Pilgrim, Unpublished Data) and mustelids (wolverine, fisher, marten, mink, and skunk; Riddle et al. *Submitted*).

The results from our geographic range and blind tests were that the methods we developed to identify felids were correct 100% of the time when DNA was of sufficient quality to be analyzed, and were consistent across the geographic range of the target organisms.

Additionally, we developed methods that allow the identification of individual lynx (Schwartz et al. 2002; Section 2). While not essential to the NLS, we attempt to individually identify all lynx samples produced in the NLS.

Survey design

Management structure

The National Lynx Survey is funded through 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 3 primary leaders: Dr. Kevin S. McKelvey, James J. Claar, (Region 1, USDA Forest Service) and Dr. L. Scott Mills. Dr. McKelvey has responsibility for general oversight

and design of the entire survey effort, Mr. 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.

Scope of inference

While extensive, the surveys cannot cover the entire historical range of the lynx. The formal scope of inference is to the area covered by the grid, and a buffer of undefined size around it, but if a grid is centered on, and occupies the majority of, a habitat island, it is reasonable to infer the results to the island. We therefore suggest that grids be centered on large contiguous areas of designated lynx habitat or, where habitat is more extensive, that multiple grids be representatively placed within large contiguous areas of habitat (McKelvey et al. 1999; Section 3).

Field protocol

The goal of the 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. No matter how conceptually sound a survey may be, this ability needs to be demonstrated. To give us the best chance of achieving both high and quantifiable detection probabilities we evaluate detection at the level of a large grid of stations such that differences in probability of detection at the station level will have relatively little effect on presence/absence sampling at the level of the grid, and we test the probability of detection directly by implementing the survey in as many areas as possible where lynx are known to be present.

In Kluane, we had a 26% rate of detection at single stations baited with the best lure tested (beaver castoreum and catnip oil; BCC), and a 45% rate of detection for a transect of 5 stations, even though 4/5 of these stations were baited with less effective lures (McDaniel et al. 2000; Section 2). There was, therefore a significant increase in detection rates associated with using transects rather than single stations. Additionally, using transects minimizes potential detection differences based on micro-placement of a single station. We therefore chose transects similar to those applied in Kluane as the basic sampling unit. We knew of no evidence for the use of particular habitat features by lynx in the summer (Apps, 2000; McKelvey et al. 2000b; Squires and Laurion 2000), but placed transects perpendicular to the terrain so that if organisms used terrain features (ridges, valleys, side-hill movement) preferentially, transects would intersect these features.

Because we wanted, as much as possible, to be sure that we detected lynx when present, each presence/absence grid consisted of multiple transects. We place 25 5-station transects on a grid at a density of 1 transect per every 4 square miles (2-mile intervals

between transects). Stations are 100 m apart, and therefore each transect is 0.4 km long. Additionally, we specify that the survey be run in each location for 3 years.

Though the goal was to achieve high rates of detection, we did not know, for the period of survey, what the detection likelihood would be in the contiguous U. S. To determine this, we placed grids in areas in which we knew lynx were present in order to directly determine the ability of a grid to detect lynx when present. We were limited because we knew of so few locations that contained lynx. In Northwest Montana, we knew that approximately 20 lynx were found in the Clearwater drainage in the area around Seeley Lake, MT because our research group was conducting a large radio-telemetry study in the area (Squires and Laurion 2000). Based on forest surveys, and based on John Weaver's unpublished work, we felt that lynx were likely widespread in the Kootenai National Forest, but knew nothing about their densities. In the Okanogan National Forest in northwest Washington State, we also knew that lynx occurred based on ongoing camera surveys. In Wyoming, we knew of a tiny group, probably no more than 5 individuals that existed in the northern portion of the Wyoming range (Squires 2002; Section 5. Squires and Laurion 2000). Lastly, we knew that lynx existed in northern Maine. Additionally, it was thought that lynx might occur in Glacier National Park, in the Pioneer Range in Southwest Montana, and within the Colville National Forest in northeastern Washington. We therefore attempted to place grids in all of these areas. The Kootenai and Colville National Forests declined to participate, but we placed surveys in all of the other locations, and have currently run them for at least one year.

Whenever an index (in this case an index of lynx presence) is generated, regularizing the methodology is essential. Accordingly we took the following 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 (McKelvey et al. 1999; Section 3). 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 (McKelvey et al. 1999, Section 3).

Additionally the field protocol was extremely 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 makeup and skills could not successfully carry out the protocol.

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 (See Pilgrim et al. 2002, Section 4 and Mills et al. 2000, Section 2 for details). There, the hair

was unboxed, cataloged, separated into morphologically distinct groups where necessary, and DNA from each hair sample was extracted (See Pilgrim et al. 2002; Section 4).

Participants in the National 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 (Hanvey 2002; Section 3).

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. Species identification of lynx and other felids is based on Mills et al. (2000; Section 2). These 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 (Pilgrim unpublished), coyote, wolf/dog, foxes (Paxinos et al. 1997), and mustelids (Riddle et al. *Submitted*) 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 were present we would be 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 (Pilgrim et al. 2002; Section 4).

We consulted extensively with the USFWS Forensic lab in Ashland, OR concerning preserving 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.

Data Management

All data recorded in the inventory, macroscopic examination, and DNA analysis is recorded in a Microsoft (ACCESS) database. Final lab result reports are prepared containing two parts. The first part is a summary sheet of the vials received, the samples that contain quality DNA, and a list of the number of “hits” by particular species. The

second part is the detailed vial list for each transect, station, and hit, with associated species identifications.

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 John Squires to find lynx in preparation for trapping and subsequent radio-tracking (Squires 2002; Section 5). 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 NF's, the only heretofore unknown lynx locations associated with the NLS to date.

Check-backs and validation

There are 2 potential errors that can effect 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, creating 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. Therefore, the probabilities of misidentification given careful lab methods are virtually non-existent, and we have never misidentified a sample of known origin, blind or otherwise. The extreme reliability of these assays is the primary strength of the method, and one of the primary reasons we chose DNA analysis. The only ways that false positives can be created are through contamination or if the samples themselves have been altered. We control contamination by extracting hair in a separate lab from the PCR amplification and through a variety of quality controls within the lab (Pilgrim et al. 2002; Section 4), and check for contamination by using negative controls for every reaction. Additionally there are a number of procedures we use to prevent sample tampering. First, field protocol dictates that no station should be visible from a road, and we emphasize in training the need to keep the transect locations secret. Mailed boxes are not opened until they are in the hair lab, and both labs are locked. From the time the hair samples enter the lab through PCR amplification and final reporting, the samples and the resulting DNA are only handled by 2-3 people. We match the samples to the field reports and note discrepancies. Lastly, we conduct extensive follow-up surveys. In the highly unlikely case where a false positive result occurs through the DNA sampling, the follow-up surveys will produce negative results. Based on these controls and follow-up procedures, we feel that the probability of producing false positives is extremely low, and the probability that false positive results will trigger conservation responses is non-existent. These beliefs are supported by the survey results to date. Even though we have processed more than 1200 hair samples with sufficient DNA to amplify, we have only found 4 samples 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 in both areas this winter.

The second error, failing to detect lynx when they are, in fact, present cannot be entirely eliminated. A given lynx may choose not to rub; a given sample may not amplify; a careless employee may not notice a hair sample and leave it in the woods.

One way to help reduce the chances of failing to detect lynx is to standardize the field protocol. In this regard we took a number of steps. First, we developed a very simple field protocol, and we provide uniform training and materials across the country. Additionally, we take careful notes concerning each incoming sample and on the field reports. We look for evidence that the survey has not been properly implemented and the samples not properly handled. Additional checks occur in the lab where we evaluate the amplification rate and look for common contaminants such as evidence of pet hair (we identify house cats and wolf/dogs). If a survey has not followed protocol, we inform the Forest, and the survey does not count toward the 3-year protocol.

There are many ways to improve the likelihood that a survey will detect organisms, and we have used a variety of methods (Table 1). It was to this end that we tested a variety of lures (McDaniel et al. 2000; Section 2), and found that one lure worked better than the others tested. Similarly, one can seek to improve the amplification rate (we average about 80%) by changing how hair samples are treated and processed. The methods we use produce a higher proportion of positive identifications on hair samples than do other potential methods such as DNA sequencing, and we run multiple assays on each sample to ensure that we maximize the amplification rate. The positive control also ensures that every reaction has the ability to identify the target organism if it occurred as within the amplified samples.

The best way to determine the likelihood of detecting lynx if present, however, is to carry out the survey in areas where lynx are known to exist and thereby directly test the efficacy. In general, in those areas where we know lynx exist, we obtain samples in a single year, and where we have sampled for multiple years (Okanogan, Lolo), we obtain samples each year (Table 2). Only in the Bridger/Teton, an area with very few lynx (Squires 2002; Section 5, Squires and Laurion 2000), have we so far failed to detect lynx. In 2001, we added a test grid in northern Maine, and at this time (01/02) have not analyzed those samples. At the conclusion of the survey, we will evaluate the results from the test grids, determine the likely efficacy of the survey, and evaluate the need for further survey efforts.

Survey changes and ongoing research

During the first 3 years, we modified the survey in a variety of minor ways to address problems that were encountered in the field (Hanvey 2002; Section 3). For instance, we found that people were having difficulty seeing hairs on our original rub-pads. We therefore changed the color and texture of the carpet to make seeing hair easier, and include a magnifying glass in each kit. We have found that hair samples from lynx can

often be obtained if snow tracks are followed to a day bed or kill site, and that we can frequently amplify DNA from these samples. We are still collecting data, evaluating the efficacy of this method, and determining whether we can identify individuals from samples collected in this manner. However, the method appears to have great potential, and we now follow all encountered lynx tracks and collect hair samples when conducting follow-up snow track surveys (Squires 2002; Section 5).

Cited literature not included in Sections 1-5

Apps, C. D. 2000. Space-use, diet, demographics, and topographic associations of lynx in the southern Canadian rocky mountains: a study. pgs 351-371 in Ruggiero et al. editors. Ecology and conservation of lynx in the United States. University Press of Colorado, Boulder Colorado.

Diefenbach, D. R., M. J. Conroy, R. J. Warren, W. E. James, L. A. Baker, and T. Hon. 1994. A test of the scent-stations survey technique for bobcats. *Journal of Wildlife Management* 58:10-17.

Foran, D. R., K. R. Crooks, and S. C. Minta. 1997. Species identification from scat: an unambiguous genetic method. *Wildlife Society Bulletin* 25:835-839.

McKelvey^a, K. S., K. B. Aubry, J. K. Agee, S. W. Buskirk, L. F. Ruggiero, and G. M. Koehler. 2000. Lynx conservation in an ecosystem management context. pgs 419-442 in Ruggiero et al. editors. Ecology and conservation of lynx in the United States. University Press of Colorado, Boulder Colorado.

McKelvey^b, K. S., K. B. Aubry, and Y K. Ortega. 2000. History and distribution of lynx in the contiguous United States. pgs 207-264 in Ruggiero et al. editors. Ecology and conservation of lynx in the United States. University Press of Colorado, Boulder Colorado.

Riddle, A. E., K. L. Pilgrim, L. S. Mills, K. S. McKelvey, and L. F. Ruggiero. Identification of mustelids using mitochondrial DNA and non-invasive sampling. Submitted for review to Conservation Genetics.

Sargeant, G. A.; D. H. Johnson, and W. E. Berg, 1998. Interpreting carnivore scent-station surveys. *Journal of Wildlife Management* 62:1235-1245.

Squires, J. R. and T. Laurion. 2000. Lynx home range and movements in Montana and Wyoming: preliminary results. pgs 337-349 in Ruggiero et al. editors. Ecology and conservation of lynx in the United States. University Press of Colorado, Boulder Colorado.

Weaver, J. L. 1997.. Lynx survey and habitat assessment, Kootenai National Forest, Montana. Year 1 Progress Report, June 1996-May 1997. Unpublished report.

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.

Avoiding false positive results	Detecting lynx when present
<p>Geographic range tests of DNA methods Test results consistent</p> <p>Blind tests of DNA methods 100% success</p> <p>Quality controls in the lab Careful documentation of samples, reactions Positive and negative controls on each reaction Total separation between extraction and PCR</p> <p>Follow-up surveys for all lynx identifications outside of test grids</p>	<p>Use of a method that allows representative surveys of roadless areas.</p> <p>Testing the efficacy of the method In Kluane lynx detected on 45% of transects We use the best lure tested</p> <p>Saturation of the sample areas with 125 stations in 25 transects</p> <p>Conducting the survey for 3 years If protocol is not followed, the local survey doesn't count towards the 3 years</p> <p>Complete standardization of all materials and training used in the survey</p> <p>Geographic range tests of DNA methods Test results consistent</p> <p>Blind tests of DNA methods 100% success</p> <p>Multiple DNA extractions if PCR is unsuccessful About 80% amplification rate</p> <p>Positive controls on every reaction</p> <p>Running multiple test grids to directly evaluate survey efficacy</p>

Table 2. Positive lynx hits from the National Lynx Survey 1998, 1999, 2000; the unauthorized, planted lynx samples from the Gifford-Pinchot and Wenatchee are omitted. Blank spaces indicate where years in which the surveys were not run. 1998 was only a pilot study year. In the Bridger/Teton National Forest, the 2000 sample suffered quality control problems due to a mid-season crew turnover. According to our protocol, that year's survey does not count towards the 3-year survey protocol, and is not included.

	1998	1999	2000
Lolo test	1	2	11
Okanogan test	3	5	1
Boise NF		2	0
Shoshone NF		2	
Glacier NP			8
WA-DOT			3
Bridger/Teton		0	NA
Total	4	11	23