Summary of the Superior National Forest's Canada lynx (*Lynx canadensis*) DNA database and population monitoring 2023 February 12, 2024

DAN RYAN – USDA Forest Service, Superior National Forest, Laurentian Ranger District, 318 Forestry Rd., Aurora, MN 55705
DAVE GROSSHUESCH – USDA Forest Service, Superior National Forest, Kawishiwi Ranger District, 1393 Hwy 169, Ely, MN 55731
CHERON FERLAND - USDA Forest Service, Superior National Forest, Supervisors Office, 8901 Grand Avenue Place, Duluth, MN 55808
KRISTY PILGRIM – USDA Forest Service, National Genomics Center for Wildlife and Fish Conservation, 800 E. Beckwith, Missoula, MT 59801
SHANNON BARBER-MEYER – Pacific Whale Foundation
300 Ma'alaea Road Ste 211, Wailuku, HI 96793

Introduction

Snow tracking and other methods used to obtain genetic samples have confirmed presence of Canada lynx (*Lynx canadensis*) across northeastern Minnesota since December 2000. In 2008, the Superior National Forest (Superior NF) created, and continues to maintain, a database of genetically confirmed Canada lynx (hereafter lynx) to document their occurrence, persistence and reproduction in Minnesota. Genetic samples (typically scat but also hair and tissue) have been collected primarily as part of the Superior NF's survey and monitoring program. Also included in this database are samples collected during an independent genetic research project, a radio telemetry project, mining project surveys, and from specimens that were surrendered to resource agencies (e.g., from animals that had been trapped, shot or killed in vehicle collisions). These samples were submitted to the USDA Forest Service Rocky Mountain Research Station's National Genomics Center for Wildlife and Fish Conservation for testing. Samples that were identified as lynx using mitochondrial DNA analysis were further evaluated using nuclear DNA analysis methods to determine sex (Pilgrim et al. 2005) and individual identification. Further testing was used to determine Canada lynx-bobcat (*Lynx rufus*) hybridization (Schwartz et al. 2004). Field observations combined with DNA analysis have been used to document lynx reproduction.

Summary

The current database contains 2,943 samples that have been submitted for DNA testing. Mitochondrial DNA analysis has identified 2,813 (95.6%) of them to species, 2,533 (86.3%) of those as lynx. Nuclear DNA analysis has determined 611 unique lynx genotypes, 290 female (47.5%), 319 male (52.2%) and 2 of indeterminable sex (0.3%).

Reproduction has been documented on the Superior NF every year since 2001. Since 2010 we have identified a minimum of 84 family groups producing a total of 170 presumed kittens, 93 female (54.7%) and 77 male (45.3%) (Figure 3). Of the 525 individuals that were identified prior to this survey season and were not originally detected as a result of a mortality, 163 (31.0%) are known to have persisted into a second year. Fifteen individuals have persisted for over 6 years, the longest, a female, over 10 years.

During the 2022/2023 survey season 317 samples were collected and submitted for testing. Two hundred and ninety-eight (94.0%) were identified as lynx, 274 of those (86.4%) were able to be genotyped identifying 118 individuals: 55 female and 63 male. Sixty-five individuals (55.0%: 22 female and 18 male) were previously recorded in this database (recaptures), and 53 individuals (45.0%: 20 female and 33 male) were new to the database this year including 15 kittens.

Field observations suggest that there were at least 10 family groups with as many as 18 kittens found in the survey area. DNA analyses confirm 9 family groups with 15 individuals (8 female, 7 male) genetically consistent with being offspring. Of the 45 individuals detected during the 2022/2023 survey season whose age can be estimated and that were not kittens, 32 (71.1%) have persisted for 2 years or more (Figure 8). Twenty-seven (60.0%) of the 45 have been detected into their third year or more indicating recruitment into the population. Two females have persisted in the survey area for over 9 years. There are 38 individuals new to the database this year whose age could not be estimated.

The DNA database also contains 44 samples that have been identified as F1 Canada lynx-bobcat hybrids. There are 13 unique lynx-bobcat hybrid genotypes, 5 (38.5%) female and 8 male (61.5%). One Canada lynx-bobcat hybrid, a male, was first detected in February 2011 and was at least 9 years old when he was trapped in December of 2019. There were no Canada lynx-bobcat hybrids detected during the 2022/2023 survey season.

The USFWS maintains a “take” database of all reported incidents of take in Minnesota. Between 2001 and 2023 there have been 77 reported incidents of lynx take within the state. Fifty-four of these incidents have resulted in mortalities to the animal. There have been 8 incidents of shooting, 35 trapped, and 20 that have been hit by a car or truck, snowmobile, or a train. There were 14 incidents of mortality where the cause was unknown. Two of the statewide reported instances fell under the allowable take within the Superior NF’s Forest Plan 2011 Incidental Take Statement issued by the USDA Fish and Wildlife Service (USDI FWS 2011, USDI FWS 2021).

We continue to work with researchers at the University of North Carolina to refine the dynamic occupancy model as described in Hostetter and Gardner (2016). Initial results of this work were published by Hostetter et al. (2020). We also continue to build on the data set used to generate core area population estimates described in Barber-Meyer et al. (2018) to produce ongoing annual estimates and assess potential trends over time. We have two survey seasons of data implementing the Spatial Capture Recapture (SCR) survey method in 2019/2020 and 2022/2023. A grant has been secured to analyze this data and it should get started in 2024.

This genetics database contains all known samples submitted by the Superior NF to the Wildlife Genetics Laboratory since the year 2000. Other contributors to this database are Chippewa National Forest, Franconia Minerals Corporation, Leech Lake Band of Ojibwe, 1854 Treaty Authority, Minnesota Department of Natural Resources, Natural Resources Research Institute (NRRI) of the University of Minnesota-Duluth, PolyMet Mining Inc., Steve Loch, US Fish and Wildlife Service, US Geological Survey, Voyageur’s National Park and Wolf Ridge ELC.

Canada lynx DNA Database 2000-2023

Survey Effort

For the purposes of this report, the primary survey area is generally considered to be the proclamation boundaries of the Superior National Forest (see attached map). Survey techniques over the years have been predominantly on an ad hoc basis. Survey effort has varied dependent upon funding, personnel availability and suitable snow conditions; biologists usually survey areas on their Districts as time and snow conditions allowed. Prior to 2014 records and GPS tracks were not stringently maintained, nor was there consistency between surveyors in how those tracks were collected. However, as part of a recent research effort by North Carolina State University (NCSU) to develop an occupancy model (see DNA Database Research below) and continued development of a monitoring model, we summarized our survey effort into miles of occupancy surveys and trailing miles (miles in which surveyors were following lynx tracks) during survey seasons 2014/2015 – 2022/2023. During surveys season 2012/2013 and 2013/2014 only the trailing miles were recorded and not included in the NCSU work but are included in Table 1 below. These 2 parameters give an index of survey effort by Superior NF personnel/volunteers in collecting genetic samples for this database.

Year	Occupancy miles	Trailing miles	Samples collected	Number lynx samples	Individuals identified	Unknown Individuals**
2013	NA	41.6	149	122	35	16 (46%)
2014	NA	45.6	198	162	68	45 (66%)
2015	1,970	43.1	135	114	49	24 (49%)
2016	2,044	52.8	127	113	38	7 (18%)
2017	2,279	70.2	144	130	42	7 (17%)
2018	2,601	76.8	210	187	68	26 (38%)
2019	2,064	95.6	157	153	59	18 (30%)
2020*	5,032	131.9	221	209	82	22 (27%)
2021	3,532	61.3	171	145	66	9 (14%)
2022	3,794	80.1	236	211	79	27 (34%)
2023*	5,384	165.6	317	298	118	56 (47%)

Table 1. Survey effort for monitoring and DNA collection.

*2020 and 2023 were intensive survey years made possible through a funding agreement with Polymet Mining, Inc.

** New individuals that were not known to be kittens. Percent of all individuals identified that were new.

Species Identification

To date there are 2,943 samples contained in the database, of which 2,813 (95.6%) have been identified to species (Figure 1). Of the samples for which species results were obtained, 2,533 (90.0%) were identified as lynx. Two thousand one hundred and eighty-seven of those samples (86.3%) were able to be genotyped (meaning an individual identification was made), 346 (13.7%) could not be identified to individual (identified as lynx only). One-hundred thirty of all the samples contained in the database (4.4%) did not contain enough quality DNA to make a species determination.

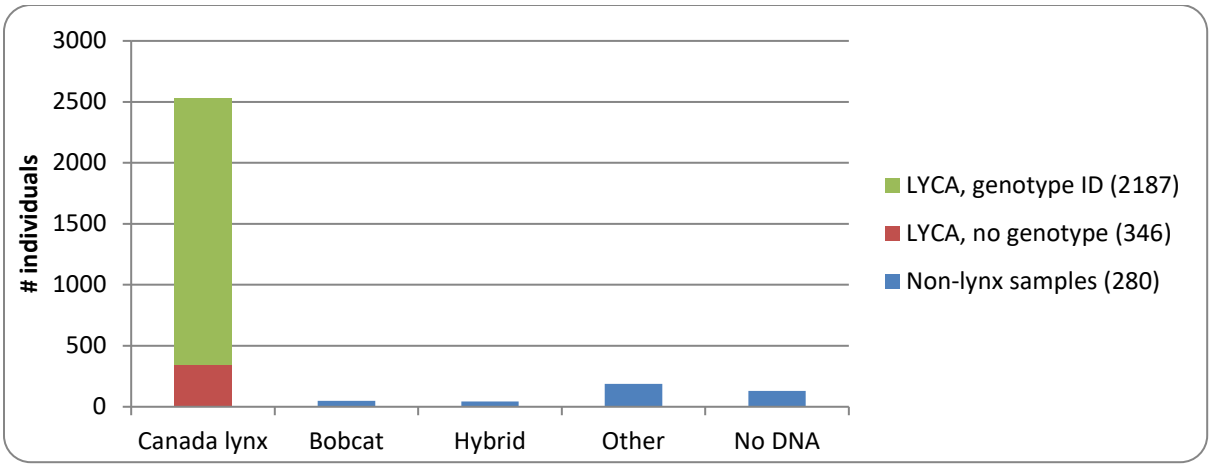

Figure 1. Species identification results 2000-2023 (*n* = 2,813)

Of the 2,533 lynx samples:

- 611 individual lynx genotypes were identified, of which there are:
  - 290 females (47.5%), 319 males (52.2%) and 2 (0.3%) undetermined sex (Figure 2)

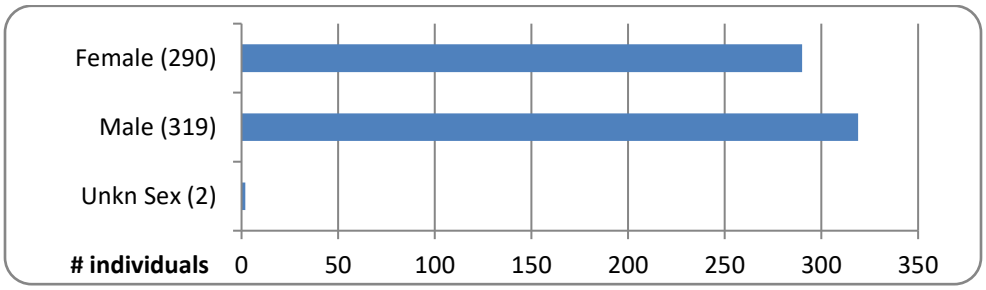

Figure 2. Total Canada lynx individuals detected 2000-2023 (*n* = 611)

Reproduction

Field observations and DNA analysis have been used to document reproduction of lynx in Minnesota every year since 2001. Areas that contain tracks of family groups (adults and kittens (animals presumed to be <1 year old)) are continually monitored during the survey season to attempt to collect DNA from all individuals. However, genetic samples from each member of the family group may not always be obtained, nor is every family group likely detected each year, so numbers presented here under-represent the total numbers of family groups and kittens. These figures represent only those family groups and kittens for which DNA analysis has shown a parent-offspring relationship.

Field observations of family groups combined with DNA analysis since 2010 have identified a minimum of 84 family groups producing a total of 170 presumed kittens, 93 female (54.7%) and 77 male (45.3%) (Figure 3). Overall, 216 kittens have been identified since 2001 from 61 different mothers and 40 different fathers.

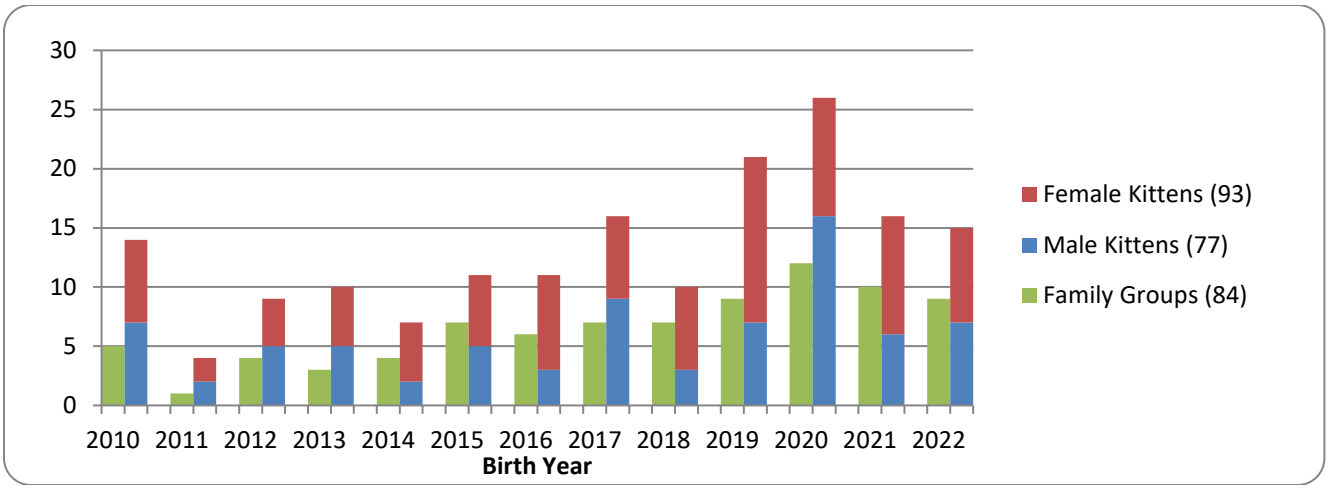

Figure 3. Family groups and known annual reproduction, birth years 2010-2023

Persistence

Snow tracking and other methods used to obtain genetic samples have confirmed persistence of lynx on the Superior NF and elsewhere across northeastern Minnesota since January 2002 (Figure 4). Of the 525 individuals that were identified prior to this survey season and were not originally detected as a result of a mortality, 163 (31.0%) are known to have persisted into a second year. There is 1 individual, a female, which has persisted for over 10 years who was last detected in February 2021. When first detected she was of an unknown age so may be older than the 10 years of persistence in the area. There are 15 individuals that have been detected over 6 years or more since monitoring began.

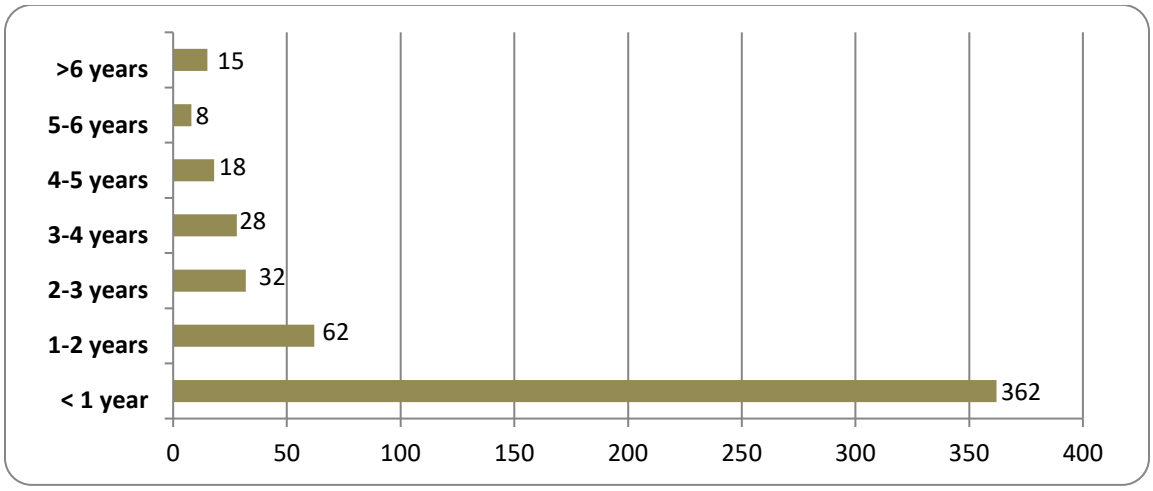

Figure 4. Persistence of individuals 2000-2023 (*n* = 525)

Survivorship/Recruitment

Although not an accurate representation of true kitten survivorship, the persistence of kittens in the database into their second year and longer can be an index of survivorship and recruitment into the overall population. Of the 204 individuals that have been identified as kittens (113 female and 91 male) in the database prior to 2022/2023 and were not initially detected as a mortality, 64 of them (31.4%: 35 female and 29 male) have been recaptured into their second year or beyond and are assumed to have been recruited into the northeastern Minnesota (NE MN) sub-population (Figure 5).

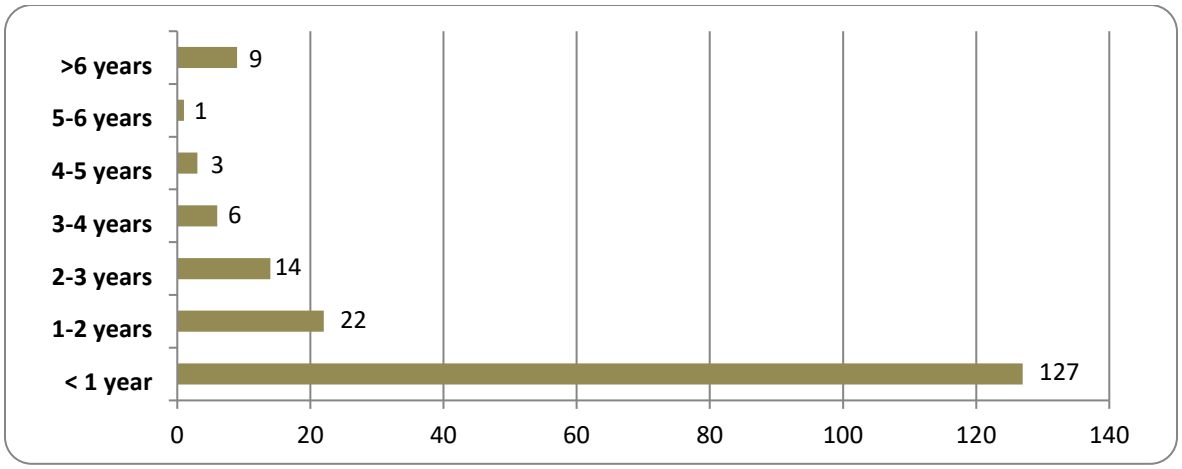

Figure 5. Persistence/recruitment of kittens 2001-2023 (*n*=204)

It is presumed that not all surviving kittens are detected within the survey area, and other surviving kittens emigrate to other sub-populations. It is not uncommon to find the second year "kittens" in their mother's home range, and even interacting with the new families of their mothers.

Distribution and Dispersal

Lynx detections are distributed over 11 counties in Minnesota and Canada. The majority occur in St. Louis, Lake and Cook counties in northeastern Minnesota where essentially all field data collection efforts have been focused (Table 2). The attached map at the end of the report represents locations of samples genetically confirmed as lynx within the State of Minnesota since they were listed as a threatened species under the Endangered Species Act (March 24, 2000).

County	No. of individual lynx detections by County	% of total individual lynx (*n*=609)
Cook	121	19.9
Lake	353	58.0
St. Louis	117	19.2
All other	18	3.0

Table 2. Distribution of individual lynx detections in Minnesota by county

Dispersal and movement of individuals both within and out of the core survey and monitoring area has been documented. Maximum movement distance is 196 miles for males and 46 miles for females.

2022-2023 Monitoring Results

Species Identification

Three hundred and seventeen samples were collected and submitted for analysis during the period of May 2022 through April 2023. Two hundred and ninety-eight samples (94.0%) were identified as lynx, and genotypes were obtained from 274 of these identifying 118 unique individuals, 55 female (46.6%) 63 male (53.4%) (Figure 6). Sixty-five individuals (55.1%: 35 female and 30 male) were previously

recorded in this database (recaptures), and 53 individuals (44.9%: 20 female and 33 male) were new to the database this year including 15 kittens.

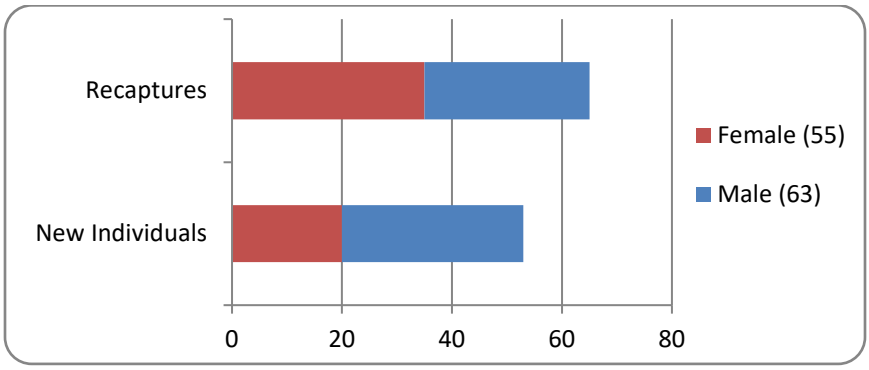

Figure 6. Canada lynx individuals detected 2023 (*n* = 118)

Reproduction

Field observations during the winter 2022-2023 survey season suggest that there were at least 10 family groups with as many as 18 kittens found in the survey area. DNA analysis did confirm 9 family groups in the survey area with a total of 15 kittens genotyped; 8 female (53.3%) and 7 male (46.7%), genetically consistent with being offspring (Figure 7). Family groups are determined by comparing the genotypes of samples collected from animals using the same area for parent-offspring relationships. For the 9 family groups we collected genetic samples from, litter sizes ranged from 1-2 kittens, with an average of 1.7 kittens/litter.

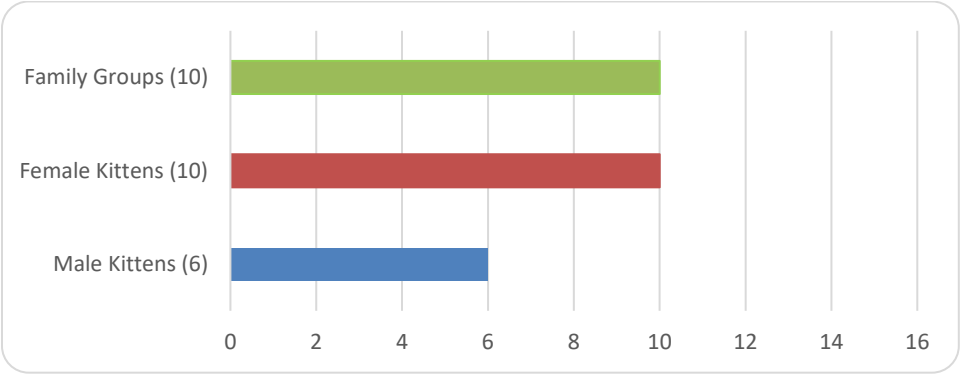

Figure 7. Family groups and known reproduction birth year 2022

Three of the mothers are from previous Minnesota born litters. Seven of the 9 family groups had a father identified within the database.

Persistence

Persistence age distribution of the current year's individuals may be representative of the overall age structure of the NE MN sub-population. Of the 45 individuals detected during the 2022/2023 survey season whose age can be estimated and that were not kittens, 32 (71.1%) have persisted for 2 years or more (Figure 8). Six of these individuals have been detected over 6 years or more. There are two breeding female that has persisted in the database and remained in the survey area for over 9 years. There are 38 individuals of an unknown age that are new to the database this year and are therefore

not considered in persistence analysis. These 38 were travelling alone, tied to a family group but were unrelated, or were part of a family group for which parent-offspring determinations could not be made.

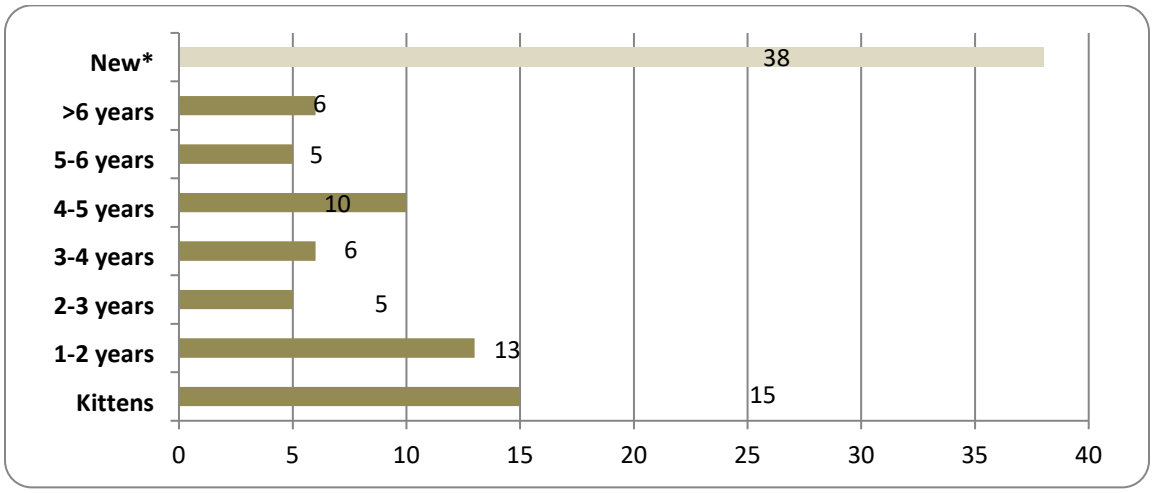

Figure 8. Persistence of individuals 2023 (*n* = 80)
* Individuals new to the database of an unknown age

Canada Lynx – Bobcat Hybridization

A Canada lynx-bobcat hybrid was first detected in Minnesota from a tissue sample obtained from an animal killed by a train in December 2001. This sample along with 2 other Minnesota samples obtained in November and December 2002 represent the first verified hybridization between Canada lynx and bobcat in the wild (Schwartz et al. 2004). Subsequently, genetic samples from other areas have been tested for hybridization resulting in additional hybrid detections in the State of Maine and New Brunswick, Canada (Homyack et al., 2008). The earliest recorded hybrid in Minnesota comes from a specimen that was reportedly harvested in 1997. All samples submitted that are identified as lynx or bobcat are routinely tested for possible hybridization. As part of this monitoring effort, hybrids have been detected in Cook, Itasca, Lake and Pine Counties in Minnesota, and in Polk County, Wisconsin. One hybrid, a male, had persisted for almost 9 years before he was trapped in December of 2019. All hybrids are F1 and thus far there have been no known offspring from either male or female hybrids. All are a result of female lynx and male bobcat mating.

Although not annually, Canada lynx-bobcat hybrid animals have been detected on a regular basis (last detection was December 2019) during surveys for lynx. To date the database contains 44 hybrid samples. Forty-three of these have been genotyped representing 13 individuals, 5 female and 8 male (Figure 9).

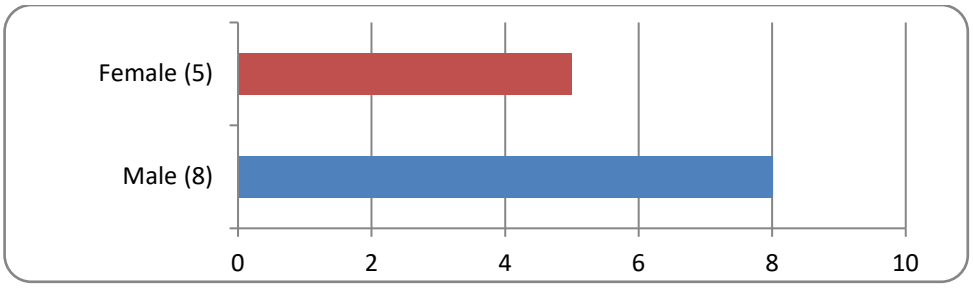

Figure 9. Total Canada lynx-bobcat hybrid individuals detected (*n* = 13)

Take

Section 9 of the Endangered Species Act (ESA) prohibits the take of endangered and threatened species without special exemption. Take is defined in Section 3 (19) of the Endangered Species Act as "to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct" of a federally listed species (16 U.S.C. Chapter 35 Section 1532). The USDI Fish and Wildlife Service (USFWS) maintains a database of reported incidents of "take" of Canada lynx that have occurred in Minnesota since the year 2001. There have been 77 incidents of reported take of Canada lynx since 2001 in Minnesota (Figure 10). Fifty-four of these incidents have resulted in mortalities to the animal. There have been eight incidents of shooting (all mortalities), 35 trapped (15 mortalities and 20 released alive), and 20 that have been hit by a car or truck, snowmobile or a train (17 mortalities, 3 unknown outcomes (carcasses not recovered)). There were also 14 incidents of take that resulted in the mortality of an animal but the cause is unknown. These include cases of likely predation, recovery of decomposed animals or remains, or the recovery of a radio collar that was no longer attached to a study animal.

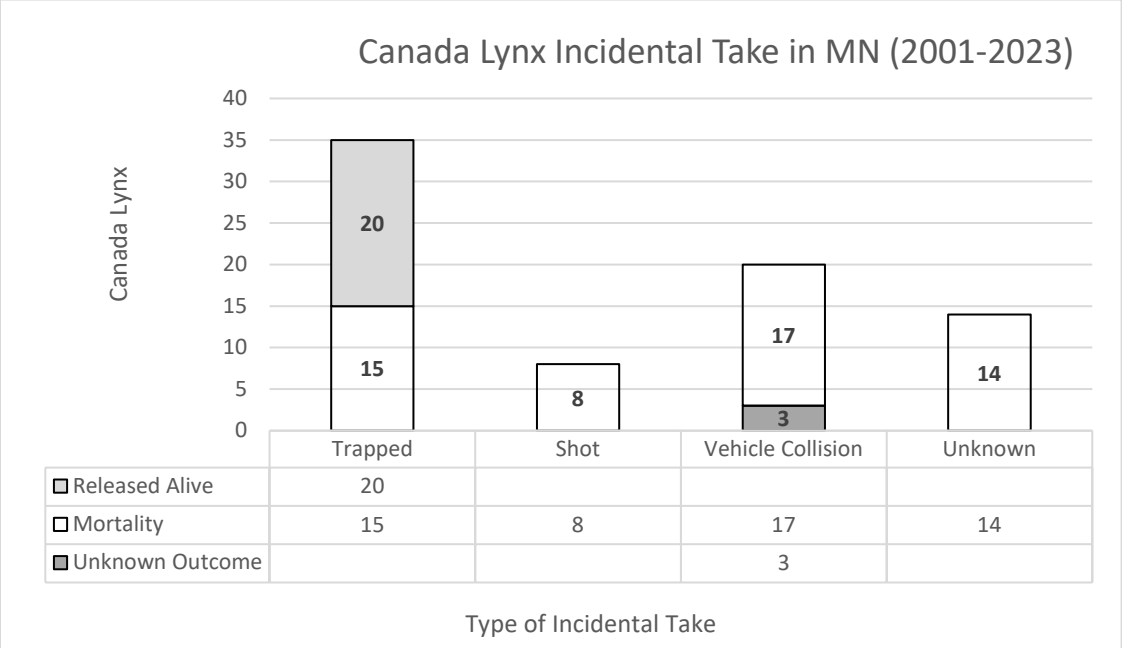

Figure 10. Reported Canada lynx take in Minnesota 2001-2023 by type and outcomes (*n* = 77)

Incidental Take - Superior National Forest Plan Implementation

Under the terms of ESA, taking that is incidental to and not intended as part of an agency action is not considered to be prohibited taking under the Act provided that such taking is in compliance with the terms and conditions of an Incidental Take Statement issued by USFWS. The risk of incidental take of Canada lynx is not completely eliminated by provisions in the Superior National Forest Land and Resource Management Plan (Forest Plan). On-going implementation of the Forest Plan is expected to result in the incidental take of Canada lynx. Historically, take that occurred due to vehicle collisions on **all roads on all ownerships within the proclamation boundary of the Forest** was considered incidental to the implementation of the Forest Plan (USDI FWS 2011). According to the original Incidental Take Statement (ITS) of the 2011 Biological Opinion (which "revises and supplants, the 2004 Biological Opinion"), "The Service expects no more than one lynx would be taken annually on the Superior National Forest and no more than 10 would be taken over the [generally] 10-year life of the Forest Plan due to vehicle collision on all roads on all ownerships within the Superior National Forest proclamation boundary" (USDI FWS 2011). In 2021, the USFWS issued a statement which clarified that **"Incidental take due to vehicle collisions will only be counted for the ITS when it occurs on USFS jurisdiction roads."** (USFWS 2021)

Since 2011 there has been one reported incident of lynx take due to vehicle collisions within the Superior NF's proclamation boundaries that took place on USFS jurisdiction roads. Additionally, there was one reported instance of lynx mortality along a USFS jurisdictional road that took place in 2005 prior to the current ITS. There has also been one mortality due to snowmobile on the National Forest.

In accordance with the 2011 Biological Opinion Reasonable and Prudent Measures, the Superior National Forest is required to "Document and report to the Service annually any known lynx mortality within the National Forest Proclamation boundaries in Minnesota due to vehicle collision, accidental trapping, or poaching". All reports of lynx mortality are sent directly and immediately to USFWS when they are received by Forest Service personnel.

DNA Database Research

Non-invasive genetic capture-mark-recapture

In 2018, we published a paper in the Canadian Wildlife Biology and Management journal that utilized these DNA data to estimate abundance, trend and density estimates for lynx in northeast Minnesota (Barber-Meyer et al. 2018). Since then, we have continued to use the analytical framework as described in this publication to monitor lynx populations in northeastern Minnesota. Figure 11 shows a population estimate chart generated from these data updated with information from 2022/2023 regarding the larger core areas consistently surveyed since 2015. Figure 12 shows a population estimate chart reflecting the smaller core areas that have been consistently surveyed since 2012.

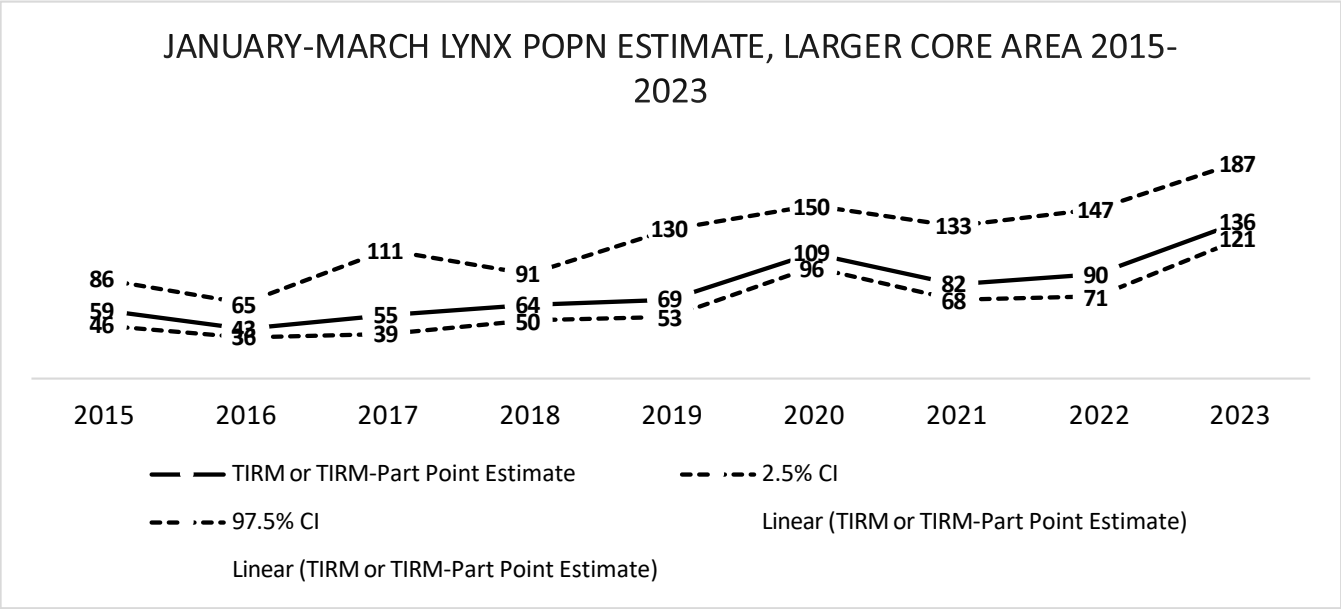

Figure 11. Lynx population point estimates and 95% confidence intervals generated using capture-mark-recapture analysis of genetic samples for larger core areas consistently surveyed from January-March of 2015-2023 in the Superior National Forest, Minnesota, USA. Adapted from Barber-Meyer et al. 2018.

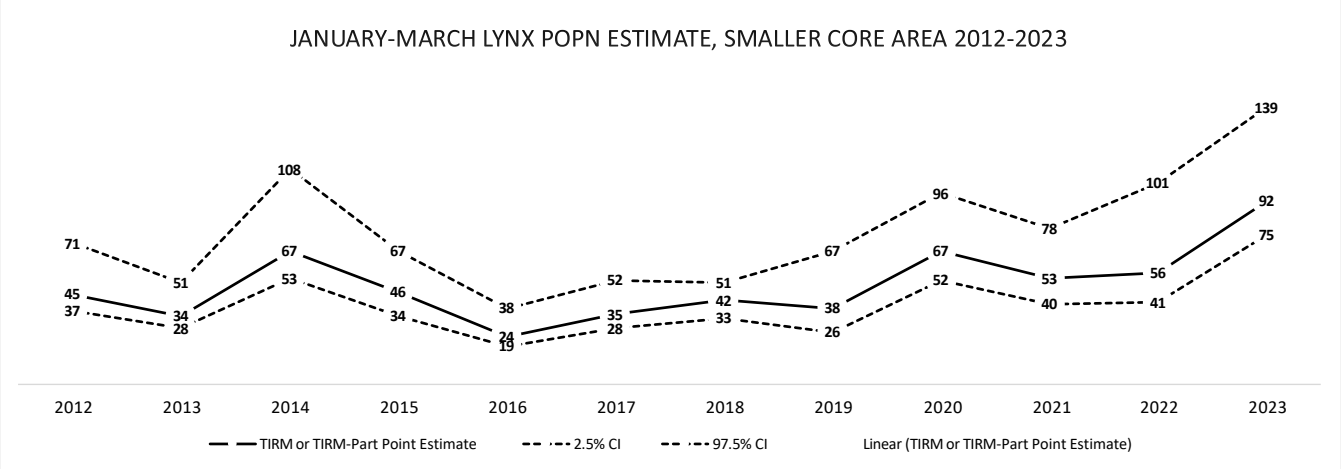

Figure 12. Lynx population point estimates and 95% confidence intervals generated using capture-mark-recapture analysis of genetic samples for smaller core areas consistently surveyed from January-March of 2012-2023 in the Superior National Forest, Minnesota, USA. Adapted from Barber-Meyer et al. 2018.

Multi-Season Occupancy Surveys

In 2016 we collaborated with researchers from North Carolina State University and USDI Fish and Wildlife Service to develop an occupancy model for lynx in northeastern Minnesota (Hostetter and Gardner 2016). We continued to work with researchers to improve our methods which resulted in the publication of the results in Diversity and Distributions in March 2020 (Hostetter et al. 2020). We continue to work with the authors to refine the model and resulting outputs adding probability analysis for colonization and persistence of cells used in the occupancy analysis. The maps in Figure 13 show

the outputs of this model using survey effort and number of detections from survey seasons 2014/2015 to 2022/2023.

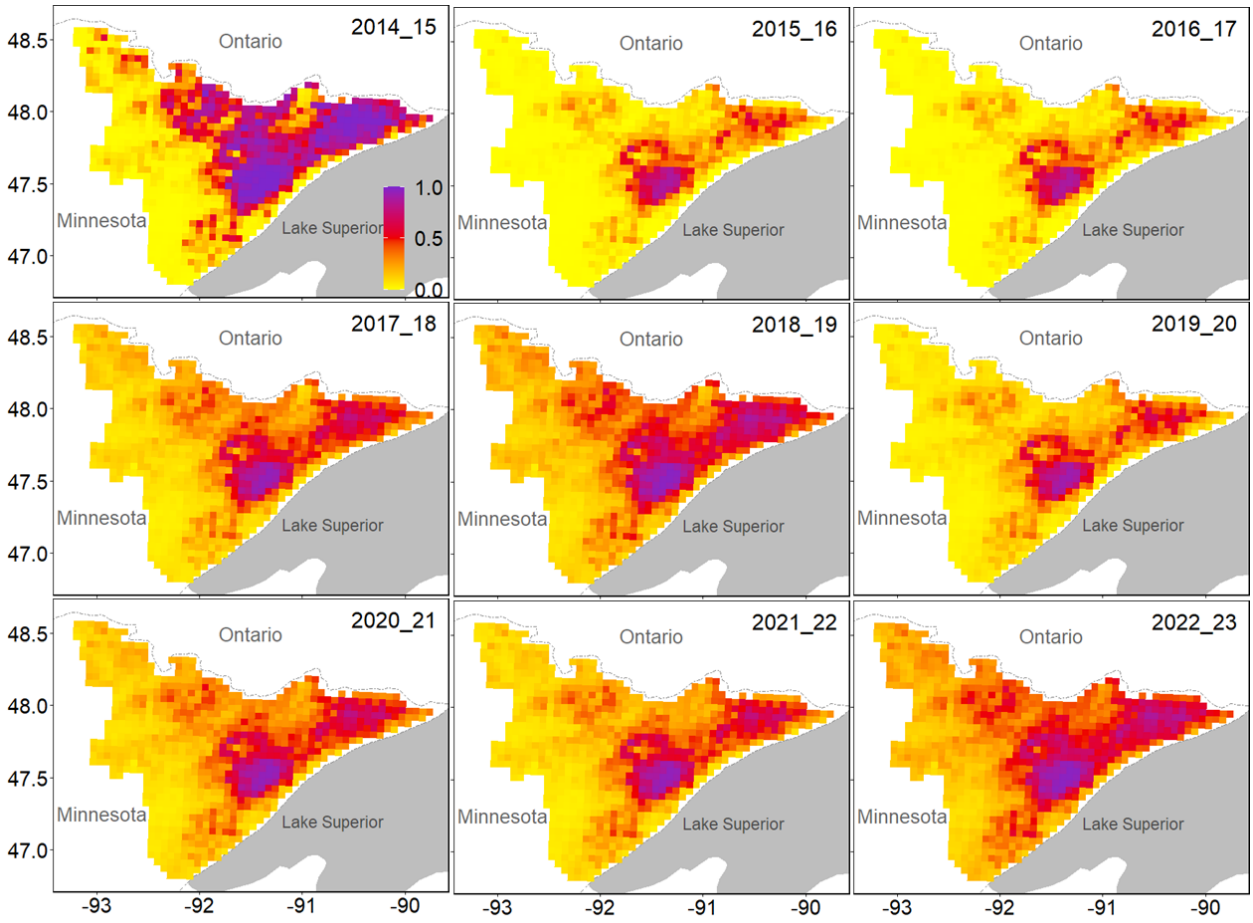

Figure 13. Lynx winter-specific occupancy probabilities (medians). Grid cells are 5×5 km and encompass Superior National Forest and designated lynx critical habitat in Minnesota, USA

Figure 14 shows that colonization probability (probability an unoccupied grid cell becomes occupied) was low in all years (<0.20) while persistence probability (probability an occupied grid cell remains occupied) was substantially lower in the first (median <0.30) relative to subsequent years (medians >0.90).

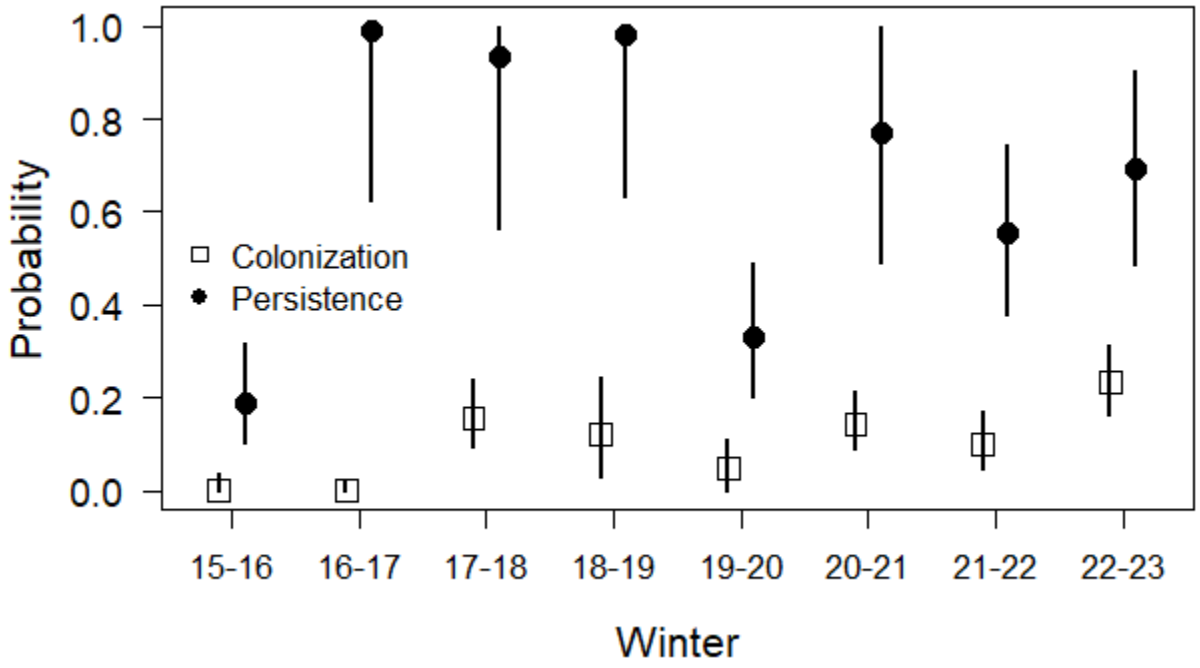

Figure 14. Annual lynx average colonization and persistence probabilities (median, 95% credible intervals) across Superior National Forest and designated lynx critical habitat in Minnesota, USA.

Figure 15 shows the proportion of those cells that were surveyed as being occupied during any given survey season.

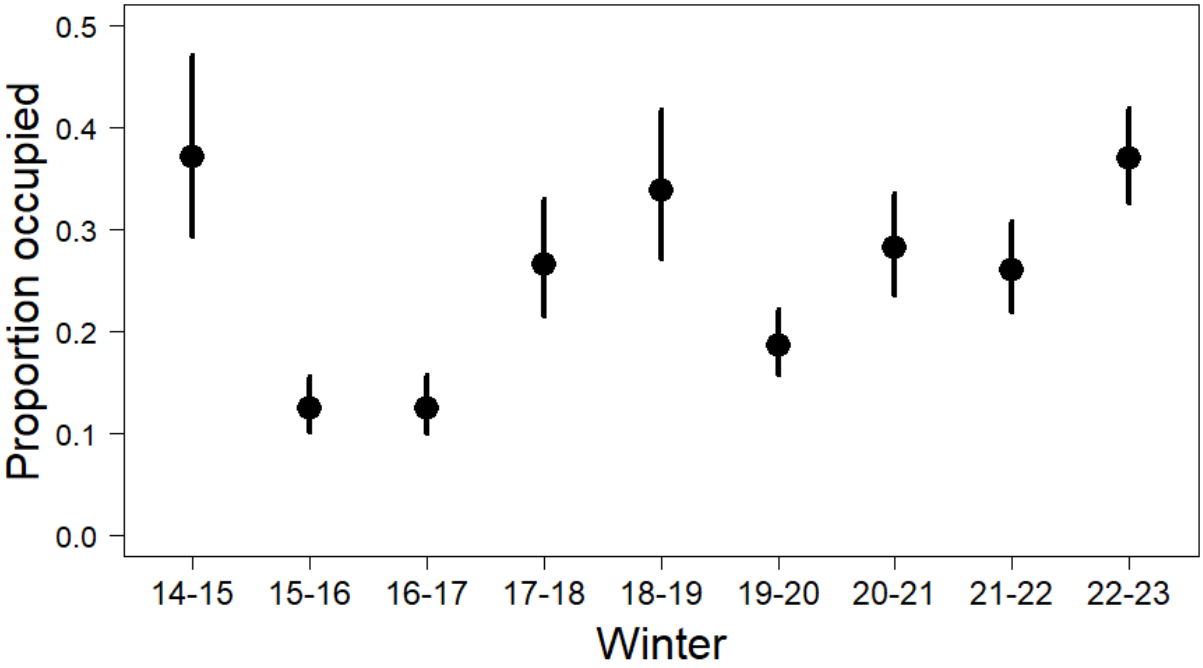

Figure 15. Proportion of sites occupied (median, 95% credible intervals)

Acknowledgements

Thanks to this year's surveyors; H. Becker, T. Brannock, N. Malinowski, Gary Olson, R. Pennesi, P. Robertsen, S. Swanson, J. McDonald, B. Peterson, and D. Welch. We thank all those folks that have surveyed and collected DNA over the years. They've put on a lot of miles across some difficult country in some tough conditions with what some would call the inexplicable ambition to collect a turd. Thanks to the University of Minnesota Wildlife Club that supplied 16 students over two weeks to assist with lynx trailing. Fond du Lac Tribal and Community College students also assisted with trailing and sample collection. Thanks to Dr. M. Schwartz and I. Ortloff at the RMRS National Genomics Center for all their work and support in conducting the DNA analysis. Thank you to Dr. N. Hostetter for his continued work in conducting the analyses of the occupancy survey data. Thanks to T. Smith and M. Kosterman at the USFWS Field Office in Bloomington, MN for their work in gaining additional funding to support this work over the years. Additional funding to conduct surveys and DNA analysis was provided through an agreement with Polymet Mining, Inc. during survey years 2020, 2021, 2022 and 2023. And thanks to Steve Loch for his total dedication to this project from the very beginning.

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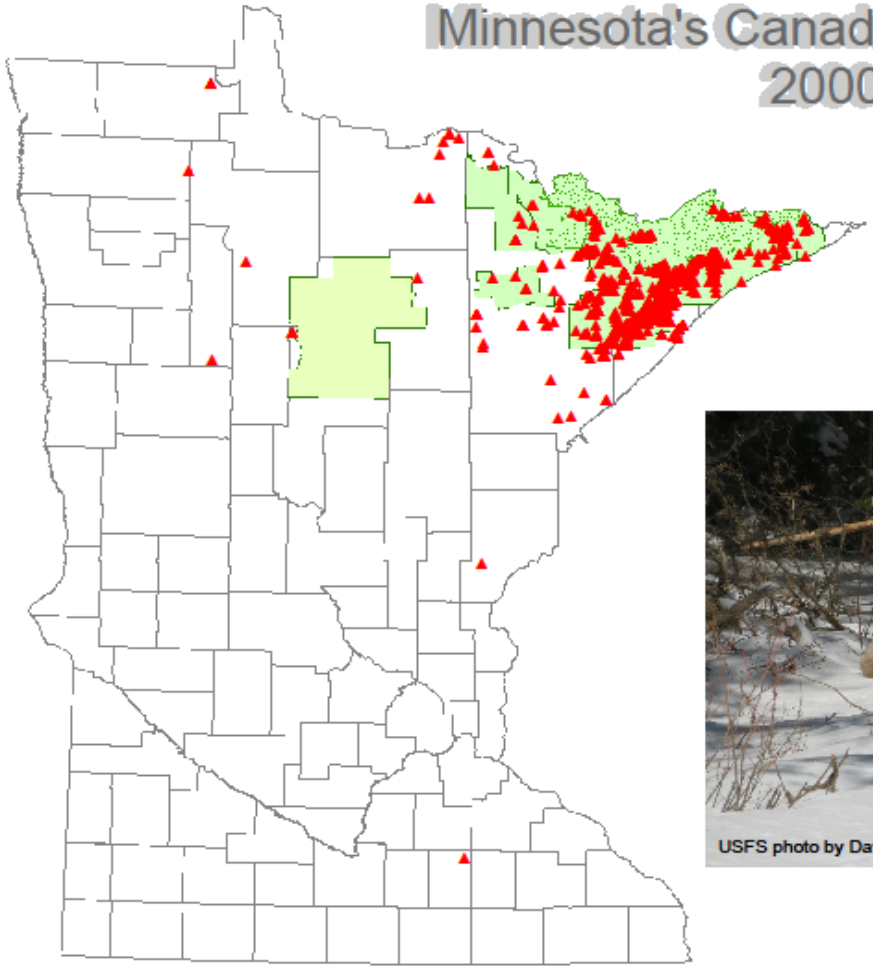
USDI Fish and Wildlife Service. 2021. Email from M. Kosterman to C. Ferland on 3/23/2021, Re:(EXTERNAL) RE: Forest Plan ITS.

Minnesota's Canada lynx DNA locations 2000-2023



- Lynx DNA locations
- Superior NF
- BWCAW
- Chippewa NF

USFS photo by Dave Grosshuesch

50 25 0 50 Miles

tjc 9/27/2021