

# Summary of the Superior National Forest's 2017 Canada lynx (*Lynx canadensis*) DNA database

October 12, 2017

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## 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 Laboratory 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 within the State since 2002.

## Summary

The current database contains 1,624 samples that have been submitted for DNA testing. Mitochondrial DNA analysis has identified 1,325 of them (81.6%) as lynx. Nuclear DNA analysis has determined 333 unique lynx genotypes, 162 female (48.4%), 169 male (51.3%) and 2 of indeterminable sex. Since 2010, 30 family groups have been identified producing 65 kittens that survived to the winter following their birth, 36 female (55.4%) and 29 male (44.6%). Of the 302 individuals that were not originally detected as a result of a mortality, 67 (22.2%) are known to have persisted into a second year. Five individuals (1.7%: 3 female and 2 male), have persisted for over 6 years.

During the 2016-2017 survey season 144 samples were collected and submitted for testing. One-hundred thirty (90.3%) were identified as lynx and 42 unique genotypes were determined, 24 female (57.1%) and 17 male (40.5%) and 1 of indeterminable sex. Twenty-three individuals (54.8%: 14 female and 9 male) were previously recorded in this database (recaptures), and 19 individuals (45.2%: 10 female, 8 male and 1 indeterminable sex) are new to the database this year. Field observations suggest that there were at least 8 family groups with as many as 17 kittens found in the survey area. DNA analysis confirm 6 family groups with 11 individuals (8 female, 3 male) genetically consistent with being offspring. Of the 31 individuals identified that were not kittens, 24 (77.4%) have persisted in to their second year, 11 (35.5%) have persisted into their third year or more. Three individuals (9.7%:1

male and 2 female) have persisted on the Forest for over 6 years. There are 6 individuals new to the database this year whose age could not be determined.

To date there have been 70 reported incidents of lynx “take” in the database maintained by USFWS. Fifty-three of these incidents have resulted in mortalities to the animal. There have been 7 incidents of shooting (all mortalities), 34 trapped (17 mortalities and 17 released alive), and 13 that have been hit by a vehicle or a train (all mortalities). There were also 16 incidents of take that resulted in the mortality of an animal but the cause is unknown.

The database also contains 43 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 detected during this year’s surveys; a recapture who was first detected in February 2011 and has persisted for over 6 years.

This 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 Steve Loch, Natural Resources Research Institute (NRRI) of the University of Minnesota-Duluth, Franconia Minerals Corporation, PolyMet Mining Corporation, Minnesota Department of Natural Resources, Voyageur’s National Park, US Fish and Wildlife Service, Chippewa National Forest, US Geological Survey, Wolf Ridge ELC and Leech Lake Band of Ojibwe.

# Canada lynx DNA Database 2000-2017

## 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. However, as part of a recent research effort by North Carolina State University (NCSU) to develop an occupancy model (see Current Research below), we summarized our survey effort in to miles of occupancy surveys and trailing miles (miles in which surveyors were actually following lynx tracks) during survey seasons 2014/2015, 2015/2016, and 2016/2017. 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 in collecting genetic samples for this database.

Year	Occupancy miles	Trailing miles	Samples collected	Number lynx samples	Individuals identified
2013	NA	41.6	149	122	35
2014	NA	45.6	198	162	68
2015	1,970	43.1	135	114	49
2016	2,044	52.8	127	113	38
2017	2,279	70.2	144	130	42

Table 1. Survey effort for DNA collection.

## Species Identification

To date there are 1,624 samples contained in the database, of which 1,546 (95.2%) have been identified to species (Figure 1). Of the samples for which species results were obtained, 1,325 (85.7%) were identified as lynx. One-thousand one-hundred of those samples (83.0%) were able to be genotyped (meaning an individual identification was made), while 225 (17.0%) did not amplify (identified to species only). Seventy-eight (4.8%) did not contain enough quality DNA to make a species determination.

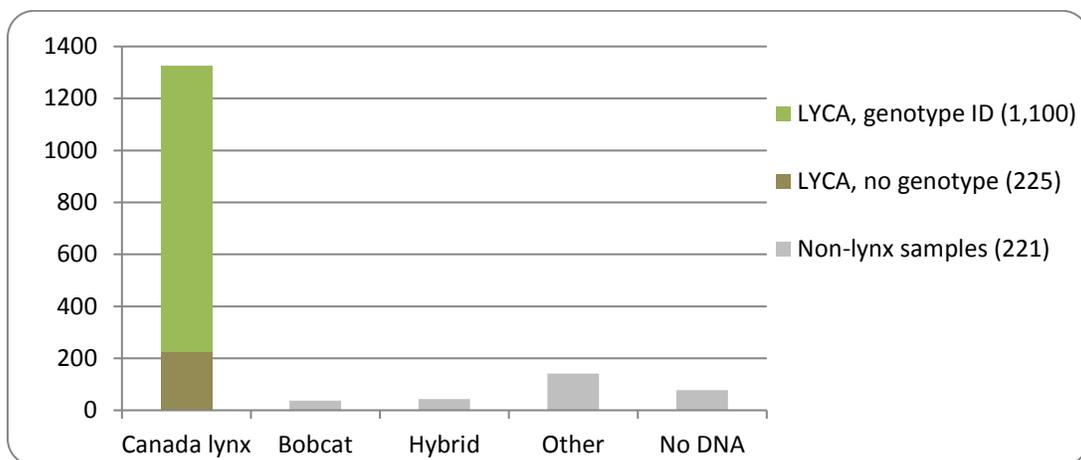


Figure 1. Species identification results 2000-2017 (n = 1,546)

Of the 1,325 lynx samples:

- 333 individual lynx genotypes were identified, of which there are
  - 169 females (50.8%), 162 males (48.6%) and 2 (0.6%) undetermined sex (Figure 2)

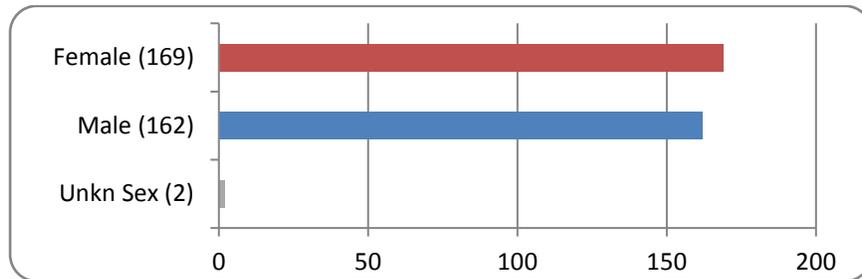


Figure 2. Total Canada lynx individuals detected 2000-2017 ( $n = 333$ )

## Reproduction

Field observations and DNA analysis have been used to document reproduction of lynx in Minnesota since 2002. Areas that contain tracks of family groups (adults and kittens (animals presumed to be <1 year old)) are continually monitored during the survey season in an effort 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 likely 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 31 family groups producing a total of 65 presumed kittens, 36 (55.4%) female and 29 (44.6%) male (Figure 3). Overall, 103 kittens have been identified from 27 different mothers. Seventy-two of the 103 have an identified father within the database, from 17 different fathers.

Of note in these data are: Mother S390 (detected 4 winters 2012-2015) has had at least 13 kittens from 4 litters; mother L31 (detected 5 winters 2005-2009) has had at least 11 kittens from 3 litters. Father L28 (detected as an adult 5 winters 2007-2011) has sired at least 15 kittens over 5 years with 5 different mothers; father L10 (detected 3 winters 2003-2005) has sired at least 10 kittens from 3 litters.

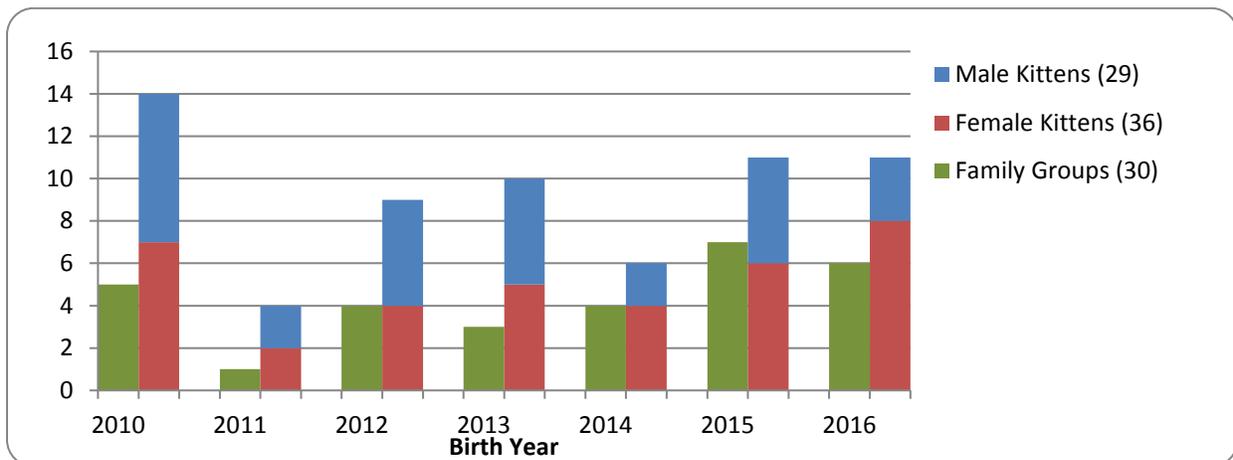


Figure 3. Family groups and known annual reproduction 2010-2016

## 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 302 individuals that were not originally detected as a result of a mortality, 67 (22.2%) are known to have persisted into a second year. There are 5 individuals (1.7%) that have been detected over 6 years, 1 that has been detected over 5 years, 6 that have been detected over 4 years, 8 that have been detected over 3 years, 13 that have been detected over 2 years, and 34 that have been detected over 1-2 years. The number of detections of an individual ranges from just once to 41 times.

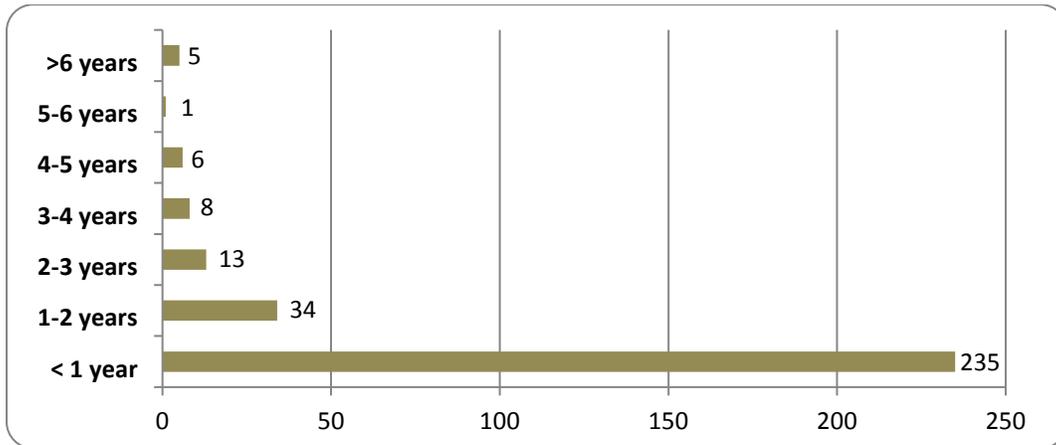


Figure 4. Persistence of individuals 2000-2017 ( $n = 302$ )

## Survivorship/Recruitment

Although not an accurate representation of true kitten survivorship, the persistence of kittens in the database in to their second year and longer can be an index of survivorship and recruitment in to the overall population (Figure 5). Of the 91 individuals that have been identified as kittens in the database prior to 2016/2017, 26 of them (28.6%: 13 female and 13 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. It is also presumed that not all surviving kittens are detected within the survey area, and other surviving kittens emigrate to other sub-populations. Figure 5 shows only those detected as kittens prior to the 2016/2017 survey season.

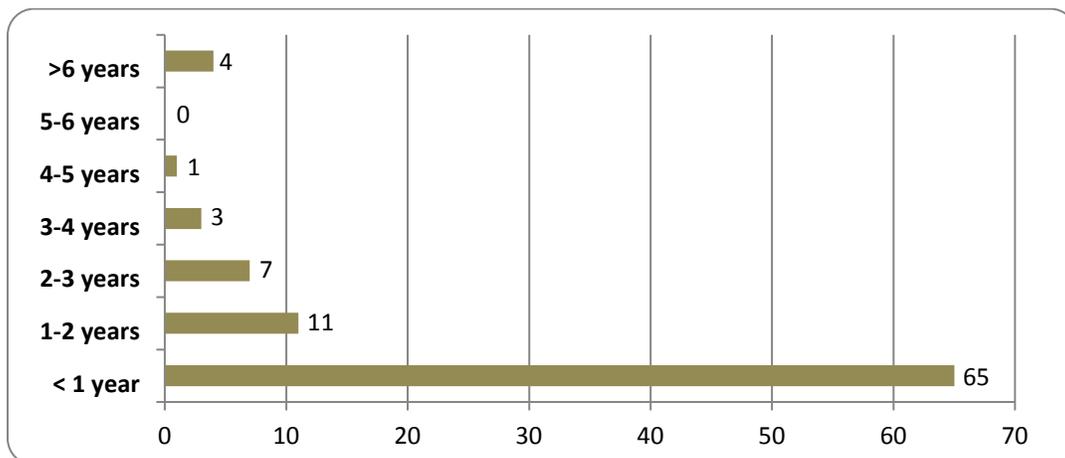


Figure 5. Persistence/recruitment of kittens 2001-2017 ( $n=91$ )

Field observations in combination with genotypes of individuals yield some interesting relationships in the NE MN sub-population's lineage and recruitment from the 2016/2017 samples. For example, Loch132, identified as a kitten in 2010, has produced 6 kittens from 4 litters since that time. She had known litters in 2012, 2013, 2014 but did not have a known litter in 2015. In that year her normal territory was occupied by one of her kittens (S551) who had a kitten of her own in 2015. Loch132 was back in her territory with a kitten again in 2016. Male Loch270, identified in 2011, has fathered 4 families of lynx (3 with Loch132) producing 6 kittens, including with Loch132 again in 2016/2017.

Two breeding females from 2015/2016 (S551 and S571) were not found to have kittens this year in the territories they occupied last year. Both territories had different breeding females with kittens. They were, however, found within the Forest and may have had families in different locations that were not surveyed.

### Distribution and Dispersal

Lynx detections are distributed over 12 counties in Minnesota. 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). There are 19 lynx samples in the database that do not have an accurate enough confirmed location to be represented in this table. The attached map 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 lynx samples	% of lynx samples
Cook	200	15.3%
Lake	889	68.1%
St. Louis	198	15.2%
All other	19	1.5%

Table 2. Distribution of lynx samples 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.

## 2016-2017 Monitoring Results

### Species Identification

One-hundred forty-four samples were collected and submitted for analysis during the period of June 2016 through April 2017. One-hundred thirty samples (90.3%) were identified as lynx, and genotypes were obtained from 113 of these identifying 42 unique individuals, 24 female (57.1%) 17 male (40.5%) and 1 (2.4%) of indeterminable sex (Figure 6). Twenty-three individuals (54.8%: 14 female and 9 male) were previously recorded in this database (recaptures), and 19 individuals (45.2%: 10 female, 8 male, and 1 of indeterminable sex) were new to the database this year including 11 kittens.

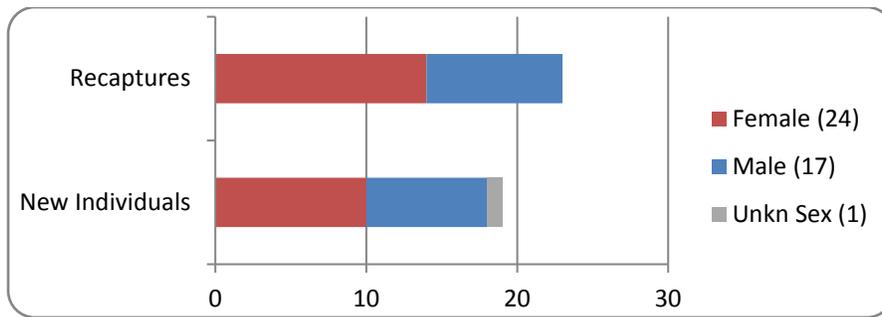


Figure 6. Canada lynx individuals detected 2017 ( $n = 42$ )

## Reproduction

Field observations during the winter 2016-2017 survey season suggest that there were at least 8 family groups with as many as 17 kittens found in the survey area. DNA analysis confirm 6 family groups in the survey area with a total of 11 individuals, 8 female (72.7%) and 3 male (27.3%), genetically consistent with being offspring (Figure 7). Two additional family groups were reported but could not be verified either through site visits or DNA collection. Of the 6 family groups we collected genetic samples from, one family group consisted of a candidate mother and at least 3 presumed kittens (2 female, 1 male) consistent with being her offspring. Three family groups consisted of a candidate mother with at least 2 kittens, two with 1 female and 1 male, the other with 2 females. There were also 2 family groups with at least 1 kitten each, both female, consistent with being the offspring of the candidate mother each was associated with.

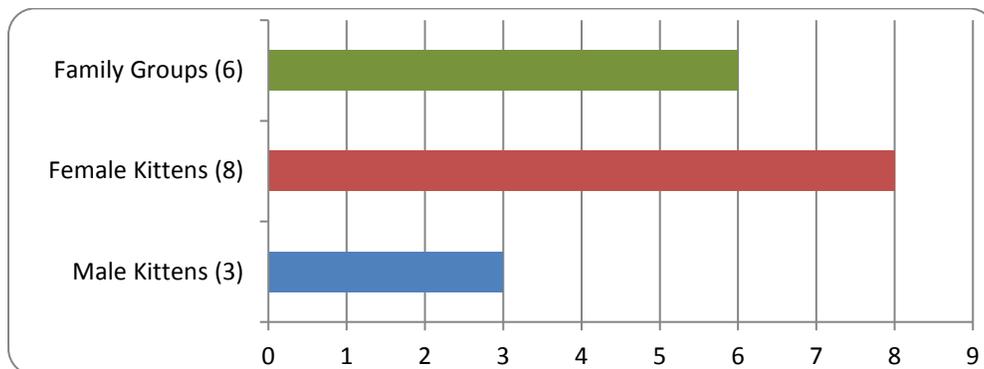


Figure 7. Family groups and known reproduction 2017

Five of the 6 mothers are previously known in the database. Three of them have had known litters in previous years and have produced a total of 12 kittens.

## Persistence

Persistence distribution of the current year's individuals may be representative of the overall age structure of the NE MN sub-population. Of the 30 individuals detected during the 2016/2017 survey season that were not kittens, 15 (50.0%: 8 female and 7 male) have persisted at least into their second year (Figure 8). Three individuals have been detected over a 6 year period, 1 over a 5 year period, 3 over a 4 year period, 4 over a 2 year period and 7 over a 9 year period. There are 8 individuals that are new to the database this year that are not assumed to be kittens and therefore are not considered in persistence analysis. By using field observations and genetic analysis 1 of these 8 was determined to

be an adult female with kittens, and 1 an adult male that fathered a current year's litter. Six are of unknown age; either they were travelling alone or tied to a family group but were unrelated.

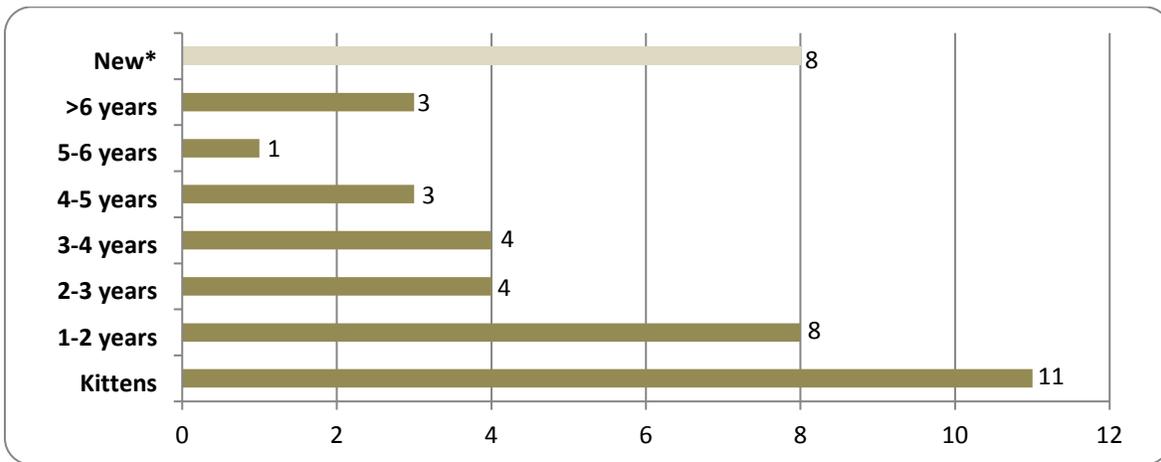


Figure 8. Persistence of individuals 2017 ( $n = 42$ )  
 \* Individuals new to the database of an unknown age

## Take

Section 9 of the Endangered Species Act (ESA) prohibits the take of endangered and threatened species without special exemption. 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. 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). There have been 70 incidents of reported take of Canada lynx since 2001 (USFWS 2017) (Figure 9). Fifty-three of these incidents have resulted in mortalities to the animal. There have been 7 incidents of shooting (all mortalities), 34 trapped (17 mortalities and 17 released alive), and 13 that have been hit by a vehicle or a train (all mortalities). There were also 16 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.

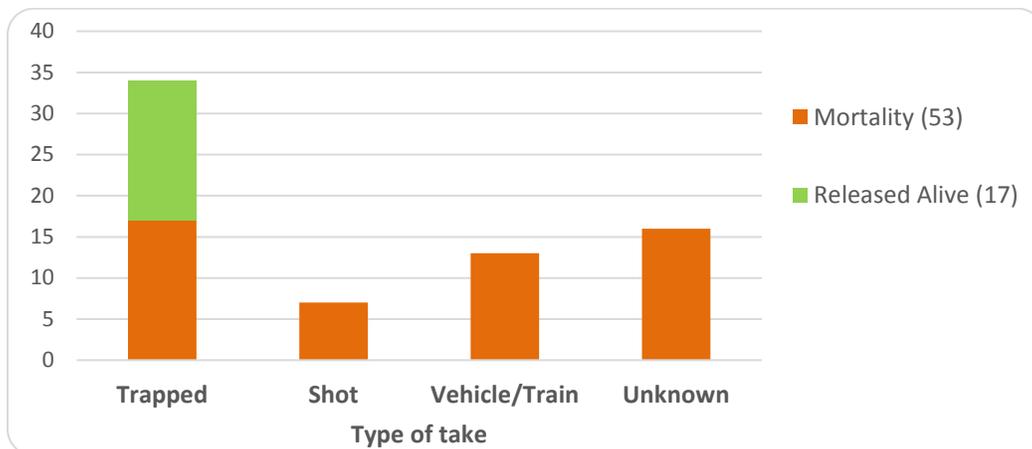


Figure 9. Reported Canada lynx take in Minnesota 2001-2017 by type and outcomes ( $n = 70$ )

## **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 Forest Plan is expected to result in the incidental take of Canada lynx over the life of the Forest Plan. According to the 2011 Biological Opinion Incidental Take Statement– “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.” (USFWS 2011)

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 know lynx mortality within the National Forest Proclamation boundaries in Minnesota due to vehicle collision, accidental trapping, or poaching”. All mortality reports are sent directly and immediately to USFWS when they are received by Forest Service personnel. USFWS maintains a state-wide mortality database which houses these and all other lynx take reports. Mortalities that have occurred due to vehicle collisions on all roads on all ownerships within the proclamation boundary of the Forest is considered incidental to the implementation of the Forest Plan since 2005, There have been 3 reported incidents of lynx take due to vehicle collisions within the Superior NF’s proclamation boundaries in the USFWS database; one each in years 2005, 2014 and 2017.

No take incidents have been reported within the proclamation boundaries of the Chippewa NF.

## **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). The earliest recorded hybrid in Minnesota comes from a specimen that was reportedly harvested in 1997. Hybrids have been detected in Cook, Itasca, Lake and Pine Counties in Minnesota, and in Polk County, Wisconsin.

Although not annually, Canada lynx-bobcat hybrid animals have been detected on a regular basis during surveys for lynx. To date the database contains 43 hybrid samples. Forty-two of these have been genotyped representing 13 individuals, 5 female and 8 male. During the 2016/2017 survey season, 1 Canada lynx-bobcat hybrid, a male, was detected; a recapture who was first detected in February 2011 and has persisted for over 6 years. 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.

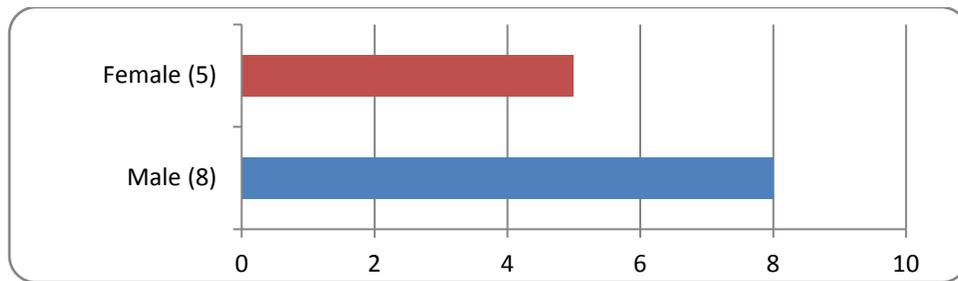


Figure 10. Total Canada lynx-bobcat hybrid individuals detected ( $n = 13$ )

## Acknowledgements

Thanks to Steve Loch for his total dedication to this project since the beginning. Thanks to this year's surveyors; S. Abrahamsen, J. Alfonso, D. Anderson, T. Anderson, C. Beal, H. Becker, A. Belleman, A. Denasha, M. Grover, T. Klotz, M. Knott, J. Lind, S. Malick-Wahls, N. Malinowski, A. Roberts, P. Robertsen, C. Sandeno, K. Severs, S. Swanson. Additional samples were obtained and provided by Dr. S. Barber-Meyer (USGS). We also 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 Kristy Pilgrim, Dr. Mike Schwartz and Cory Engkjer at the RMRS Wildlife Genetics Lab for all their work and support in conducting the DNA analysis. And to Tam Smith at the USFWS Field Office in Bloomington, MN for her work in gaining additional funding to support this work over the years.

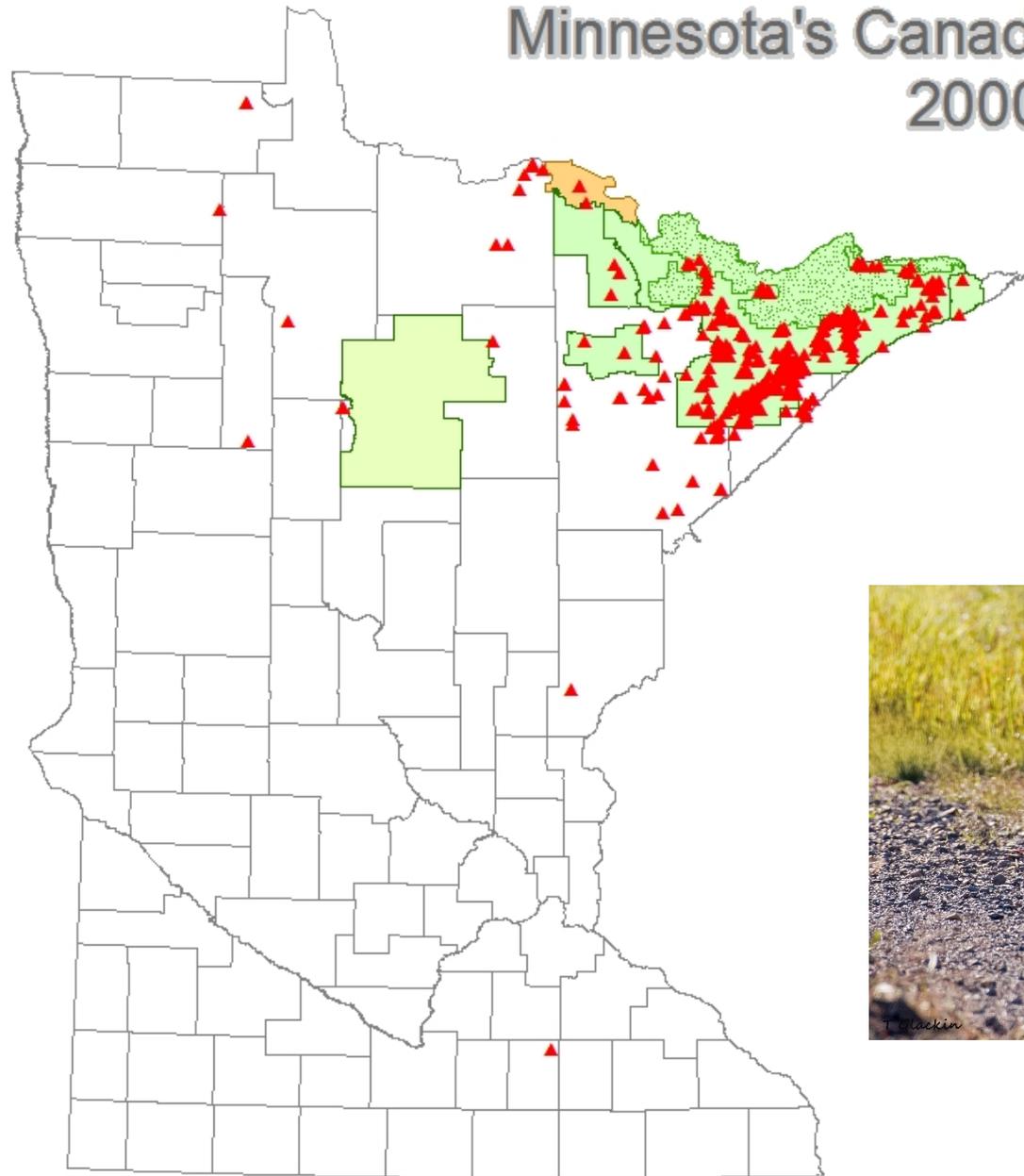
## Current Research

Hostetter, N.J. and B. Gardner. 2016. Detection/non-detection surveys to estimate Canada lynx occupancy in Superior National Forest and critical habitat in Minnesota. Report to USDI Fish and Wildlife Service. 21 pp.

## Literature Cited:

- Pilgrim, K.L., K.S. McKelvey, A.E. Riddle and M.K. Schwartz. 2005. Felid sex identification based on noninvasive genetic samples. *Molecular Ecology Notes*. 5: 60-61.
- Schwartz, M.K., K.L. Pilgrim, K.S. McKelvey, E.L. Lindquist, J.J. Claar, S. Loch and L.F. Ruggiero. 2004. Hybridization between Canada lynx and bobcat: Genetic results and management implications. *Conservation Genetics*. 5: 349-355.
- USDI Fish and Wildlife Service. 2011. Biological Opinion issued in concurrence of reconsultation of implementation of the 2004 Forest Land and Resource Management Plan for the Superior National Forest. September 16, 2011.
- USDI Fish and Wildlife Service. 2017. LYCA Incidental Take 2001-Present 5OCT2017 Excel Workbook. Unpublished data.

# Minnesota's Canada lynx DNA locations 2000-2017



- ▲ Lynx DNA locations
- Superior NF
- BWCAW
- Chippewa NF
- Voyageur's NP

