

**Lower Churchill Labrador
Island Transmission Link -
Newfoundland Marten
Environmental Effects
Monitoring Program**

**Field Report for Winter 2014-
Newfoundland Marten Hair Snag
Trapping and Off Highway
Vehicle Track Densities**



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Draft Interim Report

February 10, 2015

LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

Table of Contents

| | | |
|------------|------------------------------------|-----------|
| 1.0 | INTRODUCTION | 1 |
| 2.0 | STUDY OBJECTIVES..... | 2 |
| 3.0 | METHODS | 2 |
| 3.1 | HAIR SNAG TRAPPING | 2 |
| 3.2 | LABORATORY DNA ANALYSIS | 3 |
| 3.3 | OHV SURVEY | 3 |
| 3.4 | STUDY TEAM | 4 |
| 4.0 | RESULTS | 5 |
| 4.1 | SURVEY EFFORT AND CONDITIONS | 5 |
| 4.2 | WINTER OHV TRACK SURVEY | 7 |
| 4.3 | HAIR SNAG LAB RESULTS..... | 7 |
| 5.0 | SUMMARY..... | 11 |
| 6.0 | REFERENCES..... | 12 |

LIST OF APPENDICIES

| | |
|------------|---------------------------------------|
| Appendix A | Research Permit |
| Appendix B | Field Photos from Winter Surveys 2014 |
| Appendix C | Field Data from Winter Surveys 2014 |
| Appendix D | Lab report from hair snag samples |

LIST OF TABLES

| | | |
|-----------|---|---|
| Table 3.1 | Furbearer Survey Team, April 17 – May 8, 2014 | 4 |
| Table 4.1 | Trapping Effort, Success and Weather | 5 |

LIST OF FIGURES

| | | |
|------------|--|----|
| Figure 4-1 | Marten Hair Snag Deployments – April 17, 2014 | 6 |
| Figure 4-2 | OHV Survey in the Main River Area, Northern Peninsula..... | 8 |
| Figure 4-3 | OHV Observations – April 17, 2014 | 9 |
| Figure 4-4 | Marten Hair Snag Results | 10 |



LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

INTRODUCTION
February 10, 2015

1.0 INTRODUCTION

During the environmental assessment for the Labrador-Island Transmission Link (the Project), furbearers were identified as a Valued Environmental Component (VEC) for the Environmental Impact Statement (EIS) due to their economic, cultural, and ecological importance (Nalcor 2012). Some furbearer species are harvested by provincial residents, may serve as ecosystem indicators as they are predators or keystone species; and one species in Newfoundland, the Newfoundland population of American marten (*Martes americana atrata*) is a species at risk. Newfoundland marten are listed as threatened and are protected both federally under the *Species at Risk Act* (COSEWIC 2007) and provincially under the Newfoundland and Labrador *Endangered Species Act* (Government of Newfoundland and Labrador 2004). Newfoundland marten were recommended for this special status a result of a substantial population decline (COSEWIC 2007). Following this designation a Recovery Plan was developed by the Newfoundland Marten Recovery Team (2010), which identified threats to this insular population including habitat loss and mortality from snaring and trapping. Critical habitat (delineated by 8 km² blocks based on female territory sizes) within core areas for this species in Newfoundland was identified from evidence of occurrence and habitat quality data. This critical habitat presently has a gradient of partial to full protection.

Newfoundland marten are sensitive to habitat alteration and have a limited and discontinuous distribution in Newfoundland. As a result, it was included as a key indicator species for the EIS. Habitat alteration and loss during the construction phase was predicted as the greatest potential Project effect on furbearers. The Project overlaps critical Newfoundland marten habitat identified within the Main River core area. This core area has a protection rating (referred to as Group 2 Habitat) which requires development and forest harvesting to be managed through the *Environmental Protection Act* and resource planning process. All land-based traps, locking snares, and small game snares are legally prohibited under this level of protection (The Newfoundland Marten Recovery Team 2010).

Based on monitoring requirements and commitments during the Environmental Assessment, the Furbearer Environmental Effects Monitoring Program (EEMP) will examine the presence and/or distribution of marten. The scope of work for this component involves:

- assessment of the cleared RoW as a barrier to Newfoundland marten distribution;
- determining the efficacy of watercourse buffer zones, brush piles, windrows, and any applied modified vegetation management techniques as travel corridors; and
- assessment of snowmobile access provided by the cleared RoW.

This interim report describes the methods and results from the first winter hair snagging efforts conducted between mid-April to early May 2014.



LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

STUDY OBJECTIVES

February 10, 2015

2.0 STUDY OBJECTIVES

The primary objectives of the marten hair snagging program were to collect and monitor potential environmental effects during Project construction. This work is a part of the Environmental Mitigation and Management Plan associated with the Labrador-Island Transmission Link Project. These surveys will aid in the development of a program for monitoring the effectiveness of mitigation measures, and facilitate compliance with regulatory requirements and commitments made in the EIS (Nalcor 2012).

The objectives of this field program were:

- to determine the presence and distribution of marten in the core habitat areas within the proposed RoW; and
- to determine the density/access points for OHV (Off-highway Vehicle) use along the RoW.

3.0 METHODS

This wildlife research has been authorized by a permit issued by the Department of Environment and Conservation, Wildlife Division (NLWD) to Stassinu Stantec (Appendix A).

Stassinu Stantec Geographic Information System (GIS) personnel worked with the NLWD personnel to produce a map folio of the Main River valley and the Labrador-Island Transmission Link RoW indicating primary marten habitat (i.e. core marten habitat blocks) and transects to be surveyed for the OHV use during this late winter field program. Centroids from core marten block habitat were identified as locations for hair snag trap deployment. The Study Area for this program includes primary marten habitat throughout the Main River valley and the transmission line RoW.

3.1 Hair Snag Trapping

Hair snag traps were constructed and deployed using guidelines provided by the NLWD. A triangular shaped trap was constructed from three boards that were wired together. Suitable Newfoundland Marten habitat was selected using mapping provided by the Wildlife Division and by assessing suitable habitat during an aerial survey. The 17 identified trap locations were accessed via helicopter and on foot April 17, 2014. Each hair snag trap was mounted horizontally to a large living coniferous tree with screws. Four sticky pads and bait were placed as per the NLWD guidelines in each trap. A GPS waypoint and digital photo was taken at each trap location (Appendix B). Traps were checked (and re-baited if necessary) on three occasions (approximately once per week) during the survey period (Table 3.1).



LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

METHODS

February 10, 2015

3.2 Laboratory DNA Analysis

A set of 37 envelopes containing hair samples was delivered to the Genomics and Proteomics (GaP) Facility of the CREAT Network at Memorial University of Newfoundland. One sticky pad per envelope was processed. DNA was extracted from approximately 20 roots using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Toronto, Ontario, Canada) following the manufacturer's Tissue Protocol, except that DNA was re-suspended in two consecutive 75 µL elutions, for a total volume of 150 µL of DNA. Hair roots were digested overnight.

DNA from hair samples were screened twice at the following 11 microsatellite loci using standard operating protocols developed in the GaP Facility: Ma1, Ma2, Ma7, Ma9, Ma10, Ma11, Ma14, Ma18, Ma19 (Davis and Strobeck 1998); MP0085, MP0114 (Jordan et al. 2007). Alleles were called independently by two readers.

Sex determination of samples was carried out by amplifying an intron within the zinc-finger gene that is present on both sex chromosomes using primers LGL331 and LGL335 (Shaw et al. 2003) with standard operating protocols developed in the GaP Facility. Samples with two bands (zinc finger X and Y) were identified as male, and those with one band (two copies of zinc finger X) as female. Agarose gels were read independently by two readers.

Complete genotypes were run through GENECAAP version 1.3, a Microsoft Excel macro that compares each individual multi-locus genotype with all other genotypes within the data set to locate matching genotypes (Wilberg and Dreher 2004) and thus identify individuals within a set of samples. (Complete report can be found in Appendix D)

3.3 OHV Survey

All OHV tracks were recorded with locational data during an aerial survey on April 17, 2014. Other parameters such as: snow depth, start and end times of each transect were also recorded. The flight track file and digital photos were stored for future reference.

Track densities were calculated for each species using the following formula:

$$\text{track density} = \frac{\# \text{ tracks observed}}{\text{transect length (48.3km)} \times \text{field of view (400m)}}$$

Prior to initiating the field program, all personnel reviewed the Project specific Health and Safety Plan, and attended a Lower Churchill Project orientation (March 2014). On the first day of field effort the field teams reviewed Stantec's Risk Management Strategy (RMS) 1. An RMS 2 form was reviewed daily throughout the field program to ensure safety hazards were identified and managed.



LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

METHODS

February 10, 2015

3.4 Study Team

The Study Team for the furbearer field program included Stantec personnel and pilots from Universal Helicopters Newfoundland Limited LP or Canadian Helicopters (Table 3.1).

Table 3.1 Furbearer Survey Team, April 17 – May 8, 2014

| Name | Position / Role during each Visit | | | | Organization |
|--------------|-----------------------------------|-----------------|-----------------|-----------------|-------------------------|
| | April 17 | April 24 | May 1 | May 8 | |
| Chris Gosse | Pilot | Pilot | | | Universal Helicopters |
| Glen Piercy | | | Pilot | Pilot | Canadian Helicopters |
| Tony Parr | Field Lead | Field Lead | Field Lead | Field Lead | Stantec Consulting Ltd. |
| Tina Newbury | Field Biologist | | Field Biologist | | Stantec Consulting Ltd. |
| Stacey Camus | | Field Biologist | | | Stantec Consulting Ltd. |
| Wayne Tucker | | | | Field Biologist | Stantec Consulting Ltd. |

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RESULTS

February 10, 2015

4.0 RESULTS

4.1 Survey Effort and Conditions

Weather and snow conditions during surveys were ideal for winter tracking observations on all survey dates with the exception of April 24, 2014 which had recently experienced freezing rain (Table 4.1; Figure 4-1). The trapping effort captured marten hairs in the majority (59-82%) of the hair snags during each sampling period. The laboratory analyses of the hair samples were used to determine whether or not more than one Newfoundland marten had visited the trap site.

Table 4.1 Trapping Effort, Success and Weather

| Date | Activities | Trap success | Flying time | Weather |
|----------|--|------------------------------|-------------|---|
| April 17 | Established sampling locations and set hair snag traps | 17 traps activated | 4.4 | -4 °C, winds NE 35 Km/h, 65% cloud, no precipitation |
| April 24 | Winter ground trap checking | 10 traps positive for Marten | 4.1 | -2°C, winds NE 40 Km/h. 100% cloud cover, freezing rain |
| May 1 | Winter ground trap checking | 14 traps positive for Marten | 3.8 | -5 °C and winds 20 km/h. 0-5% cloud cover; No precipitation |
| May 8 | Winter ground trap checking | 13 traps positive for Marten | 3.8 | -3°C, no wind, no precipitation, 0% cloud cover, no precipitation |



LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

RESULTS
February 10, 2015

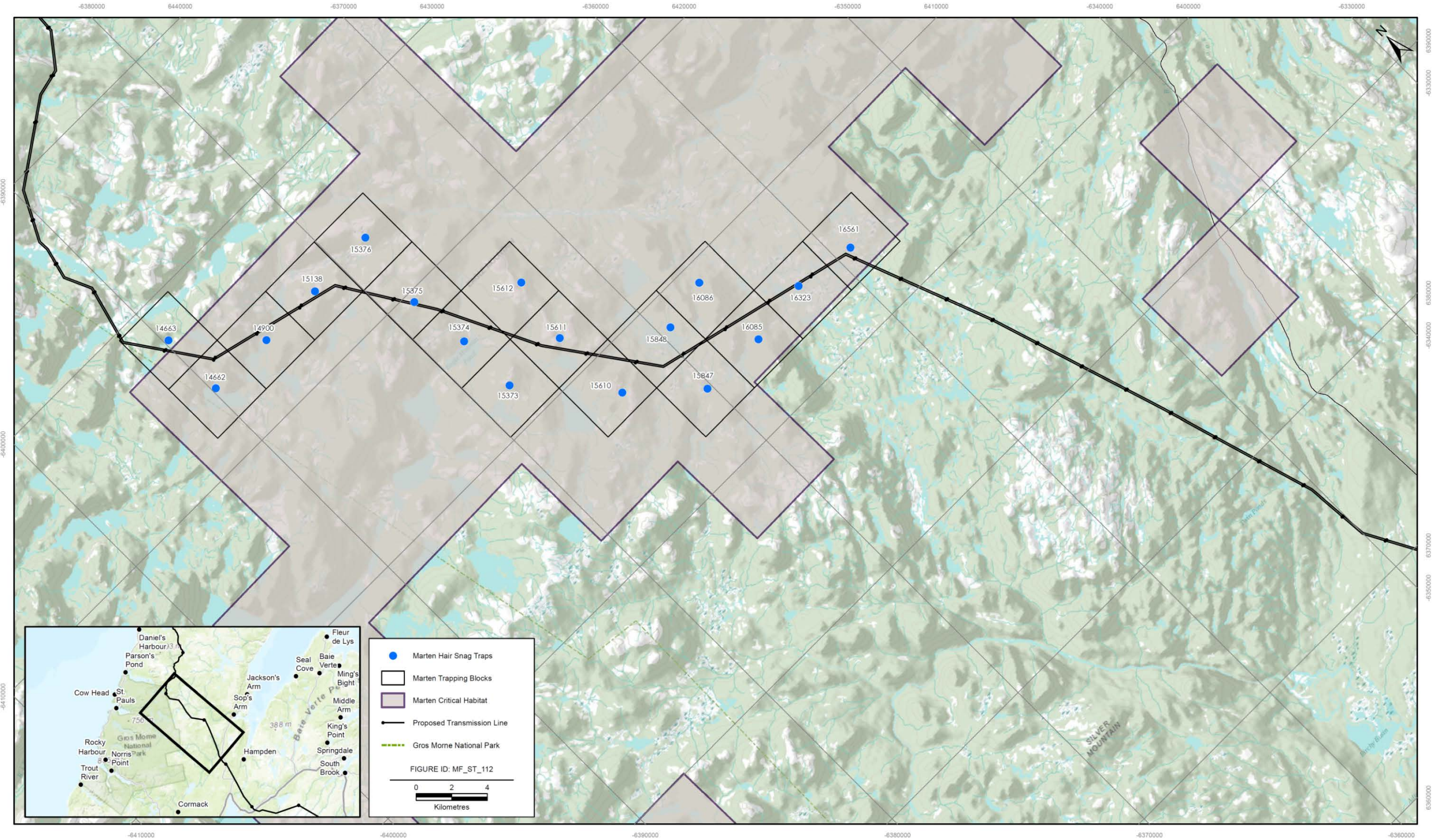


Figure 4-1 Marten Hair Snag Deployments – April 17, 2014



**LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND MARTEN
ENVIRONMENTAL EFFECTS MONITORING PROGRAM****RESULTS**

February 10, 2015

4.2 Winter OHV Track Survey

Snowmobile activity was quantified by developing track densities along the proposed RoW in the core area (Figure 4-2). This approach was chosen based on the landscape and technology limitations of trail cameras and counters. Snowmobile track density along the RoW would provide an index of use. A transect was identified along the RoW and surveyed by two scientists during the aerial survey. The transect start and end points were defined as the point where the RoW enters and exits the Main River core area at two other points (Appendix C). Winter 2014 survey data will serve as baseline/pre-construction data.

A total of 48.3 km of linear transect was surveyed via helicopter during the first day of hair snag trap placement. OHV tracks were recorded at 26 locations along the RoW during the survey (Figure 4-3).

4.3 Hair snag lab results

Out of the 37 submitted samples (individual envelopes containing trap samples), 32 were suitable to generate complete genotypes. From these 32 samples, 18 individual marten were identified. Ten of the individuals were captured multiple times; eight individuals were captured only once. Of the 18, sex was determined for 12 (67%). Five individuals were male, and the remaining seven were female.

From this breakdown we can see that some individuals are hitting multiple sites and are crossing the proposed ROW (See figure 4-4).



LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

RESULTS

February 10, 2015

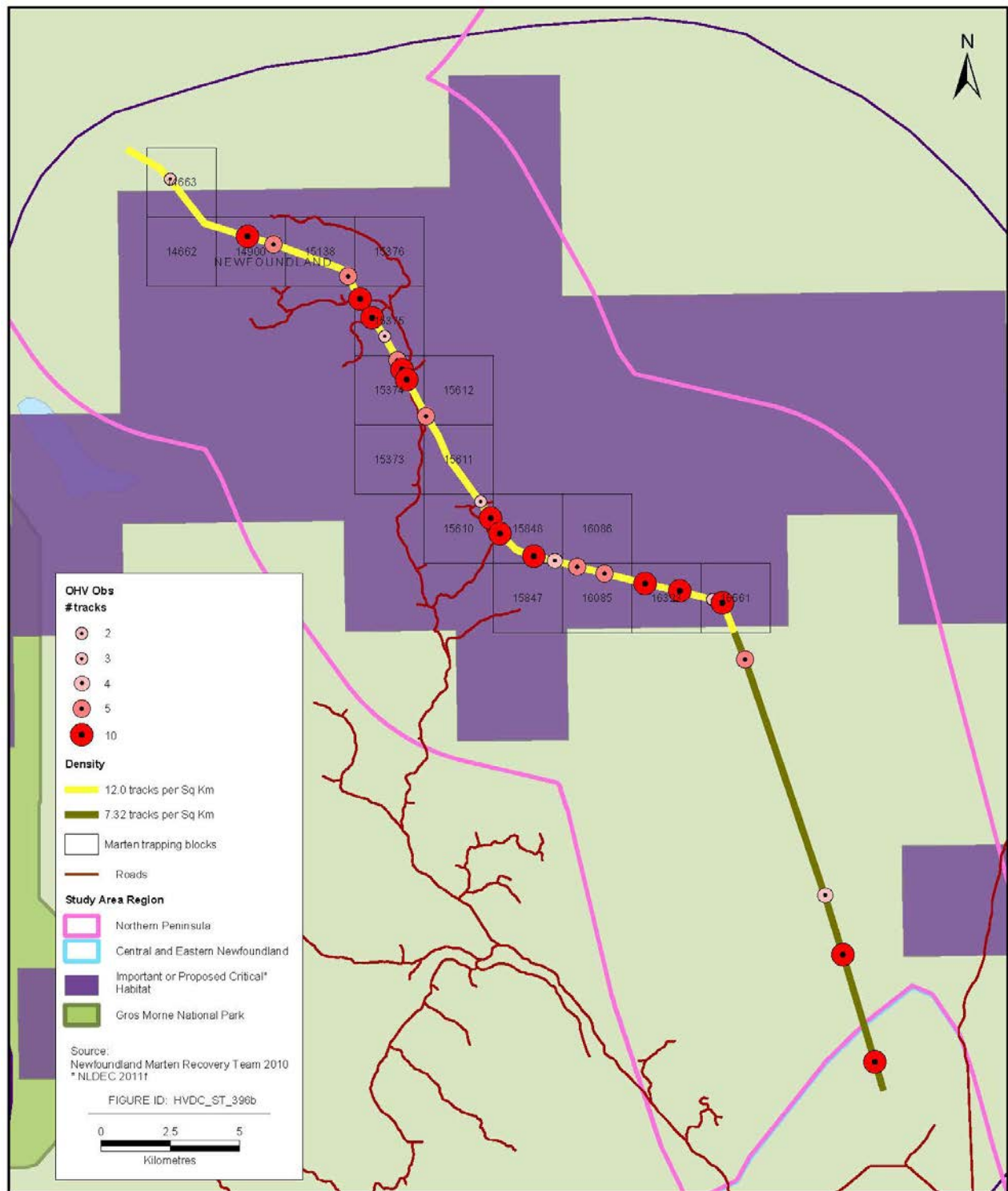


Figure 4-2 OHV Survey in the Main River Area, Northern Peninsula

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RESULTS
February 10, 2015

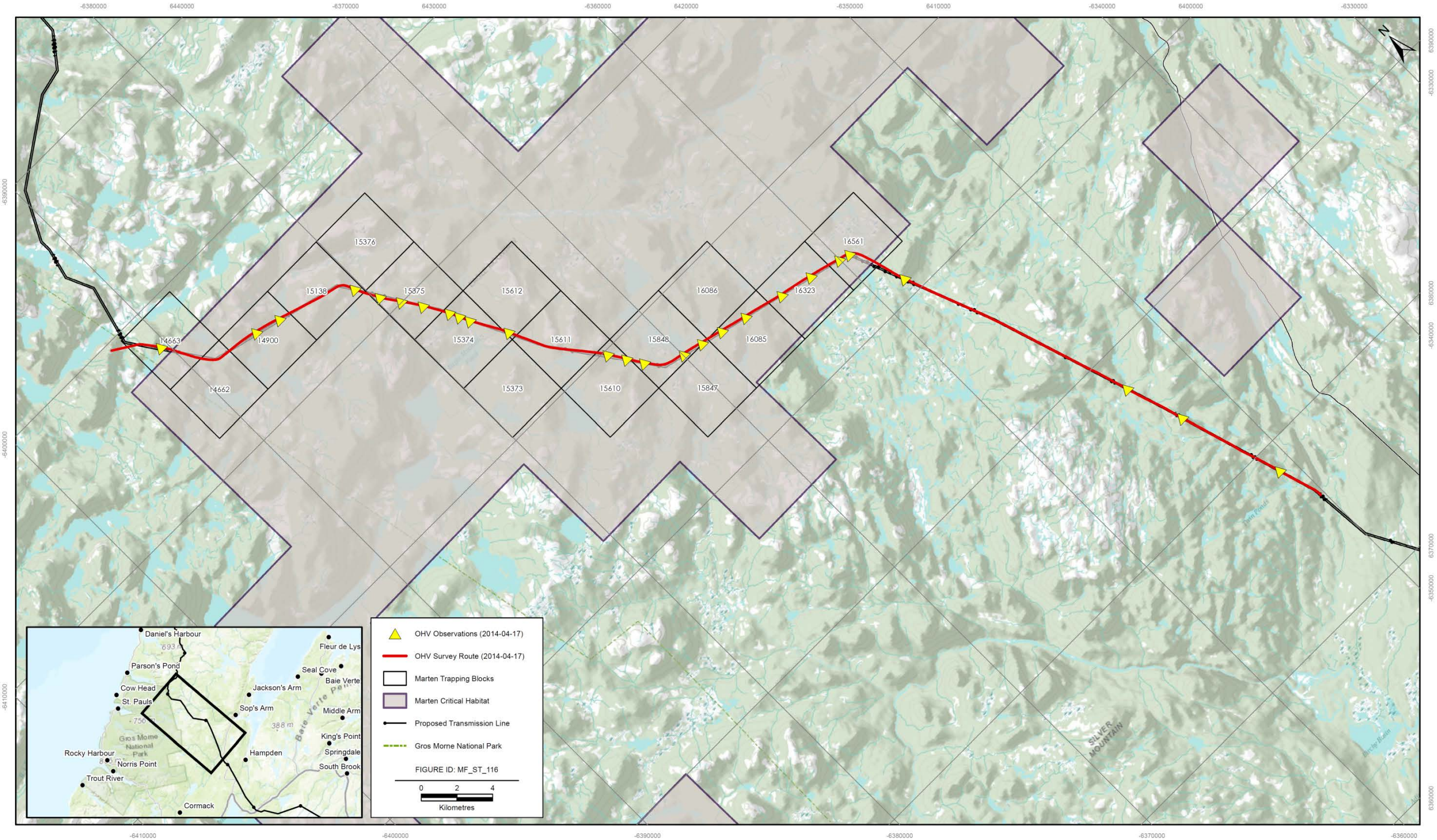


Figure 4-3 OHV Observations – April 17, 2014



LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

RESULTS

February 10, 2015

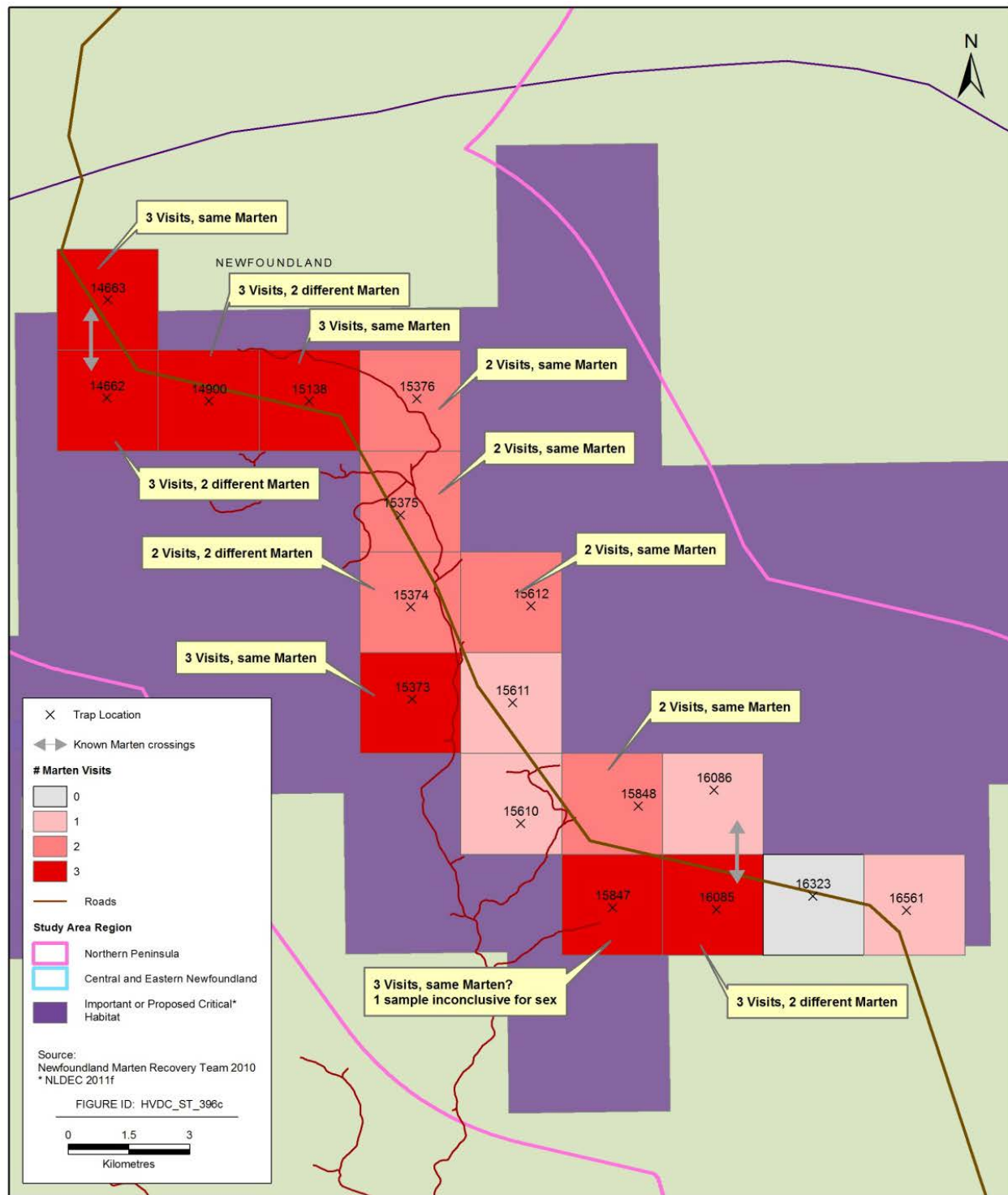


Figure 4-4 Marten Hair Snag Results



**LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND MARTEN
ENVIRONMENTAL EFFECTS MONITORING PROGRAM****SUMMARY**

February 10, 2015

5.0 SUMMARY

Newfoundland marten presence in the Study Area was confirmed through the observation of tracks and collected hair samples. This species was expected in the Main River area based on the results of previous studies (Gosse and Hearn 2005), available habitat, and proximity to identified core and critical habitat ranges by NLWD.

Survey results provide preliminary information on the abundance, distribution, and habitat use by Newfoundland marten in the Main River watershed in the vicinity of the Project. Results from the lab analysis indicate that Marten are active in the proposed ROW survey blocks. The ability to identify and track individual Marten indicates that 18 Marten are crossing the ROW. (Figure 4-4).

This document will be used in combination with results of future surveys and other components of the EEMP to assess the movement and distribution patterns of Newfoundland marten in relation to Project activities.



LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

REFERENCES

February 10, 2015

6.0 REFERENCES

- Davis C and Strobeck C. 1998. Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family mustelidae. *Molecular Ecology* 7(12):1776-8.
- Gosse, J.W and B.J. Hearn. 2005. Seasonal Diets of Newfoundland Martens, *Martes americana atrata*. *The Canadian Field-Naturalist* 119: 43-47.
- Jordan MJ, HIGLEY J, Matthews SM, Rhodes OE, Schwartz MK, Barrett RH, Palsbøll PJ. 2007. Development of 22 new microsatellite loci for fishers (*Martes pennanti*) with variability results from across their range. *Molecular Ecology Notes* 7(5):797-801.
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- Nalcor Energy (Nalcor). 2012. Labrador-Island Transmission Link Environmental Impact Statement. Volume 2B – Biophysical Assessment. 343 pp.
- The Newfoundland Marten Recovery Team. 2010. Recovery Plan for the Threatened Newfoundland Population of American Marten (*Martes americana atrata*). Newfoundland and Labrador Wildlife Division, Corner Brook, NL.
- Wilberg MJ and Dreher BP. 2004. Genecap: A program for analysis of multilocus genotype data for non-invasive sampling and capture-recapture population estimation. *Molecular Ecology Notes* 4(4):783-5.



LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND
MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

APPENDIX A

Research Permit



GOVERNMENT OF
NEWFOUNDLAND AND LABRADOR

Department of
Environment and Conservation
Wildlife Division

**A PERMIT TO CONDUCT RESEARCH ON, AND POSSESS
SPECIMENS OF A THREATENED SPECIES UNDER THE
ENDANGERED SPECIES ACT OF
NEWFOUNDLAND AND LABRADOR**

Date: April 14, 2014

Endangered Species Permit Number: 2014/15-14

Issued To: Perry Trimper, Stantec Consulting Ltd.
P.O. Box 482, Station C, Happy Valley-Goose Bay, NL A0P 1C0
Tel: (709) 896-5860
Facsimile: (709)896-5863

Permit To: Collect and possess hair specimens from American Marten

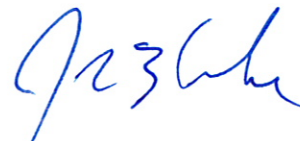
Expiry Date: May 31, 2014

CONDITIONS:

1. The permit holder may designate other individuals to collect and possess hair specimens on his/her behalf. The permit holder is responsible for the training of any designated individuals and must ensure designated individuals follow all regulations related to this permit.
2. Names and contact information for all individuals participating in research activities or privy to information collected on Newfoundland Marten shall be provided to the Wildlife Division, Department of Environment and Conservation prior to commencement of field work. Additional names or deletion of names can be provided to Wildlife Division on an ongoing basis. The permit holder must advise all individuals that their information will be provided to the Wildlife Division and may be further disclosed as permitted or required by law.
3. A copy of this permit shall be retained in the field at all times by at least one person on the permit personnel list and is to be provided to a Fish and Wildlife Enforcement Officer or other person of delegated authority upon request.

2 – ESA Permit 2014/15-14

4. Hair collection may only take place within currently identified Newfoundland Marten critical or core habitat in the vicinity of the right of way for the Labrador-Island Transmission corridor for the Lower Churchill project.
5. Any changes to the survey design or methodology outlined in the initial permit request will require prior approval before implementation.
6. A final report must be submitted to the Wildlife Division by June 30, 2014. This report must detail the location of surveys, methods employed, number of samples/specimens taken, location of samples/specimens, individual genetic identification/information for each sample and additional relevant ecological information. The location of all hair snags and associated collection data must be submitted in digital format along with the final report. The permit holder is responsible to obtain any and all permissions which may be required to release this information to the Wildlife Division.
7. All marten samples are to be transferred to an appropriate laboratory for analysis or to the Wildlife Division, Department of Environment and Conservation. Any samples remaining after analysis must be destroyed or provided to the Wildlife Division.
8. Any unusual wildlife observations or any adverse effects observed during the Project are to be reported immediately to the Wildlife Division.
9. This permit does not absolve or relieve the permit holder from any other laws, permits, regulations or orders.
10. This permit does not relieve the permit holder from the requirement to acquire permission to access private property.
11. Under the discretion of the Director of Wildlife, this permit can be revoked without notice.



JOHN BLAKE
Director

LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND
MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

APPENDIX B

Field Photos from Winter surveys 2014



Field Photos from Winter Surveys 2014



Photo 1

Site 14663 Habitat photo

Field Photos from Winter Surveys 2014



Photo 2 Site 14663 Tree Selection, and trap setup

Field Photos from Winter Surveys 2014



Photo 3 Site 14663 Final trap setup



Photo 4 Site 14663 positive hit for Marten hair sample

Field Photos from Winter Surveys 2014



Photo 5 Site 15612



Photo 6 Site 15612 Tree Selection, and trap setup

Field Photos from Winter Surveys 2014



Photo 7

Site 15612 positive hit for Marten hair sample



Photo 8

Site 14900

Field Photos from Winter Surveys 2014



Photo 9 Site 14900 tree selection



Photo 10 Site 14900 trap setup

Field Photos from Winter Surveys 2014



Photo 11 Site 14900 positive for Marten hair sample

Field Photos from Winter Surveys 2014



Photo 12 Typical habitats for NL marten hair snag trap locations within the study area

Field Photos from Winter Surveys 2014



Photo 13 Typical habitats for NL marten hair snag trap locations within the study area

LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND
MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

APPENDIX C

Field Data from Winter surveys 2014



Transects

| Waypoint | UTM_Zone | Easting | Northing | Track | #_Tracks | Notes | Region | Total_Tracks_Obs | Survey_Distance | Survey_Area (Km ²) | Track Density |
|----------|----------|---------|----------|-------|----------|-------------------------|----------|------------------|-----------------|--------------------------------|---------------|
| 276 | 21 U | 470824 | 5526853 | OHV | 2 | | Critical | 153 | 30 | 12 | 12.75 |
| 277 | 21 U | 473630 | 5524788 | OHV | 10 | | Critical | 153 | 30 | 12 | 12.75 |
| 278 | 21 U | 474565 | 5524525 | OHV | 5 | | Critical | 153 | 30 | 12 | 12.75 |
| 279 | 21 U | 477258 | 5523362 | OHV | 5 | | Critical | 153 | 30 | 12 | 12.75 |
| 280 | 21 U | 477685 | 5522517 | OHV | 10 | Road (Major route) | Critical | 153 | 30 | 12 | 12.75 |
| 281 | 21 U | 478116 | 5521862 | OHV | 10 | Road (Major route) | Critical | 153 | 30 | 12 | 12.75 |
| 282 | 21 U | 478563 | 5521191 | OHV | 2 | | Critical | 153 | 30 | 12 | 12.75 |
| 283 | 21 U | 479052 | 5520331 | OHV | 5 | | Critical | 153 | 30 | 12 | 12.75 |
| 284 | 21 U | 479214 | 5519984 | OHV | 10 | Major route | Critical | 153 | 30 | 12 | 12.75 |
| 285 | 21 U | 479384 | 5519611 | OHV | 10 | Road (Major route) | Critical | 153 | 30 | 12 | 12.75 |
| 286 | 21 U | 480076 | 5518320 | OHV | 5 | Road | Critical | 153 | 30 | 12 | 12.75 |
| 287 | 21 U | 482032 | 5515211 | OHV | 3 | Road | Critical | 153 | 30 | 12 | 12.75 |
| 288 | 21 U | 482401 | 5514619 | OHV | 10 | Major route | Critical | 153 | 30 | 12 | 12.75 |
| 289 | 21 U | 482752 | 5514068 | OHV | 10 | Major route | Critical | 153 | 30 | 12 | 12.75 |
| 290 | 21 U | 483975 | 5513260 | OHV | 10 | Major route | Critical | 153 | 30 | 12 | 12.75 |
| 291 | 21 U | 484729 | 5513076 | OHV | 4 | | Critical | 153 | 30 | 12 | 12.75 |
| 292 | 21 U | 485545 | 5512873 | OHV | 5 | Road | Critical | 153 | 30 | 12 | 12.75 |
| 293 | 21 U | 486522 | 5512626 | OHV | 5 | | Critical | 153 | 30 | 12 | 12.75 |
| 294 | 21 U | 488002 | 5512257 | OHV | 10 | Major route | Critical | 153 | 30 | 12 | 12.75 |
| 295 | 21 U | 489228 | 5511989 | OHV | 10 | Major route | Critical | 153 | 30 | 12 | 12.75 |
| 296 | 21 U | 490393 | 5511685 | OHV | 2 | | Critical | 153 | 30 | 12 | 12.75 |
| 297 | 21 U | 490789 | 5511571 | OHV | 10 | Major route | Critical | 153 | 30 | 12 | 12.75 |
| 298 | 21 U | 491582 | 5509538 | OHV | 5 | along transmission line | outside | 29 | 18.3 | 7.32 | 3.96 |
| 299 | 21 U | 494468 | 5501007 | OHV | 4 | | outside | 29 | 18.3 | 7.32 | 3.96 |
| 300 | 21 U | 495131 | 5498854 | OHV | 10 | Road (Major route) | outside | 29 | 18.3 | 7.32 | 3.96 |
| 301 | 21 U | 496281 | 5494992 | OHV | 10 | Road (Major route) | outside | 29 | 18.3 | 7.32 | 3.96 |

Survey field of view = 400 meters

Total area surveyed is 48.3 X 0.4 = 19.32 km²
 Total number tracks = 182

Over all track density is 182/19.329 = 9.42

Trap Locations

| Type | Ident | | Lat | Long | Altitude | Time | Ltime |
|----------|-------|-------|---------|----------|----------|----------------------|-----------------|
| WAYPOINT | 259 | 14663 | 49.8925 | -57.4001 | 462 | 2014-04-17T11:53:50Z | 4/17/2014 7:53 |
| WAYPOINT | 260 | 14662 | 49.8706 | -57.4003 | 496 | 2014-04-17T12:07:52Z | 4/17/2014 8:07 |
| WAYPOINT | 261 | 14900 | 49.8700 | -57.3651 | 465 | 2014-04-17T12:24:27Z | 4/17/2014 8:24 |
| WAYPOINT | 262 | 15138 | 49.8701 | -57.3305 | 509 | 2014-04-17T12:40:17Z | 4/17/2014 8:40 |
| WAYPOINT | 263 | 15376 | 49.8708 | -57.2934 | 467 | 2014-04-17T12:56:04Z | 4/17/2014 8:56 |
| WAYPOINT | 264 | 15375 | 49.8448 | -57.2988 | 519 | 2014-04-17T13:11:48Z | 4/17/2014 9:11 |
| WAYPOINT | 265 | 15374 | 49.8243 | -57.2951 | 410 | 2014-04-17T13:28:53Z | 4/17/2014 9:28 |
| WAYPOINT | 266 | 15373 | 49.8037 | -57.2946 | 519 | 2014-04-17T13:42:37Z | 4/17/2014 9:42 |
| WAYPOINT | 267 | 15612 | 49.8246 | -57.2537 | 484 | 2014-04-17T13:59:09Z | 4/17/2014 9:59 |
| WAYPOINT | 268 | 15611 | 49.8031 | -57.2598 | 487 | 2014-04-17T14:14:33Z | 4/17/2014 10:14 |
| WAYPOINT | 269 | 15610 | 49.7761 | -57.2569 | 509 | 2014-04-17T14:27:57Z | 4/17/2014 10:27 |
| WAYPOINT | 270 | 15848 | 49.7801 | -57.2165 | 338 | 2014-04-17T14:42:22Z | 4/17/2014 10:42 |
| WAYPOINT | 271 | 16086 | 49.7837 | -57.1904 | 407 | 2014-04-17T15:18:53Z | 4/17/2014 11:18 |
| WAYPOINT | 272 | 15847 | 49.7574 | -57.2253 | 361 | 2014-04-17T15:35:50Z | 4/17/2014 11:35 |
| WAYPOINT | 273 | 16085 | 49.7571 | -57.1895 | 414 | 2014-04-17T15:53:25Z | 4/17/2014 11:53 |
| WAYPOINT | 274 | 16323 | 49.7601 | -57.1562 | 318 | 2014-04-17T16:08:49Z | 4/17/2014 12:08 |
| WAYPOINT | 275 | 16561 | 49.7570 | -57.1241 | 318 | 2014-04-17T16:24:51Z | 4/17/2014 12:24 |

LOWER CHURCHILL LABRADOR ISLAND TRANSMISSION LINK - NEWFOUNDLAND
MARTEN ENVIRONMENTAL EFFECTS MONITORING PROGRAM

APPENDIX D

Lab Report from Hair Snag Samples 2014



Revised

**Microsatellite and Sex Identification of Individual
Newfoundland Marten (*Martes americana atrata*)**

Prepared for: Tony Parr, B.Sc.
Environmental Technologist
Stantec Consulting

Prepared by: Genomics and Proteomics Facility
CREAIT Network
Memorial University of Newfoundland

12 November 2014

Summary

On 11 June 2014, the Genomics and Proteomics Facility of the CREAT Network at Memorial University of Newfoundland received a set of 37 envelopes containing hair samples. Envelopes contained 2 – 4 sticky pads; we processed one sticky pad per envelope. Samples from two envelopes were not processed due to sample quality. Thirty-five hair samples were screened with 11 microsatellite loci to identify individual Newfoundland marten, and sex of each individual was determined.

Findings

- We processed a total of 35 samples of which 32 have complete data sets (genotypes).
- Of the 32 complete genotypes, 18 individual Newfoundland marten (seven female, five male and six unknown) were identified.
- Ten individuals were recaptures; the remaining eight individuals were captured once.

The purpose of this work was to identify individual Newfoundland marten (*Martes americana atrata*) by screening DNA extracted from hair samples with a suite of microsatellite loci.

On 11 June 2014, the Genomics and Proteomics (GaP) Facility of the CREAT Network at Memorial University of Newfoundland received a set of 37 envelopes containing hair samples delivered by Tony Parr, B.Sc., Environmental Technologist, Stantec Consulting (Table 1).

One sticky pad per envelope was processed. DNA was extracted from approximately 20 roots using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Toronto, Ontario, Canada) following the manufacturer's Tissue Protocol, except that DNA was re-suspended in two consecutive 75 µL elutions, for a total volume of 150 µL of DNA. Hair roots were digested overnight. Two samples were not processed due to sample quality (only a single hair completely covered in glue was present).

DNA from hair samples were screened twice at the following 11 microsatellite loci using standard operating protocols developed in the GaP Facility: Ma1, Ma2, Ma7, Ma9, Ma10, Ma11, Ma14, Ma18, Ma19 (Davis and Strobeck 1998); MP0085, MP0114 (Jordan et al. 2007). Alleles were called independently by two readers.

Sex determination of samples was carried out by amplifying an intron within the zinc-finger gene that is present on both sex chromosomes using primers LGL331 and LGL335 (Shaw et al. 2003) with standard operating protocols developed in the GaP Facility. Samples with two bands (zinc finger X and Y) were identified as male, and those with one band (two copies of zinc finger X) as female. Agarose gels were read independently by two readers.

Complete genotypes were run through GENECAAP version 1.3, a Microsoft Excel macro that compares each individual multi-locus genotype with all other genotypes within the data set to locate matching genotypes (Wilberg and Dreher 2004) and thus identify individuals within a set of samples.

We were able to generate complete genotypes for 32 samples (91%; Table 2).

From the 32 samples that had complete genotypes, we defined 18 individuals (Table 3).

The overall probability that two first order relatives will share the same genotype by chance (P_{SIB}) was $p = 0.009$, and therefore, we are confident in an analysis that screens 11 microsatellite loci.

Ten of the individuals were recaptures. The remaining eight individuals were captured only once (Table 3).

We were able to identify sex for 12 individuals (67%). Five individuals are male, and the remaining seven are female (Table 2 and 3).

Table 1. GaP Facility inventory for hair samples detailing (where available) sample ID, block ID, sample collection date, crew which collected sample, any comments about the sample provided by crew, and GaP comments about the sample during DNA extraction.

| Sample ID | Block ID | Sample Collection Date | Crew | Stantec comments about sample | GaP comments about extraction |
|-----------|----------|------------------------|---------------------|-------------------------------|--|
| 1 | 14662 | 24-Apr-14 | TP & SC | None provided | 25 hairs, roots visible, 4 sticky pads |
| 2 | 14663 | 24-Apr-14 | TP & SC | None provided | >50 hairs, roots visible, 2 sticky pads |
| 3 | 14900 | 24-Apr-14 | TP & SC | None provided | >50 hairs, roots visible, 4 sticky pads |
| 4 | 15138 | 24-Apr-14 | TP & SC | None provided | >50 hairs, roots visible, 4 sticky pads |
| 5 | 15373 | 24-Apr-14 | TP & SC | None provided | 30 hairs, roots visible, 2 sticky pads |
| 6 | 15375 | 24-Apr-14 | TP & SC | None provided | >50 hairs, roots visible, 4 sticky pads |
| 7 | 15376 | 24-Apr-14 | TP & SC | None provided | >50 hairs, roots visible, 4 sticky pads |
| 8 | 15847 | 24-Apr-14 | TP & SC | None provided | >50 hairs, roots visible, 2 sticky pads |
| 9 | 15848 | 24-Apr-14 | TP & SC | None provided | >50 hairs, roots visible, 4 sticky pads |
| 10 | 16085 | 24-Apr-14 | TP & SC | None provided | >50 hairs, roots visible, 4 sticky pads |
| 11 | 14662 | 01-May-14 | TN & TP | None provided | 25 hairs, roots visible, 4 sticky pads |
| 12 | 14663 | 01-May-14 | TN & TP | None provided | >50 hairs, roots visible, 4 sticky pads |
| 13 | 14900 | 01-May-14 | TN & TP | None provided | >50 hairs, roots visible, 3 sticky pads |
| 14 | 15138 | 01-May-14 | TN & TP | None provided | >50 hairs, roots visible, 4 sticky pads |
| 15 | 15373 | 01-May-14 | TN & TP | None provided | 20 hairs, roots visible, 4 sticky pads |
| 16 | 15374 | 01-May-14 | TN & TP | None provided | >50 hairs, roots visible, 2 sticky pads |
| 17 | 15376 | 01-May-14 | TN & TP | None provided | 10 hairs, one root visible, 3 sticky pads |
| 18 | 15611 | 01-May-14 | TN & TP | None provided | 20 hairs, roots visible, 4 sticky pads |
| 19 | 15612 | 01-May-14 | TN & TP | None provided | 30 hairs, roots visible, 4 sticky pads |
| 20 | 15847 | 01-May-14 | TN & TP | None provided | >50 hairs, roots visible, 4 sticky pads |
| 21 | 15848 | 01-May-14 | TN & TP | None provided | >50 hairs, roots visible, 4 sticky pads |
| 22 | 16085 | 01-May-14 | TN & TP | None provided | 30 hairs, roots visible, 4 sticky pads |
| 23 | 16086 | 01-May-14 | TN & TP | None provided | >50 hairs, roots visible, 4 sticky pads |
| 24 | 14662 | 08-May-14 | W. Tucker & T. Parr | #2; Snow 110 cm | 20 hairs, few roots visible, 4 sticky pads |
| 25 | 14663 | 08-May-14 | W. Tucker & T. Parr | #1; Snow 80 cm | >50 hairs, roots visible, 4 sticky pads |

Table 1 continued.

| Sample ID | Block ID | Sample Collection Date | Crew | Stantec comments about sample | GaP comments about extraction |
|------------------|-----------------|-------------------------------|---------------------|--------------------------------------|--|
| 26 | 14900 | 08-May-14 | W. Tucker & T. Parr | #3; Snow 85 cm | >50 hairs, roots visible, 4 sticky pads |
| 27 | 15138 | 08-May-14 | W. Tucker & T. Parr | #4 | 20 hairs, roots visible, 4 sticky pads |
| 28 | 15610 | 08-May-14 | W. Tucker & T. Parr | #11; Snow 130 cm | >50 hairs, roots visible, 4 sticky pads |
| 29 | 15316 | 08-May-14 | W. Tucker & T. Parr | #5; Snow 165 cm | 20 hairs, roots visible, 4 sticky pads |
| 30 | 15373 | 08-May-14 | W. Tucker & T. Parr | #8; Snow 130 cm | 10 hairs, roots visible, 4 sticky pads |
| 31 | 15374 | 08-May-14 | W. Tucker & T. Parr | #7 | >50 hairs, roots visible, 4 sticky pads |
| 32 | 15612 | 08-May-14 | W. Tucker & T. Parr | #9; Snow 155 cm | 30 hairs, roots visible, 4 sticky pads |
| 33 | 15847 | 08-May-14 | W. Tucker & T. Parr | #14; Snow 120 cm | >50 hairs, roots visible, 4 sticky pads |
| 34 | 16085 | 08-May-14 | W. Tucker & T. Parr | #15; Snow 165 | 30 hairs, roots visible, 4 sticky pads |
| 35 | 16561 | 08-May-14 | W. Tucker & T. Parr | #17 | >50 hairs, roots visible, 4 sticky pads |
| N/A | 15375 | 01-May-14 | TN & TP | None provided | Didn't process, only one hair entirely covered in glue |
| N/A | 15848 | 08-May-14 | W. Tucker & T. Parr | #11; Snow 110 cm; Squirrel? | Didn't process, only one hair entirely covered in glue |

NB: Crew information is as recorded on the individual envelopes.

Table 2. Microsatellite genotypes and sex identification results for all hair samples (N = 35) detailed in Table 1. '-' indicates no data available.

| Sample ID | Sex | Microsatellite genotypes (in basepairs): | | | | | | | | | | | | Ma1 | Mp0085 | Mp0114 | Ma14 | | | | | | |
|-----------|--------|--|-----|-----|------|------|------|-----|-----|-----|-----|-----|-----|-----|--------|--------|------|-----|-----|-----|-----|-----|-----|
| | | Ma11 | Ma9 | Ma2 | Ma19 | Ma18 | Ma10 | Ma7 | | | | | | | | | | | | | | | |
| 1 | - | 108 | 108 | 146 | 147 | 175 | 181 | 212 | 214 | 167 | 169 | 181 | 181 | 204 | 204 | 225 | 228 | 134 | 136 | 162 | 170 | 209 | 209 |
| 2 | Male | 108 | 108 | 146 | 147 | 175 | 175 | 214 | 214 | 165 | 167 | 181 | 181 | 204 | 204 | 225 | 225 | 134 | 134 | 162 | 162 | 203 | 209 |
| 3 | Female | 108 | 108 | 146 | 147 | 181 | 181 | 214 | 214 | 169 | 169 | 181 | 181 | 204 | 206 | 225 | 225 | 136 | 136 | 162 | 162 | 203 | 209 |
| 4 | - | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 214 | 167 | 169 | 181 | 181 | 204 | 206 | 225 | 228 | 134 | 136 | 162 | 162 | 209 | 209 |
| 5 | Female | 108 | 108 | 146 | 147 | 177 | 181 | 212 | 214 | 169 | 169 | 180 | 181 | 204 | 206 | 225 | 225 | 134 | 136 | 162 | 162 | 199 | 209 |
| 6 | Male | 108 | 108 | 146 | 146 | 175 | 181 | 210 | 214 | 167 | 169 | 181 | 181 | 206 | 206 | 225 | 225 | 134 | 136 | 162 | 170 | 199 | 199 |
| 7 | Female | 108 | 108 | 146 | 146 | 175 | 181 | 210 | 214 | 169 | 169 | 181 | 181 | 206 | 206 | 225 | 225 | 136 | 136 | 162 | 170 | 199 | 199 |
| 8 | - | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 214 | 167 | 169 | 180 | 180 | 204 | 204 | 225 | 225 | 136 | 136 | 162 | 170 | - | 209 |
| 9 | - | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 210 | 167 | 169 | 180 | 181 | 204 | 206 | 225 | 225 | 136 | 136 | 162 | 162 | 199 | 209 |
| 10 | - | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 214 | 167 | 167 | 180 | 181 | 204 | 204 | 225 | 225 | 136 | 136 | 162 | 162 | 199 | 209 |
| 11 | Male | 108 | 108 | 146 | 147 | 175 | 181 | 212 | 214 | 167 | 169 | 181 | 181 | 204 | 204 | 225 | 228 | 134 | 136 | 162 | 170 | 209 | 209 |
| 12 | Male | 108 | 108 | 146 | 147 | 175 | 175 | 214 | 214 | 165 | 167 | 181 | 181 | 204 | 204 | 225 | 225 | 134 | 134 | 162 | 162 | 203 | 209 |
| 13 | Female | 108 | 108 | 146 | 147 | 175 | 181 | 214 | 214 | - | 169 | 181 | 181 | 204 | 206 | 225 | 225 | 136 | 136 | 162 | 162 | 203 | 209 |
| 14 | - | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 214 | 167 | 169 | 181 | 181 | 204 | 206 | 225 | 228 | 134 | 136 | 162 | 162 | 209 | 209 |
| 15 | Female | 108 | 108 | 146 | 147 | 177 | 181 | 212 | 214 | 169 | 169 | 180 | 181 | 204 | 206 | 225 | 225 | 134 | 136 | 162 | 162 | 199 | 209 |
| 16 | Male | 108 | 108 | 147 | 147 | 177 | 177 | 214 | 214 | 169 | 169 | 180 | 181 | 204 | 206 | 225 | 225 | 136 | 136 | 162 | 170 | 199 | 209 |
| 17 | - | 108 | 108 | - | - | 177 | 177 | - | 214 | 169 | 169 | 180 | 181 | 204 | 206 | 225 | 225 | 136 | 136 | 162 | 170 | 199 | - |
| 18 | Female | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 214 | 167 | 167 | 180 | 181 | 204 | 204 | 225 | 225 | 136 | 136 | 162 | 162 | 203 | 209 |

Table 2 continued.

| Sample ID | Sex | Microsatellite genotypes (in basepairs): | | | | | | | | | | | | | | | | | | | | | |
|-----------|--------|--|-----|-----|-----|-----|-----|------|-----|------|-----|------|-----|-----|-----|-----|-----|--------|-----|--------|-----|------|-----|
| | | Ma11 | | Ma9 | | Ma2 | | Ma19 | | Ma18 | | Ma10 | | Ma7 | | Ma1 | | Mp0085 | | Mp0114 | | Ma14 | |
| 19 | Female | 108 | 108 | 147 | 147 | 175 | 181 | 214 | 214 | 169 | 169 | 181 | 181 | 204 | 204 | 225 | 225 | 134 | 136 | 162 | 162 | 209 | 209 |
| 20 | - | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 214 | 167 | 169 | 180 | 180 | 204 | 204 | 225 | 225 | 136 | 136 | 162 | 162 | 203 | 209 |
| 21 | Male | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 210 | 167 | 169 | 180 | 181 | 204 | 206 | 225 | 225 | 136 | 136 | 162 | 162 | 199 | 209 |
| 22 | - | 108 | 108 | 147 | 147 | 181 | 181 | 210 | 212 | 167 | 169 | 180 | 181 | 204 | 206 | 225 | 225 | 134 | 136 | 162 | 170 | 199 | 209 |
| 23 | - | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 214 | 167 | 167 | 180 | 181 | 204 | 204 | 225 | 225 | 136 | 136 | 162 | 162 | 199 | 209 |
| 24 | - | 108 | 108 | 146 | 147 | 175 | 175 | 214 | 214 | 165 | 167 | 181 | 181 | 204 | 204 | 225 | 225 | 134 | 134 | 162 | 162 | 203 | 209 |
| 25 | - | 108 | 108 | 146 | 147 | 175 | 175 | 214 | 214 | 165 | 167 | 181 | 181 | 204 | 204 | 225 | 225 | 134 | 134 | 162 | 162 | 203 | 209 |
| 26 | Female | 108 | 108 | 146 | 147 | 175 | 177 | 214 | 214 | 167 | 169 | 181 | 181 | 204 | 206 | 225 | 225 | 134 | 136 | 162 | 170 | 199 | 209 |
| 27 | - | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 214 | 167 | 169 | 181 | 181 | 204 | 206 | 225 | 228 | 134 | 136 | 162 | 162 | 209 | 209 |
| 28 | - | 108 | 108 | 147 | 147 | 175 | 181 | 212 | 214 | 167 | 169 | 180 | 181 | 204 | 206 | 225 | 225 | 134 | 136 | 170 | 170 | 209 | 209 |
| 29 | - | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 214 | 169 | 169 | 180 | 181 | 206 | 206 | 225 | 225 | 136 | 136 | 162 | 162 | 199 | 209 |
| 30 | Female | 108 | 108 | 146 | 147 | 177 | 181 | 212 | 214 | 169 | 169 | 180 | 181 | 204 | 206 | 225 | 225 | 134 | 136 | 162 | 162 | 199 | 209 |
| 31 | Male | 108 | 108 | 146 | 146 | 175 | 181 | 210 | 214 | 167 | 169 | 181 | 181 | 206 | 206 | 225 | 225 | 134 | 136 | 162 | 170 | 199 | 199 |
| 32 | - | 108 | 108 | 147 | 147 | 175 | 181 | 214 | 214 | 169 | 169 | 181 | 181 | 204 | 204 | 225 | 225 | 134 | 136 | 162 | 162 | 209 | 209 |
| 33 | - | 108 | 108 | 146 | 147 | 181 | 181 | 210 | 214 | 167 | 169 | 180 | 180 | 204 | 204 | 225 | 225 | 136 | 136 | 162 | 162 | 203 | 209 |
| 34 | Female | 108 | 108 | 147 | 147 | 181 | 181 | 210 | 212 | 167 | 169 | 180 | 181 | 204 | 206 | 225 | 225 | 134 | 136 | 162 | 170 | 199 | 209 |
| 35 | - | 108 | 108 | 146 | 147 | 175 | 181 | 214 | 214 | 167 | 169 | 180 | 181 | 204 | 204 | 225 | 225 | 134 | 136 | 162 | 162 | 199 | 203 |

Table 3. Individual Newfoundland marten identified in this molecular study (including sex results) with samples having identical genotypes identified. '-' indicates no data available.

| | Sex of individual | Sample IDs with matching genotypes | | | |
|----|--------------------------|---|----|----|----|
| 1 | Male | 1 | 11 | | |
| 2 | Male | 2 | 12 | 24 | 25 |
| 3 | Female | 3 | | | |
| 4 | - | 4 | 14 | 27 | |
| 5 | Female | 5 | 15 | 30 | |
| 6 | Male | 6 | 31 | | |
| 7 | Female | 7 | | | |
| 8 | Male | 9 | 21 | | |
| 9 | - | 10 | 23 | | |
| 10 | Male | 16 | | | |
| 11 | Female | 18 | | | |
| 12 | Female | 19 | 32 | | |
| 13 | - | 20 | 33 | | |
| 14 | Female | 22 | 34 | | |
| 15 | Female | 26 | | | |
| 16 | - | 28 | | | |
| 17 | - | 29 | | | |
| 18 | - | 35 | | | |

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