STUDY PLAN FOR MINK INJURY DETERMINATION

INVESTIGATION OF MINK ABUNDANCE AND DENSITY RELATIVE TO POLYCHLORINATED BIPHENYL CONTAMINATION WITHIN THE HUDSON RIVER DRAINAGE

HUDSON RIVER NATURAL RESOURCE DAMAGE ASSESSMENT

HUDSON RIVER NATURAL RESOURCE TRUSTEES

STATE OF NEW YORK

U.S. DEPARTMENT OF COMMERCE

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* Names of certain individuals and affiliations have been removed to maintain confidentiality

EXECUTIVE SUMMARY

Natural resources of the Hudson River have been contaminated through past and ongoing discharges of polychlorinated biphenyls (PCBs). The Hudson River Natural Resource Trustees – New York State, the U.S. Department of Commerce, and the U.S. Department of the Interior – are conducting a natural resource damage assessment (NRDA) to assess and restore those natural resources injured by PCBs.

Many species of mammals rely on the Hudson River, including its floodplain, for habitat, food, and as a breeding ground. Mammals that depend on the river for food and habitat include otter, muskrat, raccoon, beaver, and mink. The Hudson River NRDA Plan identified mink and otter health as an area of biological injury investigation. Mink are the subject of this Final Study Plan for an injury determination effort as part of the Hudson River NRDA

Based on the results of preliminary investigations conducted by the Trustees, including the mink and otter work conducted in the upper Hudson River drainage during the 1998-1999 and 1999-2000 trapping seasons, input from a panel of mammal experts, review of the existing mink and otter toxicology literature, and considering factors such as the life history of mink, preliminary results of the mink PCB-feeding study, and goals of the NRDA, the Trustees have determined that it is appropriate to conduct further investigations focused on mink to be initiated in the year 2012.

Pursuant to the Hudson River NRDA Plan, the Trustees have developed this Final Study Plan for a mink injury determination effort. A Draft Study Plan for this work was peer reviewed and made available to the public for review and comment. All comments received on the Draft Study Plan, as part of the peer and public review process, have been considered. The Trustees evaluated peer and public comments and, where warranted, incorporated these comments in the Draft Study Plan to produce the Final Study Plan. A Responsiveness Summary, responding to public comments on the Draft Study Plan, will be provided by the Trustees in the near future.

The objective of the study is to estimate abundance and density of mink in areas within the Upper Hudson River drainage where elevated levels of PCBs have been found, and to compare that estimate of mink abundance and density to that in a reference river. The Trustees will assess the following potential injury to mink: reduced abundance and density in areas contaminated by PCBs; or, reduced occupancy in areas contaminated by PCBs. In the future the Trustees may propose additional work to supplement this effort.

The purpose of this work is to inform the Trustees regarding injury to mink and guide their future efforts to identify pathways and specific injuries to mink from PCBs, as defined in regulations written by the U.S. Department of the Interior contained in Title 43 of the Code of Federal Regulations Part 11, Natural Resource Damage Assessment. This work will also be used to help determine whether future studies will be performed, and if so, to help in their design.

Pursuant to the Hudson River NRDA Plan, the results of the work conducted pursuant to this Study Plan will be peer reviewed upon completion of the study, and the results then released to the public.

Table of Contents

EXECUTIVE SUMMARY

1.0 BACKGROUND 1

2.0 INTRODUCTION 2

3.0 PURPOSE AND OBJECTIVE 2

4.0 METHOD 3

5.0 QUALITY ASSURANCE/QUALITY CONTROL 4

6.0 SPECIAL PROVISIONS 4

7.0 LITERATURE CITED 4

APPENDIX A: STUDY PLAN INVESTIGATION OF MINK ABUNDANCE RELATIVE TO POLYCHLORINATED BIPHENYL (PCB) CONTAMINATION WITHIN THE HUDSON RIVER DRAINAGE

STUDY PLAN FOR MINK INJURY DETERMINATION—INVESTIGATION OF MINK ABUNDANCE AND DENSITY RELATIVE TO POLYCHLORINATED BIPHENYL CONTAMINATION WITHIN THE HUDSON RIVER DRAINAGE

1.0 BACKGROUND

Past and continuing discharges of polychlorinated biphenyls (PCBs) have contaminated the natural resources of the Hudson River. The Hudson River Natural Resource Trustees – New York State, the U.S. Department of Commerce, and the U.S. Department of the Interior – are conducting a natural resource damage assessment (NRDA) to assess and restore those natural resources injured by PCBs (Hudson River Natural Resource Trustees 2002).

Many species of mammals rely on the Hudson River, including its floodplain, for habitat, food, and as a breeding ground. Mammals that depend on the river for food and habitat include otter, muskrat, raccoon, beaver, and mink. The Hudson River NRDA Plan identified mink and otter health as an area of biological injury investigation. Mink are the subject of this Final Study Plan for an injury determination effort as part of the Hudson River NRDA.

Injury means a measurable adverse change, either long- or short-term, in the chemical or physical quality or the viability of a natural resource resulting either directly or indirectly from exposure to release of a hazardous substance, such as PCBs, or exposure to a product of reactions resulting from the release of a hazardous substance. An injury to a biological resource, such as mink, has resulted from the release of a hazardous substance, such as PCBs, if the concentration of the substance is sufficient to cause the biological resource or its offspring to have undergone at least one of the following adverse changes in viability: death, disease, behavioral abnormalities, cancer, genetic mutations, physiological malfunctions (including malfunctions in reproduction), or physical deformations.

Mink are small carnivorous mammals that are associated with aquatic habitats of all kinds including rivers, lakes, and wetlands (USEPA 1993). They are opportunistic hunters, feeding on any animal material they can find and kill (Linscombe et al. 1982). Mink appear to select prey primarily based on its availability (Gilbert and Nancekivell 1982) and vulnerability (Eagle and Whitman 1987). The mink diet includes other small mammals such as mice, rats, rabbits and muskrats, aquatic prey including frogs, fish, and crayfish, and terrestrial prey including birds, reptiles such as snakes, insects, and other invertebrates. Mink are exposed to PCBs directly through their diet. Mink are also exposed to PCB-contaminated water and soil or sediments as they build dens and forage for food.

The Trustee agencies have assessed PCB concentrations in mink from the Hudson River. PCB concentrations in liver (normalized for the amount of fat, or lipids, in each sample) from mink collected from the Hudson River between Hudson Falls and Troy were as high as 139 ppm (Mayack & Loukmas, 2001).

Analysis of mink collected from 1998 to 2000 for hepatic PCB burdens as Aroclors indicated concentrations were elevated for animals collected from the main channel of river sections contaminated with PCBs or tributaries entering those sections. Maximum PCB levels in mink exceeded criteria for reproductive impairment and criteria for potential health impairment (Leonards *et al.* 1994; Smit *et al.* 1996). Approximately half the mink collected during 1998-2002 within 6 km of the main-stem of the Hudson River had elevated levels of PCBs in their livers; mink with elevated levels of PCBs in their livers were not recovered beyond 5 km from the main-stem Hudson River. In addition to elevated contaminant burdens, a lower take of mink relative to trapping effort was evident for trap sites located within 6 km of PCB-contaminated sections of the Hudson River between Hudson Falls and Troy compared to sites at least one home range from the river or upstream of Hudson Falls (Mayack and Loukmas, 2001).

Those preliminary investigations of mink exposure to PCBs were undertaken to assist the Trustees in determining the extent to which mink in the Hudson River are contaminated with PCBs, to determine if additional pathway and injury assessment studies focused on mink should be conducted as part of the Hudson River NRDA, and for potential use in the design of future studies to assess the health of Hudson River mink.

In January 2002, the Trustees assembled an expert panel to review the exposure and effects information compiled by the NYSDEC for mink and otter, and to provide guidance to the Trustees on appropriate next steps for determining whether PCBs are causing adverse biological effects in Hudson River mammals, particularly mink and otter. The Hudson River NRDA Plan noted that the Trustees planned to build upon the existing mink and otter studies, potentially conducting further studies to determine PCB effects in mink and otter from the Hudson River.

The Trustees are engaged in two such studies. The first study is a laboratory study; the second study is this field study.

Regarding the laboratory study, in 2006, the Trustees initiated a mink-PCB feeding study (Hudson River Natural Resource Trustees, 2006) as part of the mink injury determination. The results of that study are currently undergoing peer review. Pursuant to the Hudson River NRDA Plan, the results of that study will be released to the public after peer review is complete. Regarding the field study, on August, 2, 2010, the Trustees released a Draft Study Plan entitled, "Investigation of Mink Occupancy Relative to Polychlorinated Biphenyl Contamination within the Hudson River Drainage" (Hudson River Natural Resource Trustees, 2010). Following peer and public review of that plan, the Trustees determined that revisions to that plan were appropriate, resulting in the March 19, 2012 Draft Study Plan (Hudson River Natural Resource Trustees, 2012) being released for further peer and public review, culminating in this Final Study Plan.

2. INTRODUCTION

Based on the results of preliminary investigations conducted by the Trustees, including the mink and otter work (Mayack and Loukmas, 2001), input from a panel of mammal experts, review of the existing mink and otter toxicology literature, and considering factors such as the life history of mink, preliminary results of the mink PCB-feeding study (Hudson River Natural Resource Trustees, 2006), and goals of the NRDA, the Trustees have determined that it is appropriate to conduct further investigations on mink to be initiated in the year 2012.

Pursuant to the Hudson River NRDA Plan, the Trustees developed a Draft Study Plan (Hudson River Natural Resource Trustees, 2012) for a mink injury determination effort. As this investigation evaluates injury endpoints, the Trustees performed a peer review of that Draft Study Plan, and made it available to the public for review and comment.

In accordance with the Hudson River NRDA Plan, the Trustees are now issuing this Final Study Plan for a mink injury determination effort. Changes made as a result of the peer review process have been incorporated into the Final Study Plan. A Responsiveness Summary responding to public comments on the Draft Study Plan will also be released. After the study is completed, the results will be peer reviewed and released to the public.

When ready, that information will be available on the following Trustee websites:

- http://www.fws.gov/contaminants/restorationplans/HudsonRiver/index.html;
- http://www.dec.ny.gov/lands/25609.html; and,
- http://www.darrp.noaa.gov/northeast/hudson/.

3. PURPOSE AND OBJECTIVE

The purpose of this work is to inform the Trustees regarding injury to mink and to guide their future efforts to identify pathways and specific injuries to mink from PCBs, as defined in regulations written by the U.S. Department of the Interior (Title 43 of the Code of Federal Regulations Part 11, Natural Resource Damage Assessment). This work will also be used to help determine whether future studies will be performed, and if so, to help in their design.

This Final Study Plan describes a field study designed to assess the abundance and density of mink in areas of the Hudson River drainage contaminated with PCBs (within 5 km of the main stem) as compared with areas with no documented or minimal contamination (within 5 km of the main stem of the Mohawk River, a reference river). The study will be conducted in the Upper Hudson River drainage (between Fort Edward and 50 km south of Fort Edward). The Mohawk River drainage (between Herkimer and 50 km east of Herkimer) will serve as a reference river, and will be evaluated similarly (sampling within 5 km of the main stem).

The objective of the study is to estimate abundance and density of mink in areas within the Upper Hudson River drainage where elevated levels of PCBs have been found, and to compare that estimate of mink abundance and density to that in a reference river. The Trustees will assess the following potential injury to mink: reduced abundance and density in areas contaminated by PCBs; or, reduced occupancy in areas contaminated by PCBs.

To investigate mink abundance and density, the Trustees will conduct a spatial capture-recapture study based on two sources of data: mink scat samples and mink hair samples. Scats obtained from mink using dogs specialized in mink-scat search will be analyzed for DNA. The Trustees will also conduct a pilot study to evaluate hair-snare collection devices as a means to collect mink hair samples, providing an additional source of DNA material. The Trustees' goal is to use those techniques to obtain individual identification of mink to estimate mink abundance and density using spatial capture-recapture (SCR) models. If sufficient data are not available to evaluate abundance and density of mink, mink occupancy within the upper Hudson River drainage will be estimated and compared to that of a reference river.

4. METHOD

On behalf of the Trustees, beginning in 2012, Principal Investigators (PIs) will conduct a study to estimate abundance and density of mink in areas within the Upper Hudson River drainage where elevated levels of PCBs have been found, and to compare that estimate of mink abundance and density to that in a reference river. This study will be conducted pursuant to a work plan entitled "Investigation of Mink Abundance Relative to Polychlorinated Biphenyl (PCB) Contamination within the Hudson River Drainage" contained in Appendix A.

This study will enable the Trustees to assess the following injuries to mink: reduced abundance and density in areas contaminated by elevated levels of PCBs; or, reduced occupancy in areas contaminated by PCBs.

5.0 QUALITY ASSURANCE/QUALITY CONTROL

This study is being conducted in accordance with the Quality Assurance Management Plan for the Trustees' Hudson River NRDA (Hudson River Natural Resources Trustees, 2002).

As noted in the Trustees' Responsiveness Summary for the NRDA Plan (Hudson River Natural Resource Trustees, 2003), for each data collection effort that is part of the Hudson River NRDA and is identified in the NRDA Plan, the Trustees will develop a project-specific QA Plan which may be an independent document or may be incorporated into the project Study Plan. Such a QA Plan, in combination with the information on QA management described in the NRDA Plan (Hudson River Natural Resource Trustees, 2002), will ensure that the requirements listed in the National Contingency Plan and applicable EPA guidance for quality control and quality assurance plans are met. The work plan (Appendix A) for the investigation includes project-specific QA plan provisions.

Strict Chain of Custody procedures will be used throughout the study.

6. SPECIAL PROVISIONS

Permits will be required from the National Park Service to conduct a portion of the field study on National Park Service land. Permission will be required to enter private lands or lands under the jurisdiction of State agencies or authorities other than New York State Department of Environmental Conservation to conduct a portion of the field study on those lands.

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Appendix A

STUDY PLAN INVESTIGATION OF MINK ABUNDANCE RELATIVE TO POLYCHLORINATED BIPHENYL (PCB) CONTAMINATION WITHIN THE HUDSON RIVER DRAINAGE

STUDY PLAN FOR MINK INJURY DETERMINATION — INVESTIGATION OF MINK ABUNDANCE AND DENSITY RELATIVE TO POLYCHLORINATED BIPHENYL CONTAMINATION WITHIN THE HUDSON RIVER DRAINAGE

Study Plan Investigation of Mink Abundance Relative to Polychlorinated Biphenyl (PCB) Contamination within the Hudson River Drainage

Hudson Natural Resource Damage Assessment

Hudson River Natural Resource Trustees

State of New York

U.S. Department of Commerce

U.S. Department of the Interior

June, 2012

Principal Investigator

Principal Investigator

Quality Assurance Coordinator

INVESTIGATION TEAM ACKNOWLEDGEMENT OF WORK PLAN REVIEW AND COMPLIANCE

By my signature, I acknowledge that I have read this Work Plan and understand it, and will comply with it in performing this work.

Name (printed):_____ Name (printed):_____

Signature:_____ Signature:_____

Initials:_____ Initials:_____

Date:_____ Date:_____

Title:_____ Title:_____

Name (printed):_____ Name (printed):_____

Signature:_____ Signature:_____

Initials:_____ Initials:_____

Date:_____ Date:_____

Title:_____ Title:_____

Table of Contents

Summary 5

Background 5

Objective 6

Mark-Recapture Experiments 7

Introduction 7

Study Design 8

Study sites 11

Analysis 14

Genetic analysis 14

Statistical Analysis: Abundance and density estimation 16

Analysis of hair data 16

Analysis of scat data 18

Alternative statistical approaches 19

Quality Assurance/Quality Control 22

Project Management 22

Data Generation and Acquisition 23

Study Documentation 25

Personnel Experience and Training 26

Assessment and Oversight 26

Data Validation and Usability 27

Chain of Custody Procedures 27

Special Provisions 29

Staff 29

Facilities 30

Expected Products 30

Literature Cited 31

Appendix 1: Hair-snare testing experiment 38

Appendix 2: Stream-road intersections in the Hudson and Mohawk River study areas 43

Appendix 3: Standard operating procedure for selection and evaluation of sites for hair and scat surveys 45

Appendix 4: Standard operating procedure for acquiring site permission for the pilot study 55
Appendix 5: Standard operating procedure for field assessment and operating an optical clinometer ... 56
Appendix 6: Home range analysis and analysis of mink use of stream- road intersections 61
Appendix 7: Standard Operating Procedure for Monitoring Hair Snares 67
Appendix 8: Datasheet for mink hair collection 70
Appendix 9: Standard Operating Procedure for photographic collection 71
Appendix 10: Dog training and sampling protocol 72
Appendix 11: Standard Operating Procedure for Scat Survey and collection 77
Appendix 12: Datasheet for mink scat collection 79
Appendix 13: Organizational chart 80
Appendix 14: Chain of Custody 81
Appendix 15: Laboratory form 84
Appendix 16: Field supplies 86

Summary

Natural resources of the Hudson River have been contaminated through past and ongoing discharges of polychlorinated biphenyls (PCBs). The Hudson River Natural Resource Trustees (*i.e.*, New York State, the U.S. Department of Commerce, and the U.S. Department of Interior) are conducting a natural resource damage assessment (NRDA) to assess and restore those natural resources injured by PCBs.

Hudson River mink have been exposed to elevated, and potentially injurious, levels of PCBs: maximum PCB levels in mink and otter collected during 1998-2002 within 6 km of the main-stem of the Hudson River had PCB concentrations in their livers that exceeded criteria for reproductive impairment and criteria for potential health impairment. However, mink recovered beyond 6 km from the main-stem of the Hudson River did not have elevated PCB concentrations in their livers. In addition to elevated contaminant burdens, a lower take of mink relative to trapping effort was evident for trap sites located within 6 km of PCB-contaminated sections of the Hudson River between Hudson Falls and Troy, compared to sites >6km from the river, or upstream of Hudson Falls.

This study will use a mark-recapture approach to estimate the abundance and density of mink in areas within 5 km of the upper Hudson River, where elevated levels of PCBs have been reported, and to compare these to estimates of mink abundance and density of a reference river (the Mohawk River). If data are not sufficient to evaluate abundance and density, mink occupancy will be compared instead. The work will help inform the Trustees regarding injury to mink and will be used to help determine whether, and how, future studies may be performed.

Background

The loading of polychlorinated biphenyls (PCBs) in the Hudson River was the highest of any major river system in the United States (Horn *et al.* 1979). An electrical capacitor manufacturing plant located at Hudson Falls, New York, and its sister plant, located approximately 2 km downstream at Fort Edward, New York, discharged PCBs into the Hudson River starting in 1947. Between 1966 and 1974, these plants purchased 35,000 metric tons of PCBs, representing approximately 15% of domestic sales (Horn *et al.* 1979). EPA estimates that between the 1940s and 1977, the General Electric Company discharged up to 1.3 million pounds of PCBs to the Hudson River (EPA 1991). Historic discharges, continuing releases from fractured bedrock, and erosion of contaminated soils and sediments have contaminated river water, sediments, floodplains, fish, wildlife, and other biota with PCBs.

Analysis of a small number of mink (*Neovison vison*, formerly *Mustela vison* (Wilson and Reeder, 2005)) and river otters (*Lontra canadensis,* formerly *Lutra canadensis* (Wilson and Reeder, 1993)) collected from the Hudson River region of New York State between 1982 and 1984 suggests that high levels of PCB contamination were present in populations of these mustelids (Foley *et al.* 1988). The maximum PCB level in mink was greater than levels known to cause reproductive failure in ranched mink (Foley *et al.* 1988). This degree of contamination suggests that reproductive impairment and a consequent decrease in abundance may be present

in wild populations of mink and river otters occupying riparian habitats of the Hudson River drainage. Although PCB levels in mustelids collected from the Hudson River region between 1982 and 1984 were the highest of any collected from eight regions (ecozones) of New York State, the number of animals collected in this region was too small to quantify the extent of contamination in these populations.

To further assess the extent of contamination and its effect on mink, river otters, and muskrats (*Ondatra zibethicus*), contaminant burdens were examined in these species collected from the Hudson River drainage. The analysis of these animals (collected from 1998-2000) for hepatic PCB burdens as Aroclors indicated concentrations were elevated for mink and otters (Mayack and Loukmas 2001). Maximum PCB levels in mink and otter exceeded the criteria of Leonards *et al.* (1994) for reproductive impairment by factors of 2.8 and 8.6, respectively. Maximum PCB levels in mink and otters exceeded criteria of Smit *et al.* (1996) for potential health impairment by factors of 6.6 and 20.5, respectively. Levels of PCBs in mink and otters from uncontaminated or less-contaminated reaches or tributaries were generally below no-effect levels for adverse toxicological effects as defined by Smit *et al.* (1996). Approximately half of the mink collected during 1998-2002 within 6 km of the main-stem of the Hudson River had elevated levels of PCBs. However, mink with elevated levels were not recovered beyond 5 km from the main-stem of the Hudson River (Mayack 2008). Furthermore, the dioxin-like toxicity equivalency quotient relative to total PCB concentration was approximately an order of magnitude greater for mink than otter.

To further investigate the apparent decrease in abundance of mink in contaminated areas, a preliminary assessment of methods to determine mink occupancy, which used scent stations equipped with track plates to monitor mink activity, was undertaken within the Hudson River drainage (Mayack 2005a): 82 scent stations (*i.e.*, 32 and 50 within the upper Hudson River and Mohawk River drainages, respectively) were placed at two nominal distances (*i.e.*, < 6 km, and > 6 km) from the main-stem of each river. Typically, track plates and scent attractant were placed in open-ended enclosures between September 26, 2000 and October 11, 2000 and checked and replaced weekly, from October 3, 2000 to April 5, 2001. Results from this study indicated that approximately half the stations (41- 57%) in each distance-drainage category were never visited by mink during either monitoring period, with the exception of the near-Hudson category (*i.e.*, <6 km from the Hudson), which had a considerably higher percentage of stations never visited by mink (77%) during the fall-early winter period.

Objective

This study's objective is to estimate the abundance and density of mink in areas within the upper Hudson River drainage where elevated levels of PCBs have been reported, and to compare these to estimates of mink abundance and density of a reference river.

If data are insufficient to evaluate the abundance and density of mink, as an alternative approach, mink occupancy within the upper Hudson River drainage will be compared to occupancy near a reference river.

Sampling procedures will include the non-invasive DNA analysis of scats obtained from mink, collected using dogs specialized in mink-scat search techniques. This study also includes a pilot effort to evaluate hair-snare collection devices as an additional source of DNA material. The goal is to use those techniques to obtain individual identification to estimate abundance and density using spatial capture-recapture (SCR) models.

Mark-Recapture Experiments

Introduction

The use of non-invasive genetic methods by genotyping hair, feather, feces, or sloughed skin represents an alternative to traditional marking methods, and is becoming increasingly popular in conjunction with mark-recapture methods to estimate population size (Bellemain *et al.* 2005, Boulanger *et al.* 2004a, Boulanger *et al.* 2004b, Creel *et al.* 2003, Eggert *et al.* 2003, Mills *et al.* 2000, Mowat and Paetkau 2002, Prugh *et al.* 2005, Taberlet *et al.* 1999, Wilson *et al.* 2003). Spatial capture-recapture models have proven useful for a wide variety of species, including Andean cats (Reppucci *et al.* 2011), Pampas cats (Gardner *et al.* 2010, Reppucci *et al.* 2011), mountain vizcachas (*Lagidium viscacia*) (Reppucci *et al.* 2011), culpeo foxes (*Lycalopex culpaeus*) (Reppucci *et al.* 2011), brown and black bear (Gardner *et al.* 2009, 2010,Woods *et al.* 1999, Mowat and Strobeck 2000), stoats (Efford *et al.* 2009), wolverine (Royle *et al.* 2011), tigers (Royle *et al.* 2009), leopard (O'Brien and Kinnaird 2011), aardwolf (O'Brien and Kinnaird 2011), spotted and striped hyena (O'Brien and Kinnaird 2011), European wildcat (Kéry *et al.* 2011), and jaguars (Sollmann *et al.* 2011).

Among the non-invasive hair sampling approaches, the hair-snare method has been used to study elusive species (Depue and Ben-David 2007, Gardner *et al.* 2010, Mowat and Paetkau 2002, Raphael 1994) and has great potential because it is a low-cost alternative to direct capture and intensive tracking (*i.e.*, radiocollar) methods. However, the success of such hair collecting devices is unknown for several species, including mink. We have therefore conducted a preliminary experiment using farm mink to evaluate the hair-snare design that maximizes hair collection (Appendix 1).

Using scat detection dogs to collect mink scats can significantly increase the number of scats collected, and has been used effectively on black bears, fishers, and bobcats (Harrison 2006, Homan *et al.* 2001, Long *et al.* 2006, Reed *et al.* 2011, Smith *et al.* 2001). Maximizing fecal-sample collection is of major importance because typically only 30-60% of fecal samples lead to species identification (Rozhnov *et al.* 2008, Harrington *et al.* 2010) and there is potential for fewer to yield individual identification.

The unique genetic profile of each individual identified through the non-invasive sampling technique is then used to build capture histories, which are in turn used in mark-recapture models to estimate parameters related to encounter probability and population size. However, classic capture-recapture models have a number of practical deficiencies which make

estimates of population size difficult to interpret in most practical situations. For example, ordinary capture-recapture models do not accommodate the existence of heterogeneous encounter probabilities due to the juxtaposition of individuals with traps, and they do not allow for modeling of trap-specific effects related to type of trap, whether it is baited, or the number of days in operation. Finally, ordinary capture-recapture models do not allow the estimation of density because there is no explicit notation of "sampled area". Indeed, in traditional mark-recapture models, the population size N is usually defined as the number of individuals present in an arbitrary sampling area of unknown size, and unless the population is homogeneously located in a well-defined geographic study area, it is difficult to relate the estimates of abundance to appropriate estimates of absolute population density.

Recently, spatial extensions of the traditional mark-recapture models (*i.e.*, spatial-capture-recapture, SCR, models) have been developed to resolve these deficiencies of traditional mark-recapture models. The development of SCR models has been proven especially useful for animals using individual home ranges and sampling situations where encounter locations represent fixed points in space because they overcome edge effects that can be problematic with traditional mark-recapture models (Otis *et al.*1978). The SCR framework represents a very flexible and wide array of models that extend classic mark-recapture models to account for a spatial component.

In SCR models, a spatial model of the population and a spatial model of the detection process are fitted to the capture histories: the first model describes the distribution of animal home ranges in the landscape while the observation model links the probability of detecting an individual at a specific trap and the distance between the trap and the animal's home range center, which is a latent variable since unknown. SCR models can be fitted using standard likelihood or Bayesian methods.

Study Design

The proposed study is designed to assess the abundance and density of mink in areas of the Hudson River drainage contaminated with PCBs (within 5 km of the main stem) as compared with areas with no documented or minimal contamination (within 5 km of the main stem of the Mohawk River, a reference site). The study will be conducted in the upper Hudson River drainage (between Fort Edward and 50 km south of Fort Edward). The Mohawk River drainage (between Herkimer and 50 km east of Herkimer) will serve as a reference area, and will be evaluated similarly (sampling within 5 km of the main stem) (Appendix 2).

We will utilize collected hair and scat to identify individuals based on genetic analyses. Site selection will follow protocols outlined in Appendices 3 and 4. Site selection for the scat study will also consider non-hazardous areas in which a scat-detection dog can work. Scat detection will be conducted at stream-road intersections on tributary streams entering the main stem of both the Hudson and Mohawk rivers (Appendix 2). One scat detection dog will survey a river (i.e., either the Hudson or Mohawk), with both detection dogs simultaneously surveying different sites. Each week, we will switch the dog that is assigned to each river. We will re-visit scat-detection sites on 3 occasions between April 30th and July 14th, 2013.

During the pilot study in summer 2012, we will sample 50 sites with both methods in each river drainage, focusing on the middle 1/3 of each study area (Appendices 3 and 4). During the pilot season we will not use *a priori* habitat associations to select sites. We will collect habitat information using GIS as well as field measurements (Appendices 3 and 5) and subsequently evaluate the relationship between these habitat variables and sites where we detect mink. This represents a variation (i.e., not in an occupancy context) of a two-phase sampling design (e.g., Pacifici *et al.* 2012) whereby we allocate effort during the second field season in 144 sites in each area that will represent a more efficient use of resources and allocation of effort to increase the amount of information collected and to improve the estimation of abundance.

For the hair-snare pilot study, two hair-snare devices will be deployed at each selected site (*i.e.*, the same sites used for scat collection). Hair snares will be visited every 7 days in the order they were installed in the field between June 1 and July 14, 2012, for a total of 5 sampling occasions. Sites for both hair snares and scat detection dogs will be within 5 km of the main stem of the Hudson and Mohawk rivers. The study will be conducted over a two-year period using the same sites each year.

Mink abundance estimates in the Hudson and Mohawk River drainages will be obtained using SCR analysis. Given the nature of the target species and the study design/data requirements, the SCR approach was chosen over conventional mark-recapture techniques. SCR models produce not only abundance estimates, but also density estimates because animal movement and location are modeled and allow the direct estimation of the area effectively covered by the search area. The SCR approach relies on several assumptions, and the study should be designed in a way that avoids or minimizes the violation of those assumptions:

- Observations made at an occupied site are independent: violation of this assumption will be avoided by rotating the dog-teams between the 2 rivers so that previous detection at a site does not cause the observer to sample with an increased effort because they remember and know that a mink most likely occupies this site. We will train the dog in sites outside the study area prior to the data collection so that it develops an efficient search strategy prior to the study. A behavioral response to account for the learning behavior of the dog (or handler) can also be accommodated in the SCR model.

- The study areas are demographically closed (*i.e.*, no recruitment or mortality): data collection will occur after the breeding season, which extends from late February through the first week in April (Enders 1952). Therefore, there will be no recruitment to the population. Data collection will occur prior to the juvenile dispersal period that begins late July to mid-August (Gerell 1970, Dunstone 1993, Niemimaa 1995), so we are sampling resident individuals. Finally, sampling will occur before any mortality associated with the fall trapping season to satisfy this assumption. The occupancy status for each site is also assumed independent of each other, so the data collection will occur at a time when home ranges are reported to be stable (Dunstone 1993).

The study design is constrained by three factors: 1) the number of sites that a technician or a dog-team can visit in a day, 2) the number of technicians and dog-teams available, given fiscal constraints, and 3) the duration of each sampling occasion, which is constrained to the season that includes only resident individuals. The number of temporal replications will be

limited by the duration of the sampling period, between the end of the breeding season in April and the onset of independence (*i.e.*, juvenile dispersal) in late July to mid-August (Gerell 1970, Dunstone 1993, Niemimaa 1995), and the length of time needed between sampling occasions. Approximately 3 weeks between sampling occasions will allow enough time for scats missed in one occasion to degrade (Sanchez *et al.* 2004, Jeffrey Loukmas, pers. comm.) before the next sampling occasion (to avoid detecting scats from a previous occasion, hence violating the closure assumption). We expect that each dog can survey 8 sites per day, which results in a total of 144 sites monitored every 3 weeks per river.

Approximately 7 days between sampling occasions will be required for the hair-snare to maximize the chance of a capture while simultaneously preventing DNA degradation (Foran *et al.* 1997), while allowing enough time to visit all subsequent traps. Moreover, given that two technicians will be available to work on the hair-snare pilot study, and we estimate that each technician can survey 8+ sites a day, the hair-snare pilot study will include 100 sites monitored weekly (*i.e.*, 50 sites per river).

Precision and accuracy of the abundance and density estimate will depend on the detection probability of mink, which in turn depends on intensity of spatial sampling, the arrangement of spatial sample units, and the frequency of temporal sampling. Given the elusive nature of mink and the short duration of the sampling season due to the reproductive cycle, spatial replication will likely be the determining factor to ensure reasonable precision in the abundance estimate. Field *et al.* (2005) concluded that 2-3 repeat surveys per site would generally be sufficient, unless site occupancy is high, or detection probability is low. During a preliminary site-survey in November, 2011 (Table 3, Appendix 3), we searched for scats at 36 sites randomly chosen in the Hudson River study area: scats were detected at 2 sites, resulting in a crude estimate of a detection probability of 0.05. In the Mohawk River study area, we sampled 60 sites, and scats were detected at 6 sites, giving a crude estimate of detection probability of 0.10. Homan *et al.* (2001) and Smith *et al.* (2001) reported that the detection probability of a detection dog would typically be 2 - 4 times greater than that of humans. Consequently, we can expect the detection probability using detection dogs to range from 0.10 - 0.20 in the Hudson study area and 0.20 - 0.40 in the Mohawk study area. Mackenzie *et al.* (2002) concluded that a reasonable bias can be achieved with a minimum of 2 sampling occasions with site occupancy > 0.7 and detection probability > 0.3. Given that sites will be selected based on habitat suitability for mink during the full-scale study in 2013, we might expect that the site occupancy value will be sufficient to ensure a reasonable bias in abundance estimates in the Mohawk study area. Tyre *et al.* (2003) concluded that "when detection probabilities are high, it is better to survey more units rather than increasing the number of surveys per sampling unit, but as detection probability decreases more surveys per unit should be conducted". Mackenzie and Royle (2005) recommend that when the detection probability is < 0.5, that > 3 sampling occasions should be used. We expect that the site selection based on habitat suitability for mink, in conjunction with the use of detection dogs, will significantly increase the detection probability.

Study sites

Introduction

Mink are indigenous to North America and occupy a variety of riparian, lake shore, wetland, and coastal habitats throughout the non-arid portions of the continent (Dunstone 1993). Furthermore, the mink as an introduced species has successfully established populations in Europe and Asia despite mustelid competitors (*e.g.,* Eurasian otter [*Lutra lutra*], polecat [*Mustela putorius*] and European mink [*Mustela lutreola*]) often with severe reductions in populations of indigenous prey species (Dunstone 1993, Aars *et al.* 2001). Much of the success of mink in occupying a diverse array of habitats throughout its range in newly colonized regions is related to the ability to meet metabolic needs by exploiting a variety of aquatic, semi-aquatic, and terrestrial species as prey (Hamilton 1959 [for mink in New York State], Eagle and Whitman 1987, Dunstone 1993). As an opportunistic predator, mink select prey according to seasonal and habitat-related abundance (Gerell 1967, Wise *et al.* 1981, Chanin 1981, Gilbert and Nancekivell 1982, Dunstone and Birks 1987). Mink can readily exploit alternate prey as prey abundance shifts seasonally, with habitat type or with perturbations affecting population level (Gerell 1967). The abundance of a prey species, however, is only one of several factors potentially affecting prey selection by mink. Prey vulnerability, the dispersion or concentration of prey within habitat, the availability of alternate prey, and prey availability relative to specific metabolic needs for reproduction and sexual dimorphism affect the relative importance of prey species (Errington 1954, Gerell 1967, Erlinge 1969, Burgess and Bidder 1980, Birks and Dunstone 1985, Arnold and Fritzell 1990, Ben-David 1997, Shier and Boyce 2009). Furthermore, niche overlap between mink and other mustelids may result in competition that alters habitat use and prey selection by mink (Erlinge 1972, Clode and Macdonald 1995, Bonesi *et al.* 2006). Thus, predicting the capability of a specific habitat to meet the metabolic needs for mink requires the evaluation of other factors that affect mink-prey interactions in addition to prey abundance.

Site Selection

The distribution of mink use-sites is associated with habitats composed of plant species such as willow and alder trees (Mason and Macdonald 1983). Mink association with specific wetland habitats may be related to the use of these habitats by their prey (Arnold and Fritzell 1990); however, the selection of specific cover types and habitat structure may be independent of prey availability when prey densities are high (Ben-David *et al.* 1996). The availability of den sites may be a factor influencing the distribution of mink within specific habitats (Racey and Euler 1983, Yamaguchi *et al.* 2003). Furthermore, human modification of vegetative cover or habitat structure affecting the availability of prey or den sites may influence mink distribution (Dunstone and Birks 1983, Racey and Euler 1983, Yamaguchi *et al.* 2003). Although to adequately predict the ability of a specific habitat to support the occupancy of mink would require an assessment of the ability of the habitat to meet metabolic requirements, the association of mink with aquatic habitats and specific vegetative cover suggests that a degree of predictability of the suitability of habitat for mink occupancy could be gained from a model

based on the presence of aquatic or wetland habitats, the degree of physical complexity, and extent of vegetative cover (e.g., U.S. Fish and Wildlife Service habitat suitability index [HSI], Allen 1984). During the pilot season we will not use *a priori* habitat associations to select sites. We will collect habitat information using GIS as well as field measurements (Appendices 3 and 5) and subsequently evaluate the relationship between these habitat variables and sites where we detect mink. This represents a variation (i.e., not in an occupancy context) of a two-phase sampling design (e.g., Pacifici *et al.* 2012) whereby we allocate effort during the second field season in areas that will represent a more efficient use of resources and allocation of effort to increase the amount of information collected and to improve the estimation of abundance.

The number of potential stream-road intersections within 5 km of the main stem of the Hudson and Mohawk rivers along a 50 km river-segment (from Fort Edward south for the Hudson River and from Herkimer east for the Mohawk) are 682 and 754, respectively (Appendix 2). Sites will be ranked using the results of the simulation study, and 144 of the highest ranked sites in both rivers will be selected and sampled according to their suitability for the detection dogs. A hypothetical home range for mink in the study area was determined as 0.83 km^2 using results from a telemetry monitoring study (General Electric, as reported to NYSDEC, Appendix 6). This home range estimate is smaller than values for mink home range typically found in the literature (Dunstone 1993, Gerell 1970, Niemimaa 1995), but provides an estimate for the targeted study area, rather than from a study area with different habitat and prey availability. Spatial capture-recapture models contain a spatial parameter that corresponds closely with the typical size of individual home ranges. In general, the precision of density estimates is closely associated with the precision with which this home range parameter is estimated. Therefore, based on home range size estimation from the analysis of telemetry data (General Electric, as reported to NYSDEC, Appendix 6), the study areas in the Hudson and Mohawk river drainages will be most likely divided into standard 1 km x 1 km grid cells. For a given total amount of effort, precision is affected by the spatial arrangement of sample units. Therefore, we will evaluate efficient allocation of spatial units using a simulation study. We will use a simulation study to compare random and systematic allocation of the 50 units of two hair-snare devices and of the 144 sites for the scat detection survey in order to maximize spatial replication (*i.e.*, detection probability).

Hair Snare Device

Hair snares will be based on a design selected during a field trial using farm mink in a captive facility (Appendix 1). The field trial suggested that the most efficient device is made of a corrugated plastic shield folded into a triangle using zip-ties. Hair will be collected using 2 gun brushes (of 0.45 caliber) mounted inside the device. A sardine will be placed in the middle of the device using an alligator clip. Winkler's Brown Beauty mink gland lure (Sterling Fur Company) will be placed in a hardware-cloth pocket (6 x 6 cm) and placed adjacent to the hair snare device.

Monitoring visitation

Hair-snares

Hair snares will be visited every 7 days in the order they were installed in the field (Appendix 7). The 7-day period will be short enough to prevent DNA degradation, while allowing enough time to visit all subsequent traps (Foran *et al.* 1997). Gun brushes with hair will be unscrewed from the device and the gun brush will be placed in a microtube (*i.e.*, falcon tube) by technicians wearing a new pair of surgical gloves for every gun brush handled.

In the field, a data sheet will be completed, including the uniquely identified site number, uniquely numbered device, the gun brush number, GPS location of device, collection date, sample number, and the collectors initials will be recorded if hair is present and/or if animal signs are present around the device (even in the event of no hair present on the gun brushes) (Appendix 8). Any unusual observations will be reported (*e.g.*, devices missing, devices moved or destroyed, etc.) and photos (Appendix 9) will be taken to document these observations, (*e.g.*, animal signs around the devices, how hair was caught in the gun brushes, etc.). Gun brushes with hairs will be removed and a thorough search of the inside of the device will ensure that no hair is adhered to the side walls of the device. Any hair-snares containing hair must be clean after hair collection. Hair-snares will be visually inspected for hair that may be adhered to the side walls, and if present, will be rinsed in the adjacent stream. Field technicians will only be required to unscrew gun brushes presenting hair and put each brush in a separate microtube. Typically, hair is identified to species or genera by macro- or microscopic examination. Therefore, each brush with hair will be examined by a Principal Investigator or designee to determine if hair can be identified as mink before being analyzed genetically. If it appears that the hairs are mink, all hairs on one brush will be analyzed as one sample. Genotyping accuracy depends both on DNA quality and quantity, so all hairs will be processed rather than archiving a subsample. Previous noninvasive studies on black bears have used a minimum of 5 hairs with root follicles per sample (Triant *et al.* 2003; Dreher *et al.* 2007, 2009). We will first identify the samples to species and then to individual.

In several ways, the pilot study during 2012 will inform us if the hair-snare device will be appropriate for the full-scale study. During the pilot study we will first identify hair samples to species using the mitochondrial mink-specific marker and then to individual based on microsatellites. The molecular species ID will be used to check microscopic hair ID and dropped from later hair analyses if the visual IDs are accurate. The proposed hair-snare design can potentially capture more than one individual, but has the potential for increased sampling with little risk of compromising data quality. For example, the 2012 pilot study will establish whether hair tufts tend to be deposited in a highly clumped pattern and will test whether single tufts are indeed from single individuals. On the basis of a 12 locus genotype the probability of falsely interpreting a mixed sample as an individual sample is very low because one or more loci will show 3-4+ alleles (2 are expected in diploid heterozygotes). If average expected heterozygosity per locus is 0.5 in wild American mink populations (Belliveau *et al.* 1999), then a hair mixture from two mink will be doubly-heterozygous and identifiable at 25% of loci genotyped. If results in the pilot study determine that hair tufts are often not from single individuals, then we may consider other designs such as a single-capture trap design (*e.g.* Bremmer-Harrison 2006) for the full scale study.

Scat collection

Two detection dogs will survey the study areas. However, in order to minimize any effect related to different search performance between the dogs, each dog will alternate daily between the 2 study areas. Each of the 144 sites will be visited every 18 days in each river drainage. Given that mink usually stay within 10 m of the nearest water source more than 88% of the time for males and 95% of the time for females (Yamaguchi *et al.* 2003), sites will be sampled using a transect that closely follows the stream as far from the stream-road intersection as permitted, given landowner's permission (Appendix 10).

Mink scat will be identified in the field based on size and habitat context. During the 2012 pilot study all scat samples will be confirmed as mink using the mtDNA marker. If field identification error proves low then this step may be eliminated for subsequent field seasons because species ID is also likely to be evident from microsatellite genotypes (not all loci will amplify) and also from the genotypes themselves (large genetic distance from mink samples). Non-mink samples will not be analyzed further. In the field, GPS location, sample number, collection date, collection time, and collector's initials will be recorded for all scats found by the dog (Appendices 11 and 12). Any unusual observation will be reported, and photos will be taken to document each fecal sample found. Scats will be placed in 10x16cm waxless paper bags, and only one scat will be stored in each bag. Field technicians will wear a new pair of gloves to collect each sample. After scat is collected, scats in paper bags will be placed in a warm, dry place and allowed to dry for 1-4 days. Scats will be kept out of direct sunlight. After drying, scats will be stored in falcon tube vials filled with 96% ethanol. Each vial will have an outside label identifying collection information as well as a duplicate label on the inside of the tube.

Given the results of mink distribution in Appendix 6, sampling at stream/road intersections should not bias the density estimates because mink are not distributed farther from stream-road intersections than random expectation. Even if a "road effect" did exist, this will not necessarily lead to bias in density estimates if we can effectively model this in the encounter probability model.

Analysis

Genetic analysis

The PIs have designated a Lead Geneticist to have primary responsibility for conducting or overseeing all genetic analyses to identify individual mink using either hair or scats.

The use of feces as a source of DNA leads to several difficulties because of the small amount and likely degradation of host DNA (intestinal epithelial cells), and the presence of unwanted sources of DNA (*e.g.*, from prey or parasites). Consequently, only ~50% of the samples (Rozhnov *et al.* 2008, Harrington *et al.* 2010) are likely to yield acceptable data. Hair

follicles are usually a more reliable source of non-degraded DNA, but low yields (*e.g.*, from <10 follicles) can lead to genotyping error.

All genetic analyses involving samples and reagents prior to PCR, including DNA extractions and setting up PCR reactions, will be performed in a laboratory where no post-PCR work is ever performed. This minimizes opportunities for cross-contamination of samples. Additionally, all PCR reaction set-ups will be done in a UV irradiation hood. UV irradiation kills DNA on pipets and other materials at a PCR bench before assembling reactions.

Species identification

Extracted DNA will be amplified with PCR primers capable of amplifying a portion of the mitochondrial cytochrome b gene from stoat, weasel, polecat, mink, pine marten, otter, and fox (Cytochrome-b-ups—TRGGAG ACC CAG ACA A; Cytochrome-b-dow—ATVCYH CGT TGT TTT GA). These primers were designed on an alignment of the cytochrome b genes from each of the species of interest and were checked to confirm amplification of the appropriate fragment from a sample of genomic DNA from each species. The resultant amplified DNA fragments were sequenced using the reverse oligo as sequencing primer, using ABI "Big Dye" dye terminator chemistry and an ABI 3730 instrument (Applied Biosystems, Foster City, CA, USA). DNA sequences will be subjected to a "BLAST" search (http://www.ncbi.nlm.nih.gov) to determine the species of origin. Samples that yield no mitochondrial amplification or the wrong species ID will not be analyzed further.

Individual identification

Because mink microsatellites previously used in population studies have moderate heterozygosity and some show Hardy-Weinberg deviations (possibly indicating technical genotyping problems), PCR optimization and selection of loci will be achieved in a preliminary study using both high quality samples and scat/hair samples. At least 15 loci will be evaluated and probably 10-12 of the best loci will be analyzed in every sample. Too few loci risks inferring a recapture by mistake, and with too many there is increased opportunity for genotyping errors. One measure of whether loci include enough power to prevent false positive recaptures is the Probability of Identity (PID). Because related individuals may be in the mink samples, we will use the smallest set of loci that achieves a $P_{\text{ID-sib}} \leq 0.001$ (as recommended in McKelvey and Schwartz 2004). $P_{\text{ID-sib}}$ values will be estimated using the program GIMLET 1.3.3 (Valière 2002).

One method used to assure quality genotype data is to perform 5-8 replicate PCR reactions for every locus in every individual. However, in addition to the cost and need for adequate DNA to apply this method, it has been criticized because it was not associated with evaluation of genotyping error - it just used redundancy to supposedly minimize it. To get complete multi-locus genotypes and assure the accuracy of recaptures (*i.e.*, two samples show identical genotypes) we will use a stepwise approach described by McKelvey and Schwartz (2004). During the pilot phase, all samples will be extracted once and amplified twice for each locus so that locus-specific genotyping error rates can be estimated. Samples with incomplete data, or that show near-identity with another sample (i.e., potentially a recapture but with slight differences

caused by genotyping error) will be re-extracted and re-amplified. Repeated genotyping will be done with questionable samples until errors are eliminated as demonstrated by finding agreement across multiple replicate genotypes (i.e., majority rule). Ultimately, the overall genotyping accuracy will be assessed from the distribution of pairwise genotype relatedness among individuals; low error data is expected to yield a bimodal or gapped distribution consisting of recaptures (i.e., zero differences) and a larger relatedness mode with variance as expected for a randomly mating population. Samples that show higher than average relatedness (low number of allelic differences) after repeated genotyping will be analyzed in terms of parentage to decide between alternative hypotheses of related individuals (parent offspring or sibling) versus recapture with error. Collected scats identified as mink scats will be stored in 96% ethanol before being sent to the Lead Geneticist's. Before isolation of DNA, samples will be dried or centrifuged to remove the alcohol. DNA will be extracted from approximately 0.3g of scrapings from the inside of the scat sample using QIAamp DNA stool mini kit (QIAGEN, Valencia, CA, USA).

Statistical Analysis: Abundance and density estimation

Databases of capture histories of each animal at each trap and each sampling occasion and available covariates will be prepared for field data and for laboratory data for both the hair-snare and scat experiments.

Analysis of hair data

SCR models integrate individual encounter information with auxiliary spatial information (trap locations and individual encounter locations) in order to make inferences about typical space usage patterns and density of the species under study. In SCR models, the probability of encounter is defined as a function of the distance between the trap and the animal's activity center. In traditional SCR models, this distance is Euclidian (*e.g.*, Gardner *et al.* 2010, Royle *et al.* 2011). However, for a species such as the mink known to use river and stream corridors, the Euclidian assumption for the distance trap/activity center may not be appropriate. In the proposed model, the detection function is assumed to decrease according to a bivariate normal kernel, but depending on the species and study design it could be modeled using a number of other standard "distance functions" (*e.g.* Borchers and Efford 2008). Consequently, a non-Euclidian distance that accommodates the linear structure of river and stream networks, or where the distance from the trap to activity center is measured as a function of a cost that decreases proportionally with the proximity of the river (*i.e.*, cost distance) will be developed.

It follows that the SCR model is a closed population mixed-model: it is a linear model including an unobserved random effect because the individual's activity centers are unknown (*i.e.*, distances from the traps to activity centers are latent variables). We therefore specify a probability distribution for the activity centers, and the standard assumption is that they are uniformly distributed in space (Efford 2004, Borchers and Efford 2008, Royle and Young 2008), although this assumption can be relaxed to allow for landscape covariates to influence expected

density of individuals. Models will be fitted using likelihood methods which are more efficient for calculations involving least-cost path distance.

Data from the hair study will consist of encounter histories for each trap and each individual mink on $K = 5$ capture occasions. Capture occasions will be 7-day intervals. Trap-specific capture histories will consist of sequences of 0's and 1's for each trap: 0 if the individual was not captured in the trap on an occasion and 1 if the individual was captured. We can summarize the encounter histories by individual and trap-specific frequencies such that y_{ij} is the number of times that individual *i* was encountered/captured in trap *j* out of *K* sampling occasions. Hair snare devices used in the analysis will be devices that were operational for the whole study period and devices that were badly damaged, destroyed or stolen during a sampling occasion will be disregarded.

We consider models for individual encounter probability $p_{i,j}$ that are functions of distance between the trap *j* and each activity center *i* as following. One possible model is based on a bivariate normal hazard function (Royle *et al.* 2009):

$$p_{i,j} = 1 - \exp\left[-\lambda_0 \times \exp\left(\frac{-d_{i,j}^2}{\sigma^2}\right)\right]$$

where λ_0 is the expected number of captures in a trap, given that an individual's activity center is located precisely at that trap;
$d_{i,j}$ is the shortest path with the lowest cost (*e.g.*, using the cost distance) between the location of trap *j* and the activity center of individual *i* using Dijkstra's algorithm (Dijkstra, 1959);
σ is a scale parameter corresponding to the standard deviation of locations under a bivariate normal model.

To include effect on the detection probability, we could for example express this model for detection probability as a linear model on the complementary log-log scale:

$$\text{cloglog}(p_{i,j,k}) = \log(\lambda_0) - \beta_1 \times d_{i,j}^2 + \beta_2 \times x_{i,j,k}$$

where β_1 and β_2 are coefficient associated respectively with the distance between the location of trap *j* and the activity center of individual *i* and with a behavioral response, e.g. $x_{i,j,k} = 1$ if animal *i* has previously been captured in trap *j* at occasion *k*.

The analysis will be conducted using an integrated likelihood approach by treating the random effect, i.e. the activity centers (the sum of which is the abundance *N*), as a nuisance parameter and eliminating it from the likelihood by integration. Consequently, we will carry out a maximization of the likelihood using a Newton-type algorithm in the R package nlm().

Analysis of scat data

For the study based on scats, the model considers the situation where the study area is not sampled uniformly. The sample unit is a 1 km x 1 km cell that will be sampled at selected road-stream intersections by a dog handler following line transects while the detection dog works using a random path around the transect line. The path taken by the dog handler will be well defined by a GPS track file. This model was developed by Royle *et al.* (2011), and is an extension of the Royle and Young (2008) model to accommodate the use of detection dogs in search-encounter surveys. Additionally, this model assumes that any individual encountered is captured and uniquely marked at each sampling occasion.

Data from the scat spatial-capture-recapture study will consist of a binary encounter event y_{ij} for each individual mink i = 1, 2, …, n and trap j out of K = 3 sampling occasions. Capture occasions will be 3-week intervals, and sampling events for each site will consist of one visit per site using a detection dog every 3 weeks for the scat experiment. When dogs detect a scat, the scat will be collected and its location u_{ij} recorded. Similar to the SCR model for the hair data, the spatial coordinates u_{ij} of each individual during the survey are only known for y = 1 and will be regarded as random effects for which we assume a distribution. We will use the Royle *et al.* (2011) Bayesian hierarchical formulation in which the model is analyzed conditional on u, using Markov chain Monte Carlo methods (MCMC), and can be split into 3 distinct models:

1) An observation model

The encounter probability p_{ij} will be expressed as closeness to the line segment by an average distance between the individual location and the track line X such as:

$$\text{logit}(p_{ij}) = \beta_0 + \beta_1 \times dist(u_{ij}, X)$$

It is reasonable to assume that some other factors may influence the encounter probability and Royle *et al.* (2011) developed one model where the hazard $h(u_{it}, X)$ of encountering individual i at a point x on occasion j can be expressed such that:

$$\log(h(u_{it}, x)) = \beta_0 + \beta_1 \times dist(u_{ij}, x)$$

The total hazard to encounter anywhere along the survey path, for an individual located at u_{ij}, say $H(u_{it})$, is obtained by integrating over the surveyed line, and will be evaluated numerically by a discrete sum where the hazard is evaluated at the set of points x_j along the surveyed path. The encounter probability then becomes:

$$p_{ij} = 1 - \exp(-H(u_{ij}))$$

2) An ecological process model for the variables u_{ij}

The spatial coordinates u_{ij} of each individual during the survey are only known for $y = 1$ and are regarded in the present model as random effects for which we assume a distribution. The coordinates u_{ij} can be seen as the outcome of a movement process conditional on the activity center s_i and a legitimate choice of distribution for the variables u_{ij} is consequently the bivariate normal distribution such that: $u_{it}|s_i \sim Normal(s_i, \sigma^2 I)$ where I is the 2x2 identity matrix.

3) An ecological process model for the variables s_i

However, similar to the model used for the hair data, the centers of activity, s_i, are latent variables for which we will specify a probability distribution for the activity centers, the standard assumption being that they are uniformly distributed in space (Efford 2004, Borchers and Efford 2008, Royle and Young 2008).

We obtain an estimate of the total population size, *N*, which inhabits the prescribed state-space (or any partitioning of the state-space), and density, *D*, is estimated by dividing *N* by the area of the state-space. Statistical evidence of a difference in population size or density between the two areas will be highlighted by the confidence intervals: if they do not overlap, the population sizes will be considered significantly different. Another option would be to include area as an effect to model the detection probability and therefore analyze the Hudson and the Mohawk data in a single model rather than in separate analyses.

Alternative statistical approaches

Data generated under the proposed sampling strategies could potentially be analyzed by a wide variety of existing approaches. However, the following alternatives will only efficiently allow the estimation of abundance. These alternative approaches include closed-population mark-recapture models, the Cormack-Jolly-Seber (CJS) model and occupancy models. We present the description of these alternative approaches for two reasons; first, if we do not collect sufficient data on individual mink, we could use the occupancy approach as an alternative, and second, the closed-population and CJS models could be used as comparisons to other studies that use similar approaches.

1) Huggins Closed-population mark-recapture models

In the Huggins models (Huggins 1989), the likelihood is conditioned on the number of animals detected and the abundance drops out of the likelihood. The major advantage of Huggins models compared to classic closed-capture models (Otis *et al.* 1978) is the fact that the capture probabilities can be modeled using covariates. However, the full conditional likelihood is the product of individual likelihoods and numerical methods are required to find the maximum likelihood estimate for each parameter. In the simplest model with no covariate and no time

effect on the capture probability *p* and the recapture probability *c*, for *k* capture occasions, referred to as model M_0, individual likelihoods are constructed as following:

Encounter history	probability of the encounter history	Conditional likelihood
111	pcc	$\dfrac{pcc}{1-\prod_{j=1}^{k}(1-p)}$
110	$pc(1-c)$	$\dfrac{pc(1-c)}{1-\prod_{j=1}^{k}(1-p)}$
101	$p(1-c)c$	$\dfrac{p(1-c)c}{1-\prod_{j=1}^{k}(1-p)}$
011	$(1-p)pc$	$\dfrac{(1-p)pc}{1-\prod_{j=1}^{k}(1-p)}$

Covariates could then be added to model the first capture probability *P* and the recapture probability *C*, using a logistic type of model:

$$\text{logit}(P_{ij}) = a + a_j + \beta_1 W_{i1} + \beta_2 W_{i2}$$

$$\text{logit}(C_{ij}) = a + a_j + \nu + \beta_1 W_{i1} + \beta_2 W_{i2}$$

Where W_{i1} = 1 if the ith animal is a male and 0 if it is a female

W_{i2} = 1 if the ith animal is a young and 0 if it is an adult

a is the intercept, a_j the time effect, β_1 denotes the effect of being male, β_2 denotes the effect of being young, ν denotes the effect of behavioral response.

This model is referred to as model M_{tbh} and classic closed-population submodels can be formulated as following:

M_{bh} by setting $a_1 = a_2 = ... = a_k = 0$
M_{tb} by setting $\beta_1 = \beta_2 = 0$
M_{th} by setting $\nu = 0$
M_h by setting $\nu = 0$ and $a_1 = a_2 = ... = a_k = 0$
M_t by setting $\nu = 0$ and $\beta_1 = \beta_2 = 0$
M_b by setting $\beta_1 = \beta_2 = 0$ and $a_1 = a_2 = ... = a_k = 0$

M_0 by setting $\beta_1 = \beta_2 = 0$, $a_1 = a_2 = ... = a_k = 0$ and $\nu = 0$

2) The CJS model

The CJS model (Cormack 1964) is based on a likelihood component of the Jolly-Seber model and contains the recapture data (number of animals seen at time t and seen again at a subsequent time) conditional on the numbers of newly and previously marked animals released at each occasion. The CJS model is a product of *T*-1 conditionally independent multinomial distributions that specify a probability for each possible capture history given the number of animals exhibiting each capture history:

$$P_3\left(\{m_{ij}\} \mid \{R_t\},\{\phi_t\},\{p_t\}\right) = \prod_{t=1}^{T-1} \frac{R_t}{(m_{t,t+1})!(m_{t,t+2})!...(m_{t,T})!(R_t - r_t)!} (\phi_t p_{t+1})^{m_{t,t+1}}$$
$$\left[\phi_t(1-p_{t+1})\phi_{t+1}p_{t+2}\right]^{m_{t,t+2}...}\left[\phi_t(1-p_{t+1})...\times\phi_{T-1}p_T\right]^{m_{t,T}} \chi_t^{R_t - r_t}$$

where R_t denote the number of releases at time *t*, r_t the number of R_t captured again later, ϕ_t the apparent survival at time *t* and χ_t the probability that an animal alive and in the study population at time *t* is not caught or observed again at any time after capture occasion *t*.

Under the CJS model, all parameters are based on the time index only and there are 2*T*-3 identifiable parameters: $\phi_1, \ldots, \phi_{t-2}$ and $p_2, .., p_{t-1}$. The initial capture probability p_1 cannot be estimated and the final survival and capture probability cannot be estimated separately but only as a product $\phi_{t-1}p_t$.

The CJS model provides a flexible framework for conditional open-population modeling and permits the modeling of apparent survival and capture probability. River and time effect could be tested on detection probabilities.

A Horvitz-Thompson estimator can be used to estimate abundance using capture probabilities estimated under the CJS and Huggins models:

$$\hat{N}_i = \sum_{i=1}^{n_j} \frac{I_{ij}}{\hat{p}_{ij}}$$

Where $\hat{N}_i$ is the abundance at time *i*, n_j is the number of animals caught at time *j*, I_{ij} a binary index indicating if animal *i* was caught at time *j* and $\hat{p}_{ij}$ the capture probability of animal *i* at time *j*.

3) Occupancy model

In the event that the genetic analysis fails to identify the animals at the individual level, we might consider the use of occupancy models and use occupancy as a surrogate for abundance and density. Occupancy is a binary variable indexed by space that takes the value 1 if the cell *i* is occupied and 0 if it is not. Logistic regression is the most widely used method to model the

observations z_i of such stochastic process with the assumption of a binomial distribution for the observations: $z_i \sim Bin(1, \pi_i)$. The interest is usually to assess how the success probability π_i varies as a function of covariates:

$$\text{logit}(\pi_i) = \beta_0 + \beta_1 x_i$$

where $\beta_0, \beta_1, ..., \beta_p$ are the coefficient of variation corresponding to the explanatory variables x_i.

Under such model, the likelihood of the observations can be expressed as following:

$$L(\beta \mid z) \propto \prod_{i=1}^{M} \pi_i(\beta)^{z_i} (1 - \pi_i(\beta))^{J_i - z_i}$$

$L(\beta \mid z)$ can then be maximized using numerical optimization methods.

Quality Assurance/Quality Control

Project Management

This study is being conducted in accordance with the Quality Assurance Management Plan for the Trustees' Hudson River NRDA (Hudson River Natural Resources Trustees 2002). The study team is organized based on tasks and levels of responsibility to ensure good communication between all personnel (Appendix 13). The NYSDEC Hudson River NRDA Case Manager, working under the direction of the Hudson River Trustee Council, has overall project oversight responsibility. The Quality Assurance Coordinator manages communications from the QAC with the project team, especially the Principal Investigators (PIs). The NYSDEC Case Manager is responsible for ensuring that adequate coordination and communication occurs amongst the Trustees, the Quality Assurance Coordinator, the Principal Investigators, and the NYSDEC Project Coordinator. The Principal Investigators are responsible for the project's design, statistical analysis, reporting to the Trustees, and providing guidance and technical expertise as needed to the Field Teams through the Field Lead. The Field Lead guides the field study (under the PIs' direction) from afar (in addition to making site visits), working with the NYSDEC Project Coordinator and Quality Assurance Coordinator to ensure that the study is consistent with the overall QA objectives of the NRDA. The NYSDEC Project Coordinator also supports implementation of the study plan developed by the PIs, facilitating the acquisition of access to NYSDEC facilities and support staff, and helping oversee the actions of the Field Teams.

The Study Plan and Standard Operating Procedures for this study were developed to provide detailed and explicit instruction for the Field Teams to follow when collecting study data. The plan will be reviewed, commented on, and approved by key parties to the study before the beginning of formal identification of study sites. Reliance on a detailed, explicit, and fully reviewed study plan ensures that:

- Study objectives, methods, procedures, and details are reviewed thoroughly

  before sampling. Data will be collected in a systematic and consistent way throughout the study.

- Every member of the study team adheres to the requirements of the plan. Each

  field team member is required to sign a statement (Appendix 14)

  that they have read the Study Plan and associated Standard Operating Procedures and understand them.

Events can arise during field data collections that require changes to the procedures being used. In these circumstances, deviations from the plan will be conducted only after consultation with the PIs or designee. Deviations from the work plan will be carefully documented, as will a detailed explanation as to why the deviations were necessary.

Data Generation and Acquisition

Data developed in this study must meet standards of precision, accuracy, completeness, representativeness, comparability, and sensitivity, and be consistent with sound scientific methodology appropriate to the data quality objectives.

Precision is defined as the level of agreement of repeated independent measurements of the same characteristic. The proposed hair-snare and scat-based studies permit species and individual identification through genetic analysis. For the scat studies, the dogs will be trained in the field prior to sampling and evaluated for their ability to locate mink scats in a natural setting. If the dog fails to locate 5% of placed scats it would be considered "unsuitable". However, a failure to locate a scat will be defined by the dog entering the scent cone (i.e. the pattern formed by scent molecules dispersing outwards from their source) but not locating the scat. If the dog does not enter the scent cone during the search and misses the sample, this is not considered a failure. The 5% threshold for missed scats might be revised if environmental conditions are difficult during the dog evaluation (*e.g.*, windy conditions which lead to the scent cone being spread and thus the scat harder to locate). We will record a number of observations during the dog evaluation: the environmental conditions, the number of trials (*i.e.*, sites where we placed scats), the number of scats placed in each location, and the number of scats recovered and missed (*i.e.*, when the dog entered the scent cone, but did not locate the scat) by the dog. All genetic analyses involving samples and reagents prior to PCR, including DNA extractions and setting up PCR reactions, will be performed in a laboratory where no post-PCR work is ever performed. This minimizes opportunities for cross-contamination of samples. Additionally, all PCR reaction set-ups will be done in a UV irradiation hood. UV irradiation is an easy way to kill DNA on pipets and other materials at a PCR bench before assembling reactions. Individual identification will be achieved by 5-8 replicate PCR reactions for every locus in every individual. Field personnel (2 technicians to check the hair snares and collect the data for the hair study and 2 dog teams, *i.e.*, 2 dogs and 2 handlers, for the scat study) will be trained by the Field Lead or the

NYSDEC Project Coordinator in the operation of GPS units, data collection, and the transfer of data to computers prior to the study. Data downloads will be verified daily by the Field Lead or the NYSDEC Project Coordinator. Data forms will be filled out in the field on-site and electronically transferred daily after the field work. Electronic records, including files for photographic data, will be kept up to date (i.e., daily) and backup electronic or paper copies created. Photographs will be kept under the chain of custody and copies will be made for working files (Appendix 9).

Accuracy is defined as the agreement of a measurement with its true value. For the parameters related to identification of animals, accuracy means that the identified animal has the agreed-upon characteristics that uniquely distinguish the species from other species. Each team member will use the same reference sources and agreed upon characteristics for the identification of hair and scat. The same agreed-upon characteristics from reference sources will be used for identification of hair and scat by team members. Measures will be taken to minimize and quantify genotyping error rates. To get complete multi-locus genotypes and assure the accuracy of recaptures (two samples show identical genotypes) we will use a stepwise approach described by McKelvey and Schwartz (2004). All samples will be extracted once and amplified twice for each locus. Samples with incomplete data or that show near-identity with another sample will be re-extracted and re-amplified. Repeated genotyping will be done with questionable samples until the distribution of pairwise genotype relatedness is bimodal, consisting of recaptures (zero differences) and a larger relatedness mode with variance as expected for a randomly mating population.

Completeness is defined as the percentage of the planned monitoring actually completed. Sites that were not visited by a detection dog at any of the 3 sampling occasions (*e.g.*, because of lack of time, bad weather preventing the dogs to work, etc.) will not be included in the study. Similarly, any site that was not visited at any of the sampling occasions or any hair-snare missing at any of the sampling occasions will lead to the suppression of the sites for the analysis. We will strive for at least 95% of the sites being sampled, but the pilot study will help to inform the total number of sites required to determine the completeness goal for the field effort in 2013. Data forms will be filled out each day correctly and completely and 100% will be checked daily by the Field Lead or the NYSDEC Project Coordinator. Data forms will be legible and accurate. Files for photographic record will be downloaded and reviewed during each monitoring visit and duplicate files archived. All photographic files will have location, time and date stamps (Appendix 9).

Sensitivity is defined as the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest. Because mink microsatellites previously used in population studies have moderate heterozygosity and some show Hardy-Weinberg deviations (possibly indicating technical genotyping problems), PCR optimization and selection of loci will be achieved in a preliminary study using both high quality samples and scat/hair samples. At least 15 loci will be evaluated and 10-12 of the best loci will be analyzed in every sample. Using too few loci risks inferring a recapture by mistake, and with too many there is increased opportunity for genotyping errors. One measure of whether loci include enough power to prevent false positive recaptures is the Probability of Identity, P_{ID}. Because related individuals may be in the mink samples, we will use the smallest set of loci that show (1) the highest genotyping accuracy and (2) achieve a $P_{ID\text{-}sib} \leq 0.001$ (as recommended in McKelvey and

Schwartz 2004). $P_{ID\text{-}sib}$ values will be estimated using the program GIMLET 1.3.3 (Valière 2002). Preliminary evaluations of different hair snare designs will demonstrate which one will maximize hair collection and should be used in the study. Instruments used for field measurements (GPS, temperature, etc.) will have a level of resolution necessary to meet the degree of precision needed for each measurement and requirements are described in Appendices 3 and 5.

Representativeness is defined as the degree to which the data accurately reflect the encounter history at the hair-snare device and at each site sampled for scats during each monitoring period. The use of detection dogs to increase detection probability and record the presence of animals at sites should ensure representative data for animal presence for each monitoring period at each site.

Comparability is defined as the measure of confidence with which results from this study may be compared to another similar data set. Because of the nature of the study, there cannot be a duplication of effort in the same area at the same time. However, the same sampling protocol employed in the Hudson drainage area will be used to sample an area of the Mohawk drainage similar in size and landscape to the Hudson area. Thorough documentation of methodology used in this study will permit similar methodology to be employed in future studies, thus ensuring more comparability of this study with future work.

Study Documentation

All study activities will be documented in field notebooks, data forms, or personal digital assistants (PDAs) as appropriate. Electronic files will be downloaded and hardcopies printed. All hardcopies will be placed into three-ring binders. To the extent possible, information will be recorded on pre-formatted data sheets on rite-in-the-rain paper. The use of pre-formatted data sheets is a quality assurance/quality control measure designed to:

- Ensure that all necessary and relevant information is recorded for each sample and each sampling activity,
- Serve as a checklist for the field teams to help ensure completeness of the data collection effort,
- Assist the field teams by making data recording more efficient, and
- Minimize the problem of illegible field notebook entries.

Each field team will have a single field data recorder responsible for recording information in field notebooks or on data forms. Assigning this responsibility to a single person will help ensure that documentation is complete and consistent throughout the sampling event. The field data recorder is also responsible for the care, custody, and disposition of the field notebook and data forms and for downloading electronic files and providing hardcopies.

Field notebook and data sheet entries will be made in ink. Corrections will be made with a single line through the error accompanied by the correction date and corrector's initials. Each

completed data sheet will be reviewed, corrected (if necessary), and initialed by the field data recorder. Following completion of the study, field notebooks, data sheets, and electronic-file originals including files for photographic data will be stored at the NYS DEC Hale Creek Field Station.

Personnel Experience and Training

Site selection will be conducted by personnel capable of using maps, GIS information, and photo imagery in identifying potential sites for hair and scat sampling. Personnel will have experience in contacting landowners and in obtaining access and written permission to privately owned lands. Personnel will also have knowledge, based on trapping experience, in selecting sites likely to be visited by mink in riparian areas. Field teams will receive explicit instructions in the execution of Standard Operating Procedures (SOP) for evaluating sites (Appendix 5) and preparing appropriate databases and preliminary reports. Field teams will receive explicit instructions in the execution of SOPs for monitoring hair snares (Appendix 7) and conducting scat-dog detection (Appendix 11). Field teams will have signed an acknowledgement that they read the study work plan and will be instructed before beginning monitoring, and the instruction will be repeated or refreshed during the monitoring period as necessary. Field-crew members will be trained in the collection of field data, operation of cameras, GPS units, and procedures used in setting up and maintaining hair snares as well as the collection of scat by the PI or the PI's designee, or the NYSDEC Project Coordinator.

Assessment and Oversight

The QC management plan specifies that studies that generate data will be audited to ensure that the project-specific plans are being properly implemented. Several mechanisms for internal audits of the data generation process will be used. These mechanisms include:

- A project management structure that defines clear lines of responsibility and ensures communication between field teams and the PI or designee. Clear responsibilities and communication can serve as a means of providing internal audits of monitoring data.
- A requirement that field notebooks and data forms be reviewed by the PI or designee, or the NYSDEC Project Coordinator.
- The use of pre-formatted data sheets that serve as a checklist for monitoring procedures, thereby helping to ensure that data collection is complete.

The site selection and hair and scat sampling phases of the study will not begin until this Work Plan has been approved by the Quality Assurance Coordinator or the QAC's designee. The QAC or designee will conduct a field audit of procedures and documentation of the study. The

site selection and analysis of habitat-related variables for those sites will be conducted and accepted by the Trustees prior to the initiation of the deployment and monitoring of hair collection devices or scat detection dogs for summer 2013.

Data Validation and Usability

This study employs standard, repeatable methods based on the scientific literature for collecting data. The study plan has been extensively reviewed for the adequacy of the study design and methods. The original field notebooks will be maintained by NYS DEC and archived at the Hale Creek Field Station. All materials related to the study will be archived until approval for any disposal is approved in writing by the Trustees. Final reports can be reviewed against original records to ensure that the data present in the reports represent compete and accurate information.

The PI or designee will validate that biologists and technicians are collecting data as described in the study plan, and are completing data forms properly, by performing periodic checks during the study. Additionally, the PI, PI's Designee, or the DEC Project Coordinator will verify 100% of the manual transcriptions from the field forms to the electronic data sheet.

Data analysis (*i.e.*, estimating abundance and density) will be performed using the software R, WinBUGS or other commercially available statistical software.

Chain of Custody Procedures

Strict Chain of Custody (COC) procedures will be used throughout the study. The purpose of the COC is to assure the integrity of each photographic data file and genetic sample (*i.e.*, hair or scat), and clearly identify who was responsible for these records. The collection of site evaluation data and photographic and genetic samples will be fully documented on field (Appendices 8 and 12 and laboratory data forms (Appendix 15), which clearly identify the team member(s) responsible, as well as the date and time. The COC form (Appendix 14) will be used to maintain records of sample transfer between personnel other than immediate team members. The immediate team members are personally responsible for the care and custody of electronic files, microtubes (*i.e.*, falcon tubes) with hair, or waxless paper bags with scat that are in their possession. Files or genetic samples are in custody of the immediate team member if any of the following occur:

- The electronic card containing the file, device with a duplicate file, or genetic microtube or paper bag with scat is in the individual's physical possession;
- The electronic card or device, the genetic microtube, or paper bag with scat is within view after being in possession;
- The electronic card or device, genetic microtube, or paper bag with scat is in a locked or sealed container that prevents tampering after being in possession;

- or, The electronic card or device, genetic microtube, or paper bag with scat is in a designated secure area.

A completed COC form will accompany any transfer of electronic cards containing files, devices with duplicate files, genetic microtubes, or paper bags containing scat. The COC form will contain the following information:

- Project name
- Unique identification for each file, genetic microtube, or paper bag with scat
- Name and signature of individual relinquishing custody
- Name and signature of individual accepting custody
- Shipping date and mode of shipment.

Other information such as monitoring date and location may be on the COC form or on accompanying documentation. Each shipping container containing electronic cards, genetic microtubes, or paper bags containing scat will be accompanied by an original COC form for the items in the container. All sections of the COC form will be completed. All items included in the catalog will be clearly listed. Indication of the number of containers per shipment (*e. g.*, 1 of 3) will be listed on the form if more than one container is shipped. Once the form is completely filled out, it will be placed securely inside the container. Field personnel will maintain a copy of the COC to keep with shipping invoices. The container will be sealed with custody seals. Custody seals are used to detect unauthorized tampering with the contents until the time of receipt. Signed and dated gummed paper seals may be used for this purpose. The seals will be attached so that they must be broken to open the shipping container. Each container will be sealed with strapping or other tape. All electronic cards containing files, devices with duplicate files, genetic microtubes, or paper bags containing scats will be kept in a locked or otherwise secure location, or with custody seals at all times until shipped.

An air bill, Federal Express shipping label, etc. can be used to document the transfer of electronic cards containing files, devices with duplicate files, genetic microtubes, or paper bags containing scat from the field team to an intermediate storage location or archive. Scat samples in ethanol will be transported to the Lead Geneticist's laboratory by the Field Lead at the conclusion of the field work in 2012 and 2013. Additionally the Field Lead will periodically transport samples during the field season.

Containers with electronic cards containing files, devices with a duplicate files, genetic microtubes, or paper bags containing scat will be opened only by a person authorized to receive these records. The containers will first be inspected for integrity of the chain-of -custody seals or other signs of tampering. The receipt of each record will be verified on the COC forms. The signed COC form will be photocopied, and the photocopy will be mailed to the sending party. Electronic cards containing files, devices with duplicate files, genetic microtubes, or paper bags containing scats will be stored in a secure area according to procedures documented for each facility.

Special Provisions

Permits will be required from the National Park Service to conduct a portion of the field study on National Park Service land. Written permission will be required to enter private lands or lands under the jurisdiction of State agencies or authorities other than New York State Department of Environmental Conservation to conduct a portion of the field study on those lands.

Staff

The study team is organized based on tasks and levels of responsibility to ensure good communication between all personnel (Appendix 13). The NYSDEC Hudson River NRDA Case Manager, working under the direction of the Hudson River Trustee Council, has overall project oversight responsibility. The Quality Assurance Coordinator manages communications from the QAC with the project team. The NYSDEC Case Manager also provides direction to the Principal Investigators (PIs). The NYSDEC Case Manager is responsible for ensuring that adequate coordination and communication occurs amongst the Trustees, the Quality Assurance Coordinator, the Principal Investigators, and the NYSDEC Project Coordinator. The Principal Investigators are responsible for the project's design, the statistical analysis, and the formal reporting back to the Trustees. The Principal Investigators provide guidance and technical expertise, as needed to the Field Teams through Field Lead. The Field Lead guides the field study and the genetic analysis (under the PIs' direction) from afar, working with the NYSDEC Project Coordinator and Quality Assurance Coordinator to ensure that the study is consistent with the overall QA objectives of the NRDA. The NYSDEC Project Coordinator also supports implementation of the study plan developed by the PIs, facilitating the acquisition of access to NYSDEC facilities and support staff, and helping oversee the actions of the Field Teams. The NYSDEC Project Coordinator should be included in consultations that require deviations from the work plan.

The PIs have primary responsibility for project design, data interpretation (including statistical analyses), final report preparation and scientific publication resulting from the investigation.

The Field Teams have primary responsibility of for the initial identification of potential sites for the study, landowner information, and the development of methods for extraction of data from global information systems used to characterize habitat at potential hair-snare sites. Field work will include on-site assessments for sites. Field work with regard to site selection will include securing permission from landowners and recording environmental variables.

The PIs have designated a Lead Geneticist to have primary responsibility for conducting or overseeing all genetic analyses to identify individual mink using either hair or scats.

The NYSDEC Project Coordinator has the primary responsibility of coordinating field activities and data transfer between the Field Teams and NYSDEC. This may include the

supervision of supplemental field teams composed of NYSDEC staff if additional field teams are deemed necessary for the study. The NYSDEC Project Coordinator will also assist in the extraction of relevant data from geographical information systems supporting the study and will assist in verifying accuracy of data transferred from field forms to electronic data sheets.

Technicians designated by the NYS DEC will support field work efforts including the construction of hair snares and the weekly monitoring of hair snares, collection of scat samples, preparation of materials for archival storage, and compilation of databases and data reports that include metadata and data summaries. A list of basic field supplies is provided in Appendix 16.

Facilities

NYS DEC facilities are available for construction and storage of field apparatus, storage of supplies, and archival storage of any photographic data or data sheets.

NYS DEC Hale Creek Field Station
182 Steele Ave. Ext.
Gloversville, NY 12078
Phone 518 773 7318
Fax 518 773 7319

Expected Products

Activity updates are to be provided to the Trustees monthly. A report summarizing the progress in developing an appropriate model to estimate mink abundance will be provided to the Trustees for review prior to the initiation of field work assessing mink abundance. A report summarizing the results of the first year of field work assessing mink abundance will be provided to the Trustees before the initiation of the second and final year of field work. A final report will be prepared for the study. The contractor will provide databases and data reports that include methods, metadata, and an initial summary of field data.

Reports will be forwarded to the U.S. Fish and Wildlife Service New York Field Office, 3817 Luker Road, Cortland New York 13045, to the attention of Ms. Kathryn Jahn; where possible, electronic copies of the reports will also be provide to the Trustees (Kathryn_jahn@fws.gov). Monthly activity updates are to be provided to the same address (e-mail preferable).

Results of this study may be published in one or more peer-reviewed scientific journal articles, subject to review and approval by the Trustees.

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Appendix 1: Hair-snare testing experiment

Introduction

The use of non-invasive techniques is increasing and becoming common practice in animal population monitoring (Bellemain *et al.* 2005, Boulanger *et al.* 2004a, Boulanger *et al.* 2004b, Creel *et al.* 2003, Eggert *et al.* 2003, Mills *et al.* 2000, Mowat and Paetkau 2002, Prugh *et al.* 2005, Taberlet *et al.* 1999, Wilson *et al.* 2003). These techniques that currently focus mainly on hair, feces, and urine collections are attractive due to their relatively low cost compared to invasive methods, and can provide valuable information on species distribution, population composition, and size. Hair-snaring methods are particularly useful to sample rare species in remote areas and have notably proven useful for many species such as bears, martens, and otters (Depue and Ben-David 2007, Gardner *et al.* 2010, Mowat and Paetkau 2002), but there are no studies specifically focused on mink. Moreover, hair-snares are compact and versatile, which allows the setting of several per site to increase the trapping effort. We developed an experiment to test several hair-snare designs on captive mink in order to select the design that will maximize hair collection in the field. The aim was to test which device would be attractive enough for a mink to go into, while collecting as much hair as possible.

Devices

1- Gutter type

This device is made of 3 distinct parts mounted together: 1 middle piece with bait and gun brushes (labeled “4a” Fig.1) and 2 longer parts (labeled “5a” and “5b” on Fig.1). These 3 pieces are plastic gutter materials. The gun brushes are mounted to the plastic gutter by drilling a hole and are fastened using wing nuts (Fig.1).

Fig.1- Gutter device (left), middle part of the gutter device with gun brushes and the alligator clip for the bait (right).

We tested 2 different lengths of this device: 70 cm and 126 cm.

2- Triangular type

Fig.2- Triangular device.

The triangular device (Fig.2) is made of a corrugated plastic sheet fold into a triangle using 5 bolts and wing nuts (or, zip ties instead of wing nuts). Hair is collected by 2 gun brushes mounted on the triangle using wing nuts. Bait (*i.e.*, a sardine) is placed in the middle of the device using an alligator clip.

We tested 2 versions of this design: both were 90 cm long but the triangle was 10x10x13cm for the large version and 9 x 8 x 8 cm for the small version.

Experimental design

Devices were tested in a closed room where a mink was introduced for approximately 4 hours. The device was placed along the wall using a log to prevent it from moving, and it was baited with a sardine (Fig. 3). A camera was placed facing one entrance of the device to record mink exploring the device.

Fig.3- Experimental set up: a log prevents the device from moving and a camera is placed in the back left corner facing the device. A mink is present along the back wall, to the right of the camera.

Results

Observations for each type of device are summarized in Table 1.

Table 1- Date deployed, device type, mink ID, time, and observations for the experimental test of hair collection devices.

Date	Device	Mink ID	Time in-out	Observations
1/23/2012	Long gutter	L13X	8.45am-12.15pm	Mink dissembled the device, knocked over the camera
1/23/2012	Long gutter	K480	12.45-4.30pm	Mink did not enter but pushed the device away
1/24/2012	Long triangle	J344	8.15am-12pm	Mink went through the device when technician entered the room; Hair collected
1/24/2012	Long gutter	J344	12.20-4.30pm	Hair on brush
1/25/2012	Small triangle	L56	9.15am-12.30pm	Device is too small, mink did not enter
1/25/2012	Long gutter	L32	12.45-4.30pm	Mink did not enter
1/26/2012	Big triangle	J344	7.55-11.30am	Mink did not enter; gun brushes were placed horizontally
1/26/2012	Big triangle	J344	11.45am-4.30pm	Mink went into device; hair on brush

There were no observations of mink entering the gutter devices when brushes were mounted at the entrance. However, mink J344 probably entered the device since hair was collected in the remaining brushes (Fig.4).

Fig.4- Individual J344 checking the long gutter device (left) and the big triangle (right).

Despite striking interest, the small triangle device appeared too narrow for a mink to enter (Fig.4), but mink J344 went twice in the larger version.

Discussion

The triangle device appeared to be the best device for hair collection. However, this preliminary experiment indicated that improvements are required: in particular, a mechanism to firmly fix the device in the field is necessary; the corrugated plastic sheet was white, and this may not be appealing for mink, so a camouflaged color will be explored; the device seems to be slippery, so a nonskid surface will be added to the bottom of the device.

The pilot study will inform us if this hair-snare device can be used for the full-scale study: if we cannot individually identify different individuals captured in the same trap based on the pattern of hair tufts, or if we think we can distinguish individual mink but the pattern of heterozygosity is too high (indicating mixed hair samples) then we may consider other designs such as a single-capture trap design (*e.g.* Bremmer-Harrison 2006) for the full scale study.

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Appendix 2: Stream-road intersections in the Hudson and Mohawk River study areas

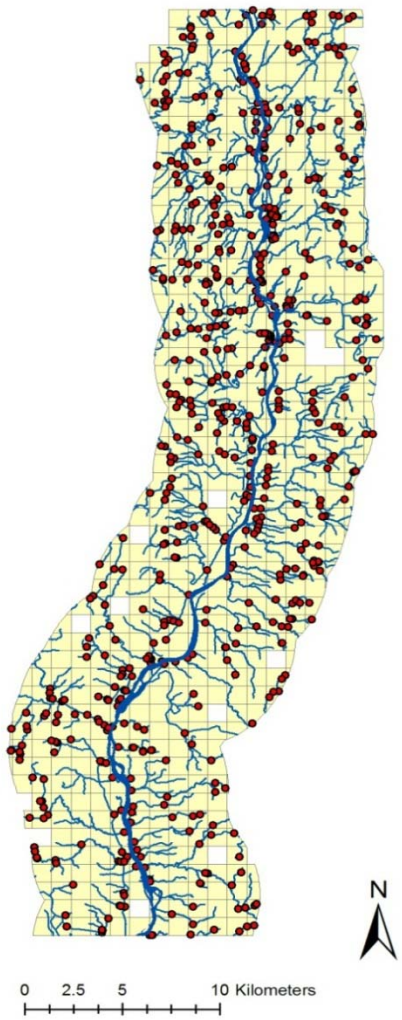


Fig.5- Stream-road intersections (red points) in a 5 km buffer in the Hudson River study area. Yellow boxes= 1 km grid cells used to approximate the home range size of mink.

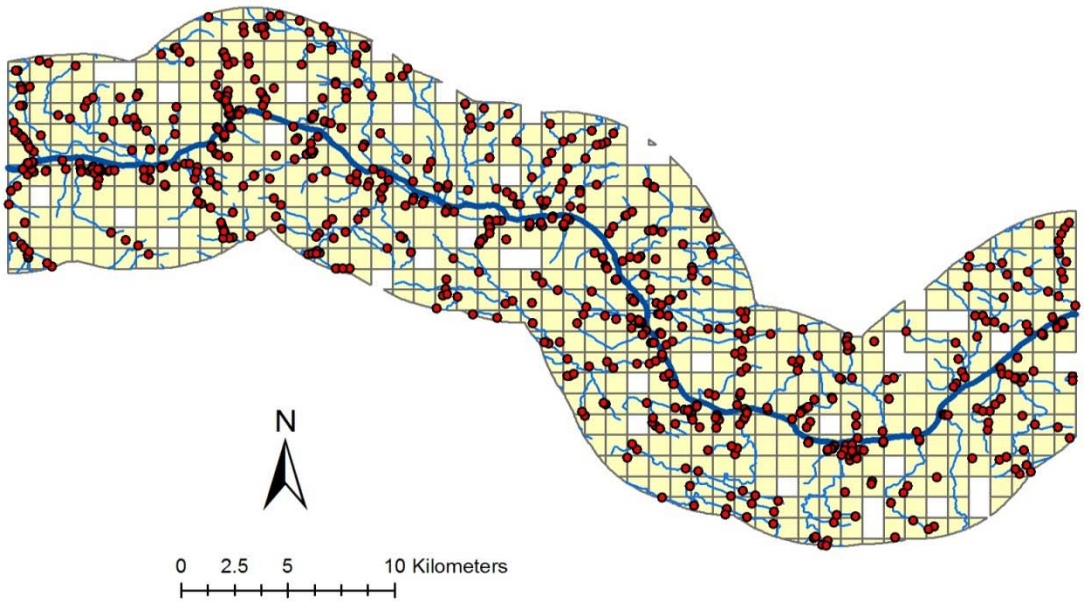


Fig.6- Stream-road intersections (red points) in a 5km buffer in the Mohawk River study area. Yellow boxes represent 1 km grid cells used to approximate the home range size of mink.

Appendix 3: Standard operating procedure for selection and evaluation of sites for hair and scat surveys

There are 682 and 754 potential road-stream intersections within 5 km of a 50 km segment of the Hudson (from Fort Edwards south) and Mohawk (between Herkimer and Amsterdam) rivers, respectively (Appendix 2). Sites will be located using available coverages in a Geographic Information System (described below), and sites within urban areas will be discarded. Landowners of potential sites will be contacted for permission to access sites, and written permission will be obtained. A database and report summarizing site locations, habitat characteristics, and landowner information will be prepared. During the pilot season, sites will be chosen based on a grid design with site selection independent of environmental-related variables. After completion of the pilot season, we will assess if environmental variables measured at each site can be used to accurately predict occupancy by mink. If so, and based on the resulting predictive model, 144 sites in each river drainage with the highest probability of occupancy will be chosen for the full scale study.

Habitat covariate measurement

Contractors will undertake GIS-based analyses using the Wildlife Management Institute database (http://rcngrants.org/spatialData) to determine the value of the following variables:

1) Strahler stream order
2) Stream length
3) Stream gradient (slope)
4) Canopy cover
5) Percent of land cover types (Table 2) from 2006 NLCD

Detailed descriptions of these measurements are documented below.

Table 2-Description of Forest/shrub cover types in the National Land Cover Database.

Forest/Shrub Cover Type	Description
Mixed Forest	areas dominated by trees generally greater than 5m tall, and greater than 20% of total vegetation cover. Neither deciduous nor evergreen species are greater than 75% of total tree cover
Deciduous Forest	areas dominated by trees generally greater than 5m tall, and greater than 20% of total vegetation cover. More than 75% of the tree species shed foliage simultaneously in response to seasonal change.
Evergreen Forest	areas dominated by trees generally greater than 5m tall, and greater than 20% of total vegetation cover. More than 75% of the tree

	species maintain their leaves all year. Canopy is never without green foliage.
Woody Wetlands	areas where forest or shrubland vegetation accounts for greater than 20% of vegetation cover and the soil or substrate is periodically saturated with or covered with water.
Emergent Herbaceous Wetlands	areas where perennial herbaceous vegetation accounts for greater than 80% of vegetation cover and the soil or substrate is periodically saturated with or covered with water.
Shrub/Scrub	areas dominated by shrubs; less than 5m tall with shrub canopy typically greater than 20% of total vegetation. This class includes true shrubs, young trees in an early successional stage or trees stunted from environmental conditions.

The GIS contractors will provide the following information for each site in a GIS shapefile:

X, Y location in meters

COUNTY - New York County where the point is located; Source: US Census 2000 Counties shapefile

Source Link: http://www.census.gov/geo/www/cob/co2000.html

TOWN - New York Town where the point is located, Source: NY State Office of Real Property Services

Source Link: http://www.mass.gov/mgis/adjstbnd.htm

ROAD NAME - Name of the closest US Census 2010 Tiger/Line road to the point (where available)

Source: US Census 2010, Source Link:
http://www.census.gov/geo/www/tiger/tgrshp2010/tgrshp2010.html

ROAD TYPE - Road classification of the closest US Census 2010 Tiger/Line road to the point

S1100 – Primary Road

S1200 – Secondary Road

S1400 – Local Neighborhood Road, Rural Road, City Street

S1500 – Vehicular Trail (4WD)

S1630 – Ramp

S1710 – Walkway/Pedestrian Trail

Source: US Census 2010, MAF/TIGER Feature Class Code (MTFCC)

Source Link: http://www.census.gov/geo/www/tiger/tgrshp2010/tgrshp2010.html

STREAM NAME - Geographic Names Information System (GNIS) name of the closest National Hydrography Dataset (NHD) high-resolution stream to the point (leave blank if unavailable)

Source: National Hydrography Dataset High Resolution Streams for NY State, September 2011

Source Link: ftp://nhdftp.usgs.gov/DataSets/Staged/States/FileGDB/HighResolution/

STREAM LENGTH - Length of the closest NHD high-resolution stream segment to the point

Source: National Hydrography Dataset High Resolution Streams for NY State, September 2011

Source Link: ftp://nhdftp.usgs.gov/DataSets/Staged/States/FileGDB/HighResolution/

STUDY AREA - Based upon the initial points files provided, define as either Hudson or Mohawk study area points.

ELEVATION

National Elevation Dataset 1 arc-second (about 30 meters) resolution digital elevation model height in meters for the point

Source: US Geological Survey National Elevation Dataset, 2009

Source Link: http://seamless.usgs.gov/

Stream Order

NHD Plus Stream Order for medium-resolution streams

Source: National Hydrography Dataset Plus Region 02 Version 01

Source Link: http://www.horizon-systems.com/NHDPlus/

Method: Link the stream order attribute table to the NHDPlus map layer data based on the unique COMID field. Assign stream order values to point-based sites based on location. All points within 40 meters of a medium-resolution stream will be given the slope of the nearest stream segment. Points that are between 40 and 100m from the nearest stream segment should be visually assessed to determine if the stream order values from the nearest medium-resolution stream line are applicable. If the stream order values were not available due to the size of the stream, the value should be assigned as -99.

NHD Plus Stream Class for medium-resolution streams

Source: National Hydrography Dataset Plus Region 02 Version 01

Source Link: http://www.horizon-systems.com/NHDPlus/

Method: Link the stream class attribute table to the NHDPlus map layer data based on the unique COMID field. Assign stream class values to point-based sites based on location. All points within 40 meters of a medium-resolution stream should be given the slope of the nearest stream segment. Points that are between 40 and 100m from the nearest stream segment should be visually assessed to determine if the stream order values from the nearest medium-resolution stream line are applicable. If the stream order values are not available due to the size of the stream, the value should be assigned as -99.

Stream Gradient

SLOPE

National Hydrology Dataset Slope of flowline (cm/cm)

Smoothing techniques should be applied to remove zero and negative slope values.

Please see the NHDPlus user guide for more information.

Source: National Hydrography Dataset Plus Region 02 Version 01 Catchment Flowline Attributes

Source Link: http://www.horizon-systems.com/NHDPlus/

Method: The flowline attribute table will be linked to the NHDPlus map layer data based on the unique COMID field. Slope values will be assigned to point-based sites based on location. All points within 40 meters of a medium-resolution stream are given the slope of the nearest stream segment. Points that were between 40 and 100m from the nearest stream segment should be visually assessed to determine if the gradient values from the nearest medium-resolution stream line are applicable. If the gradient values are not available, the value should be assigned as -999.

NAHCS (Northeast Aquatic Habitat Classification System) Full Slope

Gradient is measured as the slope of the flow line, calculated as rise over run and notated as a percentage. Six gradient classes are recognized by NAHCS team based on related stream biotic changes. See the report through the source link for more information on the modeling and description of these classes.

Source: Northeast Aquatic Habitat Classification System, 2009

Source Link: http://rcngrants.org/spatialData

FGDC and ESRI Metadata: http://www.fws.gov/r5gomp/gom/bd/NEAHCP/flowlines_nahcs.htm

Method: The gradient class values will be joined from the NAHCS flowline map layer to the NHDPlus map layer based on spatial location. They should then be joined to candidate sites based on spatial proximity. All points within 40 meters of a medium-resolution stream should be given the stream gradient of the nearest stream segment. Points that are between 40 and 100m from a stream should be visually assessed to determine if the gradient values from the nearest medium-resolution stream line are applicable. If the gradient values are not available, the value should be assigned as -999.

Simplified Slope Classes (one item for each of 5 Classes, 4 classes, 3 classes)

See the report through the source link for more information on the modeling and description of these classes.

Source: National Hydrography Dataset Plus Region 02 Version 01 Catchment Flowline Attributes

Source Link: http://www.horizon-systems.com/NHDPlus/

Method: The gradient class values were joined from the NAHCS flowline map layer to the NHDPlus map layer based on spatial location. They were then joined to candidate sites based on spatial proximity. All points within 40 meters of a medium-resolution stream were given the stream gradient of the nearest stream segment. Points that were between 40 and 100m from a stream were visually assessed to determine if the gradient values from the nearest medium-resolution stream line are applicable. If the gradient values were not available, the value was assigned as -999.

Canopy Cover

Mean of Percent Canopy Cover of National Land Cover Dataset (NLCD) cells within a 1 km^2 circle around the point

Source: National Land Cover Dataset 2001, Multi-Resolution Land Characteristics Consortium

Source Link: http://www.mrlc.gov/nlcd2001.php

Method:

The value of this field represents the average percent of canopy cover across all cells within a 1 km^2 circle around the point.

Standard Deviation of Percent Canopy Cover of National Land Cover Dataset (NLCD) cells within a 1 km^2 circle around the point

Source: National Land Cover Dataset 2001, Multi-Resolution Land Characteristics Consortium

Source Link: http://www.mrlc.gov/nlcd2001.php

Method:

The value of this field represents the standard deviation of percent of canopy cover across all cells within a 1 km^2 circle around the point.

Mean of Percent Canopy Cover of National Land Cover Dataset (NLCD) cells within 100m of point

Source: National Land Cover Dataset 2001, Multi-Resolution Land Characteristics Consortium

Source Link: http://www.mrlc.gov/nlcd2001.php

Method:

The value of this field represents the average percent of canopy cover across all cells within 100m of the point.

Standard Deviation of Percent Canopy Cover of National Land Cover Dataset (NLCD) cells within 100m of point

Source: National Land Cover Dataset 2001, Multi-Resolution Land Characteristics Consortium

Source Link: http://www.mrlc.gov/nlcd2001.php

Method:

The value of this field represents the standard deviation of percent of canopy cover across all cells within 100m of the point.

Percent Land Cover within 100m Buffer of Point

Percentage of National Land Cover Dataset (NLCD) **Open Water, Developed, Forested, Scrub/Shrub/Herbaceous, Pasture/Crops, Wetland** cells within 100m of point

Source: National Land Cover Dataset 2006, Multi-Resolution Land Characteristics Consortium

Source Link: http://www.mrlc.gov/nlcd2006.php

Method:

Out of total NLCD cells located within a 100m buffer of each stream-road intersection point, the value of this field represents the percent of the cells that were categorized as Open Water (NLCD class 11).

Field evaluation

Contractors will be required to undertake field measurement (Appendix 5) of the following variables in the selected sites per river:

1) Presence of water
2) Bank slope at 5m from stream/road intersection
3) Shoreline cover

Site Occupancy modeling for environmental variables

After the pilot study, the PI and the PI's designee will assess the relationship between mink occupancy and the environmental variables measured remotely by GIS and in the field. Linear models (LMs) will be used to explore the effect of the environmental variables x_p on the response variable Y_i, i.e. mink occupancy at site *i*, using the following formulation:

$$Y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} ... + \beta_p x_{pi} + \varepsilon_i$$

Where - $\varepsilon_i \sim N(0, \sigma^2)$

- the observations i=1,…,n are independent
- $\beta_0, \beta_1, ..., \beta_p$ are the coefficient of variation corresponding to the explanatory variables $x_{1i}, \ldots, x_{pi}$
- ε_i are the errors.

A binomial family will be specified for the response variable and diagnostic plots will be used to check model assumptions. The Akaike Information Criterion (AIC) will be used to determine models resenting the best fit to the data (lowest AIC). Models with ΔAIC≤2 will be considered of equal value (Burnham & Anderson 2002).

Site selection for the pilot study

Given the time constraint for the pilot study, 50 sites will be sampled in each river drainage. To avoid spreading the effort over a large area and having sites too far apart, we will select the 50 sites among sites present in a 10 x18 km polygon from the epicentre of each study area (near Schuylerville in the Hudson river area and near Saint Johnsville in the Mohawk river area): 222 and 219 potential sites are present within this area in the Hudson and in the Mohawk Rivers, respectively. Previous simulations (J.A. Royle, unpublished results) demonstrated that a cluster design would be ineffective for conditions we expect to encounter in surveys of mink. Therefore, different non-cluster designs will be tested through simulations. Current literature (Sollmann *et al.* 2012, Efford unpublished manuscript) suggest an optimal spacing of 1.5-2 home-range radius units. We therefore devised a sampling scheme based on trying to achieve this average spacing of sample locations over subsets of the Mohawk and Hudson river study areas. For each river drainage, the polygonal area was divided into 1km^2 grid cells and one site per cell was sampled. We tested the following fifty-cell designs over one capture occasion:

1- Random designs where cells are picked at random
2- Grouped designs with 50 adjacent cells, *i.e.* the northern or southern half of the polygonal area of the Hudson and the western or eastern half of the polygonal area of the Mohawk.

Mink activity centers were randomly placed using a density of 0.2 mink/km^2, a value of 500 m for σ, the scale parameter corresponding to the standard deviation of locations under a bivariate normal home range model (See model details p.17), and each design was tested by simulating 100 populations, subjecting each population to sampling, and then fitting the model to the resulting data

The design that produced the best results in the simulations was the grouped design for both rivers: the northern half for the Hudson River and the western half for the Mohawk River. Therefore, permission should be acquired for one site (the most central site in each of the identified cells, if possible) per cell (Appendix 4). If permission is not obtained in a one-site cell

(*i.e.* cells with only one site), or for any site of a multiple-site cell (*i.e.* a cell with more than one site), another cell will be used in its place and site permission will be obtained.

Final site selection

If environmental predictors appear significant in the best model, prediction on occupancy will be made for all the sites: sites will be ranked for probability of occupancy. The contractors will use the ranking to obtain landowner permission. Permissions will be obtained following the ranked list of sites until 144 sites have positive landowner permissions within each watershed based on this ranking.

Note also that the variables in the GIS analysis and the field evaluation will possibly be used as covariates to model the probability of detection, and to ascertain that any difference in mink abundance is not due to differences in habitat quality.

If environmental predictors do not appear significant to predict site occupancy, sites will be chosen based on a design that maximizes the number of sites that can be sampled while providing the best estimate of abundance.

Table 3- Stream-road intersections surveyed for scats in November, 2011.

.Site #	Location	Date	Site UTM	# Mink scats
1	Hudson	11/2/2011	N43 16.706 W73 35.941	
2	Hudson	11/2/2011	N43 16.022 W73 35.468	
3	Hudson	11/2/2011	N43 15.887 W73 35.349	
4	Hudson	11/2/2011	N43 15.950 W73 35.240	
5	Hudson	11/2/2011	N43 15.949 W73 35.168	
6	Hudson	11/2/2011	N43 15,574 W73 35.765	
7	Hudson	11/2/2011	N43 12.952 W73 34.968	
21	Hudson	11/4/2011	N43 02.046 W73 32.701	
22	Hudson	11/4/2011	N42 59.236 W73 33.071	
23	Hudson	11/4/2011	N42 55.912 W73 39.361	
24	Hudson	11/4/2011	N42 56.382 W73 38.081	
25	Hudson	11/4/2011	N42 57.451 W73 37.373	
26	Hudson	11/4/2011	N42 59.183 W73 36.290	
27	Hudson	11/4/2011	N43 00.115 W73 35.511	
28	Hudson	11/4/2011	N43 02.854 W73 34.655	
29	Hudson	11/4/2011	N43 08.069 W73 35.358	
30	Hudson	11/4/2011	N43 09.174 W73 34.646	
31	Mohawk	11/14/2011	N42 44.441 W74 19.994	
32	Mohawk	11/14/2011	N42 44.690 W74 19.602	7
33	Mohawk	11/14/2011	N42 44.718 W74 19.620	1
34	Mohawk	11/14/2011	N42 45.427 W74 20.122	

35	Mohawk	11/14/2011	N42 55.803 W74 18.992	
36	Mohawk	11/14/2011	N42 55.750 W74 18.638	
37	Mohawk	11/14/2011	N42 54.449 W74 21.125	1
38	Mohawk	11/14/2011	N42 49.436 W74 20.413	
39	Mohawk	11/15/2011	N42 49.250 W74 22.625	
40	Mohawk	11/15/2011	N42 49.621 W74 24.552	
41	Mohawk	11/15/2011	N42 48.886 W74 25.490	
41	Mohawk	11/15/2011	N42 48.886 W74 25.490	
41	Mohawk	11/15/2011	N42 48.886 W74 25.490	
42	Mohawk	11/15/2011	N42 48.487 W74 25.925	
43	Mohawk	11/15/2011	N42 48.452 W74 26.007	
44	Mohawk	11/15/2011	N42 48.533 W74 27.618	
45	Mohawk	11/15/2011	N42 49.539 W74 28.495	
46	Mohawk	11/15/2011	N42 51.648 W74 28.477	
47	Mohawk	11/15/2011	N42 52.628 W74 29.562	
48	Mohawk	11/15/2011	N42 49.875 W74 30.755	
49	Mohawk	11/15/2011	N42 50.852 W74 31.097	
50	Mohawk	11/15/2011	N42 51.135 W74 31.350	
51	Mohawk	11/15/2011	N42 56.356 W74 30.744	
52	Mohawk	11/15/2011	N42 57.292 W74 32.888	road kill
53	Mohawk	11/15/2011	N42 57.829 W74 27.468	
54	Mohawk	11/15/2011	N42 58.039 W74 25.200	
55	Hudson	11/21/2011	N42 48.467 W74 13.173	
55	Hudson	11/21/2011	N42 48.470 W74 13.166	
56	Hudson	11/21/2011	N42 49.023 W74 14.590	
57	Hudson	11/21/2011	N42 48.426 W74 14.014	
59	Hudson	11/21/2011	N42 49.182 W74 14.808	
59	Hudson	11/21/2011	N42 49.189 W74 14.796	
60	Hudson	11/21/2011	N42 49.684 W74 15.020	
60	Hudson	11/21/2011	N42 49.684 W74 15.019	
61	Hudson	11/21/2011	N42 53.850 W74 14.042	
61	Hudson	11/21/2011	N42 53.850 W74 14.042	
62	Hudson	11/21/2011	N43 00.358 W74 44.509	
62	Hudson	11/21/2011	N43 00.357 W74 44.510	
63	Hudson	11/21/2011	N42 59.995 W74 43.817	
63	Hudson	11/21/2011	N43 00.318 W74 43.762	
64	Hudson	11/21/2011	N43 01.045 W74 41.035	2
64	Hudson	11/21/2011	N43 01.045 W74 41.035	
65	Hudson	11/21/2011	N43 00.996 W74 42.128	1
65	Hudson	11/21/2011	N43 00.995 W74 42.016	
67	Mohawk	11/22/2011	N43 04.105 W73 40.247	
67	Mohawk	11/22/2011	N43 04.104 W73 40.248	
68	Mohawk	11/22/2011	N43 04.956 W73 36.392	1

68	Mohawk	11/22/2011	N43 04.956 W73 36.391	1
69	Mohawk	11/22/2011	N43 04.976 W73 35.915	
69	Mohawk	11/22/2011	N43 04.970 W73 35.905	
71	Mohawk	11/22/2011	N43 02.556 W73 37.536	
71	Mohawk	11/22/2011	N43 02.559 W73 37.546	
72	Mohawk	11/22/2011	N43 02.168 W73 35.536	
72	Mohawk	11/22/2011	N43 02.170 W73 35.537	
73	Mohawk	11/22/2011	N43 00.110 W73 36.457	
73	Mohawk	11/22/2011	N43 00.109 W73 36.458	
74	Mohawk	11/22/2011	N42 59.912 W73 36.617	
74	Mohawk	11/22/2011	N42 59.913 W73 36.617	
75	Mohawk	11/22/2011	N43 09.072 W73 34.528	
75	Mohawk	11/22/2011	N43 09.113 W73 34.552	
77	Mohawk	11/22/2011	N43 08.747 W73 31.537	
77	Mohawk	11/22/2011	N43 08.736 W73 31.544	
78	Mohawk	11/22/2011	N43 10.059 W73 32.573	
78	Mohawk	11/22/2011	N43 10.058 W73 32.570	
79	Mohawk	11/22/2011	N43 09.895 W73 32.354	
79	Mohawk	11/22/2011	N43 09.896 W73 32.355	
81	Mohawk	11/22/2011	N43 13.015 W73 32.792	
81	Mohawk	11/22/2011	N43 10.879 W73 32.931	
82	Mohawk	11/22/2011	N43 10.986 W73 31.668	
82	Mohawk	11/22/2011	N43 10.983 W73 31.664	
83	Mohawk	11/22/2011	N43 11.416 W73 31.428	
83	Mohawk	11/22/2011	N43 11.416 W73 31.427	
84	Mohawk	11/22/2011	N43 14.857 W73 31.752	
84	Mohawk	11/22/2011	N43 14.857 W73 31.752	

References

Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer-Verlag, New York.

Sollmann, R., Gardner, B. and J. L. Belant. 2012. How does spatial study design influence density estimates from spatial capture-recapture models? PlosOne 7(4): e34575. Doi:10.1371/journal.pone.0034575.

Appendix 4: Standard operating procedure for acquiring site permission for the pilot study

Given the time constraint for the pilot study, contractors will need to obtain permission for 50 sites in each river drainage. There are respectively 107 and 102 cells with at least one site within the 10 x 18km polygonal area of the Hudson and the Mohawk river drainages. Contractors will be required to ask permission for sites located within the cells forming the best design (each cell will be provided to the contractors as well as a map of the cells in relation to roads and streams and the potential sites within each cell):

1- If the cell has more than one site they should ask permission for the most central one. If permission is denied for this site, then they should ask permission for the next most central site. If permission has been denied for all sites within the targeted cell, they should choose a new cell that is contiguous to a cell that has already been identified as a viable cell (i.e., landowner permission has already been obtained).

2- If the cell only has one site and permission is denied, they should choose a new cell that is contiguous to a cell that has already been identified as a viable cell (i.e., landowner permission has already been obtained).

When possible, contractors should identify sites falling within the boundaries of the same landowners in order to acquire permission for additional sites that could be used as back-up in case targeting sites cannot be sampled with dogs for topological/environmental constraints.

Appendix 5: Standard operating procedure for field assessment and operating an optical clinometer

Field technicians will be required to complete the forms below for the field assessment. An electronic database including all of the variables per site will be created. Each piece of information recorded in the datasheet below will be entered in an excel spreadsheet daily with a column per type of information (*e.g.*, Stream name, Site ID, Study Area, UTM…).

Contractors will be required to undertake field measurement of the following variables in the selected 144 sites per river that have received positive landowner permission:

4) Presence of water: only sites where there is flowing water year-round will be included. The contractors will record the presence or absence of water at each potential site.

5) Bank slope at 5 m from stream/road intersection using an optical clinometer.

Assuming the field technicians will work alone, they will first need to design a sighting pole using a stake marked at two levels (Fig.7): the reference level showing the depth to which they will drive the stake into the soil and the eye level, which is the vertical measurement from the reference level to their eye level.

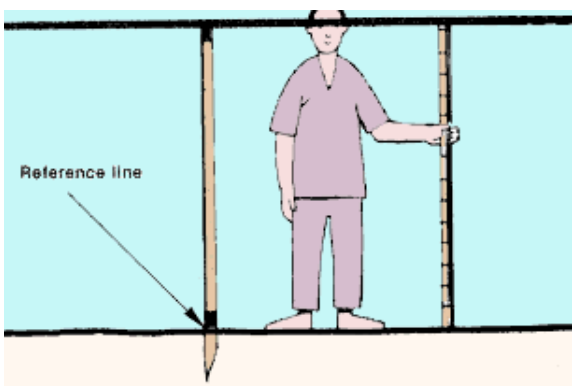


Fig.7: Procedure to mark the sighting pole at the reference level and at the eye level.

Next they will place the sighting stake at 5m away from the stream edge on the slope (point Y in Fig.8). Standing at about 1m from the stream edge, they will look through the sighting device of the clinometer.

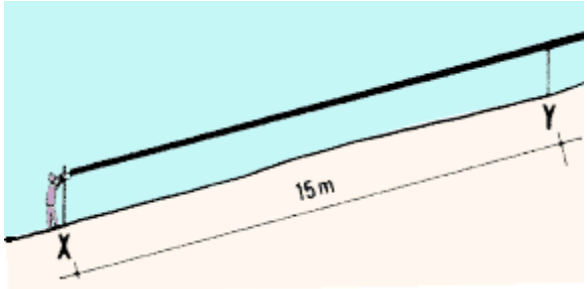


Fig.8- Clinometer field set up.

Typically slope can be read directly on a clinometer using a cross-hair and two scales. The left scale is graduated in degrees and the right scale is graduated in percent. Both scales have a positive (+) section for measuring uphill slopes and a negative section (-) for measuring downhill slopes (Fig.9). Given the differences in clinometers, technicians will have to follow the specific instructions for the particular clinometer used.

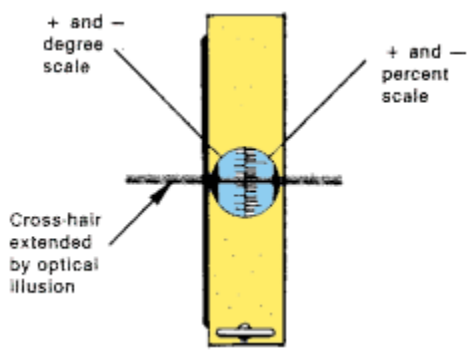


Fig.9- Clinometer reading procedure.

Keeping both eyes open, sight with one eye through the optical clinometer, moving it until the cross-hair lines up with the marked level of the marked pole. Read the graduation at the cross-hair and record the bank slope in percentage and degree at 5m.

6) Shoreline cover measured as the percentage of the shoreline within 1 m from the water edge (i.e. the high waterline), along a 30m transect starting at the stream/road intersection and following the stream upstream and downstream on both sides of the stream. Contractors will record at 15 locations (A-O) along the 30m transect the percentage of shoreline cover present in a $1m^2$ plot by visual identification using a $1m^2$ pvc square: the first plot should be recorded at the bridge and contractors will place the $1m^2$ pvc square 1m from the shore. By visual identification, contractors should record the percentage of shoreline cover present within the square as one of the categories: 0%, 1-10%, 11-30%, 31-50%, 51-75%, 76-100%. Contractors will also record, using the corresponding

abbreviation, the dominant type of cover (except when the percentage of shoreline cover is 0%) present within the square as one of the categories: logjams (L), rock crevices (RC), debris (D), exposed roots (ER), boulders (B), emergent vegetation (EV), undercut banks (UB) or other (O). Contractors will then move 1m and record the shoreline cover measurement for the second plot and continue moving 1m between measurements until they reach 15 measurements per bank on each side of the intersection.

Field technicians will be required to fill out the following form:

Data Sheet for Habitat Suitability Evaluation

LOCATION AND DATE

Stream Name:_____

Site ID:_____

Study Area: Hudson: ____Mohawk:____ (Add a X for the corresponding study area)

UTM Coordinates:_____

Date (MM/DD/YY):_____ Time:_____ AM PM

STREAM FLOW

Uninterrupted Annual Stream Flow: yes___ no___uncertain___(Add a X for the corresponding flow)

BANK SLOPE

Bank slope (%): at 5m ____

Bank slope (degree): at 5m ____

SHORELINE COVER

Fill the table using the following categories:

1- Categories of %: 0%, 1-10%, 11-30%, 31-50%, 51-75%, 76-100%
2- Cover categories: logjams (L), rock crevices (RC), debris (D), exposed roots (ER), boulders (B), emergent vegetation (EV), undercut banks (UB) or other (O).

Section (Upstream):

Left Bank

Plot	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
%															
Cover															

Right Bank

Plot	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
%															
Cover															

Section (Downstream):

Left Bank

Plot	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
%															
Cover															

Right Bank

Plot	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
%															
Cover															

Field Technician:_____ Date: _____

Project Leader:_____ Date:_____

Appendix 6: Home range analysis and analysis of mink use of stream-road intersections

Introduction

Mink can occupy a wide variety of habitat types (riverine, lacustrine, coastal) as long as an adequate source of food is available (Allen 1984). Consequently, the home range of mink will vary in size and shape due to factors such as habitat type, food and den site availability, population density, season, reproductive status, and degree of social instability (Dunstone 1993). Home ranges associated with high-density mink populations tend to be smaller than those associated with low-density populations. The linear home range (associated with riverine systems) for an adult male is generally twice as large as that for an adult female mink. The non-breeding home range for adult males ranges from 2.5-5.5 km and, for adult females, from 0.5-3.0 km (Gerell 1970, Eagle and Whitman 1987). The median length of river used by male and female mink in the United Kingdom was 3.4 km and 2.2 km, respectively and on average, mink were located within approximately 200 m of the river (Harrington and MacDonald 2008). Female home ranges tend to be more stable than those of males (Eagle and Whitman 1987), and mink of the same sex tend to have distinct, non-overlapping territories (Dunstone and Birks 1983).

Measuring an animal's home-range size, shape, and pattern of utilization is important for most research concerned with population density, habitat selection and distribution of resources, spacing of individuals, and their interactions. A variety of analytical techniques exist to evaluate home-range size and to determine patterns of home-range utilization, based on sampling animal locations within a specified timeframe. Choice of a home range estimator should depend on three factors: the objective of the study, the nature of the data, and the movement behavior of the animal in question. Here the purpose of the analysis is to refine the home range estimates available in the literature to individual mink in the study area. Moreover, the objective of the analysis is to describe the entire area used by an individual in order to maximize spatial replication rather than home range *per se*, and thus will result in more robust population size estimates. Given that a relatively large sample size (*i.e.*, number of locations per individual) is required for kernel-density analyses (Seaman *et al.* 1999), and because we have an average of only 47 relocations per individual mink (n = 13, General Electric, as reported to NYSDEC), we used the 95% minimum convex polygon (MCP) method to describe the home range. Mohr (1947) introduced the concept of "minimum home ranges", and developed the method of the minimum convex polygon (MCP) to delineate a home range boundary.

Data

We used relocations of 13 individuals from a telemetry study (General Electric, as reported to NYSDEC) between May and July in 2008-2011 to avoid any overestimation due to reproductive activities before May and juvenile dispersal in late summer (late July to August).

Method

Home ranges were calculated using the minimum convex polygon estimator in the R package adehabitatHR. We used the 95% MCP to map the maximum area potentially used by each individual mink, and to describe the structure of the home ranges we used a clustering process based on a modification of the single linkage algorithm where the home ranges are defined as the set of minimum convex polygons enclosing the relocations in the clusters.

Area-observation curves (Laundré and Keller 1984) were calculated by estimating the 95% MCP over 100 replicates for each number of relocations for each mink using a bootstrap procedure.

Given the possible concern over sampling at stream/road intersections and the fact that mink may be located farther from stream/road intersections than expected, we measured the distance of radiolocations to the nearest stream/road intersection and measured the distance of random points within the Hudson river study area to the nearest stream/road intersection to determine if mink are randomly distributed relative to stream/road intersections. We tested the null hypothesis of a random distribution of the mink in the study area relative to stream-road intersection against the alternative hypothesis that mink are located closer to stream-road intersection than randomly expected using a Wilcoxon test.

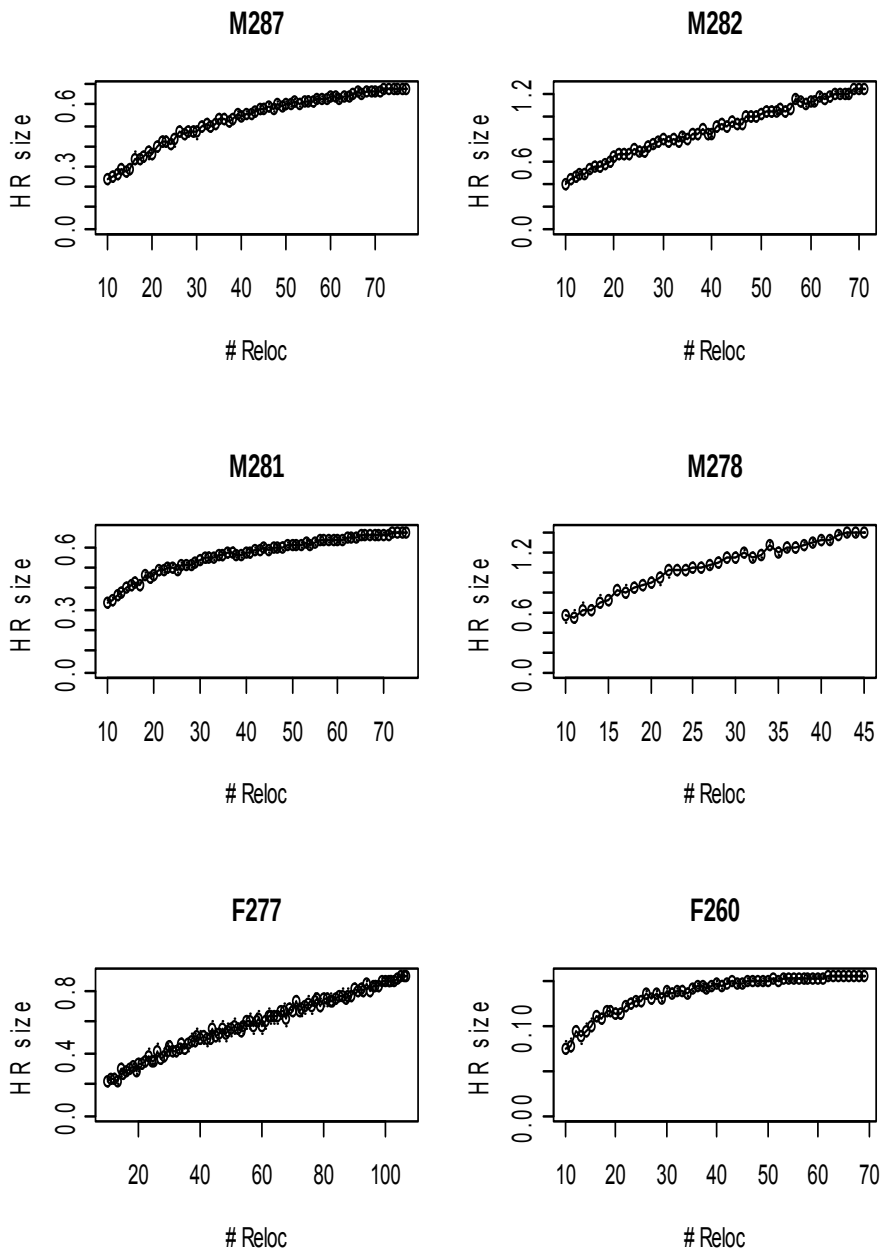
Results

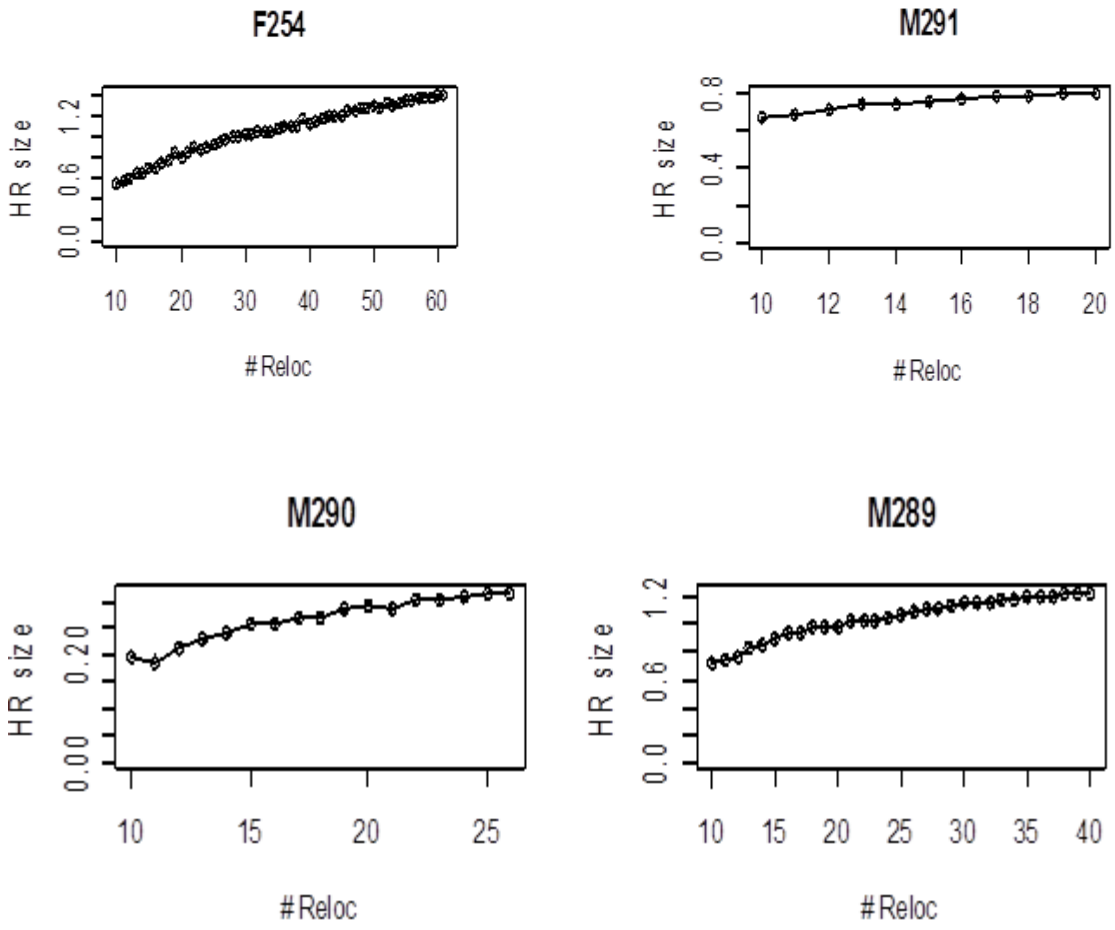
The 95% MCP home range of 13 mink is reported in Table 4.

Table 4- 95% MCP home range area (km^2), sex (F = female; M=male) and number of relocations of 13 mink in the Hudson River valley.

id	Sex	# relocations	HR size
F254	F	61	1.4
F260	F	69	0.16
F277	F	107	0.89
M278	M	45	1.42
M281	M	75	0.67
M282	M	71	1.26
M287	M	77	0.68
M288	M	31	0.61
M289	M	40	1.24
M290	M	26	0.32
M291	M	20	0.81
M295	M	35	0.35
M298	M	21	1.02

The average 95% MCP home range (males and females pooled) was estimated at 0.83 km^2 (standard deviation = 0.42). Plots of area-observation curves (Laundré and Keller 1984) based on the radiolocations suggest that home range size has not yet reached an asymptote for most individuals (Fig.10), and therefore this represents an underestimate of home range size, and therefore these estimates should only be used as a minimum estimate of size.




F254
HR size
0.0
0.6
1.2
10
20
30
40
50
60
#Reloc
M291
HR size
0.0
0.4
0.8
10
12
14
16
18
20
#Reloc
M290
HR size
0.00
0.20
10
15
20
25
#Reloc
M289
HR size
0.0
0.6
1.2
10
15
20
25
30
35
40
#Reloc

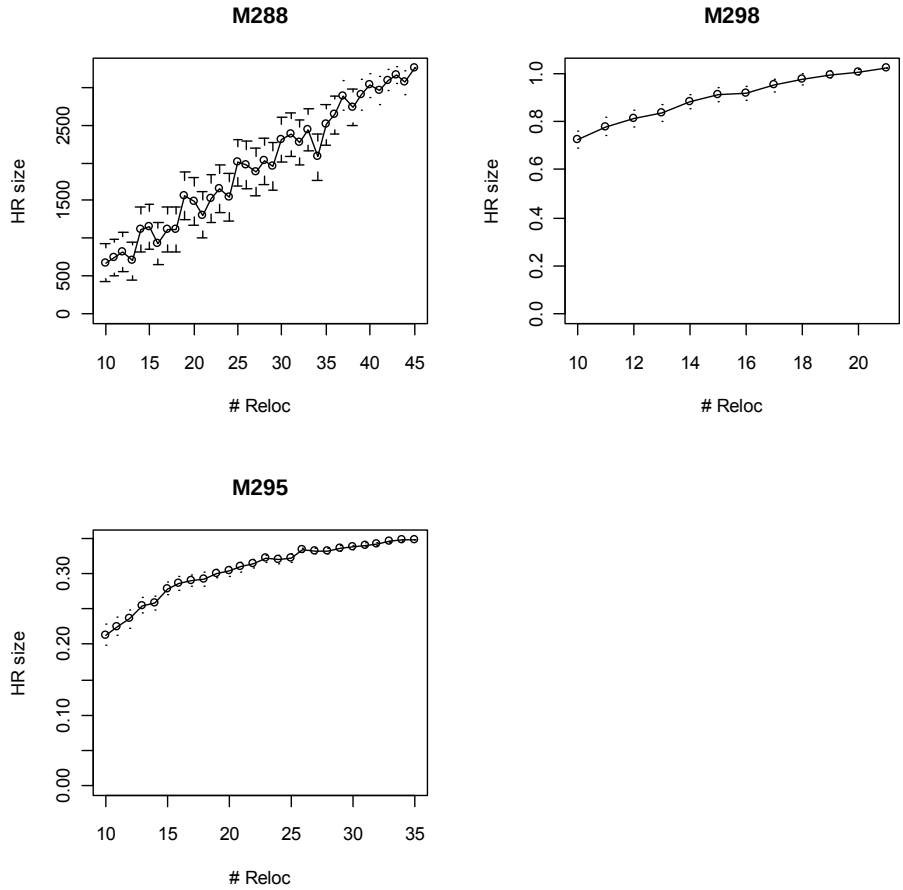


Fig.10- Area-Observation curves, displaying home range size of mink as a function of the number of radiolocations used to estimate home range. Home range size is calculated using a 95% minimum convex polygon and 100 bootstraps for each number of radiolocations. These curves should be asymptotic in order to calculate a home range.

The average distance of actual radiolocations (n = 720) to stream/road intersections was 528.41m (SD = 319.26m) and the random distance to stream/road intersections (n = 600,000) was 831.5m (SD = 853.01m). The p-value for the one-sided unpaired Wilcoxon signed-rank test was < 2.2e-16, therefore, at 0.05 level of significance, we reject the null hypothesis that mink are located randomly relative to stream/road intersections in favor of the alternative hypothesis that mink are located closer than expected to stream-road intersections.

Discussion

The purpose of this analysis was to estimate the home range and distribution relative to stream-road intersections for mink during the sampling period. The home range estimated using the available telemetry data in the study area revealed a 95% MCP home range that is significantly smaller than mink home ranges found in the literature (Dunstone 1993, Gerell 1970, Niemimaa 1995). However home ranges were estimated during a limited time interval represented by a stable social environment, when food availability was high (May to July) and

area-observation curves (Fig.10) suggest that there are not enough relocations, hence that the present estimates of home range size are most likely underestimating the true home range size. However, another study also reported small home ranges of 0.3 km for females in May and 1.5 km for males in June (Dunstone and Birks 1983). Given that no information is available on the structure of the mink population in the study area, we will assume that this sample is representative of the whole population, and we will use the current home range estimation as a crude approximation of home ranges in the study area. These home range estimates will not be used in any habitat analysis or in describing habitat requirements in any way.

Given the present results of mink distribution, sampling at stream/road intersections should not bias the density estimates because mink are not distributed farther from stream-road intersections than random expectation. Even if a "road effect" did exist, this will not necessarily lead to bias in density estimates if we can effectively model this in the encounter probability model.

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Appendix 7: Standard Operating Procedure for Monitoring Hair Snares

The sites selected for the scat collection will be equipped with hair snare devices between June and July, 2012 as well as during the 2013 field season. Two hair snare devices will be placed at each site in the Hudson study area and in the Mohawk study area. These hair snare devices will be monitored weekly. Any damaged, misplaced, or missing devices will be fixed or replaced and repositioned.

Hair-snare Installation

At each site, field technicians will place one hair snare device on each side of the river under the bridge when possible, or close to the road-stream intersection. Hair-snare devices should be located close to the water edge (no further than 5 m away) along a concrete surface (if directly under the bridge), or along a log or similar pathway. Field technicians will secure the hair-snare devices to the ground to prevent them from moving by using tent stakes attached to each end of the device using the existing eye bolts and rope. When the ground is too hard to use tent stakes, square 1 gallon bottles filled with water from the stream will be used in place of the stakes. One bottle will be attached closely to each end of the device using rope through the eye bolt. The eye-bolt in the middle of the device can also be used if more appropriate, given the field conditions. The important point is that the device is secured to the ground in some manner to ensure that it does not blow away, or that it is not easily moved by animals. The hair-snare device will be camouflaged with surrounding materials such as branches, stones, sand, leaves, or grass to mimic a tunnel. Placement of the device should try to follow natural features (e.g., logs, large branches, large stones, etc.) and blend into the landscape. One hair-snare device will be placed on opposite banks at each site (2 devices per site). Sites and hair snare devices will be uniquely identified by a number. Records of weather conditions including degree of precipitation (defined as either "none" or "rain", with a description of the type/amount of rain), temperature in (◦F), and light conditions (defined as either "overcast", "partial sun" or "sun") will be made in a dedicated field notebook and on data forms.

The design will be based on the best design from the hair-snare device testing (Appendix 1 and Fig.11): a corrugated plastic sheet (90 x 33 cm) folded into a triangle using 2 bolts (e.g., fitting '6-32' wing nuts), 3 eye bolts (e.g. ¼-20 eye bolt, i.e. also fitting '6-32' wing nuts) and wing nuts (*e.g.,* 6-32). In replace of the wing nuts and bolts, zip ties can be used instead. Ideally, the corrugated plastic sheets will be brown or green. If the only color available is white, the corrugated plastic sheets will be spray painted with camouflage paint (if used, the gallon water jugs should also be spray painted)(*e.g.* Fig.12).

Please note that the stripes of the corrugated plastic sheet should be longitudinal to make the folding possible.

Hair will be collected by 2 gun brushes (of 0.45 caliber) in each device, mounted inside the triangle using wing nuts (*e.g.,* 6-32)(Fig.11). A sardine will be placed in the middle of the device using a small alligator clip. Winkler's Brown Beauty (or similar) mink gland lure (Sterling Fur Company) will be placed in a hardware cloth pocket (6 x 6 cm) or using vegetation

(e.g. apply drops of the lure on a stick) and placed adjacent to the hair snare device (e.g. the rope used to attached the device to the peg or the bottle should be used to tie the cloth pocket, or if using a stick, the stick should be placed in the ground vertically to expose the end with the lure). A permanent marker (e.g., sharpie) will be used to designate gun brush 1 and gun brush 2 (i.e., each gun brush will have either a number 1 or number 2 associated with it). Sites ("Site #" in forms in Appendix 8) in the Hudson will be numbered sequentially, beginning with "H-1"and following in numerical order (e.g., H-2, H-3, etc.) and the same follows for the Mohawk – each site will be numbered sequentially, beginning with "M-1" and following in numerical order. A permanent marker will uniquely identify the outside of the hair-snare device. Each hair-snare device will be given a unique identifier ("Device #" in forms in Appendix 8). The first identifier will be the site number (e.g., H-1) followed by either A or B. So, at Site H-1, the first hair-snare device will be labeled H-1A and the second hair-snare device at that site will be labeled H-1B. The 2 gun brush location on each device ("Brush #" in forms in Appendix 8) will be identified as following: "site number, device number" followed by either 1 or 2, *e.g.* "H-1A-1" for the gun brush located at the location #1 on device A in site 1 in the Hudson.

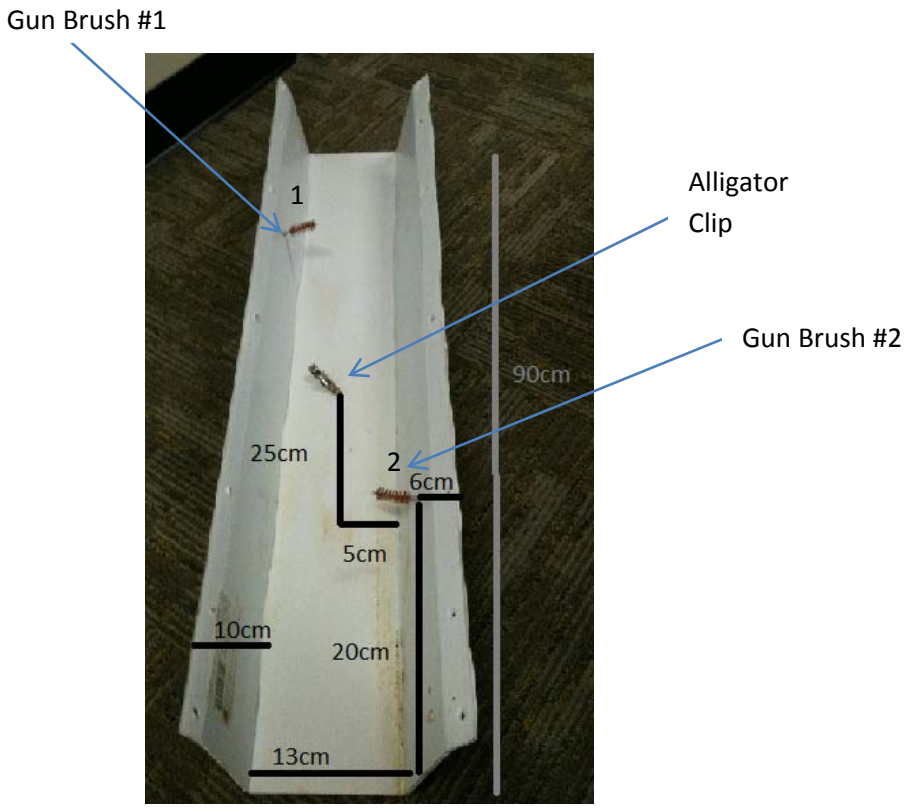

Fig.11- Dimensions for the hair-snare device.

Fig.12: Example of hair snare set up in the field.

Hair-snare monitoring

Field technicians will visit sites every 7 days in the daily order they visited them when they first installed the hair-snare devices.

Technicians will inspect each hair-snare device by unscrewing and opening the device: gun brushes presenting hair will be unscrewed from the device and the gun brush will be placed in a microtube (*i.e.*, falcon tube) by technicians wearing a new pair of surgical gloves for every gun brush handled. It is important to change surgical gloves after handling a gun brush. Field technicians will record the uniquely numbered site, the uniquely numbered device, the uniquely numbered gun brush number, GPS location of device, collection date, sample number, a description of the hair on the brushes (e.g., one hair, clumped hair, multiple colors of hair, etc.) and the collector's initials will be recorded if hair is present and/or if animal signs are present around the device (even in the event of no hair present on the gun brushes) (Appendix 8). Any unusual observations will be reported (*e.g.*, devices missing, devices moved or destroyed, etc.) and photos (Appendix 9) will be taken to document these observations, (*e.g.*, animal signs around the devices, how hair was caught in the gun brushes, etc.). Gun brushes with hairs will have been removed and placed in a falcon tube labeled with the brush number (i.e., "Brush#" in form 2 of Appendix 8, which includes site number, device number, brush number) and the date (as MMDDYYYY) and a thorough search of the inside of the device will ensure that no hair is adhered to the side walls of the device. Any hair-snares containing hair must be clean after hair collection. Hair snares will be visually inspected for hair that may be adhered to the side walls, and if present, will be rinsed in the adjacent stream. Field technicians will replace any missing or damaged device and install a new gun brush for any gun brush taken for collection.

At each site visitation, the mink lure will be changed either by impregnating the previously-used cloth pocket or a new cloth pocket or a stick, and the sardine will be replaced. Observations of mink and other animals will be recorded on the field data sheets, including scats or tracks in the vicinity of the hair snare device.

Appendix 8: Datasheet for mink hair collection

The following forms will be filled when the devices are first placed in the field (form (1)) and at each site visitation to record weather condition and any changes in the device location (form (2)) (definition of items in Appendix 7)**:**

(1)

Date MM/DD/YYYY	Site #	UTM northing	UTM easting	Device #	Device UTM northing	Device UTM easting

(2) Definition of items for the following form are defined in Appendix **7:**

Site #	Device #	Brush #	Date MM/DD/YYYY	Time	Collector's name	Photo #	Precipitation	tempera -ture	Light condition	Observations

Appendix 9: Standard Operating Procedure for photographic collection

All field staff engaged in collecting photographic records must comply with the following guidelines in the field:

1- Ensure that date and time are accurate on the camera
2- Activate the visible date and time option so that the photos have the date and time
3- Record the corresponding photographs on the field forms for the hair and scat collections (Appendices 8 and 12)
4- Do not delete photos from the camera before an official digital archive is created
5- Set camera to keep incrementing file names across multiple downloads. This avoids duplicate filenames

Additional guidelines for transferring the files to a digital archive must also be followed. These include:

6- Create 2 subdirectories on the computer to store the photographic records using the name "MINK" and "MINK_archive"
7- Within the subdirectory "MINK" and "MINK_archive" create a folder for the Mohawk and Hudson rivers called respectively "Mohawk" and "Hudson", "Mohawk_archive" and "Hudson_archive"
8- Make the "Mohawk_archive" and "Hudson_archive" folders Read-Only by right-clicking on them, choosing "Properties", checking the Read-Only box, selecting "Apply change to this folder, subfolders and files" and clicking "ok"
9- Within the "Mohawk" and "Hudson" folders in the "MINK" subdirectory, create a folder for each field work day using the name convention: "Mo- MM/DD/YYYY" (for the Mohawk) and "Hud- MM/DD/YYYY" (for the Hudson)
10- Transfer the photos to the appropriate folders in the "MINK" subdirectory on the computer by either (a) by connecting the camera directly to the computer using the appropriate cable or (b) by removing the memory card from the camera and using the memory-card slot on the computer or a card-reading device
11- Validate the transfer of photos to the "MINK" subdirectory by viewing the directory and comparing file sizes to originals
12- do not delete the photos after this transfer
13- Transfer the photos to the "MINK_archive" subdirectory in the appropriate folder
14- DO NOT EVER open the photos within the "MINK_archive" subdirectory. This archive is required to ensure that we have a full, un-edited photo record.
15- Only after a digital archive of all photos is created, you are allowed to delete photos from the memory card.

Appendix 10: Dog training and sampling protocol

Introduction

The use of non-invasive genetic methods by genotyping hair, feather, feces, or sloughed skin represents an alternative to traditional marking methods, and is becoming increasingly popular in conjunction with mark-recapture methods to estimate population size (Bellemain *et al.* 2005, Boulanger *et al.* 2004a, Boulanger *et al.* 2004b, Creel *et al.* 2003, Eggert *et al.* 2003, Mills *et al.* 2000, Mowat and Paetkau 2002, Prugh *et al.* 2005, Taberlet *et al.* 1999, Wilson *et al.* 2003). These methods have been used for species including brown and black bears (Woods *et al.* 1999, Mowat and Strobeck 2000), cougars (Sawaya *et al.* 2010), tigers (Mondol *et al.* 2009), and marmots (Goossens *et al.* 1998). Among the non-invasive feces sampling approaches, the use of detection dogs has become popular in the last few years to study elusive species, and has great potential because it can significantly increase the number of scats collected. The method has been used effectively on black bears, fishers, and bobcats (Harrison 2006, Homan *et al.* 2001, Long *et al.* 2006, Reed *et al.* 2011, Smith *et al.* 2001). The study will involve 2 detection dog teams (2 handlers and 2 dogs) working simultaneously on different sites.

Dog training

Dogs will be trained by the detection dog company with mink scats found in the study areas during a preliminary study in November, 2011 (Hudson River drainage: 2, 4, and 21 November, 2011; Mohawk River drainage: 14, 15, and 22 November, 2011; Tables 3 and 5) and during additional searches in April 2012. Scats found during this preliminary study will be analyzed genetically by the Lead Geneticist to ensure species identity (*i.e.*, that they are mink scats).

Dogs will be trained in field conditions 2-3 days prior to the sampling period in sites outside of the sampling area (Table 5). The training will consist of placements of wild mink scats (*e.g.*, a subset of the scats collected in November, 2011) at known locations in the field training sites. The dog handler will record the dog ID, date, environmental conditions, site location, number of scats placed, locations (UTM) of each scat placed, and the number and locations of missed and recovered scats. The aim of the preliminary training in field conditions is double: to test the efficiency of the dogs at detecting mink scats to get a naive estimate of the detection probability, and to allow the dog to develop an efficient search strategy prior to the study. Because the dog handler is aware of the locations of the scats, this training will also allow the handler to observe how the ability of the dog to find a scat relies on environmental conditions such as topography, vegetation and weather. This field training should therefore help the handlers develop ways to guide the dog in various field conditions, and to learn how to compensate for a possible loss in scent due to the field conditions (*e.g.*, by moving the dog downwind, hence maximizing the scat collection).

Table 5- Available sites for the dog field training in the Mohawk River drainage outside of the study area.

Date	UTM site	Mink scats collected
11/3/2011	N43 09.551 W73 51.523	
11/3/2011	N43 10.025 W73 51.328	
11/3/2011	N43 13.392 W73 50.803	
11/3/2011	N43 13.370 W73 51.039	
11/3/2011	N43 12.222 W73 51.779	
11/3/2011	N43 11.529 W73 51.361	
11/3/2011	N43 09.793 W73 48.940	
11/3/2011	N43 09.128 W73 46.423	1
11/3/2011	N43 09.128 W73 46.423	1
11/3/2011	N43 11.264 W73 41.944	
11/3/2011	N43 10.707 W73 41.969	
11/3/2011	N43 09.812 W73 41.998	
11/3/2011	N43 11.602 W73 41.062	
11/3/2011	N43 11.281 W73 43.315	1 or 2

Standard sampling procedure for dog teams

Prior to the search for scats, weather (*i.e.*, temperature, wind speed, precipitation) will be recorded for each site from the road. Temperature will be recorded using a portable thermometer or anemometer (in Fahrenheit), wind speed using a portable anemometer (in mph), and precipitation will be noted subjectively (no rain, drizzle, light rain, medium and heavy rain).

Dog teams will survey both banks of the river on each side of the stream/road intersection (*i.e.*, 4 transects per site, minimum length of each transect = 100 m). The handler will typically start walking from the stream/road intersection on a transect following the river edge, at approximately 5 m from the edge (Bonesi and Macdonald 2004). This distance of the handler/dog from the river edge seems optimal given that Yamaguchi *et al.* (2003) reported that female and male mink stayed within 10 m of the nearest water source, 95% and 88% of the time, respectively, and Reed *et al.* (2011) reported that dogs detected >75% of scats located within 10 m with a decrease in the dogs' detection rates with increasing distance of scats from the transect line. Length of transects will be subject to permission and accessibility, and will therefore likely vary within and between sites (we will account for variable length transects in the SCR model, so this is not worrisome). Dog teams will try and survey as much linear distance as possible, given landowner permission, and allowing for enough time to complete the required number of sites per day. During the transect walk, the dog will be off-leash and in-sight of the handler, unless the landowner requires that the dog be on leash. At the end of a transect, the team can either cross the river if it is safe to do so (to start a transect on the opposite bank walking towards the intersection/bridge), or walk back the same transect. In the latter case, the dogs will not be searching for scats during the walk back. The handler will collect any scat found by

himself/herself and by the dog. The handler or orienteer (*i.e.*, assistant) will record the GPS location, sample number, collection date, collection time, and collector's initials for all scats found (Appendix 12).

When several sites are sampled without any mink scat collection, the handler should hide at least one wild mink scat in the next transect without the dog's knowledge (if agreeable to the dog company). This is in an attempt to keep the dog motivated and focused on mink scats, and to make sure the dog is rewarded throughout the day (Wasser *et al.* 2004).

Any unusual observation will be reported, and photos will be taken to document each fecal sample found. Scats will be placed in 10x16cm waxless paper bags, and only one scat will be stored in each bag. Each bag will be labeled with the site number, date, time, the UTM of the scats, name of the collector, name of the dog, and the scat sample number. Handlers will wear a new pair of gloves to collect each sample. After the scat collection at each site, individual bags containing the scats will be placed in a plastic tote or cooler. At the end of each sampling day, collected scats in paper bags will be placed in a warm, dry place and allowed to dry for 1-4 days. Scats will be kept out of direct sunlight. After drying, scats will be stored in falcon tube vials filled with 96% Ethanol. Each vial will have an outside label identifying collection information as well as a duplicate label on the inside of the tube.

Site Monitoring Schedule

All sites will be numbered and a daily schedule will be organized prior to the monitoring period. The daily schedule will contain 8 sites, and each dog team is expected to work approximately 8 hours a day. The dog team will also have information on the next day schedule in the case where the team can sample more sites in a day. If, for logistical reasons (*e.g.*, bad weather) the daily schedule could not be met, the team should sample the remaining sites on the next sampling day.

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Appendix 11: Standard Operating Procedure for Scat Survey and Collection

At each site, field technicians will record the following information (form (1) in Appendix 12), regardless of whether scat is found:

1- The date (MM/DD/YYYY)
2- The site number "Site #" in Appendix 12: Sites in the Hudson will be numbered sequentially, beginning with "H-1"and following in numerical order (e.g., H-2, H-3, etc.) and the same follows for the Mohawk – each site will be numbered sequentially, beginning with "M-1" and following in numerical order.
3- GPS location ("UTM northing" and "UTM easting") of the site
4- Field conditions which include degree of precipitation (defined as either "none", "light rain", "heavy rain", "fog"), temperature in (◦F), and wind speed measured in (mph) with an hand-held anemometer: field technicians should follow the manufacturer's recommendations and directions for the specific anemometer. To record wind speed with the anemometer, field technicians should stand downwind of the anemometer while holding and orienting the instrument so that it faces the direction of the wind. The operator should hold the anemometer with an extended arm to maximize the distance between the instrument and the operator.
5- "Collectors' name+dog" defined as the names of the field technicians (including the dog handler) and the dog involved in the scat collection at the site.
6- "Observations": any observation such as tracks of mink and other animals, food remains, etc.
7- Start time of search, end time of search recorded in "hour-minute am/pm".

Additionally, for each scat found the technician in charge of the collection should record in a form (form (2) in Appendix 12):

1- the date (MM/DD/YYYY),
2- the site number "Site #" in Appendix 12: Sites in the Hudson will be numbered sequentially, beginning with "H-1"and following in numerical order (e.g., H-2, H-3, etc.) and the same follows for the Mohawk – each site will be numbered sequentially, beginning with "M-1" and following in numerical order.
3- GPS location of the scat ("UTM northing" and "UTM easting")
4- Sample #: the sample will be identified as following: "site #" (e.g. H-1…) followed by a letter in alphabetical order (first scat found at the site will be identified with "A", second scat with "B"…) followed by the date MMDDYYYY. So at site H-1, on the 31st of June 2012, the bag containing the first scat found will be labeled: "H-1A06312012".
5- Time recorded in "hour-minute am/pm"
6- Collector's initials
7- The number of photos taken
8- "location": a description of the location where the scat was found (e.g. on a log, under a rock, etc.)
9- "distance": the approximate distance of the scat to the stream/river in meters

10- “freshness”: the approximate age of the scat (defined as “old” if dry and moldy or “fresh” if shiny)
11- “Size”: scat size (both width and diameter in that order and in millimeters)
12- “color”: scat color
13- “consistency”: scat consistency (solid, runny, etc.)
14- “habitat”: a general habitat description of the immediately surrounding area, including overstory type (tree, bush, open space…), if open space, provide the approximate distance to the nearest overstory habitat, if visible.

Field technicians will place scats in 10x16cm waxless paper bags and only one scat will be stored in each bag. If multiple scats are found at a location, each scat will be placed in a separate bag. Field technicians will wear a new pair of gloves to collect each scat. Bags will be clearly labeled using a permanent marker pen with the identifier defined above for “sample #” in form (2) of appendix 12.

After scat is collected, scats in paper bags will be placed in a warm, dry place (e.g. a cooler with no ice) before being transported to a warm, dry place and allowed to dry for 1-4 days. Scats will be kept out of direct sunlight. After drying, field technicians will store scats in falcon tube vials filled with 96% Ethanol and labeled clearly with a sticker with the sample number “sample #” defined above. Each vial will have an outside label as well as a duplicate label on the inside of the cap of the tube using a sticker with the sample number “sample #” defined above.

Appendix 12: Datasheet for mink scat collection

The following form will be filled for each site visited, definition of items for the following forms are defined in Appendix 11:
(1)

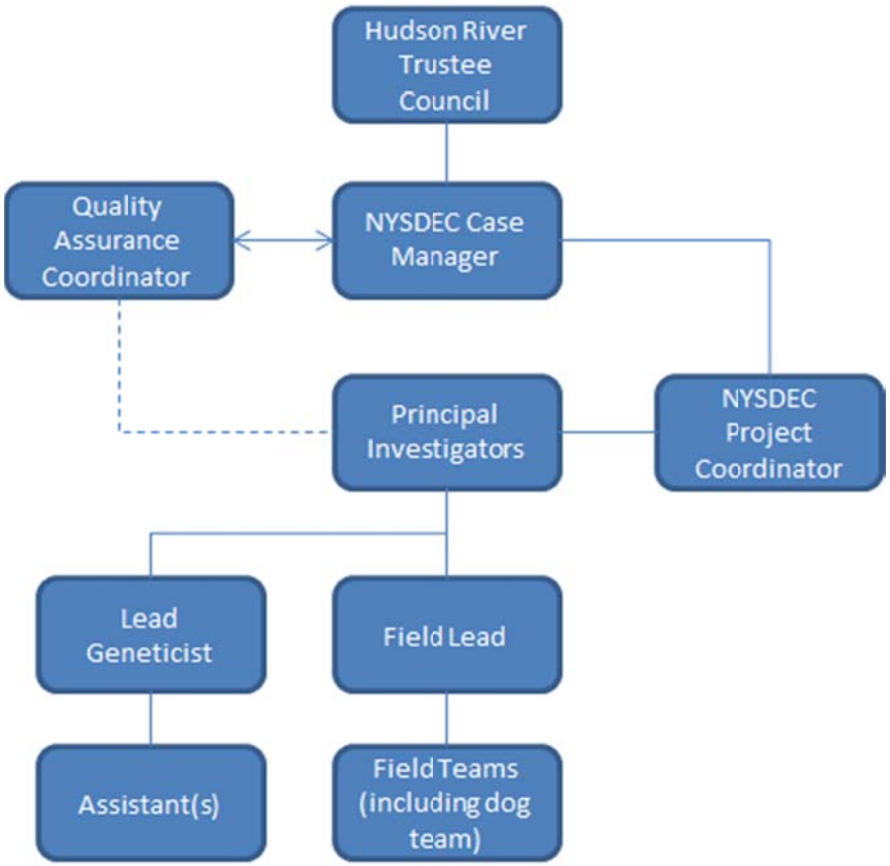
Date MM/DD/YYYY	Site #	UTM northing	UTM easting	Temperature	Wind speed	Precipitation	Collectors' Name + dog	Observations	Start time	End time

For each scat collected, the following form should be completed (definition of items in Appendix 11):

(2)

Site #	UTM northing	UTM easting	Sample #	Date MM/DD/ YYYY	Time	Collec-tor's initials	Photo #	location	distance	freshness	Size (width-diameter)	color	consisten-cy	Habitat

Appendix 13: Organizational chart



Appendix 14: Chain of Custody

A. Chain of Custody
B. Field Team Acknowledgement of Work Plan review

New York State Department of Environmental Conservation

Chain of Custody

I, _____, of _____ City

_____ State _____ Zip Code _____ have collected on

_____, 20___ from _____in the (Date) vicinity of Town of

_____, _____ County.

Item:

_____

_____ said sample(s) were in my possession and handled according to standard procedures provided to prior to collection. The sample(s) were placed in the custody of a representative of the New York State Department of Environmental Conservation on

_____, 20_____

(Date)

_____ _____

Signature Date

I, _____, have received the above mentioned samples on the date specified and have assigned identification number(s) _____ to the sample(s). I have recorded pertinent data for the sample(s) on the attached collection records. The sample(s) remained in my custody until subsequently transferred, prepared or shipped at times and dates as attested to below.

_____ _____

Signature Date

Second Recipient (Print Name)	Time and Date	Purpose of Transfer
Signature	Unit	
Third Recipient (Print Name)	Time and Date	Purpose of Transfer
Signature	Unit	
Fourth Recipient (Print Name)	Time and Date	Purpose of Transfer
Signature	Unit	
Received in Laboratory by (Print Name)	Time and Date	Purpose of Transfer
Signature	Unit	
Logged in by (Print Name)	Time and Date	Accession Numbers
Signature	Unit	

SEE REVERSE SIDE

Notice of Warranty

By signature to the chain of custody (reverse), the signator warrants that the information provided is truthful and accurate to the best of his/her ability. The signator affirms that he/she is willing to testify to those facts provided and the circumstances surrounding same. Nothing in this warranty or chain of custody negates responsibility nor liability of the signators for the truthfulness and accuracy of the statements provided.

Handling Instructions

Keep samples in a dry place.

Initial recipient (either DEC or designated agent) of samples from collector(s) is responsible for obtaining and recording information on the collection records forms which will accompany the chain of custody. This person will seal the container using packing tape, writing his/her signature, time, and date across the tape onto the container with indelible marker. Any time the seal is broken, for whatever purpose, the incident must be recorded on the chain of custody (reason, time and date) in the purpose of transfer block container, then reseal using new tape and rewriting signature with time and date.

Investigation of Mink Abundance Relative to Polychlorinated Biphenyl (PCB) Contamination within the Hudson River Drainage

Field Team Acknowledgement of Work Plan Review

Your signature below indicates that you have read and understood the study plan and associated standard operating procedures

Name (printed):_____ Name (printed):_____

Signature:_____ Signature:_____

Initials:_____ Initials:_____

Date:_____ Date:_____

Title:_____ Title:_____

Name (printed):_____ Name (printed):_____

Signature:_____ Signature:_____

Initials:_____ Initials:_____

Date:_____ Date:_____

Title:_____ Title:_____

Name (printed):_____ Name (printed):_____

Signature:_____ Signature:_____

Initials:_____ Initials:_____

Date:_____ Date:_____

Title:_____ Title:_____

Appendix 15: Laboratory form

The laboratory technician will fill the following electronic spreadsheet for the genetic analysis as data become available. Once the data have been entered, the file should be printed, the paper version should be dated and signed by the technician and then the electronic version should be saved in Microsoft Excel 2010 as a read-only file under the name "Mink_Genet_Results_MMDDYY.xlsx" (with MMDDYY being the date the file was modified and saved). To save the file as read-only with the file open in Excel, the technician must:

1. Go to the **File** menu, click **Save As**.
2. On the **Tools** menu in the **Save As** dialog box, click **General Options**.
3. Select the **Read-only recommended** check box, and then click **OK**.
4. Click **Save**.
5. If prompted, click **Yes** to replace the existing workbook.

Except when the first file is created, when new data are available, to add them to the last saved spreadsheet "Mink_Genet_Results_MMDDYY.xlsx", the technician must copy the last saved spreadsheet and only open the copy of the last saved spreadsheet. When prompt to answer the Excel message "'Mink_Genet_Results_MMDDYY-copy.xlsx' should be opened as read-only unless you need to save changes to it. Open as read-only?", the technician must select "No". Before printing and saving the new spreadsheet, the technician must go to **Review**, **Track changes**, **Highlight changes** and make sure no changes occurred to the previously-saved data. Then the technician must print the new spreadsheet, date and sign the paper version and save the electronic version as "'Mink_Genet_Results_MMDDYY.xlsx". The following steps must be repeated every time new data are entered.

1- copy the previous file
2- open the copy not as read-only
3- add data to the copy
4- print the spreadsheet when done entering the data
5- date and sign the paper version
6- save the electronic file as read-only "Mink_Genet_Results_MMDDYY.xlsx".

Site #	Date of field collection MM/DD/YYYY	Device #	Brush #	Scat Sample #	Laboratory Start Date MM/DD/YYYY	Extrac-tion source	# loci geno-typed	# Repli-cate geno-types	Ind mink ID	Date of record MM/DD/YYYY	Analyst Name

N.B.: "Ind mink ID" will be identified as following: "site #" (e.g. H-1…) followed by the letter "M" and a number (first identified mink at the site will be identified with "M1", identified mink with "M2"…) followed by the date MMDDYYYY. So at site H-1, on the 31st of June 2012, given it was the first time a mink was identified at this site, the mink identity will be labeled: "H-1M106312012".

Laboratory Technician's Name: _____ Signature:_____

Date: _____

Appendix 16: Field supplies

A- Dog teams

2 GPS units
2 digital cameras
2 memory cards for camera
2 anemometers
2 portable thermometers
20 boxes surgical gloves (25 pair count) for handling scat samples
25L ethanol
2 pairs of waders
2 pairs of hip boots
2 backpacks for field equipment
500 50 ml plastic falcon tubes for storing scat samples
500 printable labels for falcon tubes
500 10x16cm waxless paper bags
2 clip boards
Pens, sharpie markers
2 small plastic rulers for measuring dimensions of scats
Rite in the rain field data sheets

B- Pilot hair study

1 power drill
3 bottles Winkler's Brown Beauty mink gland lure
Hardware cloth for lure
Camouflage spray paint (or brown/gray/green mix) for hair collection devices (if brown corrugated plastic is not available)
300 tent stakes to secure hair collection devices
800 Sardines
Pens, sharpie markers
20 boxes surgical gloves (25 pair count) for hair samples
200 50 ml plastic falcon tubes
200 Printable labels for the plastic tubes
2 backpacks for field collection equipment
2 GPS units
2 clipboards
Rite in the rain paper for data forms

We estimate collecting an average of 2 samples for each positive detection, a detection rate of 0.20, visit of 100 sites (50 sites in the Hudson River study area and 50 sites in the Mohawk River study area) * 2 devices per site, for a total of 200 hair collection devices. We

check devices on 5 occasions. Estimated number of samples = 200*5*0.20 = 200 plastic tubes to collect the gun brushes.

For hair collection during the pilot study, 2 devices will be deployed at 50 sites per river, hence a total of 200 devices.

200 pieces of 90 x 33cm corrugated plastic
1000 6-32 wing nuts for bolts (5 per device)
1000 bolts to fit wing nuts (5 per device)
800 gun brushes (of 0.45 caliber, 4 per device)
800 6-32 wing nuts for gun brushes (4 per device)
200 small alligator clips
200 empty plastic 1Gallon bottle
120 tent stakes
600m of rope (e.g. multi-purpose type)

Considering occasional damages, misplacement or theft, additional supplies might be needed.

U.S. DEPARTMENT OF THE INTERIOR · MARCH 3, 1849

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION · NOAA · U.S. DEPARTMENT OF COMMERCE

DEPARTMENT OF ENVIRONMENTAL CONSERVATION · NEW YORK STATE