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Attachments: [2016 Wolverine Multistate Sampling Station Protocol v3.2 093016.pdf](#)

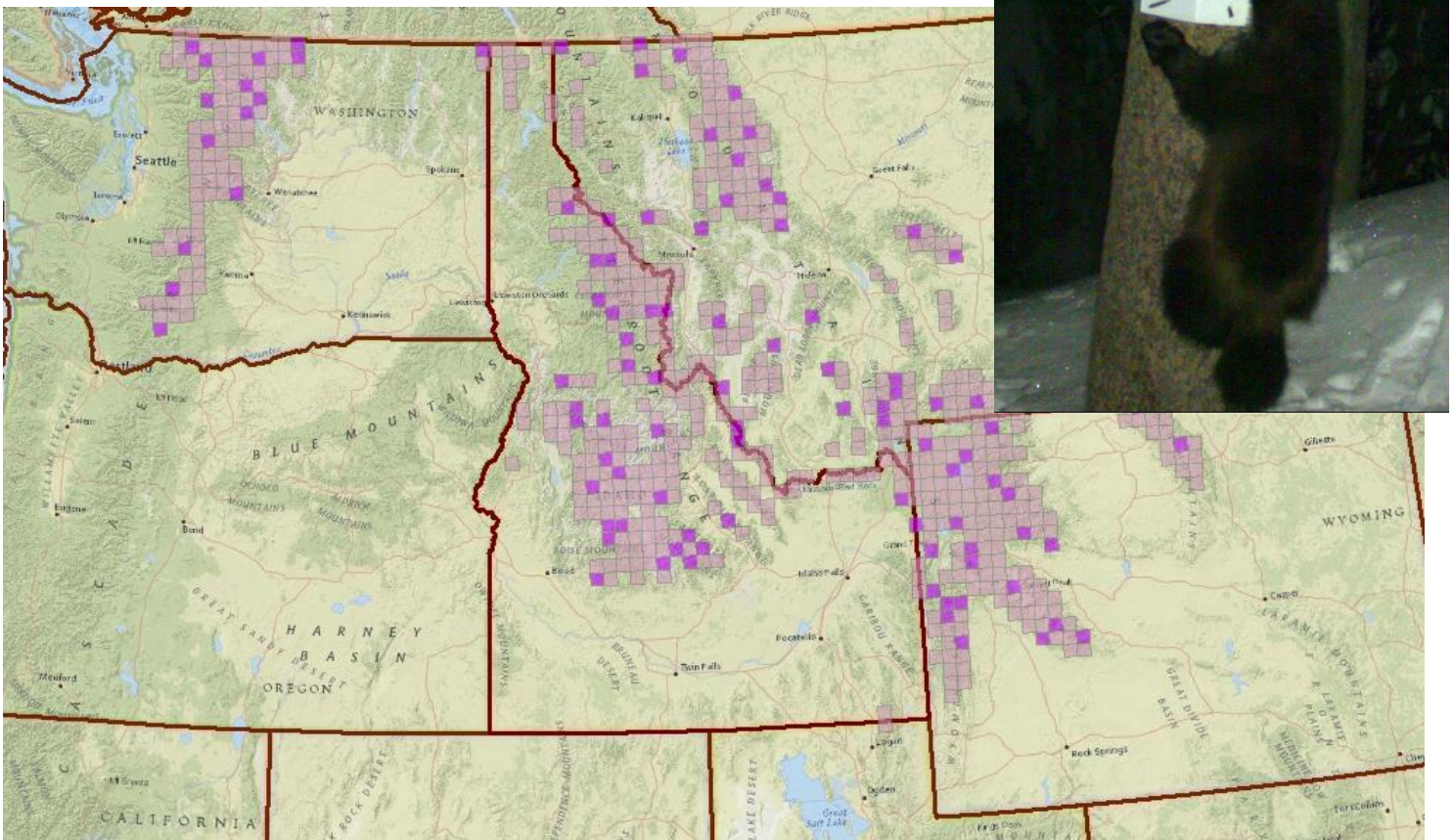
Attached. Thanks Betty.

-Bob Inman

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Western States Wolverine Conservation Project Baseline Survey

Standard Operating Procedures for Camera-DNA Stations



Western States Wolverine Working Group
Version 3.2
September 30, 2016



Washington
Department of
FISH AND WILDLIFE

Diane Evans Mack and Robert Long compiled this protocol for the Western States Wolverine Conservation Project Working Group based on decisions made by the group at a workshop in Salmon, ID, July 2015 and subsequent discussion.

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Cover photo: Camera station near McCall, ID, February 2016, Idaho Department of Fish and Game

Acknowledgements

Several individuals beyond the Working Group shared techniques and ideas incorporated into this protocol. We thank Joel Sauder, Idaho Department of Fish and Game, for his work on scent dispensers and thoughts on camera and hair snare placement. Michael Lucid, Idaho Department of Fish and Game, shared techniques for hanging and securing bait. Eric Lofroth, British Columbia, developed the long call scent lure that formed the basis for our current recipe. Meghan Riley, The Wolverine Initiative, led the field work for a pilot effort in Wyoming in early 2015. Scott Fitkin, Washington Department of Fish and Wildlife, and John Rohrer, Okanogan-Wenatchee National Forest, worked with Jeff Lewis to add lynx-detection capacity to the station design.

This project was made possible with funding from the U.S. Fish and Wildlife Service's Competitive State Wildlife Grant Program, a U.S. Fish and Wildlife Service CFDA Program award through the Great Northern Landscape Conservation Cooperative, and the National Fish and Wildlife Foundation. 110 cameras purchased by the Wildlife Conservation Society with a grant from the National Park Service were loaned to the project.

Changes from version 3.1

This protocol is essentially the same as v3.1, dated 08/01/2016, with one notable exception. Meat bait will not be placed at inaccessible stations. A scent dispenser and cow femur will be deployed in place of bait, not in addition to bait at inaccessible stations. Because of this change, additional edits were made for consistency and clarity.

INTRODUCTION

This document provides the standard operating procedures (SOPs) for deploying and maintaining baited camera stations for coordinated multi-state wolverine sampling within suitable habitat across Washington (WA), Idaho (ID), Montana (MT), and Wyoming (WY). The objective of this project is to establish a baseline of wolverine occurrence (probability of occupancy) across the 4-state area from which to assess future changes in occupancy that might be due to the influences of conservation actions, environmental change, or anthropogenic disturbance.

Sampling will occur within a spatially balanced random selection of 15 km x 15 km grid cells resulting from a Generalized Random Tessellation Stratified (GRTS) sampling procedure which ranked each cell (Stevens and Olsen 2004). At a minimum, 180 cells (ranks 1–180) will be sampled for this project, with approximately 26 grid cells in WA, 60 in ID, 46 in MT, and 48 in WY.

By providing detailed protocols we aim to ensure that field methods and results are consistent in all 4 states and across 2 consecutive winters and that we will generate a statistically reliable dataset with minimum ‘noise’. The main elements of these SOPs were developed at a workshop for wolverine coordinated multi-state sampling held July 8 & 9, 2015, in Salmon, ID.

Other forest carnivores will be incidentally detected by this survey. Mustelids (fisher, marten) readily climb for bait similar to wolverines. Lynx respond to different attractants. To increase the likelihood of detecting a lynx along with fisher and marten, we added components to the station design specific to lynx. No adjustments to where stations are located were made to accommodate these other forest carnivores.

STANDARD OPERATING PROCEDURES

Project Period and Timing of Deployment

Sampling will begin in Wyoming during winter 2015/16. Sampling will continue in winter 2016/17 in WA, ID, MT, and remaining locations in WY.

Sampling will occur during 1 December through 31 March both years. The timing of deployment will differ among stations (see below under Station Type) with the objective of having all cameras deployed and running as of 1 December. As part of the occupancy modeling design, each 1-month interval will represent an independent visit to the station (December, January, February, March).

Station Density

We will deploy 1 camera/hair snag station per 15 km x 15 km sampling unit (grid cell).

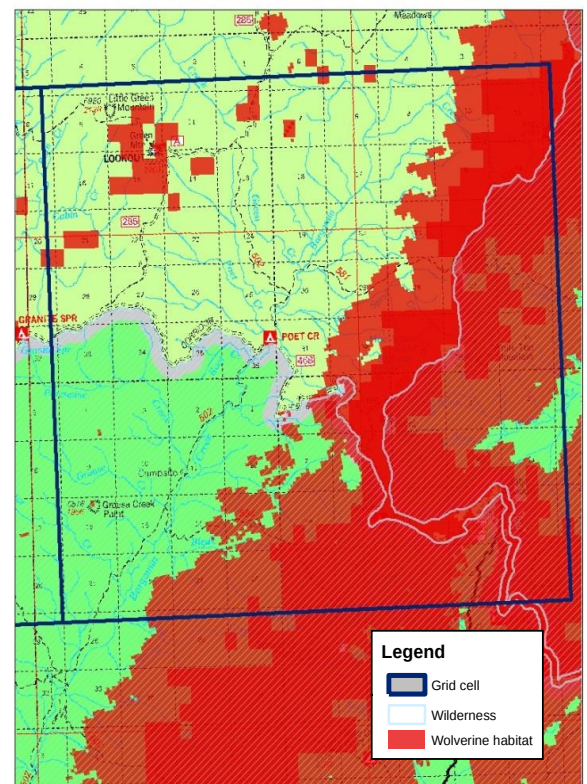
Station Location within a Grid Cell

Prior to the field season we will examine GIS layers to identify a potential suitable location at which to deploy each camera (Figure 1). The field crew will aim to navigate to this location unless conditions encountered at the time of deployment make it unreachable, in which case they will

select another location in the best quality site that can be reached. Each location will aim to be as centrally located in the cell as possible, subject to these considerations and criteria:

- in mapped wolverine habitat at a high-quality site (e.g., high elevation near tree line, a saddle between peaks, or drainage bottom)
- a preference for a location that will be accessible throughout the winter
- a preference for a non-wilderness location over a wilderness location
- >2 km from any adjacent station; the farther apart the better
- >200 m from a human-use road or trail
- >500 m from a campground

Figure 1. Configuration of wolverine habitat, wilderness area boundary, and access routes within selected grid cell to identify potential station locations.



Station Type

There are 2 types of stations based on accessibility during the December–March sampling period. Accessibility is determined in part by safety concerns for the field crews during winter (e.g., avalanche-prone areas) and not necessarily by land designation. “Accessible” stations will be revisited monthly to replenish bait and scent, collect DNA samples, exchange the SD card, and adjust the camera. “Inaccessible” stations will be deployed in late fall and not revisited until the following spring (6-9 months later). Inaccessible stations will use a scent dispenser to drip a small amount of lure daily, but otherwise will have no adjustments to account for snow accumulation and no periodic DNA collection.

It has been determined that sampling stations that occur within Wilderness Areas across the four states will be able to be accessed without the use of mechanical transport or motorized equipment. If mechanical transport or motorized equipment is later determined to be necessary for accessing a specific wilderness station, that station will be reviewed separately for consistency with legal requirements and policies.

Maps and Waypoint Lists

Field crews will be provided 2 paper maps of each grid cell selected for sampling. One will have aerial imagery as the base layer, the other will have topography as the base layer. Other spatial data included on the maps will be:

- wolverine habitat
- rivers/stream
- relevant administrative boundaries
- roads/trails
- groomed snow machine trails (if available)

Coordinates of the 4 corners of each grid cell, the center of the cell, and the projected station location will be calculated in ArcMap and uploaded to GPS units for field navigation. A hard copy list of these coordinates will be carried in the field.

Coordinates uploaded to, and collected by, GPS units will be standardized as:

geographic coordinate system: lat/long
 locations expressed as: decimal degrees (dd.dddd on the GPS)
 datum: WGS84

Equipment

Camera All stations will deploy the same type of camera, the Reconyx PC800 HyperFire semi-covert infrared model. Cameras will operate with Energizer Ultimate Lithium batteries (no alkaline batteries). Camera settings are detailed in Appendix A.

Hair snagging collars All stations will use .30 caliber gun brushes to collect DNA samples (via snagged hair). Brushes will be arrayed on 2 corrugated polypropylene plastic “collars” that attach to the bole of a tree. The larger, primary collar is designed for wolverine and follows the Figura and Knox (2008) model, except with a 5” spacing. Construction and attachment are detailed in Appendix B.

In addition, there is a second, lower gun brush collar designed to increase capacity to detect lynx at the wolverine stations. Construction and attachment are detailed in Appendix C.

Schematics of both station types are provided in Appendix D.

DNA collection is detailed in Appendix E.

Bait and Lure Accessible stations will have meat bait, a long call scent lure for wolverine, and a lynx lure. Inaccessible stations will not have bait; instead they will have a scent dispenser that drips a small amount of wolverine lure each day onto a cow femur plus the separate lynx lure sponge. Appendix F provides details on bait and lures.

A complete equipment check list is itemized in Appendix G.

Station Deployment–Revisit Schedule

I. Accessible location (assumes reliable access December–March)

Deployment Date---Accessible stations should be deployed in the tightest window possible **before 1 December**. Bait and scent lure will degrade over time, and our goal is to start the project with stations as near as possible to fresh conditions.

1. Select a suitable site (see Station Location section above).
2. Select 2 trees 4–6 meters apart. The bait tree should be ~8–25" DBH.
3. Bait the station with a 15–20 lb chunk of game (e.g., elk chunk, deer hind quarter, beaver). Firmly secure bait to the tree bole with smooth wire as high overhead as can be reached from the ground or snow surface on which you're standing. Secure with multiple wraps around the tree. Tighten wire with twists of a screw driver. See Appendix F for bait prep and attachment.
4. Wire a sponge soaked with long call wolverine scent lure above the bait. Keep this in view of the camera.
5. Attach a wolverine gun brush collar 12–18" below the bait (see Appendix B, p. 13).
6. Attach a lynx gun brush belt 18" above the ground and offset 75-80° from the wolverine gun brushes. See Appendix C.
7. Soak the lynx lure sponge with lynx lure. See Appendix C.
8. Review the camera settings and make sure they match Appendix A. Make sure the LABEL has been assigned as the station ID (grid cell #). Make sure date and time are correct for the current time.
9. Secure the camera to the bole of a tree ~4–6 meters from the bait tree. Use a knotted cord marked at 1-meter intervals to measure this distance.
10. Focus the camera midway between the ground and bait (i.e., with sufficient view to catch an animal on the ground or snow surface if it doesn't climb the tree).
11. Put the camera in 'walk test' mode to test sensor and photo positioning. Reposition the camera as needed.
12. Arm the camera and move into the field of view at the bait tree to take several test photos of yourself. Turn the camera off.
13. Carefully examine the test images¹ to confirm that the camera is positioned properly (not too high or too low, too far right or left, etc.). The image should capture an animal on the ground below the bait and on the bait itself. Reposition as needed and test again.
14. Review tips from Appendix A regarding false triggers.
15. Lock the camera into position with the cable lock.
16. Mark the location with a GPS and label the GPS point with the station ID.
17. Arm the camera.
18. Write the **station ID** and **date** on a sheet of white paper with a marker and stand in front of the deployed camera to store a reference image that includes yourself in the image.

¹ Examine test images on site with a laptop or tablet with appropriate card reader that has been tested to handle the images without damage. Some digital cameras and cell phones damage the images or metadata on the card.

Revisit the station at 1-month intervals---Revisits should be as close to the 1st of the month as possible.

1. Walk in front of the camera to photograph your presence.
2. Disarm the camera.
3. Remove the SD card and place in a secure holder for transport. It is not necessary to examine the photos in the field.
4. Insert a fresh SD card pre-labeled for the camera. Enter the CodeLoc to reactivate the camera. Ensure LABEL is set for the correct grid cell # on the new card.
5. Check battery capacity and replace if needed.
6. Examine gun brushes and remove any brushes with hair from the gun brush collar.
7. Collect gun brush samples per DNA protocol (see Appendix E). Make sure to identify which gun-brush belt (wolverine or lynx) each brush came from.
8. Replenish bait; adjust height to account for snow accumulation.
9. Replenish long call scent lure on sponge above bait with wolverine lure.
10. Adjust height of gun brush collars and replenish gun brushes.
11. For brushes that had no hair, run lighter flame over brush to re-sterilize.
12. Replenish lynx scent lure on lynx sponge with lynx lure.
13. Adjust and re-focus the camera per #10 above.
14. Test camera position per #11 & #12 above.
15. Carefully examine a test image to confirm that the camera is positioned properly.
16. Review tips from Appendix A regarding false triggers.
17. Arm the camera.
18. Write the **station ID** and **date** on a sheet of white paper with a marker and stand in front of the deployed camera to store a reference image that includes yourself in the image.

Dismantling the station (after March 31):

1. Walk in front of the camera to photograph your presence.
2. Disarm and remove the camera.
3. Remove gun brushes from the collar.
4. Collect hair samples per the DNA protocol (see Appendix E).
5. Collect and remove all materials associated with deployment in accordance with “leave no trace” principles.

II. Inaccessible location (assumes station will be deployed in fall and not revisited until late spring)

Deployment date---as late as possible in the fall when sites are still accessible by driving and/or hiking. This could be as early as the last week of September at some locations.

Follow **all** the same steps as for an Accessible location, with the following exceptions:

1. Do not use meat bait. Substitute a cow femur and scent dispenser. Fill scent dispenser reservoir to its capacity (~740 ml or 25 oz) with long call wolverine lure.

2. Use climbing steps and a climbing harness to get **8-12 ft** above ground level on the 'bait' tree to account for snow accumulation.
3. Attach a scent dispenser in its protective housing at this **8-12 ft** height.
4. **Cable** (not wire) 1 domestic cow femur bone to the bole of the tree immediately below the scent dispenser parallel to the bole of the tree using 5/8" coated cable and cable locks.
5. Position the dispenser to drip directly onto the cow bone. See Appendix F.
6. Position lynx gun-brush belt and lynx lure sponge **2 ft below the wolverine belt** to account for snow accumulation.
7. Soak the lynx lure sponge with lynx lure.
8. Mount the camera at a similar height as the dispenser and turn it 'sideways' to obtain a portrait image rather than horizontal image to better capture the base of the tree up to the bait and bone (see Appendix A, p. 9). Thoroughly test camera position, then wire tightly in place.

Dismantling the station:

1. Walk in front of the camera to photograph your presence.
2. Disarm and remove the camera.
3. Remove gun brushes from the collar.
4. Collect hair samples per the DNA protocol (see Appendix E).
5. Collect and remove **all** materials associated with deployment in accordance with "leave no trace" principles.

Warning Signs

All stations in grizzly bear areas must have at least 4 warning signs placed at the most likely approach points to minimize the chance that the public inadvertently stumbles onto a bear at our baited sites. Flag each warning sign with high visibility flagging tape. A sample sign is provided at the end of this protocol.

Pre-field Work

For either station type, as much pre-work as possible should be completed before venturing into the field. This includes:

- Construct gun-brush collars.
- Label each camera with a unique ID.
- Label 2 SD cards for each camera (same ID as the camera).
- Program SD cards with the Reconyx Professional Settings software.
- Insert programmed SD cards into cameras and manually set the current, correct date and time.
- Pre-load scent dispenser reservoirs with wolverine lure.
- Pre-drill and pre-wire bait and cow femurs.
- Print sheets of labels for DNA samples.
- Fill sample vials with desiccant (if using vials instead of envelopes).

Field Data & Data Management

Each field crew will be responsible for accurate records of every visit to a grid cell, sorting and managing camera images, logging and storing DNA samples, and entering data into a database. Data management should occur as frequently as possible to avoid a backlog of data entry or lost data and to keep SD cards in rotation.

Field Data

Each visit to a grid cell should be documented on a data form. A project data form is attached at the end of this document. In addition to details about the station itself and the status of cameras, bait, and DNA samples, also record information observed on approach to and in the vicinity of the station, including evidence of disturbance (human or animal) and tracks of species of interest (e.g., fisher, lynx, wolverine).

Camera Images

As a first step, SD cards should be backed up (copied) to a stable computer. With the volume of photo data this project will be collecting, organization and subfolder structure is critical. Each download from a camera will reside in its own subfolder. Use the following subfolder structure:

\project folder\station folder\visit folder\images

Once copied into the visit subfolder from the SD card, rename all the images in the visit subfolder using a naming convention that combines grid cell ID, date, and revisit #. See Appendix A – Photo Management for details. This will avoid confusion down the road if images are misfiled or moved.

Once a back-up copy of all the images has been downloaded to a hard drive, we will use Colorado Parks and Wildlife CPW Photo Warehouse software (<http://cpw.state.co.us/learn/Pages/ResearchMammalsSoftware.aspx>) to upload images directly from the SD card to the project database. The software will be used to sort, catalogue, rename, and facilitate identifying images. SD cards should not be cleared until the crew has triple checked that all images have been uploaded to the database and backed up on at least one additional hard drive. At least one of these hard drives should be stored at a different location.

Data Entry

Each state will maintain a database that captures field data from visits to sampling stations, photo image results, quantity of DNA samples submitted, and ultimately the presence/absence of wolverine at each station. These databases will be collated into a single project database by collaborators at the University of Montana. Each state will perform data QA/QC prior to the creation of the joint database.

Training

A training session will be held for all new and returning crew members. The training program will be developed by the state coordinators. At a minimum training will encompass overall project objectives; expectations; work schedules; participating states and contact info of key contacts; examination of field equipment and supplies; and hands-on demonstrations of camera operation,

SD card readers, data entry, and photo software. The training also will include review and field practice of snow machine operation, safety, and basic repair; trailering; winter defensive driving; and operation of avalanche beacons and SPOTs or other personal emergency locator devices. An avalanche safety course is strongly recommended.

Literature Cited

Figura, P. and L. Knox. 2008. A lightweight, portable tree-mounted hair snare for mesocarnivores. Unpublished document, California Department of Fish and Game, Redding, CA.

Stevens, D. L., Jr., and A. R. Olsen. 2004. Spatially balanced sampling of natural resources. *Journal of the American Statistical Association* 99: 262–278.

APPENDIX A

Reconyx PC800 HyperFire IR Camera Settings and Image Files

Every camera used for this project should be programmed by inserting a **32 gb class 10** SD memory card pre-programmed to the settings listed below using the Reconyx Professional Settings software. When the SD card is inserted into the camera, the settings will be transferred. Do not use anything larger than a 32-gb card.



Date/Time: Once the card is inserted, you will be prompted to enter the current date and time. Follow this step interactively on the camera and set the current date and time even if you already set a date/time on the SD card with the programming software. **Make sure the date and time are correct.** Do not change date/time for daylight savings time on any subsequent visits.

The software interface will show settings organized by 5 tabs.

Triggers Tab

Quickset = Advanced

Near Video = **5** pictures, waiting **1** second between pictures

Quiet Period = **15** seconds

Options = Use the internal motion trigger

Advanced: sensitivity = High; require **1** pulses within **1** second

Schedule = View Schedule

Set Daily Schedule = All On (lower right)

Time Lapse Tab (to take a single reference photo per day to show camera is working)

Time Lapse Interval = Hours; enter **24**

At the Specified Interval = Take one picture

Schedule = View Schedule

Set Daily Schedule = 1st select 'All Off' (lower right), then scroll to 11 am and toggle to 'Camera On' for the hour of 11 am to 12 noon.

Images Tab

Label = Cell <ID> (enter the grid cell #)

Brightness = 5th or mid

Contrast = 5th or mid

Sharpness = 5th or mid

Saturation = 5th or mid

Temperature = Fahrenheit

Time = 24 hr

Night Shutter Speed = Fast

Night ISO Sensitivity = Low
Resolution = High
Silent Image VGA Models = leave blank (no border)

Cellular Tab
N/A

Other Tab
HyperFire Models Only = leave blank
CodeLoc = check "Use CodeLoc" ; select digits **3 3 3 4** (see Note below)

Click 'OK to save all settings.

Note: the CodeLoc must be entered every time the camera is powered up or a SD card is added. The camera won't operate without entering the code. For this reason, it can be a theft deterrent. Place a small sticker on the outside of the camera housing stating "This camera protected by CodeLoc" to deter theft.

Camera Set-up Tips

Avoid false triggers. False triggers most often occur on sunny, breezy days (Reconyx Instruction Manual). Vegetation will soak up the sun's energy and it will become warmer than the ambient air temperature. When the wind moves the vegetation, the camera sees this and cannot distinguish it from a warm-blooded animal moving in the scene. Examine your set-up carefully and anticipate problems. Clear away hanging branches and lichen.

Use only wire to tightly secure hanging bait (no rope or cord that will fray or stretch over time or swing in the wind when the bait is gone).

Use the walk test and examine the photos! Move through the trigger space as if you were the size of a wolverine. Keep in mind that movement directly toward and away from the camera is less likely to trigger the camera than movement across the camera's field of view.

High contrast images—those with large amounts of shade and sun in the same image—often create deep shadows and result in underexposed areas of the frame. These can make it difficult to make out the details of animals that trigger the unit from the shade. Ideally, cameras should face evenly lit or shady areas, such as a backdrop of trees or an open gap with relatively no shade.

Attempt to aim the camera towards the north, away from the east and west where low sun angles during sunrise and sunset can cause flare and distortion in any images taken during those times. Aiming the camera north also could reduce backlighting issues if not facing a highly reflective surface (e.g., snow field).

Sideways Camera Position for Inaccessible Stations

Technique provided by Joel Sauder, Idaho Department of Fish and Game Clearwater Region, and Robert Long, Woodland Park Zoo, Washington.

Because inaccessible stations will be deployed early and not visited throughout the course of the winter, the camera, scent dispenser, and gun brush collars need to be placed high enough to stay above accumulating snow. We recommend 8-12 ft depending on anticipated snow depth for your site. This creates a large vertical space between the base of the tree at ground/snow level and the dispenser that needs to be captured by the camera. To maintain adequate coverage, orient the camera 'sideways' on the camera tree. This puts the camera's detection bands vertical rather than horizontal. This increases the detection area up and down, but allows less room for error in positioning the camera correctly side to side. Examine test images carefully to ensure that the bait tree falls within one or both of the vertical detection bands.

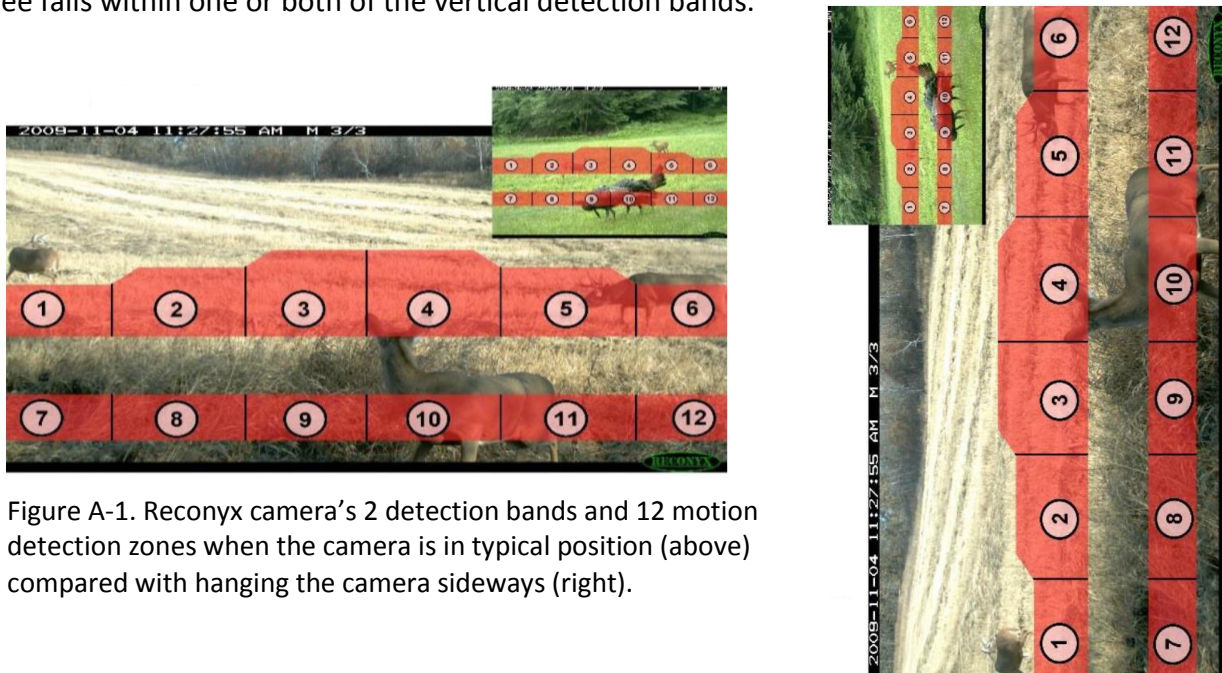


Figure A-1. Reconyx camera's 2 detection bands and 12 motion detection zones when the camera is in typical position (above) compared with hanging the camera sideways (right).

Additional hardware is needed to hang the camera sideways:

- 2 3/16 in x 3 in eyebolts
- 2 3/16 in washers
- 1 3/16 in coupling nut

Orient the 2 eyes up and down the tree, and wrap wire through each eye and around the tree separately (i.e., 2 circles of wire, one through each eye).

The camera should be placed at least 8 ft high. Angle the camera slightly downward to keep snow from piling up. Use a shim that is securely in place.



Figure A-2. Eyebolts added to allow sideways orientation of the camera.

Photo Management

Images stored on SD memory cards will be downloaded at least twice directly from the card.

The first process creates a back-up copy of the card. Images should be copied to a subfolder specific to that station and visit using the structure:

\project folder\station folder\visit folder\images

Images are assigned a sequential number by the camera, but a card from a different camera will have the same file names.

Name	Date modified	Type	Size
 IMG_0001.JPG	2/13/2014 12:53 PM	JPEG Ima...	465 KB
 IMG_0002.JPG	2/13/2014 12:53 PM	JPEG Ima...	465 KB
 IMG_0003.JPG	2/13/2014 12:53 PM	JPEG Ima...	468 KB
 IMG_0004.JPG	2/19/2014 11:29 AM	JPEG Ima...	501 KB
 IMG_0005.JPG	2/19/2014 11:29 AM	JPEG Ima...	501 KB

Thus, once the files are copied to their unique visit subfolder, rename the images to assign grid cell ID, year, date, and revisit #. Select all the files in the folder (Control A), right-click the first file name, select 'Rename', and type in the new name.

Name	Date modified	Type	Size
 313.2016.02.17.v1.JPG	2/13/2014 12:53 PM	JPEG Ima...	465 KB
 IMG_0002.JPG	2/13/2014 12:53 PM	JPEG Ima...	465 KB
 IMG_0003.JPG	2/13/2014 12:53 PM	JPEG Ima...	468 KB
 IMG_0004.JPG	2/19/2014 11:29 AM	JPEG Ima...	501 KB
 IMG_0005.JPG	2/19/2014 11:29 AM	JPEG Ima...	501 KB
 IMG_0006.JPG	2/19/2014 11:29 AM	JPEG Ima...	500 KB
 IMG_0007.JPG	2/19/2014 11:33 AM	JPEG Ima...	497 KB
 IMG_0008.JPG	2/19/2014 11:34 AM	JPEG Ima...	498 KB
 IMG_0009.JPG	2/19/2014 11:34 AM	JPEG Ima...	498 KB
 IMG_0010.JPG	2/19/2014 12:09 PM	JPEG Ima...	520 KB
 IMG_0011.JPG	2/19/2014 12:09 PM	JPEG Ima...	520 KB

313 = cell number
2016 = year
02 = month
17 = day
v1 = revisit #1

Hit enter or move the mouse outside of the file name box; all the files will be renamed with their original sequential number in ().

Name	Date modified	Type	Size
 313.2016.02.17.v1 (1).JPG	2/13/2014 12:53 PM	JPEG Ima...	465 KB
 313.2016.02.17.v1 (2).JPG	2/13/2014 12:53 PM	JPEG Ima...	465 KB
 313.2016.02.17.v1 (3).JPG	2/13/2014 12:53 PM	JPEG Ima...	468 KB
 313.2016.02.17.v1 (4).JPG	2/19/2014 11:29 AM	JPEG Ima...	501 KB
 313.2016.02.17.v1 (5).JPG	2/19/2014 11:29 AM	JPEG Ima...	501 KB
 313.2016.02.17.v1 (6).JPG	2/19/2014 11:29 AM	JPEG Ima...	500 KB
 313.2016.02.17.v1 (7).JPG	2/19/2014 11:33 AM	JPEG Ima...	497 KB
 313.2016.02.17.v1 (8).JPG	2/19/2014 11:34 AM	JPEG Ima...	498 KB
 313.2016.02.17.v1 (9).JPG	2/19/2014 11:34 AM	JPEG Ima...	498 KB
 313.2016.02.17.v1 (10).JPG	2/19/2014 12:09 PM	JPEG Ima...	520 KB
 313.2016.02.17.v1 (11).JPG	2/19/2014 12:09 PM	JPEG Ima...	520 KB
 313.2016.02.17.v1 (12).JPG	2/19/2014 12:09 PM	JPEG Ima...	521 KB
 313.2016.02.17.v1 (13).JPG	2/19/2014 12:10 PM	JPEG Ima...	514 KB

The second download from the SD card will use the CPW Photo Warehouse software to catalogue files. Refer to the software User Guide <http://cpw.state.co.us/learn/Pages/ResearchMammalsSoftware.aspx> for instructions on how to set up a new project in the database prior to downloading photos. You will define a study area, station locations, visits, and, optionally, a species list and detection details. When ready to “Import Photos” from the SD card, opt to also copy the photos to a new location. Select a destination folder located on a different hard drive than the initial backup and rename files using the same naming convention described above (cell ID.year.month.day.revisit).

SD cards should not be cleared until the crew has triple checked that all images have been uploaded to the database and backed up on at least one additional hard drive.

APPENDIX B

Wolverine Gun Brush Collar

A lightweight, portable tree-mounted hair snare for mesocarnivores

Pete Figura and Luke Knox
California Department of Fish and Game
Redding, CA
Contact: pfigura@dfg.ca.gov, (530) 225-3224

March 2008

Description

This tree-mounted hair snare design utilizes corrugated plastic, tee nuts, and gun cleaning brushes as its main components. It is light, portable, relatively inexpensive, and is easy to build and install. Samples can be collected by removing the brushes that contain hair, and replacement brushes can be threaded into place. The design has been very effective for detecting marten in northern California, and it has also detected long-tailed weasel, spotted skunk, red fox, and gray fox¹.

Parts list for each device (see Figure B-1)

- 1 - 3" x 22" piece of Coroplast™ corrugated polypropylene plastic (or equivalent), cut with long axis perpendicular to the plastic flutes
- 4 – tee nuts (8-32 thread)
- Duct tape (approximately 30" length)

Parts list for assembly (see Figure B-1)

- 4 - .30 caliber gun cleaning bristle brushes (8-32 thread)
- 3 – 1" fence staples ("u nails") or 1" roofing nails

Helpful tools for assembly

- Dark-colored Sharpie™ or equivalent marker
- Utility knife
- Hammer
- Electric drill w/ 3/16" bit
- Scrap piece of lumber



Figure B-1

¹ Dimensions and quantities specified in the following sections have been modified from the original design to accommodate larger carnivores anticipated in this wolverine project.

Assembly

1. Mark the plastic at the following distances from one end: 1", 6", 11", 16", 21".
2. At each mark location, cut through the top layer of plastic parallel to and between the flutes nearest each mark. After making these cuts, the top layer of the plastic (which will become the front of the device) will now be cut into four 3"x5" rectangles, bounded by a 1"x3" rectangle on each end (Figure B-2). These cuts permit the device to readily flex around the bole of a tree.
3. Mark the center point of each of the 3"x5" rectangles. Drill through the plastic sheet at these locations with a 3/16" bit.
4. Insert a tee nut into each of the holes. Insert the nut from the back side of the snare device, with the prongs facing forward. The fit should be snug. It is usually helpful to tap each tee nut in with a hammer. Use a piece of scrap lumber on the front side of the device to absorb the blows and protect the front end and threads of the tee nut (Figure B-3).
5. Place a piece of duct tape over the back of each tee nut, and then place a long piece of duct tape across all of the tee nuts (Figures B-4 and B-5).

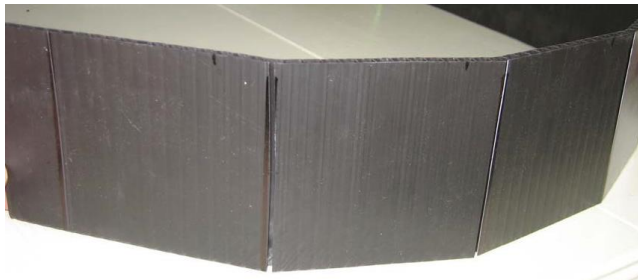


Figure B-2



Figure B-3



Figure B-4



Figure B-5

General assembly/use guidelines

1. The gun brush collar can be attached to trees of various sizes. For wolverine this should be ~8-25" DBH.
2. Attach snare device 12-18" below bait, centered under the bait (Figure B-6). Aim for ~12" (no closer) but adjust as needed to avoid branches and stobs. Attach by doubling back the one inch flaps at each end and driving a fence staple or nail through the two layers of Coroplast and into the tree. A third staple can also be driven through the center of the snare device for added stability. Alternatively, smooth wire can be used instead of staples to secure the plastic collar to the tree.
3. Thread a gun brush into each of the tee nuts (Figure B-7).

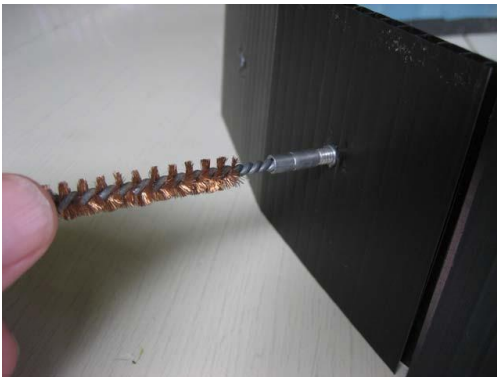


Figure B-7



Figure B-6. Wolverine bait and gun brush collar deployed. Camera positioned to view base of tree and bait. Note: gun brush collar will be painted brown for this study.

APPENDIX C

Lynx Gun Brush Strip

Methods for adding lynx detection capacity to wolverine detection stations during the 4-state wolverine project

Jeff Lewis, WDFW; Scott Fitkin, WDFW; John Rohrer, USFS

23 May 2016

Where they occur sympatrically with wolverines, lynx are commonly but incidentally detected at stations deployed to detect wolverines. In the interest of improving our ability to detect lynx during our wolverine survey efforts, we have added lynx detection capacity to our existing wolverine station protocol. Added capacity does not involve altering the location of stations to increase the likelihood of detecting lynx; capacity will only involve adding additional hair snares and lure to the existing station design.

The National Lynx Survey Protocol (McKelvey et al. 1999; see also McDaniel et al. 2000) outlined the use of small patches of carpet equipped with roofing nails and doused with a special lynx lure to collect lynx hair sample for DNA analyses. Pads were cut to size, doused with lure, sprinkled with catnip, and nailed to trees about 18 inches above the ground. These pads have been effective at collecting lynx hair (McKelvey et al. 1999, McDaniel et al. 2000, Southwestern Crown Monitoring Team 2014), however, we are testing several design simplifications/ improvements in Washington that could be implemented during the 4-state project.

As part of a test of 3 types of multi-species detection stations in the North Cascades, biologists from WDFW, USFS and Woodland Park Zoo are testing lynx hair snares that are similar to the existing gun brush belt already deployed as part of the wolverine protocol. The modification to the design for lynx is the addition of a second gun brush array and a sponge soaked with lynx lure that will be placed behind the lynx gun brush belt (Figure D-1). We will use 3 gun brushes spaced ~4" apart on the plastic, and the sponge should be positioned behind the belt and the 3 gun brushes. The 3 gun brushes are bent in the direction of the sponge to make them more likely to snag hair when a lynx rubs on the sponge. So that the lure does not run out of the sponge and down the tree where there are no gun brushes, the upper half of the sponge will be treated with lure and tacked to the tree, whereas the lower end of the sponge (untreated) will lie under/behind the gun brush belt to further secure it. A small plastic bag can be placed on the bottom half of the sponge to prevent any lure from dripping down the tree.

We will use the same lynx lure as that used in the national lynx survey (McKelvey et al. 1999), which consists of a 1:1:6 ratio of propylene glycol, glycerine, and beaver castorium, with 6 drops per ounce of catnip oil added to this mixture.

During monthly visits, lynx gun brushes will be checked, saved, replaced or sterilized similarly to the other gun brushes. Each plastic tube or coin envelope containing a gun brush with hair will be labeled to indicate that it came from the lynx belt. Also, the lynx lure sponge will be replaced each month (sponge can be prepared in advance). To ensure the lynx gun brush belt is at a proper height in relation to a walking/rubbing lynx, the belt should be moved up the tree bole to reposition it at ~18" above the snow level. At inaccessible sites, the gun brush belt for lynx will be placed higher up the bole (but at least 2 feet below the wolverine belt) to allow it to remain uncovered by accumulated snow for a greater period of time.

Literature Cited

- McDaniel, G. W., K. S. McKelvey, J. R. Squires, and L. F. Ruggiero. 2000. Efficacy of lures and hair snares to detect lynx. *Wildlife Society Bulletin* 28(1): 119-123.
- McKelvey, K. S., J. J. Claar, G. W. McDaniel, and G. Hanvey. 1999. National lynx detection protocol. US Forest Service, Rocky Mountain Research Station, Missoula, MT. 12 pp.
- Southwestern Crown Monitoring Team. 2014. Forest carnivore monitoring in the Southwestern Crown of the Continent: progress report 2012-2014. US Forest Service, Missoula, MT. 52 pp.

APPENDIX D

Camera Station Schematics

Accessible Station

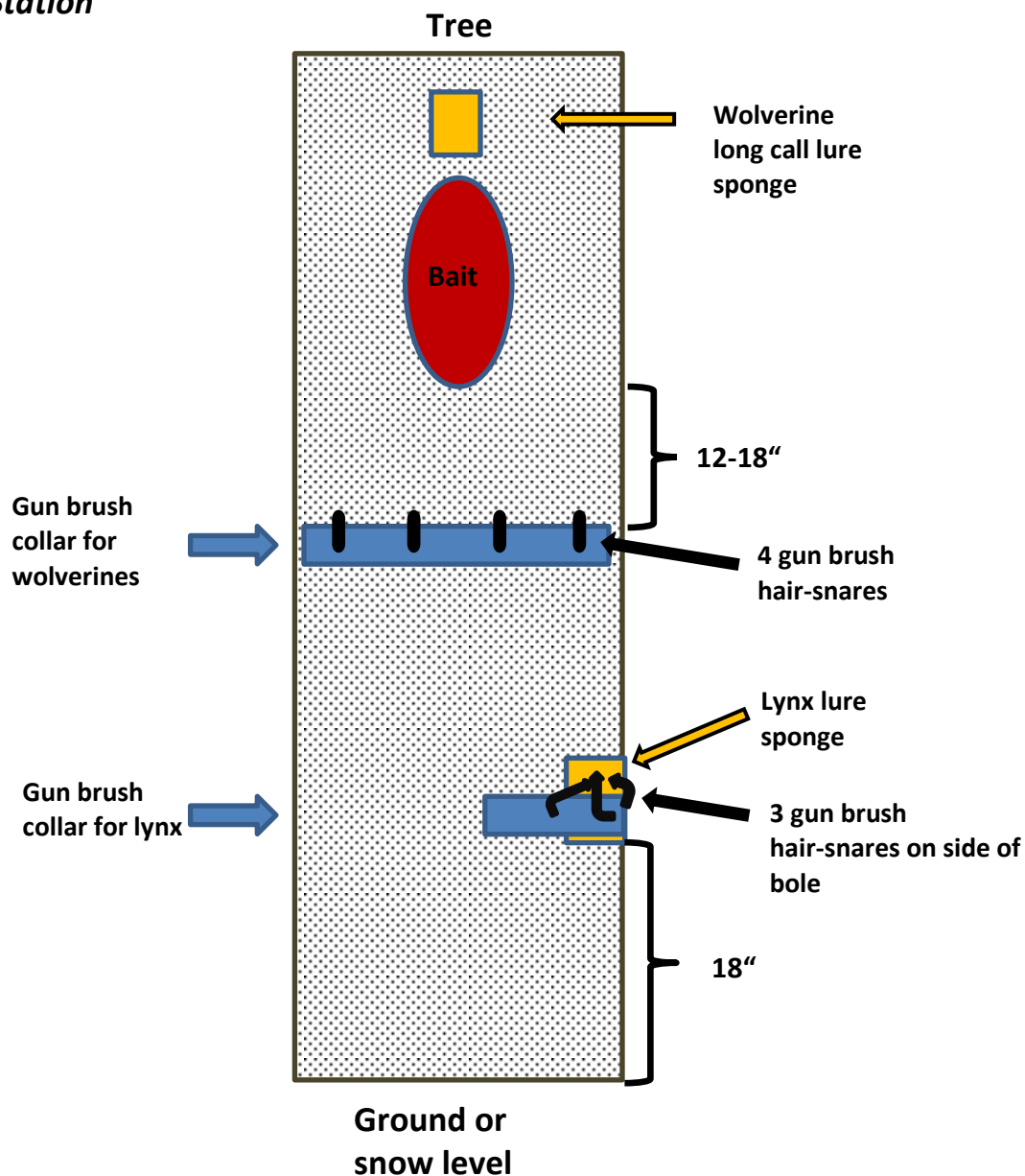


Figure D-1. Accessible station deployment includes wolverine scent lure sponge, wolverine bait, wolverine gun brush collar, lynx gun brush collar, and lynx lure sponge. The lynx lure sponge and associated gun brushes are positioned on the side of the bole (90° from wolverine brushes) to minimize hair deposition in the lynx brushes by other species climbing toward the bait. During monthly visits components are moved up the tree to stay above accumulating snow.

Inaccessible Station

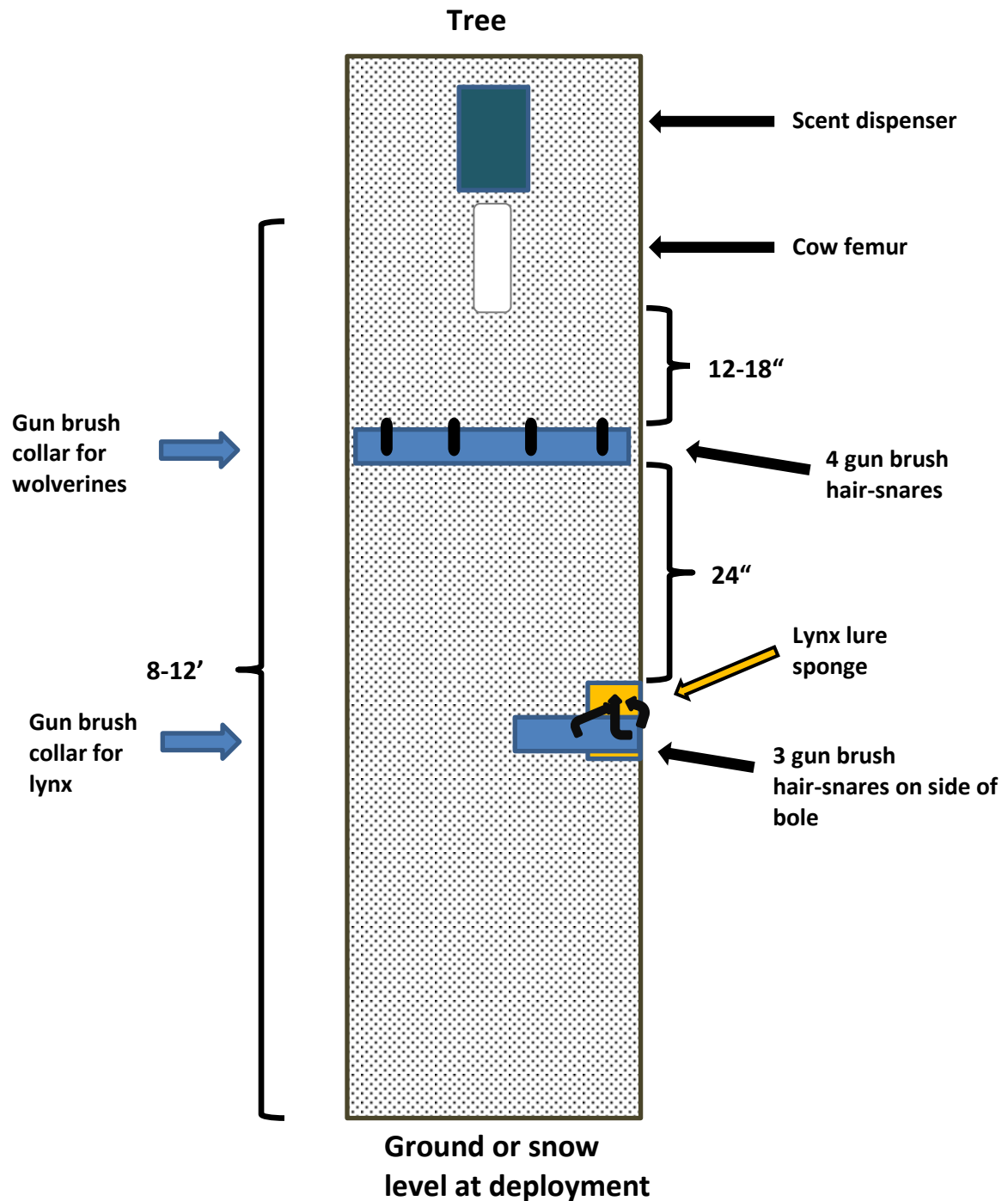


Figure D-2. Inaccessible station deployment includes a scent dispenser and cow femur instead of meat bait and wolverine lure sponge. All elements are moved higher up the tree initially to account for snow accumulation over the winter. Camera orientation is sideways.

APPENDIX E

Handling DNA Samples

This project uses the USFS Rocky Mountain Research Station National Genomics Center for Wildlife and Fish Conservation's *Protocols for Collecting and Storing DNA Samples* (rev December 2006), with modifications approved by authors Michael Schwartz and Kristy Pilgrim.

Field Collection

In most cases the entire gun brush will be collected and replaced with a fresh brush.

1. Examine all 7 brushes for hair. For any brush that contains any amount of hair, collect and label as follows:
2. Unscrew the brush and place in a separate 2 ½ x 4 ¼ coin envelope (no desiccant) *or* a 30- ml polyethylene plastic vial filled ~ ¼ full with silica desiccant beads.
3. Attach a pre-printed label to the envelope or vial and fill out the label with:
 - cell ID
 - date
 - observer
 - type of DNA (e.g., hair)
 - gun brush collar (**wolverine or lynx**)
 - position of brush in the collar (far left, far right, etc.)
 - a sequential sample number
4. Repeat steps 2 and 3 above for each gun brush with hair.
5. If using coin envelopes, place sealed envelopes into a zip lock bag holding silica desiccant beads.
6. Protect envelopes and vials from sun, moisture, and other damage during transport from the field.

Sorting and Preparing DNA Samples

In an office setting, thoroughly examine all photos from the camera corresponding to each set of 7 gun brushes from a station for a given sampling period. Prioritize viewing photos from stations that had hair in the brushes. Use the photographic record to decide which brushes will be submitted to the Genomics Lab as a "sample". Although we will ship the brushes themselves to the Genomics Lab and the Lab will remove hair from the brushes, we define what constitutes a sample before we ship brushes to the Lab based on situations described below. Keep in mind that (1) the Lab's initial test will be for species, and (2) a group of 10-15 hairs with follicles is a good genetic sample for testing.

1. If **no animals** appear in the photos, and there is clear photographic evidence that the camera **did not fail** (e.g., there is a programmed photo taken every day during the sampling period), and there are **no hairs** in any of the 7 gun brushes, then there is no sample. Burn the gun brushes to sterilize and reuse. Note in the database that those sample numbers assigned in the field were not submitted to the Lab to document why there will be sample numbers out of sequence in the sample database.
2. If there is clear photographic evidence of **1 or more species** that **is not** a carnivore (e.g., squirrel, vole, mouse), and there are **no gaps in the photo sequence** that would suggest possible camera failure, then there is no sample even if there is hair. Non-carnivore species are not analyzed. Burn the gun brushes to sterilize and reuse. Note in the database that those sample numbers assigned in the field were not submitted to the Lab to document why there will be sample numbers out of sequence in the sample database.
3. If there is clear photographic evidence of **1 or more species** that **is not** a wolverine, a lynx, or a fisher **but is** another carnivore (e.g., marten, fox), and there are **no gaps in the photo sequence** that would suggest possible camera failure, visually examine the 7 brushes and select 1 representative brush with sufficient hair to submit as the sample. If none of the 7 brushes appear to contain sufficient (10-15) hairs, combine more than 1 brush together into an envelope (or vial) to make 1 sample. Note in the database which sample numbers assigned in the field were not submitted to the Lab to document why there will be sample numbers out of sequence in the sample database. Discard hairs from unsubmitted brushes, and burn the gun brushes to sterilize and reuse.

These non-target carnivore samples will be treated differently at the Lab. Specifically, the DNA will be extracted and stored but not analyzed at this time. Highlight these samples on the list sent to the lab (see below) to clearly identify these samples.

4. If there is photographic evidence of:
 - a wolverine, a lynx, **or** a fisher
 - multiple species including a wolverine, lynx, or fisher*or if:*
 - it is unclear from the photos if a wolverine or lynx could have been present due to low-quality images or gaps in the photo sequence that indicate camera malfunction,

then each brush is an individual sample if it contains hair. Make sure the label is complete per the above instructions for labels.

Storage/Shipping

DNA samples will be shipped periodically (see below) to the RMRS genetics lab for analysis. Prior to shipment, store samples at room temperature out of direct sunlight. Occasionally check the color of the desiccant, if relevant, and add fresh desiccant as needed. Do not replace desiccant in vials as you might inadvertently dispose of hair.

DNA samples should be shipped every 2 or 3 weeks, depending on the project's collection schedule. Ship to:

Kristy Pilgrim
National Genomics Center for Wildlife and Fish Conservation
Rocky Mountain Research Station
800 E. Beckwith Ave.
Missoula, MT 59801

1. Ship samples on a Monday, Tuesday, or Wednesday. Avoid Thursday and Friday so they don't sit at a post-office in unknown conditions.
2. Send via overnight mail.
3. Send an e-mail to Kristy Pilgrim and cc Mike Schwartz letting them know the samples are coming.

kpilgrim@fs.fed.us
mkschwartz@fs.fed.us
4. Attach to the email a list of the samples being shipped.
5. Include in the package a hard copy of the list of samples so they can cross check the samples.
6. Include a hard copy of the chain of custody form in the shipment. The form is for each batch (not each sample). Fill out the top gray boxes and the first "From" line (name, release signature, release date). The form can be obtained from the RMRS website at:
http://www.fs.fed.us/rm/wildlife-terrestrial/docs/genetics/reports-protocols/CHAIN_OF_CUSTODY_RECORD.pdf
7. Expect an email confirmation from the lab when samples arrive. The lab will begin extractions immediately to preserve DNA.

APPENDIX F

Bait and Scent Lures

The presence or absence of bait and lure at camera stations influences detection of wolverines. Because station type (accessible, inaccessible) is one variable to be accounted for in analyses, it's important to reduce other potential sources of variation. Bait size relates to persistence of bait over 1-month intervals and therefore should be standardized. Scent lures and amounts also should remain consistent across stations.

Bait

Aim for a 15-20 lb chunk of wild game meat, typically from a road-killed deer or elk. Beaver carcasses also work well. Use hind quarters of deer (front quarters are not big enough), front or hind quarters of elk, or an entire beaver. Skin the meat to avoid bait hair falling onto gun brushes and swamping DNA samples.

Drill holes through the frozen meat for wiring. Make 2 holes on either side of the spinal column or leg bones about 3-4" apart (Figure F-1). Wrap wire through these 4 holes and around the spinal column several times. Use a sturdy screw driver to twist-tighten the wire around the carcass (the wiring will loosen as meat thaws). Leave wire tails 3-4 feet long on both ends (Figure F-2).



Figure F-2. Bait pre-drilled and wired.



Figure F-1. Drill 4 holes through frozen bait.

Place a large nail on the backside of the tree at the same height as the bait, and a nail above, below, and on either side of the bait ~4 inches away. Take the tails of wire extending from the bait and wrap them around the tree and the nails. Wrap 2-3 more long pieces of wire from different angles around the whole carcass, using the nails as anchor points. Twist-tighten the wire with a screwdriver so it is securely attached to the tree.



Figure F-3. Bait secured to bole of tree with smooth wire.

Cow bones and scent dispenser

Meat bait likely will not persist for 5-6 months at inaccessible stations. As a substitute for fresh bait, we will use 1 domestic cow femur bone and a scent dispenser at inaccessible stations in place of the bait described above for accessible stations. The scent dispenser will drip out a small amount of wolverine long call scent lure onto the femur each day for 6+ months. Secure a scent dispenser 8-12 ft above ground level. Drill a 3/4" hole through the cow femur and thread 5/8" cable through the hole, leaving a 3-4 ft tail. Secure the bone to the tree immediately below scent dispenser with cable locks. Use anchor nails to keep the cable from slipping. Position the scent dispenser to drip onto the bone. Make sure the scent dispenser and bone are within view of the camera.

Long call scent lure for wolverine

This project uses a recipe originally developed by Eric Lofroth for wolverine surveys in British Columbia and modified by Robert Long and Joel Sauder to perform in the scent dispenser they developed. For consistency, we will use this same long call scent lure without the dispenser during year 1 of the project in WY.

For a 2-liter (67-oz) batch of lure:

Propylene glycol	38.0 oz
Water	25.0 oz
Hawbaker's Marten lure	1.0 oz
Beaver castor oil (liquid)	2.0 oz
Skunk Quill	0.3 oz
Anise oil	0.5 oz

Saturate a ~3"x3"x3" piece of sponge thoroughly with the long call lure and wire the sponge to a high branch adjacent to the bait tree. Try to get this in view of the camera.

Lynx lure

This project uses the same recipe that was used in the national lynx survey (McKelvey et al. 1999). The formula is 1 part propylene glycol, 1 part glycerine, and 6 parts beaver castorium. To this mixture add 6 drops of catnip oil **per ounce** of mixture.

For an ~2-liter (67-oz) batch of lure:

Propylene glycol	8.0 oz
Glycerine	8.0 oz
Beaver castor	48.0 oz
Catnip oil	0.66 oz ³

Saturate a regular kitchen sponge (0.9"x6"x3") thoroughly with lynx lure. Wash the sponge prior to use to remove any soap smell. Put the bottom of the sponge in a snack-size Ziploc bag (3.25"x

³ Based on measuring drops/ounce in the Lab.

6.5”) to prevent the lure from dripping down the tree and distracting a lynx away from the hair snares. Wedge the sponge behind the lynx gun brush collar behind the 3 gun brushes.

Suggested Sources for Lure Ingredients

Item	Part #/Details	Unit	Source
Skunk Essence (100% Pure Quill)		1 oz bottle	Northland Animal Lures
Propylene glycol; food grade	SKU: 82020	40 lbs or 1 gal	The Chemistry Store
Beaver castor (liquid)	NWS011L	16 oz bottle	Wildlife Control Supplies
Hawbaker's Marten Lure	HBMARTEN	1 oz bottle ^a	Minnesota Trapline Products
Anise oil			most trapping supply sources
Glycerine		32 oz bottle	The Chemistry Store
Catnip oil		0.6 oz bottle	Amazon

^a Note that it is very difficult to get the paste out of these tiny bottles, so an alternate source may be preferred. Avoid marten lures that look like chapstick; these don't dissolve well in propylene glycol.

APPENDIX G

Equipment and Materials Checklist

- Reconyx PC800 semi-covert camera
- desiccant pak for camera
- camera cable lock & key
- camera housing
- AA lithium batteries (12 per camera)
- 32 gb class 10 SD card (1 plus 1 replacement per camera)
- wolverine plastic gun brush collar
- lynx plastic gun brush collar
- gun brushes (7 plus 7 replacement per station)
- wolverine long call scent lure
- lynx lure
- 3"x3"x3" sponge for wolverine lure
- 0.9"x 6"x 3" kitchen sponge for lynx lure
- 2 ½" x 4 ¼" coin envelopes or 30-ml vials
- labels
- desiccant beads
- ziplock bags
- bait
- smooth wire
- lighter
- map w/ habitat and grid cells
- GPS (set to dd.dddd, WGS84)
- list of grid cell coordinates
- 6-meter cord knotted at 1-m intervals
- large nails (5-6 per station)
- hammer or ax
- screw driver
- folding saw
- rite-in-rain paper
- Sharpie wide-tip magic marker
- rubber gloves
- SD card viewer, laptop, or tablet
- SPOT or other emergency safety device

Additional items for "inaccessible sites"

- screw-in climbing steps (3)
- climbing harness
- climbing safety straps
- cow leg bone (1)
- scent dispenser
- AA lithium batteries (8 per dispenser)
- 5/8" cable
- cable locks
- small wrench for cable locks
- hardware to modify camera orientation

ATTENTION

**Wildlife Monitoring Station
Beyond This Sign**

Carnivore attractant in use

Please use caution in the area

Camera/DNA Station Data Form

Station Information	Type (circle one):	Accessible	Inaccessible
---------------------	--------------------	------------	--------------

Cell/Site Number _____ Cell/Site Name _____ State _____

Drainage _____ Mtn. Range _____

Elevation _____ Lat ____ . ____ Long ____ . ____ (decimal degrees, WGS84)

Site Description (why site chosen) _____

Access and Specific Location Notes _____

Visit Type (circle one):	Initial Deployment	Revisit 1	Revisit 2	Revisit 3	Pull Camera
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Date _____ Observers _____

Initial Deployment

Camera Model _____ Camera ID# _____ CodeLoc ____ _

Batteries: Brand _____ Size _____ Type (Lithium NIMH) Expire Date _____

Correct Date & Time set? Y N Image Label set to cell ID? Y N Desiccant Pak? Y N

Bear Box deployed? Y N

Wolverine Gun Brush Collar? Y N

Lynx Gun Brush Collar? Y N

Number Gun Brushes Deployed _____

Bait Type _____ Bait Size _____ Distance Camera to Bait (m) _____

Scent Lure Type _____ Lure Placement _____

Warning Signs _____

Walk-test: Gun brushes visible in camera? Y N Bait visible? Y N

Revisit *(do not adjust camera date/time for daylight savings)*

of Photos _____ % Memory Used _____ Memory Card Exchanged? Y N

New Card: Model _____ Size _____ GB Read/Write Speed _____ MB/s Class _____ ID# _____

Battery Status _____ % Batteries Replaced? Y N

Batteries: Brand _____ Size _____ Type (Lithium NIMH) Expire Date _____

Desiccant Pak Changed? Y N

Camera Issues? Describe _____

Number of Gun Brushes Collected _____ DNA Sample ID #s: _____

Bait Status (circle one): Intact Partially Gone Gone

New Bait Type _____ Bait Size _____

Scent Lure Type _____ Lure Placement _____

All Visits

Evidence of Disturbance _____

Tracks Observed (fisher, lynx, wolverine). Document with good quality photos showing key measurements. Mark tracks with GPS and record coordinates below in decimal degrees, WGS84.

Species _____ Lat _ _ . _ _ _ _ _ Long _ _ _ . _ _ _ _ _ Dist from Station (m) _____
Photos Taken? Y N Photo Numbers _____

Species _____ Lat _ _ . _ _ _ _ _ Long _ _ _ . _ _ _ _ _ Dist from Station (m) _____
Photos Taken? Y N Photo Numbers _____

Species _____ Lat _ _ . _ _ _ _ _ Long _ _ _ . _ _ _ _ _ Dist from Station (m) _____
Photos Taken? Y N Photo Numbers _____

Additional Notes