

An assessment of the efficacy of rub stations for detection and abundance surveys of Canada lynx (*Lynx canadensis*).

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Abstract

Barbed and scented rub pads that rely on a cheek-rubbing behavioural response are a standard survey design that has been used extensively across the range of Canada lynx (*Lynx canadensis* Kerr, 1792). However, there have not been any published studies evaluating the effectiveness of rub stations for detecting lynx by comparing other simultaneous survey methods. We used a combination of paired rub stations and remote cameras at 41 sites to compare detection probabilities between the two methods and conduct a mark-recapture population estimate of Canada lynx using rub stations to further interpret our findings. The detection probability calculated using cameras approached 1.0 for most of the winter season ($\bar{x} = 0.88$), while it remained less than 0.52 for hair rub stations ($\bar{x} = 0.27$). The low and variable detection probability using hair snags, high detection probability using cameras, and the potential gender or individual bias in rubbing behaviour based on our mark-recapture analysis suggest rub stations are not the most efficient survey method available for Canada lynx. Until additional research incorporating spatial scale, seasonal timing, gender bias, and survey design is conducted, we urge caution in the use of hair stations that rely on the cheek-rubbing behaviour of Canada lynx.

Key words: Canada lynx, detection probability, *Lynx canadensis*, survey method, rub stations, hair snags, mark-recapture, British Columbia

Introduction

Surveys employing hair collection methods have been used successfully to estimate the distribution, occupancy, abundance, and/or habitat associations of a variety of carnivore species (e.g., Kendall and McKelvey 2008). The collection of hair provides DNA to identify species or individuals as well as the ability to investigate other ecological questions related to diet (stable isotopes; Mowat and Heard 2006), contaminants (Ben-David et al. 2001), or hormones (i.e., stress, reproductive; Bechshoft et al. 2015). Most surveys that collect hair are designed and adapted to the morphology and behaviour of the target species. For example, researchers have used barbed wire snags for Grizzly bears (*Ursus arctos horribilis* Ord, 1815; Mowat and Strobeck 2000), breakaway cable snares for river otters (*Lontra canadensis* (Schreber, 1777); Johnson et al. 2013), and glue pads for marten (*Martes Americana* (Turton, 1806); Mowat and Paetkau 2002).

Barbed and scented rub pads that rely on a cheek-rubbing behavioural response have been used for several wild felid species (McDaniel et al 2000; Weaver et al. 2005; Schmidt and Kowalczyk 2006). Although barbed rub pads have become the standard design and have been used extensively across the range of Canada lynx (*Lynx canadensis* Kerr, 1792), we know of only one peer-reviewed publication that reports on the success of this method. McDaniel et al. (2000) found that rub pads were somewhat effective at detecting lynx in the boreal forests of Yukon, Canada. This study, however, was primarily designed to test the effectiveness of different lures and reports survey success based on the proportion of sites with a Canada lynx detection. In addition, the same rub pads have not been as effective in other areas with known

Canada lynx populations, such as Minnesota or Maine (Crowley et al. 2005¹; Burdett et al. 2006; Moen and Lindquist 2006).

Although there have been surveys for bobcats (*Lynx rufus* (Schreber, 1777)) using multiple detection devices (Harrison 2006; Long et al. 2007; Comer et al. 2011), we are not aware of any published studies evaluating the effectiveness of rub stations for detecting lynx by comparing other simultaneous survey methods. In these comparative method studies on bobcats, there was limited detection at rub stations while cameras were found to be a relatively successful survey method. Surveys using remote cameras only (Nielsen and McCollough 2009) or using cameras paired with snow track surveys (Crowley et al. 2013) were also found to have high detection rates for Canada lynx. In addition, most Canada lynx surveys have been conducted to detect species occupancy and not designed and conducted to estimate abundance over a given area. Information on individual visits to sites would be beneficial for interpreting any biases associated with the use of rub stations as a survey method.

Accurate and unbiased measures of occupancy and/or abundance are required for monitoring the effects of human activities on wildlife populations. Surveys are the basis for informed management decisions, conservation actions, and land use practices associated with wildlife values and priorities. For Canada lynx, potential biases influencing the efficacy and reliability of survey methods that take advantage of felid rubbing behavior to collect hair/DNA are not well known. This is of concern for the conservation of Canada lynx across its range, and especially relevant in areas going through rapid and cumulative landscape change such as central British Columbia (i.e. large-scale mountain pine beetle (*Dendroctonus ponderosae* Hopkins,

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1902) epidemic and salvage harvest; Safranyik and Wilson 2006) or in areas with threatened and endangered populations at the southern periphery of their range (USFWS 2000; Nova Scotia Lynx Recovery Team 2006).

Our objectives for this study were twofold: 1) to determine the efficacy of remote rub stations for detecting Canada lynx in comparison to remote cameras; and 2) to investigate the potential implications of our findings on estimates of occupancy and population size. We use a combination of paired rub posts and remote cameras at 41 sites to compare detection probabilities between the two methods during the winter. We also use the DNA from hair collected to further interpret our findings, identify individuals, and conduct a mark-recapture population estimate of Canada lynx in our study area.

Material and Methods

Study Area

The research was conducted in the John Prince Research Forest in north-central British Columbia, Canada (Figure 1). A 16 500-ha portion of forested provincial land 45 km northwest of the town of Fort St. James, the John Prince Research Forest is co-managed by the University of Northern British Columbia and the Tl'azt'en Nation. The John Prince Research Forest is characterized by rolling terrain with low mountains (700 m to 1500 m above sea level). It represents the northern extent of contiguous Rocky Mountain Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Mayr) Franco) forests in the interior of British Columbia and is dominated by the Sub-Boreal Spruce biogeoclimatic zone (DeLong et al. 1993). The area has experienced a wide variety of logging activities over the past 50 years and contains a mosaic of old and young

coniferous forests (continuum from new harvest to old growth >250 years old) with interspersed deciduous stands. The stands have a relatively rich understory of deciduous shrubs and regenerating conifers. The research forest is traversed by many small streams that flow into either Tezzeron Lake or Pinchi Lake. Although there are many other carnivore species, the only other felid species that has been observed rarely in the study area is the Cougar (*Puma concolor* (L., 1771)). In 7 years of remote camera monitoring in the study area, a cougar has been observed on only 3 occasions.

Trail Camera and Hair Snag Surveys

In winter 2014, hair rub stations and digital passive infrared trail cameras (Bushnell Trophy Cam model 119467 and Bushnell Trophy Cam HD Max Model 119477, Bushnell Outdoor Products, Overland Park, Missouri) were paired and set for eight 1-week sessions from mid-February to early April during a time period that coincided with an estimated peak in Canada lynx breeding (Anderson and Lovallo 2003), and an increase in breeding activity behaviours such as scent-marking and cheek-rubbing (Crowley et al. 2013). A total of 41 sites were spaced 2.5 km apart and set near the center of hexagonal grid units (5.26 km²; Figure 1). At each site, a rub station was established 2 to 3 m directly in front of a remote camera (located between 0.5 and 1 m above the snow on a tree). Each rub station consisted of a small diameter log (<15 cm) that was secured in the snow with one end above the ground (45 cm) and pointing directly at the camera. During each weekly check of the site, snow was packed down around the log to maintain a consistent height above ground throughout the survey. The log acted as a protruding solid object that Canada lynx could sniff, scent-mark, and rub their faces against. Lure and a wire gun-cleaning brush (30-caliber) were placed at the end of the log to attract and

collect hair from lynx. The gun brush was attached in a vertical orientation flush against the butt end of the log with approximately one quarter of the brush length protruding above the top edge. Lure was placed on either side of the gun brush. We used a local commercial lure containing beaver castor and catnip oil as the two primary ingredients. A small piece of hanging American Beaver (*Castor canadensis* Kuhl 1820) meat (~5 cm diameter) was hung by wire directly above the end of the log (~60 cm) to serve as an additional attractant. Cameras were checked, lure and bait added, hair collected, and brushes replaced once per week. Cameras were set to take 30 seconds of video with a 1-second delay between video-recordings. This schedule allowed for nearly continuous recording of the time an animal was in view. The sensor level was set to normal, LED control for night vision was set to medium, and video sound recording was turned on.

While reviewing video-recordings, we noted the date, time, and location of Canada lynx detections. A detection using cameras was considered independent if it was >1 hour since the previous visit by a Canada lynx to the site. Based on the visitation pattern at sites (70% of visits < 5 minutes in duration), 1 hour between detections was considered a sufficient time frame to delineate an independent visit. However, to be even more conservative and as a relative comparison, we also calculated the number of detections using >24 hours to delineate an independent visit. Visits were recorded as a single detection regardless of the number of Canada lynx present in a group during that visit. We recorded every time a Canada lynx rubbed its cheeks on or near the lure/bait. Lastly, we recorded the number of lynx detections by week and site for both camera and rub station methods.

We used a single-season multimethod occupancy model in PRESENCE (Version 7.1 USGS-PWRC. <http://www.mbr-pwrc.usgs.gov/software/presence.html> ; Hines 2006) to compare

estimates of detection probabilities (P ; probability that a lynx was detected if present) between remote camera and hair snag surveys (Mackenzie 2006; Nichols et al. 2008). The multimethod approach uses the combined histories from both survey methods to estimate detection probabilities of each individual method. Detections for this analysis were based on eight 1-week survey sessions. Our primary objective was to assess the efficacy of hair stations for detecting lynx by comparing detection probabilities between the two survey methods (cameras and hair snags). For this reason, we do not report on occupancy in this paper with the intention of investigating occupancy probabilities and habitat relationships in a more detailed effort following additional years of data collection.

Genetic analysis

DNA extraction and microsatellite analysis were conducted by Wildlife Genetics International (Nelson, BC, Canada). DNA was extracted using QIAGEN's DNeasy Tissue Kits, aiming to use 10 roots clipped from guard hairs or up to approximately 30 whole underfur. Given the low sample size (34 hair samples), we did not use a separate phase of marker selection. Instead, we simply analyzed each sample with a larger-than-necessary set of markers, comprising 10 felid microsatellites and a ZFX/ZFY/SRY gender marker. Genotyping went through 3-phases of first-pass, cleanup and error-check. During genotyping, samples were removed from the analysis if they had low confidence genotype scores for >5 of the eleven markers. Marginal samples with inconclusive results were reanalyzed using a greater volume of DNA per reaction. This reanalysis resulted in most marginal samples receiving 11-locus, high confidence genotype scores. Finally, an error-checking procedure was used to re-analyze mismatching markers in similar genotypes (Paetkau 2003; Kendall et al. 2009). Here, data entry and amplification errors were identified and corrected. Following this process, there was a single case where a pair of

individuals mismatched at 1 of 11 markers suggesting that the probability of identifying a unique, but incorrect genotype was low. In this case, it was found that the error marker could be amplified more evenly at lower temperature to reduce future risk of error. Once genotyping was complete, an individual was assigned to each unique multilocus genotype.

For samples that could not be identified to individual using felid microsatellites (low confidence genotype scores for >5 of the eleven markers) we also conducted a species test to confirm samples were from Canada lynx. The species test was a partial sequence analysis of a hypervariable region of the mitochondrial *16S rRNA* gene with sequence profiles from our samples compared to profiles from reference Canada lynx samples. Species testing started with a first pass using ‘universal’ primers that amplify all mammals in proportion to their concentration in the DNA extract. If samples produced mixed results, they were re-analyzed using primers designed to bias toward Carnivora amplification.

For DNA mark-recapture, we used the CAPWIRE method (Miller et al. 2005) to generate a population estimate for Canada lynx in the research forest. This method is based on an urn model (i.e. mixing of individuals in a population) and was designed for the non-systematic collection of DNA. CAPWIRE was developed for estimating population size when data may contain multiple observations of an individual within a survey session and does a particularly good job when dealing with smaller populations (< 100) and capture heterogeneity (Miller et al. 2005). We tested for the simple even capture model (each individual is equally likely to be captured) and the two innate rates model (capture heterogeneity occurs between two segments of the population).

Results

For the detection probability analysis, there were a total of 22 and 68 lynx detections by site and week at rub stations and remote cameras, respectively. The detection probability calculated using cameras approached 1.0 for most of the winter season ($\bar{x} = 0.88$), while it remained less than 0.52 for hair rub stations ($\bar{x} = 0.27$; Figure 2). Of the total 41 sites, lynx were detected at 22 by remote camera (54%) and 9 by hair snare (22%). Lynx detection rates by cameras (sites by week) was highest in mid to late March while detection by hair stations remained highest from late February to late March (Figure 3). This pattern of detections resulted in the highest proportion of camera visited sites with a hair detection to occur from late February until the third week of March.

For remote camera detections, there were a total of 129 and 106 lynx detections using >1 hour and >24 hours to delineate an independent visit, respectively. Only 28% of lynx detections (>1 hour) by camera recorded rubbing behaviour at the site. Lynx would sometimes rub on the ground or other nearby woody debris instead of directly at the rub station (8% of lynx detections). Individuals often spent time at the site, approaching, sniffing, and/or scent marking on the post but not cheek-rubbing. In contrast, lynx would sometimes vigorously rub on the post for several minutes at a time during a visit. Lynx ate hanging bait on only 19% of lynx detections (>1 hour) and often ignored the bait while focusing their attention on the log end with scent lure. Sixty nine percent of total observed rubbing behaviours and 96% of rubbing behaviours located directly on the end of the log resulted in lynx detection using DNA.

Analysis of abundance

Genotypes identified using hair samples collected in winter 2014 resulted in 18 captures of 4 male and 3 female Canada lynx. Over the entire winter survey season, three individuals were captured once (1M: 2F), two individuals were captured two times (1M: 1F), and two individuals were captured six times (2M). Individual lynx were identified at six of the 41 sites with an average of 2 lynx per site. Of the 34 samples collected in the field, 7 samples (21%) lacked sufficient material suitable for analysis (i.e. extremely small quantity of hairs and/or hair lacking root tips). After a first pass of the remaining 27 samples with 11 markers, 9 samples (27%) were culled that had high-confidence scores for ≤ 5 of 11 markers. Among the failed samples, 8 had their scores marked down because they failed to meet strength criteria, while 1 was marked down because it amplified complex banding patterns suggestive of mixture. For the 9 failed samples, the species test analysis produced 3 distinct profiles, identifying 7 Canada lynx, 1 red squirrel (*Tamiasciurus hudsonicus* (Erxleben, 1777)) and 1 American marten. One of the seven Canada lynx samples produced mixed results, but produced a lynx result when re-analyzed using primers designed to bias toward Carnivora amplification.

Having reduced the sample size from 27 to 18, data points that were weak or difficult to read the first time (i.e. those with low-confidence allele scores) were reanalyzed using a greater volume of DNA per reaction. At the end of this 'cleanup' process, each of the 18 remaining samples had high-confidence scores for all 11 markers. With genotypes completed and checked for errors, an individual was defined for each unique 11-locus genotype. Starting with 18 successful samples, we defined 7 individuals, with 14 samples assigned to 4 male lynx and 4 samples assigned to 3 females.

Using CAPWIRE we produced a population estimate with the full set of capture samples. For the population estimate, a likelihood ratio test suggested that the two innate rates model was the most appropriate. Given the higher proportion of male captures in our sample, it is possible that the choice of the multi-strata model was specific to this source of capture heterogeneity. We generated a population estimate of 11 Canada lynx (95% CI = 7 – 17) with a corresponding density estimate of 7 lynx/100 km² (Range = 4 – 10).

Discussion

We found considerable differences between the two survey methods in estimates of detection probabilities. At the spatial scale of our study, we found that rub stations were not a very effective survey method for detecting Canada lynx. Similar to the previous year (Crowley et al. 2013), lynx camera visitations peaked in mid to late March with rubbing behaviours remaining highest from late February to mid-March. Our surveys coincided with a peak in lynx activity, yet we still had a very low number of Canada lynx detections using hair relative to the total number of visitations recorded by remote cameras.

Hair rub stations have not been effective for bobcats in other areas (Harrison 2006; Long et al. 2007; Comer et al. 2011). Comer et al. (2011) suggest limited and variable rubbing behaviour by bobcats may be explained by individual variation in response to rub pads or catnip-based lures. This observation is partially explained by a study of domestic cats that found 30% of individuals did not exhibit any response to catnip, with some cats exhibiting an avoidance response (Todd 1962). Although we used a commercial lure, catnip oil and beaver castor were the two main ingredients, and these have been found to be the most effective scent in eliciting a

rub response in Canada lynx (McDaniel et al 2000). We do not know if similar patterns of behaviour exist for Canada lynx in response to catnip or other lures, but this may partially explain our limited success with rub stations.

Cable hair snares have been used with success for bobcats as an alternative to rub stations (Stricker et al. 2012). This survey method relies on a modified body snare that releases after tightening around the animal. Observation of a Canada lynx in our study area passing through one of these modified body snares and exhibiting a negative response (i.e. jumping and thrashing; S.Crowley, pers.obs) suggest further research is needed before this can be considered a viable option for Canada lynx. It is unclear whether these snare types may result in any subsequent avoidance affecting recapture of Canada lynx.

Our hair collection station set-up was slightly different from what has been used in past studies (e.g. gun brush vs pad with nails, horizontal pole vs vertical tree mount); however, the high proportion of rubbing behaviors that resulted in successful hair/DNA collection suggests that set-up had little to do with our low detection rates at rub stations. When lynx rubbed at sites they usually left behind hair. Our low detection success using rub stations was primarily attributed to limited and variable rubbing by lynx during visits.

Our lynx density estimate (7/km²) was between but closer to the lower end (low = 2.3–3.0/100km; high = 17.0–44.9/100km²) of the lynx population cycle in the boreal forests north of our study area (Poole 1994; Slough and Mowat 1996; O'Donoghue et al. 1997), and 2-3 times the density reported south of our study area in Washington state (2.6/100 km²; Koehler 1990). Although our density estimate is not unreasonable, we suspect that it is biased low based on a low detection rate using hair stations, high detection rate of remote cameras, observations of recognizable individuals and large family groups, and temporal/spatial mismatches of individual

lynx detections during our camera survey (i.e. multiple detections at multiple sites of different lynx recorded by camera but little to no detection by rub stations during the same time period).

Our samples also indicate a potential gender or individual bias in rubbing behaviour with two males representing two thirds of all hair samples identified to individual and 2 of 3 females detected only once. Studies that rely on data from marked animals have found that males are often captured more frequently than females (e.g. Gehrt and Fritzell 1996; Boulanger et al. 2004; Lofroth et al. 2008). Heterogeneity in capture probability is typically explained by more frequent and longer movements by males (i.e. males come into contact with traps more often). Rub stations may introduce an additional bias dependent on the behavioral response of individuals, in this case rubbing action, to the lure when visiting a site. Unfortunately, we were not able to identify individual lynx using cameras and compare mark-recapture population estimates between the two methods. Unlike many wild felid species, lynx pelage patterns are more uniform and far less discernible among individuals. This is especially true for lynx in winter when this study was conducted. The combination of indistinct pelage patterns and video quality did not make it feasible to reliably identify individuals and assess population estimates with cameras.

Although our surveys were conducted during a peak in lynx activity during the winter, it is unknown if similar patterns in detection between the two survey methods occurs outside this time of year. Until additional research incorporating spatial scale, seasonal timing, gender bias, and survey design is conducted, we urge caution in the use of hair stations that rely on the cheek-rubbing behaviour of Canada lynx to collect samples for detection surveys and abundance estimates. The low and variable detection probability combined with a potential gender or individual bias in our study suggest rub stations are not the most efficient survey method available for Canada lynx. Until other survey methods are developed that can collect hair more

consistently from Canada lynx, snow track and camera surveys (Squires et al. 2004; Nielsen et al. 2009; Squires et al. 2012; Crowley et al. 2013) are likely the best option for detection surveys, and DNA/scat collection using dogs (Harrison 2006) or by following snow tracks (McKelvey et al. 2006) are likely the best options for both detection and abundance surveys.

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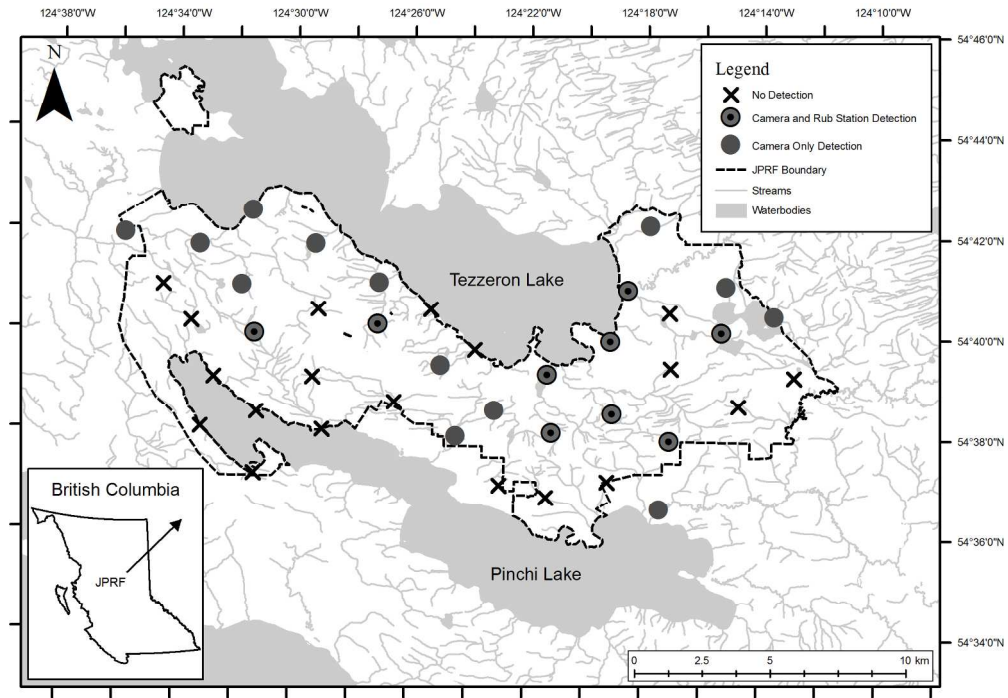
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List of Figures

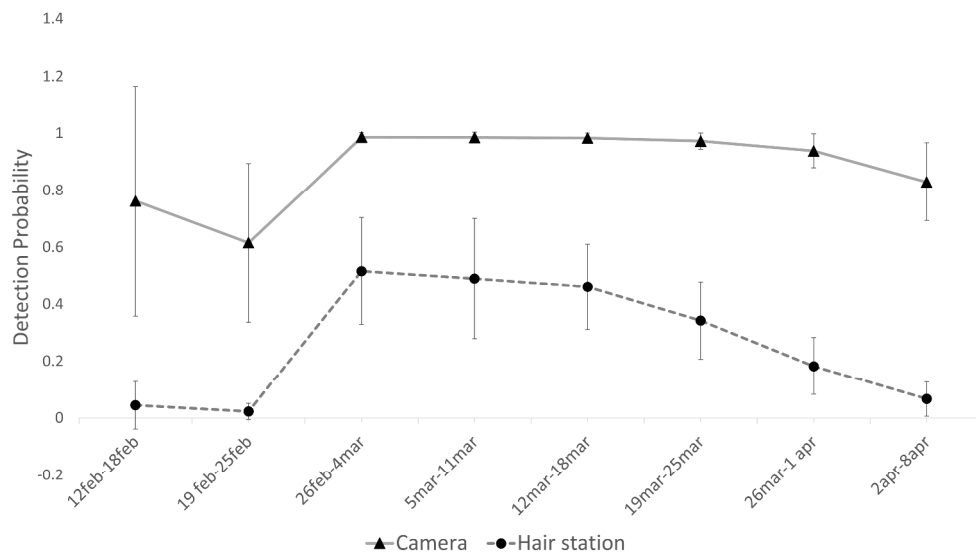
Fig. 1. John Prince Research Forest, central British Columbia, showing trail camera and hair rub station locations ($n = 41$) and detections by survey method of Canada lynx (*Lynx canadensis*), February to April 2014.

Fig. 2. Detection probabilities (\pm SE) of Canada lynx (*Lynx canadensis*) using remote cameras and hair rub stations in the John Prince Research Forest, central British Columbia, February to April 2014.

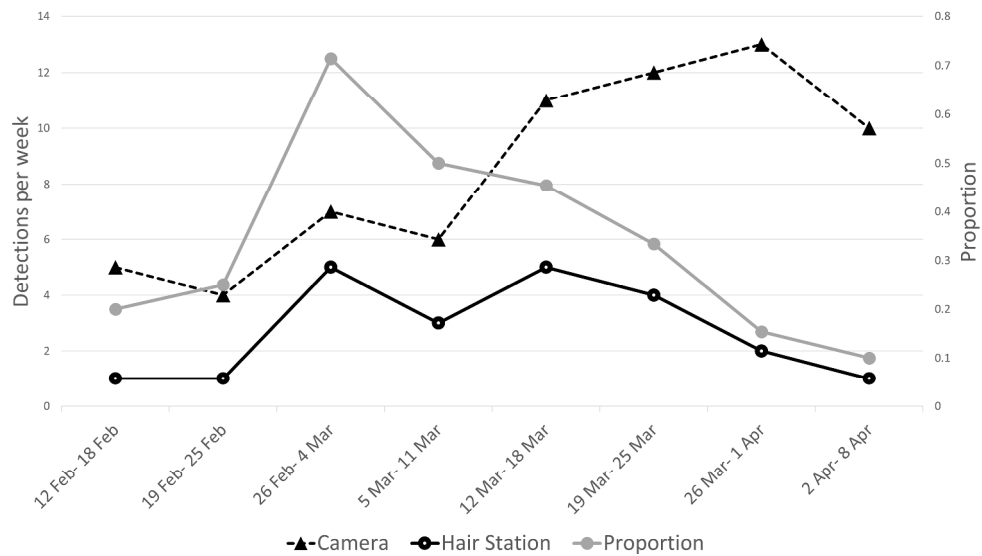
Fig. 3. Number of detections by site and week of Canada lynx (*Lynx canadensis*) using remote cameras ($n = 68$) and hair rub stations ($n = 22$) and proportion of camera visited sites with lynx detection by hair in the John Prince Research Forest in central British Columbia, February to April 2014.



199x139mm (300 x 300 DPI)



338x190mm (300 x 300 DPI)



338x190mm (300 x 300 DPI)