

ABSTRACT

SWIERS, ROBERT CLIFDEN. Non-Invasive Genetic Sampling and Mark-Recapture Analysis of a Fisher (*Martes pennanti*) Population in Northern California used as a Reintroduction Source. (Under the direction of Dr. Roger A Powell.)

We collected hair samples from forest mesocarnivores using a non-invasive occupancy protocol, obtained individual identification for sampled fishers (*Martes pennanti*), and analyzed the data with mark-recapture estimators to determine population size, survival, and growth rates for the population of fishers in a portion of the Eastern Klamath Mountains of Northern California for 2006-2011. The fisher population exists, in part, on a mixed management landscape characterized by different timber harvest practices interspersed with other public and private land. We removed 5 animals in winter of 2009-2010 and 4 animals in winter of 2010-2011 to be used as part of a founder population for a reintroduction in the Northern Sierra Nevada. Our objectives were to 1) determine abundance, survival, population growth rates, and relevant ingress and egress parameters for the fisher population for 2006-2011, to 2) determine if the study design as originally implemented had the ability to detect the removal of fishers in 2009 and 2010, to 3) determine the magnitude and direction of the effect, and to 4) make recommendations to improve the design so that it might be implemented to monitor the reintroduced fisher population. We found the Robust design estimator to be a poor fit to the survey design as implemented and instead used POPAN and Pradel open estimators for the majority of our parameter estimates. Abundance and population growth rate estimates suggest the population is stable, though survival and recruitment estimates suggest high turnover in the population. We were unable to detect removal of 9 adult fishers. Precision using open estimators was found to be low.

Recommendations to improve precision and analyze data using the Robust design, while maintaining functionality as an occupancy estimator, are offered. Concurrent surveying of all sites in the study area in future years is the strongest recommendation.

Non-Invasive Genetic Sampling and Mark-Recapture Analysis of a Fisher (*Martes pennanti*)
Population in Northern California used as a Reintroduction Source

by
Robert Clifden Swiers

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Zoology

Raleigh, North Carolina

2013

APPROVED BY:

Rob Dunn

William Kimler

Roger A Powell
Chair of Advisory Committee

DEDICATION

To my parents, Wayne and Sandra Swiers, who bore as much of the brunt as I for getting through this degree.

BIOGRAPHY

Robert spent the majority of his childhood traveling with his family as the son of a career military man. This provided him with a rounded worldview and an intense love and appreciation for the natural world. This degree contains the growth and learning that is one more step in his journey to understand as much of this world as possible.

ACKNOWLEDGEMENTS

This project was a cooperative effort of the US Fish & Wildlife Service, California Department of Fish & Game, the US Forest Service Rocky Mountain Research Station, Michigan California Timber Company and, beginning in 2009, North Carolina State University. Manpower, supplies, and logistic support were shared throughout the 7-years of the study.

Thank You to Laura Finley, Scott Yaeger and the US Fish and Wildlife Yreka office, Richard Callas, Pete Figura, Bob Hawkins, and the California Department of Fish and Game, Stu Farber of WM Beaty, Roger Powell, Ken Pollock, and Aaron Facka of North Carolina State University, Tim Shaw, Terry Salvestro, and Fruit Growers Supply Company, and Bobby Douglas and Michigan California Timber Company for all the help, support and guidance as this project was formed, implemented, and continues into the future. Each provided something valuable for my learning process that has led to this document. Thank You to Will Kimler for keeping me mindful of the past, to Rob Dunn for helping me keep the big picture in mind, and Roger Powell for being, well, himself. Thank You to the National Council for Air and Stream Improvement for providing partial funding.

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INTRODUCTION

Wildlife managers require sound data about the species and populations they manage. These data can be obtained from myriad methods, including direct wildlife surveys (camera stations, snow tracking, etc), radio and satellite telemetry, or information from visual or aural observations. It is the responsibility of the managers and researchers to choose the methods best suited to meet the objectives and goals of their particular studies (Armstrong and Seddon 2008; Ehrenfeld 2000; Romesburg 1981). Selecting the appropriate methods requires an understanding of the trade-offs between the availability of funds, risk to the study animals and researchers (Pauli et al. 2010), and the types of data required.

The growing popularity of noninvasive surveys is providing researchers with many new and efficient ways to gather information to meet study objectives and goals (Garshelis 2006; Long et al. 2008). Common types of noninvasive surveys include remote cameras (Foresman and Pearson 1998; Gompper et al. 2006), track plates to obtain tracks on a prepared surface (Slauson et al. 2008), snow tracking (reviewed by Powell 1993), hair snares to obtain genetic information from animals (Mowat and Strobeck 2000), and deploying dogs that are specially trained to detect target animals by scat left in a natural setting (Long et al. 2007; MacKay et al. 2008a). The data collected using these surveys can provide robust estimates of occupancy (MacKenzie et al. 2002; Slauson et al. 2009), abundance (Boulanger et al. 2004; Schwartz et al. 1998), demography (McKelvey and Schwartz 2004b), and population genetic structure (Schwartz et al. 1998). Following current trends in published literature and to prevent confusion, I use the term “noninvasive” when referring to surveys

conducted without the surveyor being present and that do not require the capture or handling of study animals.

In the most general sense, noninvasive techniques do not require the direct capture or handling of animals (Pauli et al. 2010), and are sometimes referred to as “remote” data collection (MacKay et al. 2008b). Data collected in this fashion allows researchers to make inference about study populations while minimizing disturbance or disruption to the normal behaviors of the animals or population (Garshelis 2006). Minimizing disturbance to study animals can be especially beneficial when researchers work with threatened or endangered species (Powell and Proulx 2003; Schwartz et al. 2007). It is appropriate to note, nonetheless, that noninvasive does not necessarily mean nonintrusive, as survey equipment can cause injury to wildlife in unanticipated ways, and the use of bait may cause a behavioral response where there otherwise might not be one. Researchers can safely assume, however, that these methods generally have fewer adverse effects than actual capture and handling (MacKay et al. 2008b).

Capture and handling of animals still has a place in research. Telemetry can be virtually noninvasive after the initial capture and can provide insight not possible through noninvasive techniques (Mech and Barber 2002). A vast amount of ecologically relevant information can be obtained through modern satellite-based telemetry methods (Cagnacci et al. 2010), and, while potentially more expensive than noninvasive methods, can often provide the best estimates of desired parameters (Choate et al. 2006).

Advances in the collection and analysis of noninvasive data over the past 25 years have provided researchers with many tools for understanding and monitoring animal

populations. These tools are helpful to managers of threatened or endangered species, as noninvasive surveys can be considerably less costly and require less manpower (Garshelis 2006; MacKay et al. 2008b; Pauli et al. 2010). A current and important trend in the analysis of noninvasive data is to estimate occupancy at different spatial scales for animals of interest (Long et al. 2007; MacKenzie et al. 2002; Slauson et al. 2009). Estimating occupancy can provide researchers and managers with further tools to investigate habitat relationships and to monitor particular parameters of interest in a population (Slauson et al. 2009). If a survey is set up appropriately, abundance can also be estimated using a mark-recapture framework (Mills et al. 2000). Conducting surveys over multiple time periods (seasons, years) can also provide researchers with a data framework to monitor surveyed populations (Boulanger et al. 2004).

Occupancy surveys determine whether one or more sites are occupied by members of a species of interest. Modern occupancy surveys originally consisted of snow-tracking animals across a pre-defined survey grid or transect (MacKay et al. 2008b). Over time, technology improved, survey methods were standardized (Long and Zielinski 2008; Zielinski and Kucera 1995), and methods for analysis became more complex (MacKenzie et al. 2002; Slauson et al. 2009). During a survey period, an animal detected at a site is considered a “presence,” and lack of detection at a site is considered an “absence” or a “non-detection”. The term “detection” refers to any instance during a survey where an animal provides the positive information that is sought by the researchers. The parameters of interest in occupancy estimation are p , the probability of detecting a member of the target species given its presence in the survey area, and ψ , the probability that a member of the target species

actually occupies the area, or site, surveyed, provided it is present. Adding and investigating various covariate parameters of occupancy models allow researchers to make inference to characteristics of the survey design (using p), and characteristics of the survey area (using ψ).

While the purpose of occupancy modeling is to allow researchers to understand characteristics of the surveyed area and detectability of members of the target species, mark-recapture studies allow researchers to estimate the abundance, survival, growth rates, and addition and subtractions of animals to populations (though capture probabilities can also be analyzed in a manner similar to detection probabilities). Mark-recapture analyses have a long and dense history in the literature (Amstrup et al. 2005; Cormack 1964; Jolly 1965; Lebreton et al. 1992; Lincoln 1930; Petersen 1896; Pollock et al. 1990; Seber 1965), predating occupancy modeling.

Both occupancy estimation and mark-recapture methods use binary data. Data are collected using 1's (detection) and 0's (non-detection) to build a site specific (occupancy) or individual specific (abundance) detection, or capture, history (Amstrup et al. 2005; Lebreton et al. 1992). The detection history of occupancy modeling is site-specific while the capture history of a mark-recapture analysis is individual-specific. This means that site occupancy can be analyzed without needing individuals to be marked with a unique animal identification (ID). Most mark-recapture surveys require individuals to be marked in some way allowing individual identifications to be known or visible. This assumption underlies almost all mark-recapture models (Amstrup et al. 2005; White and Burnham 1999). For example, a site being surveyed for occupancy by members of a certain species for four weeks

may detect a target species the first week, not the second, again on the third, and also the fourth week. That species would have a site detection history of:

$$1\ 0\ 1\ 1,$$

where the individual ID of the animals is not needed. If the purpose of the survey is mark-recapture, many sites across the surveyed area could detect a given animal in any given week. The previous mentioned site may have seen individual (1) in week 1 and 4, and individual (2) in week 3 only. If this was the only site these individuals were seen during the survey, their respective individual capture histories would be:

$$(1)\ 1\ 0\ 0\ 1\quad\quad\text{and}\quad\quad(2)\ 0\ 0\ 1\ 0,$$

providing information on two animals in a non-spatially explicit manner. These capture histories are the basis for mark-recapture analysis.

Modern advances in DNA analysis allow researchers to collect hair or other tissue samples in the field and analyze the samples in a laboratory to obtain information about study animals (Drew et al. 2003; Mills et al. 2000; Schwartz et al. 1998; Wisely et al. 2004). One important piece of information that can be obtained is the unique animal identification, allowing researchers to build individual specific capture histories for animals. Researchers can, therefore, design surveys to collect genetic information from surveyed animals allowing estimation of occupancy-, demography-, and abundance-related parameters. The first time a sample is collected from an individual it is considered a “mark,” and each subsequent collected sample is a “recapture.” One pitfall with using DNA to obtain individual IDs is that processing samples takes time, and lab analysis can be costly (Mills et al. 2000). Cameras, however, under optimal circumstances, can provide researchers with individual ID

almost immediately and at little cost after the initial equipment purchase. The surveyed animals must have individually distinguishing markings or features, however, that can be visible to a camera at a distance, such as a tiger's stripes (Karanth 1995), or ear tags (though loss of tags can present problems, see Jordan et al. 2011).

Mark-recapture estimators are of two basic forms: closed and open. Closed estimators assume that the number of individuals in the population does not change over the length of a survey, and are generally concerned with estimating abundance, N (think of the traditional Petersen-Lincoln estimator), using parameters p and c , which are the probabilities of first capture and recapture. Assumptions for closed estimators include (Chao and Huggins 2005):

population is constant over survey period,
animal marks are not lost,
animals and marks are not mis-identified, and
animals act independently.

Open estimators operate under the premise that population size can change during a survey from a combination of ingress (birth and immigration) and egress (death and emmigration; Cormack 1964; Jolly 1965; Seber 1965). Open estimators are generally used for surveys that run over longer time intervals than do surveys used for closed estimators (Cooch and White 2010; Manly et al. 2005). Open estimators are built from two main parameters p , the probability of capture, and Φ , the apparent survival probability. This latter parameter is not true survival, but includes the true survival parameter, S , and the site fidelity parameter, F . Separating these parameters requires additional data beyond individual capture

histories (Kendall 2010; Pollock et al. 2012). Assumptions for open population estimators include (Pollock and Alpizar-Jara 2005) :

equal catchability among animals,

animals have equal chance of surviving between samples,

animals are not mis-identified,

sampling is effectively instantaneous, and

migration is permanent.

An important extension of open estimators was introduced by Pollock et al. (1990) and is referred to as the Robust design. This estimator nests a closed capture estimator within an open estimator, and repeats this nested design for the desired length of the survey. One can visualize it as surveying for members of a species once a month for 4 days at the end of each month, and repeating this for many months. A two month capture history for an individual with 4 days in each month might be:

1 0 0 1 0 0 1 0,

representing captures on days 1 and 4 in the first month, and only on day 3 in the second month. Each within-month sampling period can be considered a closed period during which the population size is assumed not to vary, while between these periods the population can vary – allowing for ingress and egress (see figure pg 15-4 by Kendall 2010). One can then derive the standard estimates of abundance, survival probability, and capture and recapture probabilities, and also estimate temporary emigration, γ , for which animals can be modeled as leaving the study area but subsequently returning, a generous relaxation of the assumption of closure (Kendall et al. 1997).

Fishers

Fishers (*Martes pennanti*) are medium-sized, forest dwelling mammals and are larger than American martens (*M. americana*), the other species of *Martes* in North America. Fishers belong to the family Mustelidae and the order Carnivora (Powell 1993; Powell and Zielinski 1994). They have a distinctive weasel shape with short, stocky legs and large, rounded ears. They are dark brown in color, with black or black-tipped guard hairs over all but their facial area, though some individuals appear hoary or tan. The ventral portion may contain white or cream patches on the inner legs, stomach, and groin area (Powell 1993). The distribution of fishers has decreased substantially since European settlement. In the western United States, fishers currently occupy isolated pockets in the southern Sierra Nevada and northern Cascades, Coastal and Klamath mountains of California, and in mountainous areas of southwestern Oregon, eastern Idaho, and northwestern Montana (Davis et al. 2007; Gibilisco 1994; Lewis et al. 2012; Powell 1993; Powell and Zielinski 1994). Reintroduced populations currently occupy areas in the Southern Oregon Cascade Range (Aubry and Lewis 2003; Lewis et al. 2012), the Olympic Peninsula of Washington (Lewis 2006), and the Northern Sierra Nevada of California (Callas and Figura 2008; Lewis et al. 2012).

Section 3 of the Endangered Species Act (USDOI 1973) allows “any distinct population segment” (DPS) of a species to be listed and afforded protection. The Fish and Wildlife Service of the United States Department of the Interior has recognized two distinct population segments of fishers in the western United States: a West Coast DPS and a Rocky Mountain DPS (USFWS 2003, 2010). Two petitions to list fishers in the western United

States were denied (USFWS 1991, 1996). Listing for the Rocky Mountain DPS was found “not warranted” (USFWS 2011), while listing for the West Coast DPS was found “warranted but precluded” by higher priority listing actions (USFWS 2004). The Fish and Wildlife Service is currently under court order to provide a final listing decision for the West Coast DPS by 2014 (J.S. Yaeger, pers. comm.), and it remains listed as a “Candidate species.” The state of Washington has listed the fishers as endangered within the state (Lewis 2006). Oregon has listed the species as threatened, but California denied listing as either threatened or endangered under the California Endangered Species Act in 2008 (Callas and Figura 2008) and again in 2010. A court order in 2012 (Kahn 2012) required the California State Game Commission to reevaluate their listing decision. This reevaluation is currently in process.

Fishers can be detected easily using non-invasive surveys (Gompper et al. 2006; Long et al. 2007; Long and Zielinski 2008; Slauson et al. 2009; Zielinski and Schlexer 2009). Common survey types are remote cameras and track plates with the majority of studies in the western states being focused on distribution and habitat modeling (Carroll et al. 1999; Davis et al. 2007; Zielinski et al. 2010; Zielinski and Kucera 1995). The natural inclination of fishers to come to and to enter bait stations to leave tracks or hair, or to be photographed makes them ideal candidates for non-invasive sampling surveys. Researchers have developed genetic markers (Drew et al. 2003; McKelvey and Schwartz 2004a) that can determine an individual fisher’s identification (ID; McKelvey and Schwartz 2004b; Mills et al. 2000; Schwartz et al. 1998). With this information, researchers can determine the demographics of a fisher population and can also construct a survey to monitor the

population trends using mark-recapture analyses (Boulanger et al. 2004; Schwartz et al. 2007; Williams et al. 2009).

In 1995 the California Department of Fish & Game partnered with the USDI Fish & Wildlife Service and Timber Products Company (a private timber company, now Michigan California Timber Company, MCTC) to initiate a track-plate survey to detect fishers on a managed, forested landscape centered on the Klamath River in Northern California and Southern Oregon (Figure 1). Fishers were detected in areas south of Mt. Ashland in Oregon and around Collins-Baldy in Siskiyou County, California. Ten years later, in fall 2005, the survey was repeated using track plates and cameras. The results of this second survey and the recent availability of reliable fisher DNA markers (Drew et al. 2003) led the researchers to propose a multi-year study to document fisher presence across the study area using DNA contained in hair follicles collected from sample stations.

In 2008 the California Department of Fish and Game proposed to move native California fishers into parts of their historical range in the northern Sierra Nevada (Callas and Figura 2008). The effort was a collaboration between the California Department of Fish and Game, US Fish and Wildlife Service, Sierra Pacific Industries, and North Carolina State University. The reintroduction site was the 647 km² of the Stirling Management Area owned by Sierra Pacific Industries in Butte and Tehama counties (Figure 2). The study area is bordered by the Lassen National Forest to the North and the Plumas National Forest to the east, as well as by small private parcels and Pacific Gas & Electric land. This reintroduction was part of a research program designed to understand the ecology of fishers on managed landscapes.

The reintroduction's feasibility document (Callas and Figura 2008) stipulated that one of three source populations used to supply animals for the translocation must be monitored for effects of removal. The non-invasive, genetic surveys on the Eastern Klamath Study Area (Farber and Schwartz 2007; Farber et al. 2008), with the same two government agencies as cooperators, provided an opportunity to use the surveys, already targeted to determine abundance and population structure, for a second purpose. This had the potential to provide added conservation benefits for fishers without any retooling (Sarrazin and Barbault 1996; Seddon et al. 2007), while fitting in with the larger research program to understand fishers on managed landscapes.

Keeping with that new purpose, 5 fishers were removed (3F:1M, 1 other female died in captivity) in winter of 2009-2010 and 4 fishers were removed (3F:1M) during the winter of 2010-2011 (total 7F:2M). These removals were targeted to be from MCTC lands, meaning fishers were removed from managed industrial timberlands to be placed on a different managed landscape. The removed animals were also targeted to be adult members of the population, and prime breeders, potentially having a larger impact than traditional harvest for fur. Female fishers were also removed from lands a few drainages west of the Klamath study site in early winters of 2010-2011 (2F) and 2011-2012 (2F). These fishers were not a part of our Klamath study population; none of these fishers were detected in our surveys (M. Schwartz and K. Pilgrim, pers. comm.). No further removals of fishers are planned.

Thus, the objectives of the Eastern Klamath research were to 1) determine abundance, survival, population growth rates, and relevant ingress and egress parameters for the fisher

population for 2006-2011, and 2) to determine if the study design as originally implemented had the ability to detect the removal of fishers in 2009 and 2010. If so, we 3) would determine the magnitude and direction of the affect. We would also 4) determine problems with the study design and recommend modifications for possible implementation as a monitoring tool for the reintroduced fisher population in the Stirling Management Area. To meet objectives 1-3, we estimated abundance, survival, and population growth rates for the Klamath fisher population and ranked hypotheses comparing effects of time (to attempt to detect removal of animals in 2009 and 2010) to null hypotheses.

METHODS

Study Area

The Eastern Klamath Study Area occupied 510 km² (197 mi²) in northern Siskiyou County, California and southern Jackson County, Oregon. It was a landscape characterized by checkerboard land ownership, containing public land within the Klamath National Forest, and land managed by Michigan California Timber Company and Fruit Growers Supply Company (2 private timber companies). The Klamath River, tracked by California Highway 96, ran east-west through the middle of the study area. Mt. Ashland was at the northeastern boundary and the town of Yreka, California, was roughly 20 km east of the south-eastern border (Figure 1).

The study area was divided into 3 sections: the Mt. Ashland section in the north (207.8 km²), the Collins-Baldy section in the south (177.9 km²), and the Klamath River section in the center (124.78 km², Figure 1; Farber et al. 2008).

Survey Design

The study area was divided into 10.4 km² (4 mi²) cells, each containing 4 surveyed townships, and each containing 2 survey stations (Figure 1). Although this survey design was modified from an occupancy protocol (Zielinski and Kucera 1995), it facilitated survey logistics for estimating relevant population parameters. The Mt. Ashland section of our study area contained 42 survey stations, the Klamath River section contained 22, and the Collins-Baldy section contained 36, totaling 100 survey stations in 50 cells.

The survey in this study area was based on the occupancy protocol developed by Zielinski and Kucera (1995). The study began in fall of 2006 in the Mt Ashland and Collins-Baldy sections. The Klamath River and Highway 96 were assumed to be a barrier to fisher movement (Farber and Schwartz 2007). Analysis of the 2006 data, however, showed that fishers did, in fact, cross the suspected barrier and that the two populations were genetically homogeneous (Farber and Schwartz 2007). The Klamath section was added in 2007 (Figure 1), and surveys have been conducted in all 3 sections each fall through 2012.

Survey sites were chosen within each survey cell based on criteria believed to increase the probability of fisher detection and to facilitate easy access to survey stations. These criteria included proximity to forest roads, logging roads, and streams (annual or perennial), and ridge tops with dense canopy and good airflow to increase the dispersion of scented bait. Each survey site contained a 25x25 cm (10x10 in) tunnel that was 75 cm (30 in) long, made of Coroplast (corrugated plastic; Figure 3). The tunnel had a hardware cloth back to prevent entry or exit through the rear and had 3 pieces of 1"x2" (2x4 cm) board in the front, starting 10 cm (~4 in) from the bottom of the front of the tunnel (Figure 3). The

bottom board had a strip of non-poisonous glueboard (the type used to catch mice) attached underneath. The tunnel was baited with a can of moist cat food and a piece of raw chicken. An animal wishing to enter the tunnel to reach the bait was forced to crawl underneath the bottom board and the glue strip captured hair.

Surveys started in mid-September each year and continued through mid-December. Each site was checked once a week for 4 weeks. A survey week could be lost, however, due to 1) a tunnel being damaged by an animal (usually a bear, *Ursus americanus*) such that a fisher could not enter the tunnel and leave hair on the glue strip, 2) the removal of the glue strip by a target or non-target animal or 3) the back screen being pulled or chewed such that a fisher could enter or exit the tunnel from the back, avoiding the glue strip. Loss of a survey week resulted in the addition of a sampling week, not to exceed a total effort of 42 days (6 weeks) for any site.

Limited manpower (4 - 6 surveyors used each year) and weather required us to stagger the start of surveys. We started surveys at high elevation sites and finished at low elevation sites, making 2 sessions of data collection. We started surveys at all sites early enough, however, to allow 6 full weeks (if required) before the anticipated onset of snow deep enough to prevent access.

We installed track plates in half of the stations in 2006-2008 and in all stations in 2009-2011 and used the track plates to help identify site visitors. Track plates can also be used to assess relative abundance estimates to compare to population estimates (Foresman and Pearson 1998; Gompper et al. 2006).

We considered any hair attached to a glue strip to be a sample, regardless of evidence on track plates. This removed any possibility that an erroneous assignment could be made in the field, and allowed the lab to sort the samples as appropriate. We placed each gluestrip with hair attached (hereafter: sample) in a desiccant-filled vial in the field to preserve sample integrity. If a gluestrip was destroyed or lost, we collected any loose hairs or fragments of gluestrip found inside or around the sampling tunnel. Surveyors handled samples in the field according to the guidelines provided by Rocky Mountain Research Station of the US Forest Service in Missoula, Montana (see appendix, Figure I). Once a week, we batch-shipped samples overnight to the Rocky Mountain Research Station. Results from the DNA analysis were returned from the Research Station early in the following April. Thus, data for 2012 were not available for inclusion in my analyses.

After surveys were complete in 2009 and in 2010, we trapped fishers for the reintroduction. We placed traps where track plates had provided evidence of fishers in the northern portion of the Collins-Baldy section. Traps were cage-traps with wooden cubbies built onto the back, allowing a trapped fisher to take cover and feel secure until picked up. Bait used was raw chicken and “gusto” scent lure. Trapping continued until mid-January or until the number of fishers needed of each sex had been trapped.

Analysis

Results received from the Rocky Mountain Research Station each spring included the individual ID for each fisher sample that could be assigned to an individual, its sex, and its haplotype. I used Program MARK (Cooch and White 2010; White and Burnham 1999) to estimate demographic variables. Fishers previously marked that were removed for the

reintroduction were labeled as such in the capture histories. I followed the model naming conventions of Lebreton et al. (1992), where a parameter succeeded by “(t,s)” means that the parameter varies over time and by sex, “(t,)” for time variation only and sex combined, “(.,)” for time constant and sex combined, etc.

I derived three sets of individual capture histories, suitable for analysis using 1) Pollock’s Robust Design (Kendall 2010; Kendall et al. 1997; Pollock et al. 1990) for years 2007-2011, 2) POPAN (Schwarz and Arnason 1996) and Pradel (Schwarz and Arnason 1996) models for years 2007-2011, and 3) closed models for 2006 alone. Pollock’s Robust Design uses closed estimators within secondary sampling periods to estimate population sizes. POPAN uses open estimators to estimate population sizes as derived parameters and Pradel models use open estimators to estimate population growth rates, λ , and recruitment, f (Cooch and White 2010). We analyzed data for 2006 separately as they do not include data from the Klamath River section of the study area.

Due to the inconsistent start dates for each survey site across all years, I strung all weeks together for a given year to build capture histories for the Robust estimator. Thus, if session 1 was 5 weeks long and session 2 was 5 weeks, then the capture histories for that year for all animals were 10 weeks long. Because not all sites were run concurrently, animals had heterogeneous probabilities of encountering survey sites. We compensated for this by adding week-specific covariates to certain models each year that corresponded to the number of open sites (“trap” effort/week). Because different numbers of sites were open each week, and because this pattern repeated each year, we did not collapse the capture or

recapture probabilities (p and c , respectively), but were required to keep them time-variant within years. We designated the first week surveyed in each year as week 1.

For each set of analyses, we ranked biological hypotheses (usually referred to as “models” in mark-recapture literature) using Akaike Information Criterion (adjusted for small sample size, AIC_c). These models incorporated annually variable vs. constant survival, abundance, and population growth rate. We assumed that, based on the non-invasive study design, that fishers would not experience any negative “trap” effect. Free food and no handling stress, however, could have produced a positive trap response (trap happy fishers). We, therefore, built models for the Robust Design with and without p and c equal to investigate this. And we built models that compared random temporary emigration to Markovian temporary emigration. Random temporary emigration models individuals available for capture during secondary periods as random, and Markovian temporary emigration “remembers” whether the individual was present or not at the previous ($i-1$) sampling period (Nichols 2005; Pollock et al. 1990).

Because violations of closure were evident in the study design prior to analysis, we used POPAN models to investigate transient animals. POPAN models contain parameter N , denoting a superpopulation of animals, or the total number of animals available for capture from the beginning to the end of the study, and parameter b (or “ $pent$ ”), the probability of entrance (as well as standard p and Φ). Potential closure violations are an unfortunate consequence of sampling during the dispersal season for fishers (Powell 1993), of the inconsistent times at which given sites were run, and of fishers’ home ranges being large and overlapping the boundary of the study area (Davis et al. 2007; Fuller et al. 2001; Powell and

Zielinski 1994). We ranked hypotheses with and without time-dependent survival and probability of capture (Φ and p , respectively), and with and without entrance probabilities (b) constant. MARK implemented program RELEASE (Burnham et al. 1987) to run a goodness of fit test to check for overdispersion.

I used two Pradel variants of Jolly-Seber models to investigate closure violations. One model variant provides an estimate of the recruitment parameter, f , which is the number of recruits to the population per animal present at the previous sampling period. The Pradel λ variant provides a *realized* population growth rate parameter, λ .

I broke the 2006 data into two groups, a Mt. Ashland (21 units) and a Collins-Baldy subsection (18 units). The distance between the two study sections confound analysis spatially if grouped together. I added no covariates, therefore, I used simple closed-captures estimators (Otis et al. 1978) to estimate capture (p), recapture (c), and abundance (N) for each section. We investigated heterogeneity models and found that sample sizes were too small (9 individuals detected in the Mt. Ashland section and 13 individuals detected in the Collins-Baldy section) for finite mixture models to provide useful results.

RESULTS

Overall, analyses were not able to pick up any change in survival or population size across all years for fishers in the Eastern Klamath Study Area. With the exception of the Robust Design models, which were a poor fit due to issues with the study design, all models used to estimate survival-related parameters were the simplest available. Removal of 9 adult, breeding-age fishers did not appear to be a detriment to a stable population, at least during the period of the survey.

The top ranked hypothesis (model) from the Robust Design candidate set suggested that survival of fishers in 2007-2011 did not vary across years. The population appeared to experience random temporary emigration and the population size varied by year. Conditional probability of capture and recapture differed by week and within a given year, tracking the effort (number of sites operational) covariate (Table 1). I detected no trap response. All remaining models had $\Delta AIC_c > 4.6$ and the support for the top-ranked model was 0.88, suggesting that the other models were not competitive. Apparent annual survival of fishers was estimated to be 0.64 (± 0.08 [SE]). The estimates for annual population size ranged between 29 and 35, and the abundance estimates tracked the number of detected fishers (Figure 4). Capture and recapture probabilities ranged from 0.05 to 0.18 for 2007, 0.11 to 0.23 for 2008, 0.02 to 0.25 for 2009, 0.01 to 0.28 for 2010, and from 0.05 to 0.29 for 2011.

The top-ranked hypothesis (model) for open-population analysis using POPAN also suggested that survival of fishers in the Eastern Klamath Study Area did not vary by sex or across years. Capture probabilities did not vary by year or by sex, and fishers seemed to be entering the study population at a consistent rate from the superpopulation each year (Table 2). The goodness of fit tests of Program RELEASE (TEST2 + TEST3) found mild under-dispersion in the data (chi-squared=3.6489, df=7, p-value=0.8192), resulting in a \hat{c} of 0.5213 for the POPAN and Pradel datasets. This value was adjusted in MARK, and is reflected in the estimated parameters and the use of QAIC_c for model ranking instead of AIC_c. Support for the top-ranked model was 0.77, and all remaining models had $\Delta QAIC_c > 3.55$. Apparent survival, 0.6 (± 0.05 [SE]), was similar to that calculated using the Robust Design and

estimated capture probability was 0.56 (± 0.07 [SE]). The derived population estimates ranged from 45 to 52 (Figure 5). The superpopulation size was 133 (± 10.4 [SE]). The probability of an individual entering the population from the superpopulation, 0.165 (± 0.01 [SE]), was constant between sampling periods.

The top ranked hypothesis (model) for Pradel analyses suggested that neither survival nor capture probabilities of fishers varied by sex or across years (Table 3). Population growth rate, seniority, and recruitment were also constant across years and sex. The two Pradel models used have different parameterizations of the same model, and apparent survival of fishers was 0.60 (± 0.05 [SE]), and the constant capture probability was 0.55 (± 0.07 [SE]). Population growth rate was estimated at 1.06 (95% CI 0.97-1.15), suggesting a stable or slightly growing population. The recruitment parameter estimate of 0.45 (95% CI 0.34-0.57) suggests that for every two fishers seen in a given year, roughly one recruit will be added to the population in the next year.

Closed population analysis of the Collins-Baldy section for 2006 indicated that the population's capture and recapture probabilities did not vary by time (Table 4). This top-ranked model had high support with AIC_c weight of 0.95. For the Mt. Ashland section fishers appear to have time-specific differences in capture probabilities but no trap response (AIC_c of 0.95). The Collins-Baldy population size was estimated at 42 (± 25.9 [SE]) with a capture-recapture probability of 0.05 (± 0.03 [SE]). The Mt. Ashland population size was 9.24 (± 1.16 [SE]) with capture-recapture probabilities estimated between 0 (when no animals were detected) and 0.54 (± 0.18 [SE]).

DISCUSSION

The population of fishers in the Eastern Klamath Study Area appears to have been stable through the course of this study, and the estimators did not detect the removal of 9 fishers. All top-ranked models were models of no change in most, if not all, parameters. I found survival to be constant and in agreement between estimators (Table 5). Combined with the estimated population growth rate, this suggested a stable population. This can also be seen by the close agreement between the number of fishers detected and the lower 95% CI for each estimate (Figure 4). Also, the inverse of survival is mortality (egress; $1 - 0.60 = 0.40$). The estimate of recruitment was higher than our as hoc mortality estimate, suggesting very mild population growth. This agreed with my λ estimates. It is possible, however, that the effects of removing breeding age adults will not be realized immediately, as factors such as dispersal and recruitment may be affected further in the future.

There was disagreement of abundance estimates between the Robust Design and POPAN estimators. This is due to the sporadic temporal operation of sites across the study area each year. This left many animals “unavailable” to the closed estimator of the Robust Design in areas where they could have been detected, but were not, because no sites were in operation (K. Pollock, pers comm.). I consider, therefore, the POPAN estimates of abundance to be more reliable. The removal of 9 prime breeding adults did not affect the population size, possibly due to a high turnover as suggested by the number of animals detected vs. new animals each year (Figure 6, appendix figure II), and also the estimated recruitment parameter, 0.45 (95% CI 0.34-0.57). This removal amounts to 9.7% (5

individuals removed / 51.4 estimated by POPAN) of the population removed in 2009, and 7.75% (4 individuals removed / 51.6 estimated by POPAN) in 2010.

Being able to remove multiple breeding age adults from a population of fishers opens the door to other possible future reintroductions. The methods implemented in this study could also be used to assess the status of potential source populations for other species of concern. It is always recommended that source populations be monitored when planning a reintroduction or translocation (Armstrong and Seddon 2008; Seddon et al. 2007).

Demographic estimates are only as good, however, as the survey that collects the underlying data. Implementation of an occupancy protocol poses problems for traditional mark-recapture analysis, despite the fact that the protocol used encompasses the aspects required to meet occupancy survey objectives (Long and Zielinski 2008; Zielinski and Kucera 1995). One major issue that was not anticipated nor mitigated is the spatio-temporal misalignment which leaves a patchwork of sites operating at different times when in fact the survey requires a near-instantaneous snapshot of the population (Chao and Huggins 2005). Open models fit the data appropriately, but a substantial amount of effort is put forth and then not used once the within year captures are collapsed to “detected” or “not detected” in a year. Robust Design estimators were designed to estimate temporary emigration, allowing for the movement of animals on and off a study site while also making use of estimable parameters from open estimators. A population of fishers, sampled during the dispersal season, is a perfect opportunity to use such an estimator, provided the sample collection is designed appropriately.

Covariates may also be added to the analysis after modifications to the collection of data have been implemented. Researchers could collect data on parameters that may impact fishers' survival, including weather/snow/rain information, or previous year's winter season length. If occupancy is a requirement of the study, covariates may also be collected concerning the site. Such data would be related to habitat in nature, but should be collected to correspond to the size of the cell, bait or lure used, and other characteristics of the survey design that might affect a study animal's behavior when visiting a survey station.

Improving the study design is as simple as determining what was needed from the data, and implementing the changes needed to meet those objectives (Byron 1997; Romesburg 1981). Analysis for this study was not attempted until the study was in its fourth year, and that proved to be problematic. The survey design was meant to estimate occupancy, and there were areas of leniency in the field collection of data, most important that sites could be run in a disjunct fashion, both in space and time to allow for variation in surveyor availability. For traditional mark-recapture estimators, sites need to be run concurrently, with as little spatial separation as possible. Both objectives (occupancy and abundance/survival estimation) can be met with the same protocol, however, by understanding the tradeoffs associated with each survey design. For the current study design, all sites should be run at once to provide data to meet both occupancy and abundance/survival objectives. This changes the field logistics by requiring more effort over a shorter period of time, but would likely provide a higher precision and accuracy in estimated parameters.

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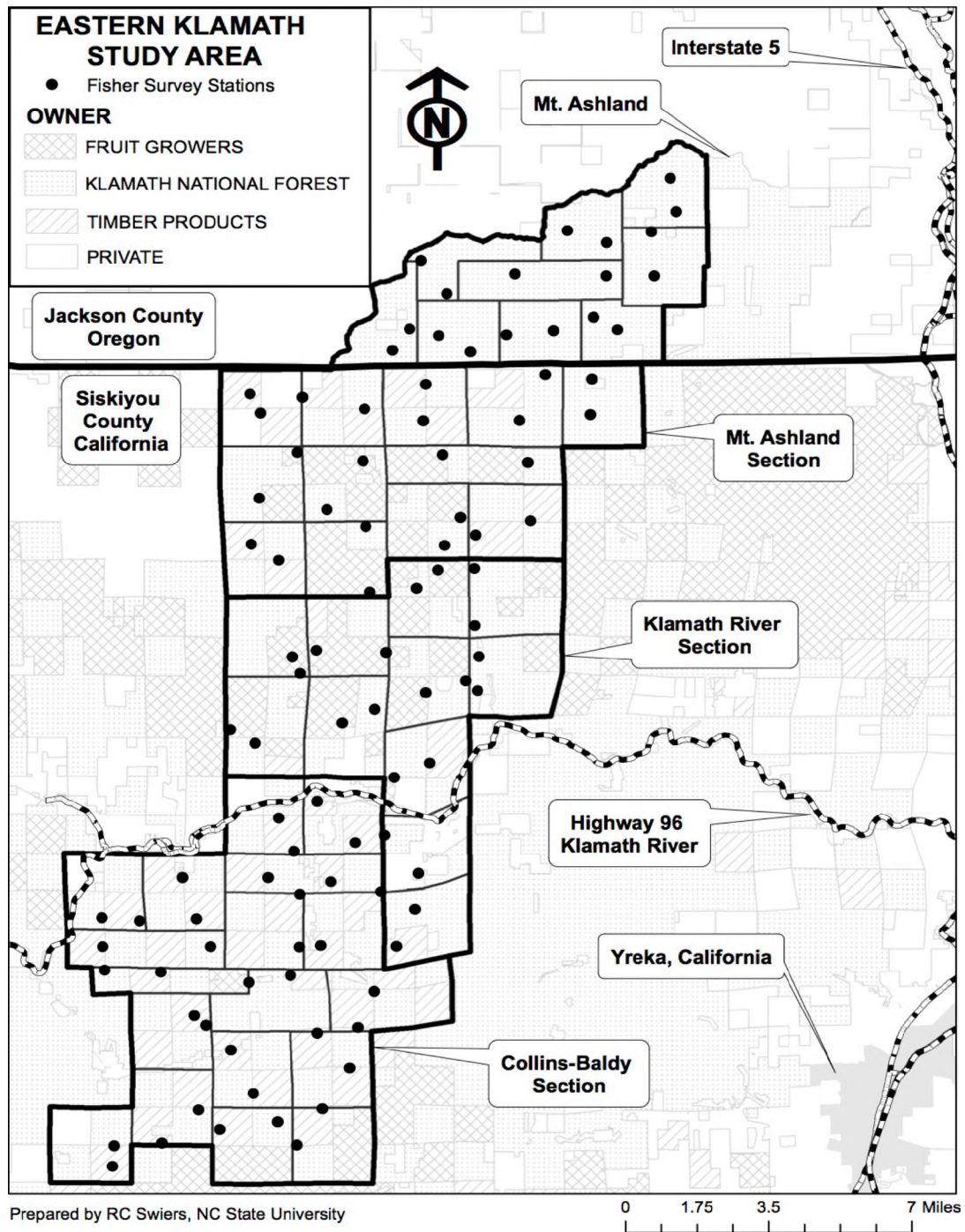


Figure 1: Fisher survey areas with Mt. Ashland area in the north, Klamath River area in the middle, and Collins-Baldy area in the south. The Klamath River and California Route 96 are shown in center.

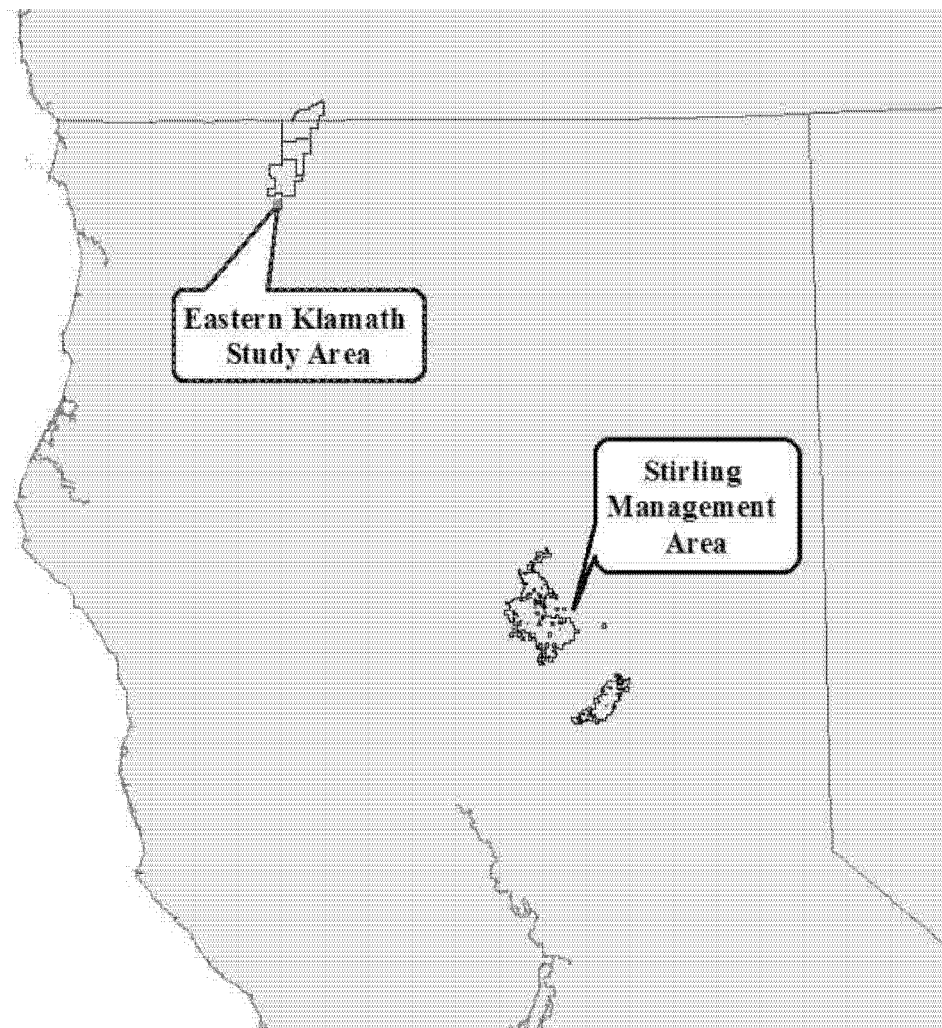


Figure 2: Eastern Klamath study area (510 km²) and Sierra Pacific Industries Stirling Management Area (647 km²) within the state of California.



Figure 3: Coroplast fisher bait tunnel showing wooden slats at entry and track plate in place on bottom of tunnel interior. The bottom slat has a gluestrip underneath to collect hairs from mammals that enter the tunnel (photo by J.S. Yaeger, US Fish and Wildlife Service).

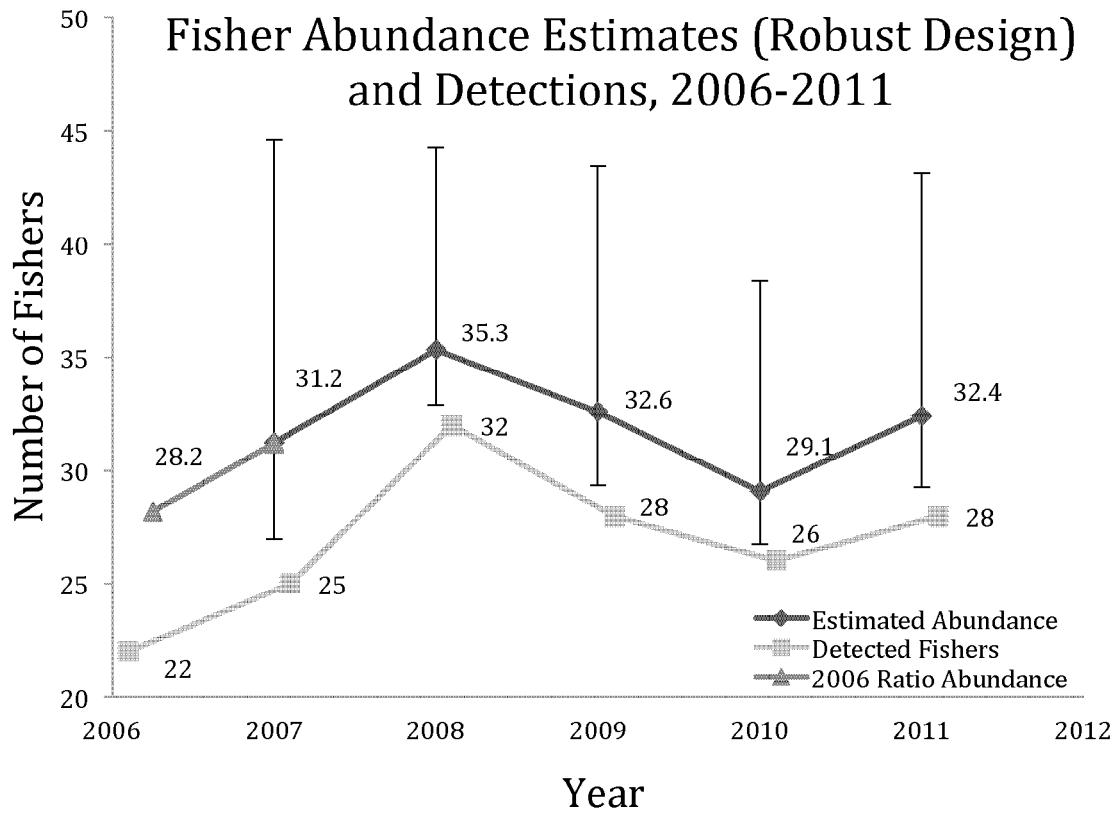


Figure 4: Estimated numbers of fishers on the Eastern Klamath Study Area 2007-2011 using Robust Design estimator. The estimate for 2006 was derived using a ratio of the sites run in 2006 vs. 2007-2011 to the mean number of fishers detected in other years.

Fisher Abundance Estimates for POPAN model, 2007-2011

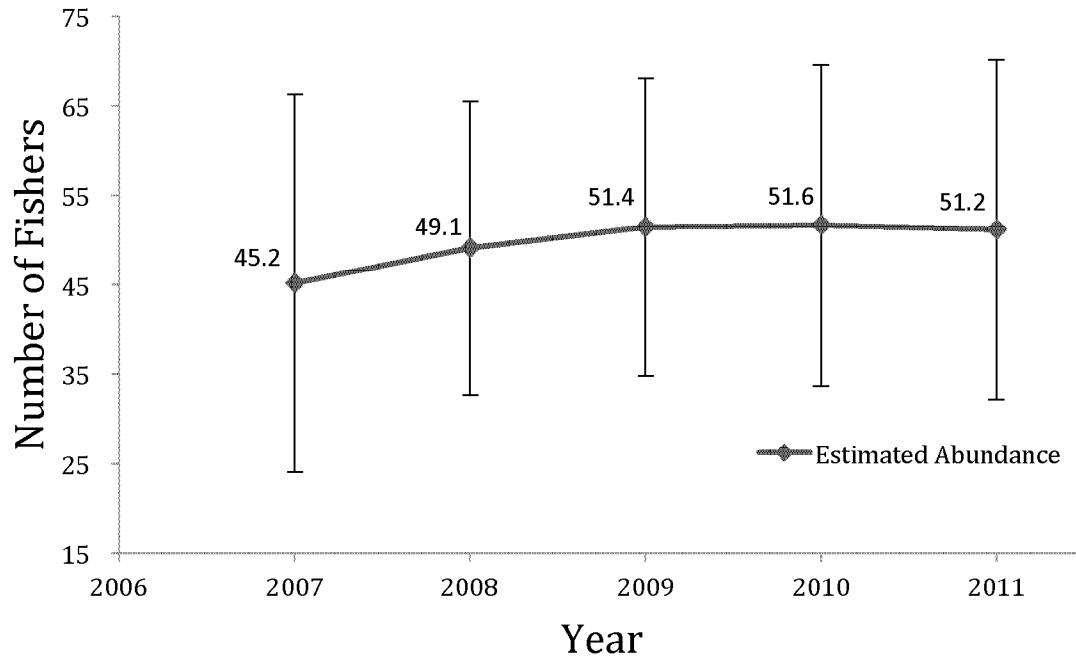


Figure 5: Estimated numbers of fishers on the Eastern Klamath Study Area 2007-2011 using POPAN estimator. Estimates are derived parameters from top-ranked model, which was the full null model.

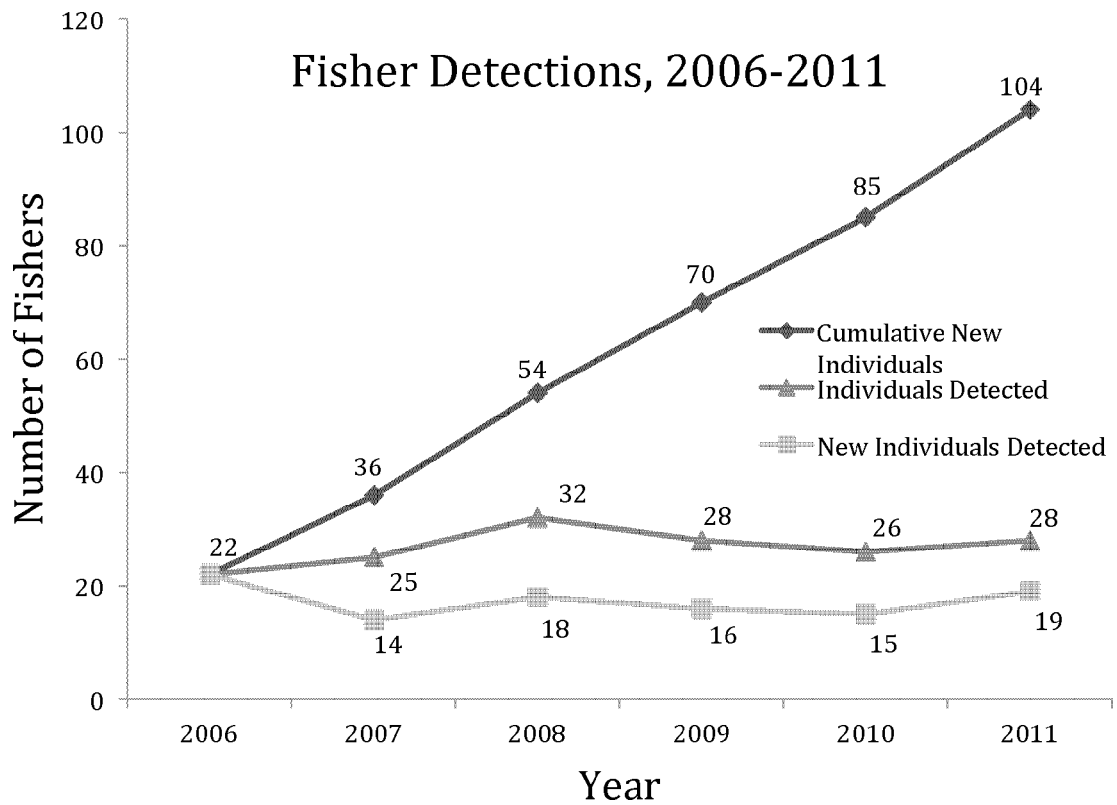


Figure 6: Total number of fishers detected each year, number of new individuals detected, and total cumulative number of fisher detected on the Eastern Klamath Study Area, 2006-2011.

Table 1: Pollock's Robust Design model selection list for Eastern Klamath Study Area fishers 2007-2011. "covar" refers to an effort covariate added to the combined capture and recapture probabilities ($p=c$). These covariates

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
S(.) $p(t)=c(t)$ covar N(t) Random Mvmnt	914.7163	0	0.87914	1	20	751.2476
S(.) $p(t)=c(t)$ covar N(t) Markovian Mvmnt	919.3455	4.6292	0.08686	0.0988	23	748.7855
S(t) $p(t)=c(t)$ covar N(t) Random Mvmnt	921.2263	6.51	0.03392	0.0386	23	750.6663
S(.) $p(t)=c(t)$ across years, covar N(t) Random Mvmnt	933.9575	19.2412	0.00006	0.0001	11	790.7921
S(.) $p(t)=c(t)$ across years N(t) Random Mvmnt	936.4906	21.7743	0.00002	0	23	765.9306
S(t) $p(t)=c(t)$ across years N(t) Random Mvmnt	943.1723	28.456	0	0	26	765.3494
S(t) $p(.) c(.)$ N(t) Random Mvmnt	991.6078	76.8915	0	0	22	823.4304
S(.) $p(.) c(.)$ N(t) Markovian Mvmnt	992.1111	77.3948	0	0	23	821.5511
S(.) $p(t)=c(t)$ N(t) Markovian Mvmnt	995.7817	81.0654	0	0	70	686.8946

Table 2: POPAN model selection list for Eastern Klamath Study Area fishers 2007-2011. QAICc represents values adjusted due to under-dispersion of the data.

Model	Model				
	QAICc	Delta QAICc	QAICc Weights	Likelihood	Num. Par
phi(t) p(.) pent(.)	417.0221	0	0.76812	1	4
phi(t) p(.) pent(.)	420.5608	3.5387	0.13092	0.1704	7
phi(.) p(.) pent(t)	422.0718	5.0497	0.0615	0.0801	7
phi(.) p(t) pent(t)	424.2382	7.2161	0.02082	0.0271	10
phi(t) p(.) pent(t)	425.5267	8.5046	0.01093	0.0142	10
phi(t) p(t) pent(t)	426.2257	9.2036	0.00771	0.01	12

Table 3: Pradel model selection lists for Eastern Klamath Area fishers 2007-2011. Lambda parameterization is shown, and recruitment compared the same models with the same ranking.

Model	Model					
	QAICc	Delta QAICc	QAICc Weights	Likelihood	Num. Par	QDeviance
Phi(.) p(.) Lambda(.)	951.8868	0	0.74998	1	3	60.7996
Phi(t) p(.) Lambda(.)	954.9394	3.0526	0.163	0.2173	6	57.3792
Phi(.) p(.) Lambda(t)	956.6793	4.7925	0.06829	0.0911	6	59.119
Phi(t) p(.) Lambda(t)	960.0815	8.1947	0.01246	0.0166	9	55.7376
Phi(t) p(t) Lambda(t)	961.4546	9.5678	0.00627	0.0084	11	52.4042

Table 4: Closed Captures model selection lists for Collins-Baldy and Mt. Ashland sections for 2006. Sexes are combined due to small sample sizes (13 for Collins-Baldy and 9 for Mt. Ashland).

2006 Collins-Baldy Closed Captures

Model	Model					
	AICc	Delta AICc	AICc Weights	Likelihood	Num. Par	Deviance
p(.) = c(.) N	33.6811	0	0.95123	1	2	19.0683
p(t) c(.) N	40.378	6.6969	0.03342	0.0351	9	9.9691
p(t) = c(t) N	42.0545	8.3734	0.01446	0.0152	9	11.6456
p(t) c(t) N	47.6218	13.9407	0.00089	0.0009	14	4.4087

2006 Mt. Ashland Closed Captures

Model	Model					
	AICc	Delta AICc	AICc Weights	Likelihood	Num. Par	Deviance
p(t) = c(t) N	64.419	0	0.95345	1	12	25.6173
p(.) c(.) N	71.8984	7.4794	0.02266	0.0238	3	54.472
p(.) = c(.) N	71.9555	7.5365	0.02202	0.0231	2	56.6567
p(t) c(.) N	78.1949	13.7759	0.00097	0.001	12	39.3932
p(t) c(t) N	78.3501	13.9311	0.0009	0.0009	20	16.4071

Table 5: Comparison of survival estimates using top Pradel, POPAN, and Robust estimators for 2007-2011.

Model	Survival			
	Estimate	[SE]	Lower CI	Upper CI
Pradel: $\Phi(\cdot)$ $p(\cdot)$ $\Lambda(\cdot)$	0.6036649	0.0488893	0.5050625	0.6945053
POPAN: $\phi(t)$ $p(\cdot)$ $\text{pent}(\cdot)$	0.5986465	0.0481638	0.5017327	0.6884165
Robust: $S(\cdot)$ $p(t)=c(t)$ covar $N(t)$ Random Movement	0.6447038	0.0801373	0.4775475	0.7827133

APPENDIX

Table I: General animal encounter information from genetic study 2006-2011.

	2006	2007	2008	2009	2010	2011	TOTALS	Mean	St Dev
Total Samples									
Collected	171	202	221	254	241	255	1344	224.0	33.0
Total fisher samples	44	74	121	74	73	85	471	78.5	24.9
Poor DNA samples	10	14	33	8	6	11	82	13.7	9.9
Total Individual IDs	34	60	88	66	68	74	389	65.0	17.9
% Individual IDs	77.3%	81.1%	72.7%	89.2%	93.2%	87.1%	<i>n/a</i>	83.4%	7.7%
Total Animals Seen	22	25	32	28	26	28	161	26.8	3.4
New Animals	22	14	18	16	15	19	104	17.3	2.9
Cumulative New									
Animals	22	36	54	70	85	104	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>
Males	9	9	18	16	13	18	83	13.8	4.2
Females	13	16	14	12	13	10	78	13.0	2.0
Ratio M:F	0.69	0.56	1.29	1.33	1.00	1.80	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>

Protocols for Collecting and Storing DNA Samples
Excerpt from Rocky Mountain Research Station
Revised Version: September 6, 2005

Introduction: The use of genetics in wildlife biology has rapidly spread. However, to ensure that studies get the most out of their genetic data, samples must be handled and stored properly. Below is an attempt to synthesize the best ways to collect tissue for field operations. There are common elements in each approach that reflect the importance of sterility, labeling and preservation of the sample.

When stored correctly DNA can be extracted from tissues that are relatively old. When improperly stored there are several dynamics that can harm DNA. First, naturally occurring enzymes found in animal cells will begin to degrade the DNA hindering future analysis. Most of these harmful enzymes require an aqueous environment to function optimally. Therefore, the goal of tissue storage is to inhibit these enzymes often by drying or freezing the sample. Second, once the cell containing DNA is broken the DNA becomes subject to a harsh environment. Abusing the sample by exposing it to freeze/ thaw (even partial thaw) cycles or excessive heat will physically degrade, damage or sheer the DNA. Fortunately, there are ways to store all types of tissues that prevent chemical and physical degradation. Below we discuss the ways to store DNA allowing for optimal data to be generated from the sample.

Collecting Data and Labeling Samples: Sample collection is useless unless the samples are well labeled. The following data should be recorded for all samples on the field form and on the vial itself:

1. Collection location
2. Collection date
3. Sample number
4. Type of sample taken (ear plug, scat, hair, blood)
5. Collector name or initials
6. Animal Sex (if known)

Non-Invasive Samples – Hair: When collecting hair, the target tissue is often not the hair shaft, but the root cell attached to the base of the hair (the follicle). The root cell can often be seen by the naked eye, and appears as the white bulb at the end of many guard or thick hairs. Therefore, it is **important that the hairs are pulled not cut**, to capture root cells. When pulling hairs from an animal aim for a tuft approximately 20-50 hairs. Below are steps recommended for best treatment of hair follicles.

1. Prior to capture
 - a. Clean the forceps with the Ethanol and wipe thoroughly with the Kim-Wipes.
 - b. Label the vial.
 - c. Label a piece of Rite in the Rain paper with pencil.
2. Sample Treatment in the field
 - a. Put on latex gloves. Pull small tuft of hair preferably with a forceps (20-50 hairs).
 - b. Place the sample gently into the vial.
 - c. Fill the vial half way with silica desiccant (pre-fill the vials)
3. Sample Treatment back at the office
 - a. Check the silica desiccant. If the color indicator is used and the silica is the original blue color the samples are in good shape. If the silica is white, pink or gray the silica desiccant is water saturated and is no longer working to preserve the samples. If any moisture is apparent, replace the silica with fresh silica desiccant, but be gentle as the root cells are fragile.
 - b. Store samples in the desiccant at room temperatures out of direct sunlight.

Figure I: Handling protocol provided by Rocky Mountain Research Station to maximize probability of sufficient genetic return and amplification of hair from follicles attached to glue strips used in bait tunnels.

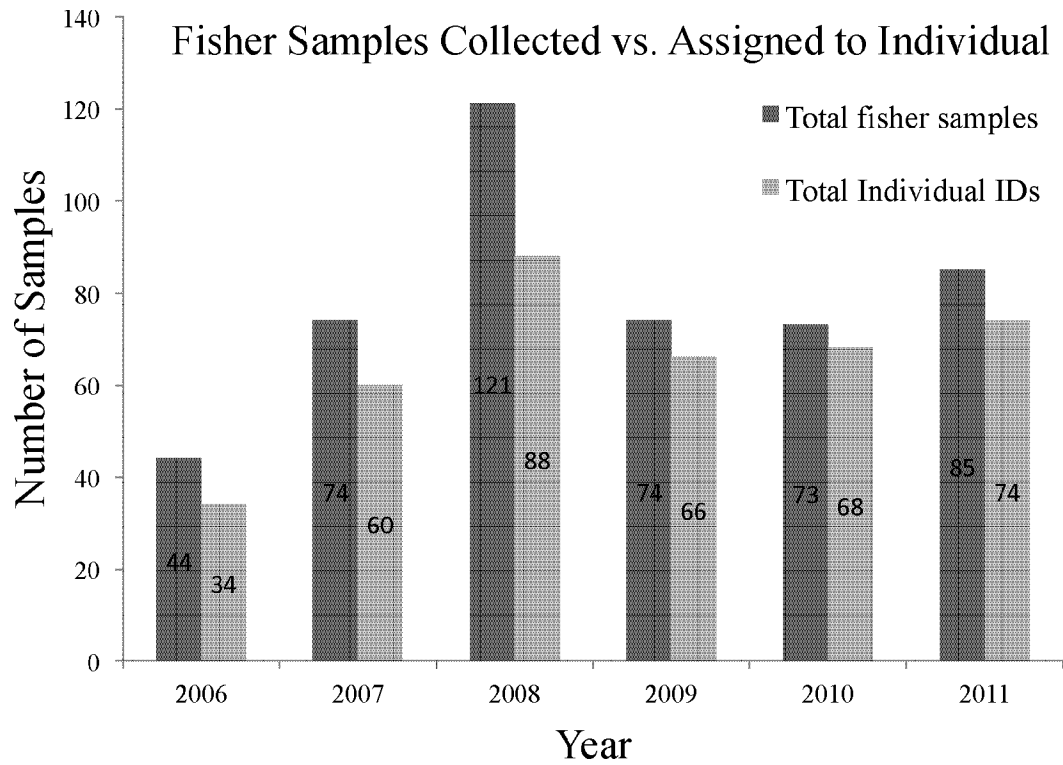


Figure II: Samples collected on the Eastern Klamath Study Area from 2006-2011 that can be assigned to the species *Martes pennanti* (fisher) or to an individual fisher.