# Fisher Ecology in the Sierra National Forest, California

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Abstract

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Fishers (*Martes pennanti*) experienced population declines throughout their range during the twentieth century. In California there is a ~400 km gap in their distribution, isolating fishers in the southern Sierra Nevada from populations in northern California. The fisher's conservation status in the southern Sierra Nevada is poorly understood, making management decisions about this species difficult. Fishers in this region are divided into at least 5 subpopulations separated by major river drainages. I compared two survey methods in the Kings River population to determine an effective strategy for monitoring fisher populations. I also used genetic data to explore the dispersal behavior of this species.

Using camera traps, I obtained estimates of demographic parameters for this population. Fishers in this region occur at lower densities than at other locations across their range, with only 10-11 animals / 100 $km^2$. Their annual adult survival rates (0.88) were comparable to those found in other studies, however there were wide confidence intervals around this estimate.

I used hair snares to perform a genetic tagging study on the same population. To complete this study, I developed 22 new microsatellite loci with samples from this population, northern California, Idaho, Minnesota, and Vermont. Only 6 of these loci were variable in the Kings River population, corroborating previous findings of the genetic isolation of fishers in the southern Sierra Nevada. I compared camera traps with hair snares for their efficacy in estimating population parameters. However, only 14 hair samples were identified as fisher hair, making estimation of demographic parameters using this method untenable.

I also used genetic methods to examine dispersal in this population. Based on the relationship between pairwise relatedness and geographic distance, I found that there was a slight difference between males and females in terms of their dispersal behavior. Overall, there was a decrease in pairwise relatedness at greater geographic distance. This relationship was slightly stronger in males than in females, suggesting greater male philopatry in this population. However, the strength of this relationship was not great. I recommend further studies of fishers, particularly at greater geographic scales, to more effectively address this question.

Chair _____ Date _____

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# Introduction

The fisher (*Martes pennanti*) is a carnivore that has experienced a significant reduction in its range over the past century, particularly in the western United States. Although it was once found throughout the North Coast, Cascade, and Sierra Nevada mountain ranges in California, it now exists in 2 isolated populations separated by over 400 km. This dissertation focuses on my efforts to develop population monitoring methods and to understand the ecology of the population that is restricted to the southern Sierra Nevada.

## FISHER NATURAL HISTORY

The fisher is a member of the family Mustelidae, which is a diverse group of carnivores with a worldwide distribution that is comprised of 65 species including weasels, skunks, badgers, otters, and martens (Nowak 1991). While there is some controversy regarding phylogenetic relationships within the family (and even the monophyly of the family itself), there is a general consensus that the genera *Gulo* and *Martes* form a monophyletic group (Sato et al. 2003, Marmi et al. 2004). Three species of this group can be found in North American: the fisher, the American marten (*Martes americana*), and the wolverine (*Gulo gulo*).

In the late Pleistocene, fishers were restricted to lower latitudes and elevations than they currently occupy (Graham and Graham 1994). Unlike American martens, however, there is no fossil record of fishers outside of the present-day eastern United

States, suggesting that they have colonized western North America relatively recently (Graham and Graham 1994). In recent history, the fisher was distributed across the entire length of Canada outside of the Plains region, while in the United States it was found in peninsular projections extending southward into the Northeast, Midwest, Rocky Mountains, Cascades, and Sierra Nevada (Gibilisco 1994).

There is a large difference in body size between the sexes of fishers; males are generally 1 m long and weigh about 4.5 kg, while females average 80 cm and weigh 2.0 kg (Powell 1993). Fishers exhibit intrasexual territoriality, where individuals defend a home range against members of the same sex, but there is considerable overlap between sexes (Johnson et al. 2000). These territories are maintained year-round except during the breeding season when males trespass on each other's territories while they search for receptive females (Leonard 1986, Arthur et al. 1989b).

**Reproductive behavior and life history**

Sexual dimorphism, such as that seen in fishers, is a trait often associated with polygyny in mammals (Kleiman 1977). Although fishers are assumed to exhibit a polygynous mating system, this has not been documented in the wild (Powell 1993). While researchers have observed cases of male-male aggression during the breeding season, no one has documented particular males monopolizing reproduction (Leonard 1986). Powell (1993) proposed that sex ratios of adults are roughly 50:50, although he admitted that this variable is difficult to measure in the wild because males are easier to trap than females.

Females give birth to a litter of 2 to 3 kits in the early spring. Mating occurs shortly after birth, although the fertilized eggs do not implant for 10 to 11 months. The father provides no parental care, while the female tends the kits for the first few months in a series of dens that are usually located in trees. At 2 to 3 months of age, juveniles become independent, though they remain on their mother's home range until they disperse when they are between 6 and 12 months of age (Arthur et al. 1993).

Dispersal distances ranged from 4 to 23 km in Maine, with males tending to disperse farther than females (Arthur et al. 1993). These short dispersal distances (relative to the size of an adult home range) were probably due to the fact that the study population was trapped, leading to more territorial vacancies. In contrast, 1 male dispersed approximately 100 km in a study in Massachusetts (York 1996). Using microsatellites, Aubry et al. (2004) determined females in southern Oregon were more related to each other than were males, suggesting females are more philopatric, which is a common pattern in mammals (Greenwood 1980, Handley and Perrin 2007).

**Population dynamics**

Estimates of population density vary across the fisher's range, with values ranging from 5 to 38 fishers / 100 $km^2$ (Powell 1993). Using radiotelemetry, Buck et al. (1983) estimated 31 fishers / 100 $km^2$ for a population in Northern California. A similar method was used by Garant and Crête (1997) in an untrapped population in Quebec, where they reported 27 fishers / 100 $km^2$. Using a combination of territory mapping and mark-resight with cameras, Fuller et al. (2001) calculated fisher densities

in north-central Massachusetts ranging from 19 to 25 individuals / 100 $km^2$, with some interannual variation and slightly higher estimates for camera captures. Two California studies using camera recapture methods yielded slightly lower density estimates: 12-17 fishers / 100 $km^2$ (M. Higley and S. Matthews, pers. comm.) and 8-17 fishers / 100 $km^2$ (J. Thompson, pers. comm.). One notable exception to the above results was a study in British Columbia that documented approximately 1 fisher / 100 $km^2$ (Weir and Corbould 2006). The variation among these estimates can be attributed to a number of factors, most importantly intrinsic differences among the populations, characteristics of the different study areas, the different methodologies used, and the inherent uncertainty associated with population estimates (Powell 1993).

Most studies of fisher population vital rates have been restricted to monitoring regional trends in commercial trapping data (Douglas and Strickland 1987), although a few more detailed studies have been conducted. Krohn et al. (1994) reported that during the non-trapping season, adult fishers had average mortality rates of 0.11 (95% CI: 0.01-0.19) in a commercially exploited population in Maine. Juvenile mortality rates were 2-3 times higher and more variable than rates for adults. Estimates of survival from the proportion of radiocollared females that survived from 1 year to the next on the Sequoia National Forest in the southern Sierra Nevada were low, averaging 0.61 (Truex et al. 1998). These survival estimates were used in a population viability analysis that predicted negative population growth for this population for all but the most optimistic combination of parameter values (Lamberson et al. 2000).

**Habitat and dietary requirements**

Relative to many other North American mammals, the fisher appears to be a habitat specialist (Buskirk and Powell 1994). They are associated with late successional forests with continuous canopy closure (Powell and Zielinski 1994, Carroll et al. 1999, Zielinski et al. 2004a), although they may use a variety of forest types for different activities. In the Sierra Nevada, fisher habitat typically consists of ponderosa pine and mixed conifer forest types, with fairly dense canopy cover and large trees (Zielinski et al. 2004a). Throughout their range, they have been observed to forage in areas of early- to mid-successional vegetation, as a vegetated understory and large woody debris appear important for prey species (Arthur et al. 1989a, Zielinski et al. 1999). However, they appear to be limited to old-growth, continuous canopy forests for resting and denning (Buskirk and Powell 1994), selecting rest structures in the southern Sierra Nevada near riparian areas and with large trees, high canopy closure, and many old forest elements including snags and downed woody debris (Zielinski et al. 2004b, Zielinski et al. 2006b). Fishers use large diameter trees and snags as rest sites, and suitable resting and denning sites may be limiting.

Fishers' primary prey throughout most of their range are snowshoe hares (*Lepus americanus*) and porcupines (*Erethizon dorsatum*; Powell 1993). However, they are considered dietary generalists and have been documented to eat a variety of small birds, carrion, and fruit (Arthur et al. 1989a). Their 2 primary prey species are either absent entirely or present in very low densities in the southern Sierra Nevada. This has resulted in a more generalized diet with an increased consumption of small mammals, such as squirrels (Sciuridae) and mice (*Peromyscus spp.*), as well as other

small vertebrates, particularly lizards (Zielinski et al. 1999). In this same study, over 20% of scats contained some plant matter, primarily manzanita berries (Ericaceae *Arctostaphylos spp.*).

**Population genetics**

Fishers have been widely reintroduced throughout their range, primarily for control of porcupines as well as for population recovery (Cook and Hamilton 1957, Berg 1982, Vinkey et al. 2006). Many populations of fishers show genetic signatures of reintroductions (Williams et al. 2000, Drew et al. 2003, Vinkey et al. 2006), and these populations generally have lower genetic diversity than corresponding source populations (Kyle et al. 2001). Fisher reintroductions have been neither as common nor as successful in the western United States. One population in southern Oregon was the result of a reintroduction, and it is isolated from other populations in the Klamath province (Aubry and Lewis 2003).

Genetic diversity and population structure vary across the fisher's range. Allozyme heterozygosity in fishers in the Northeast and Midwest United States is similar to that in other mustelids (Williams et al. 1999). However, fishers show greater levels of genetic structuring than both American martens and wolverines across their range, suggesting greater fragmentation in their distribution (Kyle et al. 2001).

Western populations of fishers have lower levels of genetic variation than their eastern counterparts (Drew et al. 2003), and there is a high level of structure at microsatellite loci in this region, suggesting low connectivity among extant

populations (Wisely et al. 2004). Fishers have particularly low genetic diversity in the southern Sierra Nevada. In the population that I studied, fishers possess only 1 mitochondrial haplotype (Drew et al. 2003) and have the lowest levels of microsatellite diversity among western populations (Wisely et al. 2004).

## CONSERVATION AND MANAGEMENT

### Range reduction

Throughout the twentieth century, fishers experienced significant range reductions across North America, which have been primarily attributed to trapping, logging, and other factors such as porcupine poisoning campaigns, road building, and increased recreational use of wildland ecosystems (Powell and Zielinski 1994). Widespread farm abandonment leading to forest regrowth, coupled with reintroductions throughout the Midwest and Northeast have led to substantial recoveries of fisher populations in these areas (Powell 1993). They have recovered to such an extent that they are now a harvested furbearer in many states and provinces.

In California, fishers once occurred throughout forested regions of the Klamath Mountains, North Coast Ranges, southern Cascades, and Sierra Nevada (Grinnell et al. 1937). In spite of a ban on trapping enacted in 1946, the species has experienced a substantial reduction in geographic range in the state and currently occurs as 2 disjunct populations, one occupying the Klamath Mountains and Coast Ranges, the other occurring in the southern Sierra Nevada (Zielinski et al. 1995). Recent surveys have confirmed that fishers were extirpated from an area of the Sierra Nevada mountains between Shasta County and Yosemite National Park (Zielinski et al. 2005). This gap

in the fisher's distribution of approximately 430 km has isolated a population inhabiting the southern Sierra Nevada from fishers in the northwest corner of the state, as this distance far exceeds the fisher's longest recorded dispersal distance of 100 km (York 1996). The southern Sierra Nevada population of fishers probably exists as 5 subpopulations, each separated by major river drainages (R. Truex pers. comm.).

**Management status**

In California, both state and federal government agencies are responsible for managing fisher populations. The State of California regards the fisher as a "species of concern." In 1990 and again in 1994, the U.S. Fish and Wildlife Service (FWS) received petitions to list western populations of the fisher under the U.S. Endangered Species Act. Both of these petitions were turned down because of "insufficient scientific information" to recommend listing (59 *Federal Register* 65884-65885). In November, 2000, the populations of the Pacific states were again proposed for listing as federally endangered in light of new information describing their fragmented distribution in this region (Center for Biological Diversity 2000). The FWS ruled that listing the fisher as federally endangered was "warranted, but precluded" due to a lack of resources to proceed with the listing process (69 *Federal Register* 18770).

Because most of the fisher's current range in California occurs on land managed by the U.S. Forest Service, this agency is the other primary stakeholder for the federal government in the state with respect to fisher management. In California, the Forest Service lists the fisher as a "sensitive species" (Macfarlane 1994), obliging the agency to prevent it from becoming federally threatened or endangered as well as

minimizing impacts on fishers in individual forest plans (16 U.S.C. 1604). A court case at the end of the twentieth century [Sierra Club v. Martin (168 F. 3rd 1, 1999)] further emphasized this role of the Forest Service, stating that it is responsible for inventorying and monitoring trends in the abundance of proposed, endangered, threatened, sensitive, and management indicator species.

## THE KINGS RIVER FISHER PROJECT

In 1993, the Forest Service initiated an adaptive management study on the west slope of the southern Sierra Nevada in the Sierra National Forest, Fresno County, CA, with the objective of studying management practices that could enhance the development of late successional forest characteristics and their associated wildlife populations (Verner and Smith 2002). This study has evolved into the Kings River Project (KRP), which seeks to restore the forest in the study area to pre-1850 conditions within an adaptive management framework (USDA 2006). Research on fishers began in 1995 with the goal of understanding their habitat requirements and population ecology within the study area (Boroski et al. 2002, Mazzoni 2002). I joined the study in 2000, at which point the focus shifted from radiotelemetry and describing resting and denning habitat to population monitoring.

My study was conducted in a 317 $km^2$ area and covered most of the extent of the Kings River fisher population, which is 1 of the 5 southern Sierra Nevada subpopulations of fishers. This population is bounded by the San Joaquin River to the north and the Kings River to the south. The study area covered an elevation gradient (1110 to 2282 m) corresponding to fisher occurrence in the region (Jordan et al. 2002),

and included a mix of public and private land. It lay mostly within the boundaries of the KRP. The most significant private land holder within the study area boundaries was Southern California Edison, a utility company. The predominant forest cover types in this area are Ponderosa Pine and Sierran Mixed Conifer (Mayer and Laudenslayer 1989).

## OBJECTIVES AND OUTLINE OF THIS DISSERTATION

We have data documenting a contraction in the distribution of fishers in California from its historic extent (Zielinski et al. 2005). However, these ongoing regional surveys are designed to detect statewide declines in abundance (Zielinski and Stauffer 1996), so we lack information about population dynamic processes occurring at the scale of forest management activities. More intensive studies are needed to validate these surveys at a local scale. Because it occurs near the northern end of the southern Sierra Nevada population, the Kings River population is crucial to the recolonization of the fisher's former range in the Sierra Nevada. I chose to focus on the dynamics of this subpopulation using noninvasive, capture-recapture methods.

The primary goals of this study were to develop different methods for noninvasively monitoring fishers and to obtain estimates of density and adult survival. I tested the efficacy of 2 capture-recapture methods: camera traps and hair snares with genetic tagging. Chapter 1 describes the camera trapping method and presents the resulting demographic parameter estimates for this population.

Prior to engaging in a genetic tagging study of fishers, I needed to develop genetic markers that could be used to individually identify fishers from samples

captured at hair snares. I describe the development of these markers in Chapter 2. In Chapter 3, I apply these markers to samples collected at hair snares and compare this method of population monitoring to camera traps.

In Chapter 4, I use genetic markers to look at dispersal within the Kings River fisher population, with emphasis on sex-based differences in dispersal in this species. In the final chapter (5), I bring together the monitoring methods comparison and dispersal study to make recommendations for future studies of fishers and to describe management implications for the species in this region.

# Chapter 1

## Monitoring fishers (*Martes pennanti*) using camera traps

### INTRODUCTION

Reliable demographic parameters are critical for effectively managing species of conservation concern (Skalski et al. 2005). Without detailed demographic data about a species, it is difficult to proceed with management actions. Wildlife surveys can be used to obtain valuable information about a sensitive species, such as its distribution (Zielinski et al. 2005), its presence or absence in particular habitats (MacKenzie et al. 2002), or its relative abundance among sites (Pollock et al. 2002). However, these studies are often insufficient for obtaining the detailed demographic information needed at the local population level (Gibbs 2000, Pollock et al. 2002), which requires more intensive methods such as capture-recapture (Seber and Schwarz 2002).

Capture-recapture studies are commonly used to obtain population parameter estimates (Otis et al. 1978, Pollock et al. 1990, Seber and Schwarz 2002). In these studies, animals are captured, given some identifying mark, and released. Subsequent sampling periods occur in which animals are either recaptured or resighted. The proportion of marked animals in this sample can be used to estimate abundance, and

the capture histories of individuals can provide estimates of population vital rates like survival and recruitment.

Camera traps are a good alternative to many traditional capture-recapture methods because cameras can detect animals at all times of day and night and do not need to be checked daily like a live trap (Kucera et al. 1995, Sanderson and Trolle 2005). Camera trapping can be used for presence/absence surveys (e.g. Zielinski et al. 2005). This technique can also be used to obtain demographic information in a capture-recapture framework when the study organisms have some form of individually identifying mark, either applied by biologists [e.g. ear tags on grizzly bears (*Ursus arctos*); Mace et al. 1994] or a naturally occurring, unique pelage or coloring pattern [e.g. stripes of tigers (*Panthera tigris*); Karanth et al. 2006].

One approach to estimating population parameters with camera traps is to use a sampling technique generally referred to as a "marking and sighting experiment" (Arnason et al. 1991). In these studies, a group of animals is captured during an initial marking phase and given distinguishing marks, such as ear tags or radio transmitters. The animals are then resighted using a different "capture" technique, but are generally not physically handled again. The resighting method can be any type of sighting, such as telemetry locations (White and Garrott 1990), band sightings (Arnason et al. 1991), or cameras (Mace et al. 1994). This type of capture-recapture sampling (hereafter referred to as "mark-resight") differs in several important ways from traditional mark-recapture sampling. From a planning standpoint, these studies can be less labor intensive because resighting often requires less effort than initial capture and handling. Because the animals are not physically restrained during resighting, the risks to

individual animals are reduced (Minta and Mangel 1989). Also, because the capture and resighting phases of the study use different techniques for "capturing" the animal, the risk of a behavioral response to trapping that affects recapture rate is reduced (Otis et al. 1978, Minta and Mangel 1989). Finally, depending on the resighting method used, additional data can be obtained such as movement and location information or activity times.

This chapter describes the development of a camera trapping protocol for mesocarnivores, using the fisher (*Martes pennanti*) as a model organism. The fisher was chosen because of concern over its status in California (Introduction). Its range has been greatly reduced in the state, and it now exists in 2 isolated populations separated by over 400 km (Zielinski et al. 2005). To verify the gap in fisher populations in California as well as to monitor trends in abundance of a variety of mesocarnivore species in the state, the U.S. Forest Service has been conducting baited track plate and camera surveys throughout the Sierra Nevada since 1996 (Zielinski et al. 2005). These regional surveys provide good presence/absence data for carnivore species of interest, and are designed to monitor changes in distribution and regional declines in abundance (Zielinski and Stauffer 1996). However, there is currently no way to relate the number animal detections at 1 of these sample locations to the number of individuals occupying the surveyed area (Zielinski et al. 2005). Consequently, we do not presently have information about densities or vital rates of fishers at the scale of forest management activities.

The goal of this study was to conduct an intensive, capture-recapture study using camera traps to estimate population parameters (density, survival, and

reproduction) for a local population of fishers. These data can be used to inform management decisions about fishers in the area and to point the way toward methods for validating the Forest Service's regional survey data with local population data.

## STUDY AREA

The study was conducted in the Kings River region of the southern Sierra Nevada. I divided the study area, which I describe in detail in the Introduction, into a trapping grid composed of 317 1 km × 1 km cells (Fig. 1-1). Three of the 317 potential cells were not used. One cell was not trapped because it was entirely within private land to which I did not have access, and 2 more cells were unused because they contained a busy campground and private summer cabins. I placed a live or camera trap into each of the remaining 314 cells (see below for details).

## METHODS

### Live trapping

Live trapping was conducted in July and August in 2002-2004. I also collected pilot data from live trapping in 2000 and 2001 over a smaller part of the study area associated with a radiotelemetry study of this population (Mazzoni 2002). One trap was placed in every other cell within the trapping grid. I attempted to place stations near the center of a given cell, though this was not always practical. Important microhabitat characteristics in trap site selection within a cell included high sawlog density (trees with > 60 cm dbh), proximity to a stream (or dry watercourse), high canopy cover, and downed woody debris. These characteristics have been shown to

be important features of habitats used by fishers in the Sierra Nevada (Mazzoni 2002, Zielinski et al. 2004a). Live traps were built by attaching Tomahawk collapsible single-door live traps (Model 207, 32” × 10” × 12”, 81.3 cm × 25.4 cm × 30.5 cm; Tomahawk Live Trap Co., Tomahawk, WI, USA) to a plywood box (Wilbert 1992). I baited traps with a piece of raw chicken securely tied to the trap and a commercial lure (“Gusto”; Minnesota Trapline Products, Pennock, MN, USA) poured onto a nearby tree or log. Each trap was open for 8 nights and was checked daily.

Because of limited resources and personnel, I did not have traps open over the entire study area during each trapping session. Instead, I divided the study area into 4 regions, using ridges between watersheds and other natural barriers to attempt to isolate each region as best as possible. I then trapped each region sequentially, starting in the northeast of the study area. After 8 trap nights, the traps were collected and moved to the next region. All traps within a region were baited and opened on the same day. I used the same rotation of trap locations every year.

I processed all live-caught fishers the first time they were captured each year. Processing consisted of taking a series of morphological measurements (see below) and marking animals. If a fisher was already marked, but had not yet been processed that year, I still sedated it and took the morphological measurements. Fishers that were processed were coaxed into a metal handling cone and sedated with a Ketamine hydrochloride and Diazepam mixture (1 mg Diazepam / 200 mg Ketamine) injected intramuscularly at a dosage of 11-24.2 mg Ketamine/kg of estimated body weight. Animals were sexed, aged, weighed, and I took a standard set of measurements: total length (cm), tail length (cm), hind foot length (cm), and ear length (cm) (Jameson and

Peeters 1988). They were also examined for injuries and ectoparasites. I measured the size (width × height) of anterior nipples ($mm^2$), which has been shown to indicate reproduction in the preceding spring (Frost et al. 1999).

Fisher pelage is not distinct enough to distinguish individual animals, so live-trapping and marking with uniquely colored ear tags was an integral part of the study design. I double-marked every fisher to reduce the likelihood of the complete loss of tags, one of the critical assumptions of capture-recapture studies (Pollock et al. 1990). Each fisher received an implanted passive integrated transponder (PIT) microchip tag (125 kHz, TX 1405L; Biomark, Boise, ID, USA) in the nape of the neck for permanent and unique identification. A unique combination of colored ear tags and reflective tape (Colored Rototag; Dalton Group Limited, Dalton House, Nettlebed, Oxfordshire, England) was fastened to each ear to identify animals resighted at camera stations. I estimated loss rate of ear tags by determining the proportion of fishers caught more than once and at least 1 year apart that had lost their ear tags.

**Camera trapping**

Camera recapture followed live trapping each year of the study. I used dual sensor remote camera systems (Trailmaster Trail Monitor, Model TM 1550; Goodson and Associates, Inc., Lenexa, KS, USA) to trigger a 35-mm camera when an infrared beam was broken (Kucera et al. 1995). The camera trap consisted of a corrugated plastic box (32” × 10 ¼” × 10 ¼”, 81.3 cm × 26 cm × 26 cm) attached to a camera with an infrared trigger oriented so that the infrared beam crossed the entrance of the box (Fig. 1-2). I placed camera traps in cells adjacent to those used for live trapping,

following the same criteria for placement within a cell as for live trapping. I baited the stations with raw chicken and a commercial lure, and they were deployed for 12 days and checked every other day.

Similar to live trapping, I did not have enough resources to deploy all of the camera traps simultaneously. For this phase of trapping, the study area was divided into 3 regions, which were trapped sequentially. Traps were moved at the end of each 12-night shift and then baited and reopened together. I subdivided the camera traps so that half of the traps within a region were opened on the first day of a trapping session, and the other half on the second day. Because they were checked every other day, I was able to double the number of traps being checked with the available personnel. As with live trapping, the same rotation pattern of trap locations was used each year of the study. In 2002, however, I did not place camera traps on the eastern edge of the study area beyond Patterson Mountain.

**Passive infrared cameras**

I placed passive infrared-triggered cameras (Trailmaster Trail Monitor, Model TM550; Goodson and Associates Inc.) outside of camera traps to estimate trap permeability, or the proportion of fishers that approached camera traps that would actually enter and trigger them (Zielinski et al. 2006a). These were set up to photograph any animals that approached the station, regardless of whether or not they entered the trap. Animals captured in this manner were not included in the population parameter estimation.

Unlike the active sensor used in the camera trap itself, the passive device was triggered by heat or motion within a broad area covered by the sensor. I placed them outside 30 camera traps during 2003 and 2004 (19% of traps) and set the passive sensors to detect animals in an approximately 2 m radius of the trap, centered on the trap entrance. The specific area covered varyied depending on site-specific characteristics such as the presence of woody debris and the availability of places for mounting the passive camera.

**Density estimation**

Traditional capture-recapture models assume that each unmarked animal that is captured is marked and then available for capture as a marked animal in subsequent capture sessions (Otis et al. 1978, Seber 1982). That is not the case for mark-resighting studies where all marking occurs prior to the resighting phase. Therefore, different parameterizations of the models that estimate abundance are necessary. The Bowden estimator (Bowden and Kufeld 1995) is an analytic estimator of abundance from mark-resighting data. It does not restrict resighting events to discrete trapping sessions, so I pooled all resighting events within each year for abundance estimation. Furthermore, the unmarked animals that are seen during resighting do not need to be individually identified with this estimator. Any animals that had ear tags from previous years, but were not captured during that year's marking phase, were treated as unmarked when estimating abundance. I estimated abundance and its 95% confidence intervals from photo data for each year of the study using the Bowden estimator implemented in Program NOREMARK (White 1996).

I wrote an ANSI-C simulation to study the impact of 2 potential violations of the assumptions of the abundance estimator: unidentifiable fishers and tag loss. Unidentifiable fishers were those for which I could not determine if they were marked or not. Typically this occurred when an animal had its head outside of the box when it triggered the camera trap. The simulation examined the effect on the abundance estimate of 2 different strategies for dealing with these unidentifiable animals: 1) count them as unmarked, or 2) exclude them from the analysis. I varied the population size, the proportion of the population marked, the probability that a marked animal would be resighted, and the proportion of captures that were unidentifiable (Table 1-1). Each combination of parameter values was simulated 1000 times.

I also simulated the effect of ear tag loss between the marking and resighting phases. The simulation allowed from 1 to 4 fishers to lose their tags each year and assumed that all captured fishers were identifiable. Fishers that lost their tags were counted as unmarked. Like the other simulation, I modified the population size, the proportion of the population marked, and the probability that a marked animal would be resighted (Table 1-1), and each combination of parameter values was run 1000 times.

Estimating density simply by dividing the estimated abundance by the size of the study area can introduce errors into the estimate because animals residing near the perimeter of the study area may have home ranges that extend beyond the edges of the trapping grid (Otis et al. 1978). Consequently, the size of the study area needs to be corrected for this edge effect. Traditionally this is done by adding a buffer strip around the perimeter of the trapping grid. I set the buffer width to the average radius

of a male fisher home range in the Kings River area (Mazzoni 2002), an approach that has been traditionally used for this correction (Dice 1938, Parmenter et al. 2003). After buffering the study area, I calculated the effective sampling area by truncating this buffered region to include only elevations between 1200 and 2300 m, which roughly corresponded to the elevational band occupied by fishers in the region (Jordan et al. 2002). All buffering and area estimation was conducted in ArcGIS 8.1.

Male home ranges in the Kings River area had an average radius of 2.64 km based on a 100% minimum convex polygon (Mazzoni 2002). Using this as a buffer and truncating for elevation produced an effective sampling area of 367 $km^2$ in 2002 and 430 $km^2$ in 2003 and 2004. Because I used data from a radiotelemetry study to estimate home range size, the calculation of effective study area does not suffer from some of the theoretical limitations inherent in calculating effective study area when using trapping data, such as the mean maximum distance between captures (Parmenter et al. 2003). I calculated density by taking abundance estimates for each year, then dividing these point estimates and the upper and lower bounds of their confidence intervals by the estimate of the effective sampling area. They were then normalized to estimate the number of fishers per 100 $km^2$.

**Survival rate estimation**

I combined live and camera capture data for all fishers from 2000-2004 to obtain a capture history for each individual. These data were then fitted to models that jointly estimated survival and capture rates for each individual (Lebreton et al. 1992, White and Burnham 1999). Candidate models allowed survival to vary by sex, year,

or an interaction between the two. I allowed recapture rate to vary by either sex or year but did not model an interaction between these parameters.

The model that best fit the data was chosen based on information-theoretic criteria (Burnham and Anderson 1998). I assessed the goodness of fit and level of over-dispersion in the data with the parameter median-$\hat{c}$, which itself was estimated using a logistic regression method (White 2002). All model selection and parameter estimation was performed in Program MARK (White and Burnham 1999).

## RESULTS

### Live trapping

I define an active trap night as one that was not lost to some form of disturbance, the most common of which were damage by black bears (*Ursus americanus*) and the trap being closed with no animal inside it. Over the 5 years of the study, I had 5590 live trap nights, 800 (14.3%) of which were lost to disturbance, leaving 4790 active live trap nights (Table 1-2). Although I could not count exactly how many trap nights I lost to bears, I estimated this number based on circumstantial evidence such as traps that were rolled away from the site and damaged. I tabulated that bear damage accounted for approximately 35% of the total number of lost trap nights, or ~5% of all trap nights.

I caught 15 mammal species in live traps (Table 1-3), and I caught fishers on between 1.0% and 2.3% of trap nights from 2000-2004 (Table 1-4). Of the non-fisher carnivores, ringtails (*Bassariscus astutus*) were the most frequently captured after fishers (Table 1-5). These data do not include occurrences of black bear disturbance

of sites, which exceeded the capture rate for fishers. The lone recorded capture of a black bear inside a live trap was of a bear cub in 2004. Fisher captures covered nearly the entire elevational range of available traps, however most fishers were caught at elevations below 1800 m (Fig. 1-3).

From 2000 to 2004, females were more commonly caught than males with the exception of 2002 (Fig. 1-4). The distribution of mean anterior nipple sizes among female fishers ranked by nipple size showed 2 groups (Fig. 1-5). The curve of this distribution shows an inflection around 10 $mm^2$, so I chose this as a cutoff between breeders and nonbreeders. However, because there is some overlap between animals that bred that year and animals that did not breed that year but had bred before (Frost et al. 1999), I excluded all animals with nipple sizes between 10 and 20 $mm^2$ from the analysis of reproductive behavior. Based on the criterion of nipple size greater than 20 $mm^2$, reproduction rate was highly variable throughout the study (Fig. 1-6). Across the 5 years of the study, annual reproductive rate was 0.44 (95% CI: 0.26-0.62).

**Camera trapping**

Times between photographs of fishers at camera traps were heavily skewed toward either short gaps (<1 h) or long ones (> 24 h). I often obtained multiple photos of the same visit of a particular animal. For calculating capture rates of all species and capture histories of fishers, I needed to determine a sufficient interval between photos to determine if a given photograph counted as a distinct capture event. To determine an appropriate minimum interval between photos, I examined data for fishers of known identity. Of the 11 occasions where more than 1 fisher visited the same trap

during a trapping session, the shortest time between captures of different individuals was 2 h 39 min ($\overline{X}$ = 49 h 58 min, SE = 49 h 45 min), so I chose 2 h as a minimum cutoff time between photos. I also used this time to calculate capture rates for other species.

I caught 18 mammal species at camera traps, including representatives of all species captured in live traps (Table 1-3). I obtained photographs of unidentifiable weasels that were either ermine (*Mustela erminea*) or, more likely, long-tailed weasels (*M. frenata*), which I counted separately from other photos of *M. frenata*. Additionally, I obtained photographs of chipmunks (*Tamias sp.*) that were not identified to species. Capture visit rates of fishers were considerably higher with camera traps than live traps (Fisher's exact test: $P < 0.001$). Elevation ranges of camera trap captures of fishers were similar to those for live-trapping ($P = 0.55$; Fig. 1-3). Among carnivores, black bears were the only species photographed more frequently than fishers (Table 1-6), accounting for 24% of all camera captures. However, in most of these cases, they disabled the station. I attributed 64% (346 out of 538) of lost camera trap nights to bear damage.

**Passive infrared cameras**

For 2003 and 2004 combined, I had 22 captures of fishers at passive infrared camera stations. Of these, 14 (64%) also resulted in a capture inside a camera trap. An additional 9 captures were recorded by active cameras inside camera traps that were not detected by the passive trap.

**Density and survival**

Over 3 years of camera trapping, 22 out of 226 (9.7%) captures were unidentifiable. Simulations showed that bias was negligible when excluding unidentifiable animals from the analysis over all parameter values, whereas abundance was overestimated by approximately 10% when these animals were counted as unmarked. Consequently, all subsequent analyses exclude these unidentifiable animals.

The abundance estimates for 2002-2004 were 49, 41, and 43 fishers respectively (95% CI: 28-89 [2002], 24-73 [2003], 29-62 [2004]). In 2002, I did not set out camera traps in the eastern end of the study area beyond Patterson Mountain, so abundance and density estimates are based only on animals caught in live traps in the rest of the study area. When dividing these estimates by the effective sampling area for each year, I obtained density estimates of 13.4, 9.5, and 10.0 fishers per 100 $km^2$ in 2002-2004 respectively (95% CI: 7.6-24.2 [2002]; 5.6-17.0 [2003]; 6.7-14.4 [2004]).

Tag loss could not be estimated directly from camera trapping data because there was no way to distinguish in a photograph between a fisher that had never been marked and one that was marked and lost its tags before recapture, so I estimated the actual rate of tag loss from the live capture data. Out of 9 fishers that I caught in live traps more than once and at least 1 year apart, 4 had lost their ear tags by the following year. Assuming that tags are lost at a constant rate, this suggests a rate of 3.7% of fishers losing their tags every month, or approximately 1-2 fishers losing tags in the 2 months between live capture and camera recapture each year. For the range of

abundance estimates and marking and resighting rates observed in this study, the simulations indicated that the abundance estimate was biased upward by approximately 10% if 2 fishers lost their tags before camera trapping.

A goodness of fit for the global model to estimate survival rates indicated a small degree of over-dispersion in the data (median-$\hat{c}$ = 1.21). As a consequence, I assessed model fit with the test statistic $QAIC_c$, which is a correction of Akaike's Information Criterion that accommodates over-dispersed data (Burnham and Anderson 1998). The best fitting model was the one that held survival and recapture rate constant between males and females and across years (Table 1-7). There was slight evidence for a difference between sexes, as the next 2 most well-supported models allowed 1 or the other parameter to vary by sex, with each model accounting for ~16% of the observed variability. Models containing an interaction between sex and year on survival rate were poorly supported. Averaging parameter estimates across all models weighted by their quasi-Akaike weights (Burnham and Anderson 1998) yielded estimates of survival around 0.88 (largest 95% CI: 0.50-0.98) and of recapture rate around 0.5 (Table 1-8). I also averaged the parameter estimates for the top 3 models only, which yielded estimates similar to those obtained from the full model set (Table 1-8).

## DISCUSSION

### Efficacy of camera traps

Camera traps had higher capture rates for fishers than live traps, and I was able to combine the 2 methods to obtain demographic estimates for fishers in the Kings

River area. I estimated density of approximately 11 fishers / 100 $km^2$. However, based on computer simulations of the tag loss process, these numbers may be biased upward by ~10%, resulting in an approximate density estimate of 10 fishers / 100 $km^2$. I also estimated annual survival rates for adults of 0.88, although these had wide confidence intervals. Based on the model selection approach, there was slight evidence for a sex-based difference in survival, but this difference did not yield different point estimates.

These methods are not limited in their utility to fishers. While fishers accounted for nearly one quarter of total camera captures, over half of the camera captures were of other carnivores. In particular, grey fox (*Urocyon cinereoargenteus*), ringtail, American marten (*Martes americana*), and spotted skunk (*Spilogale gracilis*) were commonly captured mesocarnivores using this method (Table 1-6). By making minor modifications to the lure and bait as well as trapping in different habitats, other species of mesocarnivore could be surveyed in this manner.

**Practical considerations**

In general, these traps were effective in delivering density and survival estimates for a wide-ranging, cryptic carnivore. However, there are certain practical issues to consider before planning a survey such as this. The first concern for many managers will be the cost. Each station requires a one-time capital expenditure to buy the infrared device and camera, which is higher than the cost of many other types of survey devices like live traps or hair snares. The operating costs for bait, film, minor repairs, and film developing were comparable to the equivalent costs for live trapping,

although some of these costs might be reduced as digital cameras designed for this purpose become more widespread in the marketplace. At the outset of this study, there were not mass-marketed, active infrared, digital camera systems available. Finally, labor costs should be considered; this study employed 4 people full time for 4 months every year.

One important consideration when evaluating density estimates obtained from camera trapping is the impact of ear tag loss. Because fishers do not have distinctive pelage patterns, I needed to individually mark each animal beforehand. Based on the simulation results, it is possible that actual densities reported are 10% lower than those estimated by this study. This effect may be an important consideration when reporting the results of this sort of study. Future research should examine the use of more permanent marks for camera resighting. One potential remedy for this is to use a recapture device that can read PIT tags (M. Higley, pers. comm.), which are implanted and thus unlikely to be lost. These data could be analyzed in a similar manner to camera trapping data.

Passive camera traps showed that roughly 2 in 3 (64%) fisher visits to a camera station resulted in a capture at the camera trap. This shows that fishers are relatively willing to enter the camera station to receive a bait reward. However, trap permeability could be increased. I placed strands of barbed wire across the entrance to each camera station as part of a parallel study of the efficacy of hair snares for genetic monitoring of fisher populations (Chapter 3). If these wires were not in place, it is possible fishers would have been more likely to enter the box. I did not set out any

traps without the barbed wire, so I do not have data on the effect of the wire on trap permeability.

A final issue is the problem of black bear damage to traps. In most cases of bear visits to camera traps, the bears disabled the station, and most lost trap nights could be blamed on damage caused by bears. Although it was often possible to repair the stations in the field, this was still a significant loss of trapping ability. This is an unavoidable annoyance, but it is one that should be acknowledged. I recommend incorporating a loss rate of 10-15% of trap nights for any power analysis conducted prior to commencing this sort of study. One way to avoid bear damage would be to trap during the winter when bears are not active.

## MANAGEMENT IMPLICATIONS

Estimates of fisher density vary in the literature (see Introduction for a review), which can be attributed to a number of factors, most importantly characteristics of the different study areas, the different methodologies used, and the inherent uncertainty associated with population estimates (Powell 1993). However, the 10 fishers / 100 $km^2$ estimate found in the Kings River population is lower than almost all of these published estimates. This suggests that the habitat in the Kings River region is not capable of supporting as dense a population of fishers as other areas. This finding underscores the importance of region-wide planning for fisher conservation. The relative sparseness of fishers in the southern Sierra Nevada increases the species' susceptibility to demographic events that could lead to extinction and limits the

species' ability to recolonize its historic range in the central and northern Sierra Nevada.

The point estimate of survival for this study is comparable to previous work (reviewed in Introduction), although there have been comparatively few studies estimating this parameter in fishers. The estimate reported here suggests that the population may be stable, however the uncertainty around the estimate makes it very difficult to make projections of future population trajectory with confidence. The intensive sampling period of this study combining live and camera traps was only 3 years, and longer-term studies are needed to make more precise parameter estimates.

Rangewide surveys of fishers in California have provided timely information about this species' status in the state (Zielinski et al. 2005). However, it is critical that we develop a more comprehensive understanding of what is happening at the population level. Because forest management activities take place on this smaller scale, adaptive management studies that explore the relationship between management actions and fisher population parameters are much needed. I have developed a methodology that can be used in the future to monitor the vital rates of fisher populations and that can generalized to other populations of forest carnivores.

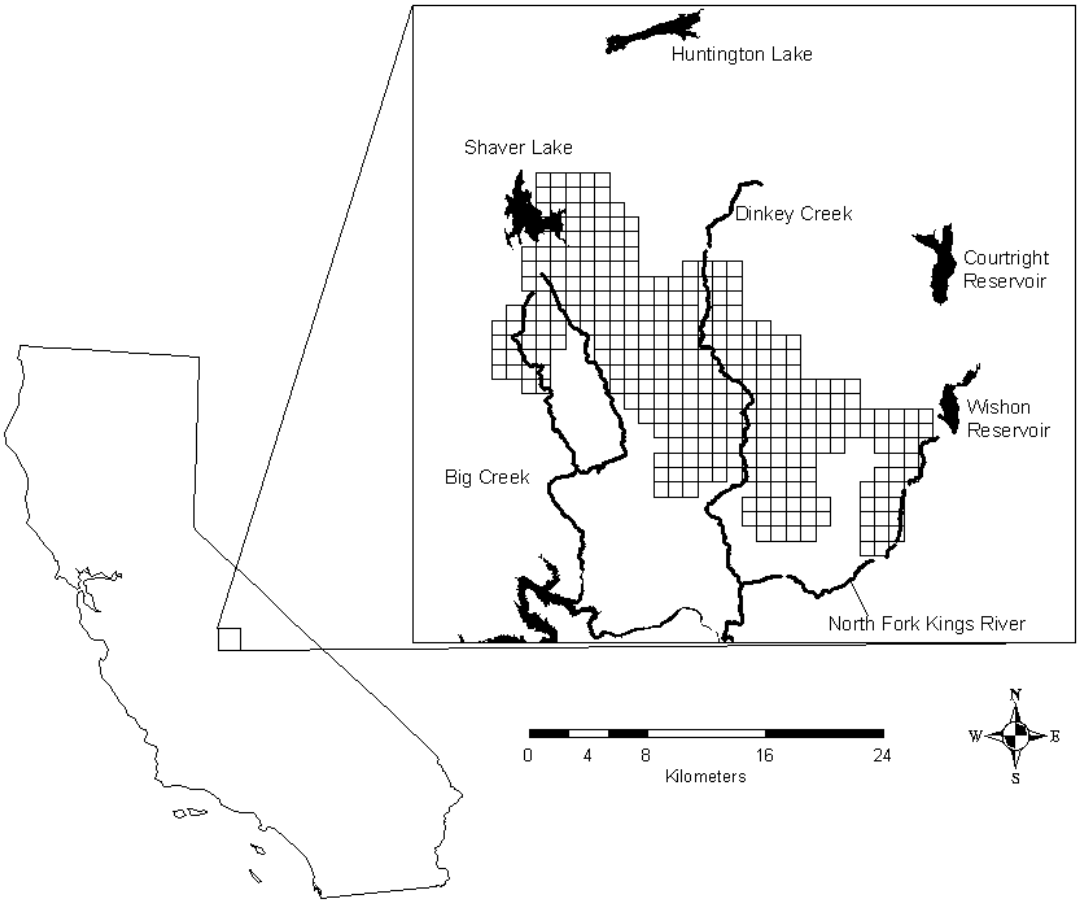


Fig. 1-1. The Kings River Fisher Project study area was located in the Sierra Nevada Mountains of Fresno County, California, USA. The study ran from 2000 to 2004. The 317 $km^2$ study area was divided into 1 km × 1 km cells. Three of the 317 potential cells were not used; one cell because it was entirely within private land to which I did not have access, and 2 more cells because they contained a busy campground and private summer cabins. Live traps were placed in every other cell, while camera traps were placed in the cells that had not been used for live trapping.

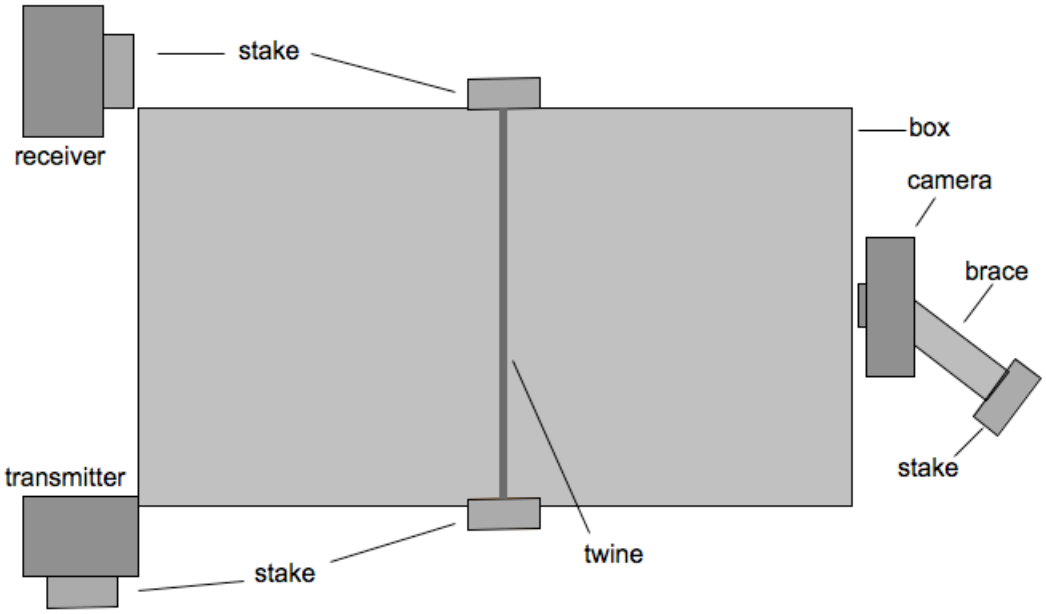


Fig. 1-2. Top view of camera trap used to monitor fisher populations in the Sierra National Forest, Fresno County, California, USA from 2002-2004. The infrared beam triggering the camera extended between the transmitter and receiver at the opening of the trap box. I closed off the back of the box with hardware cloth except for a small opening cut out for the lens of the camera. The cord connecting the receiver and the camera was buried a few cm underground.

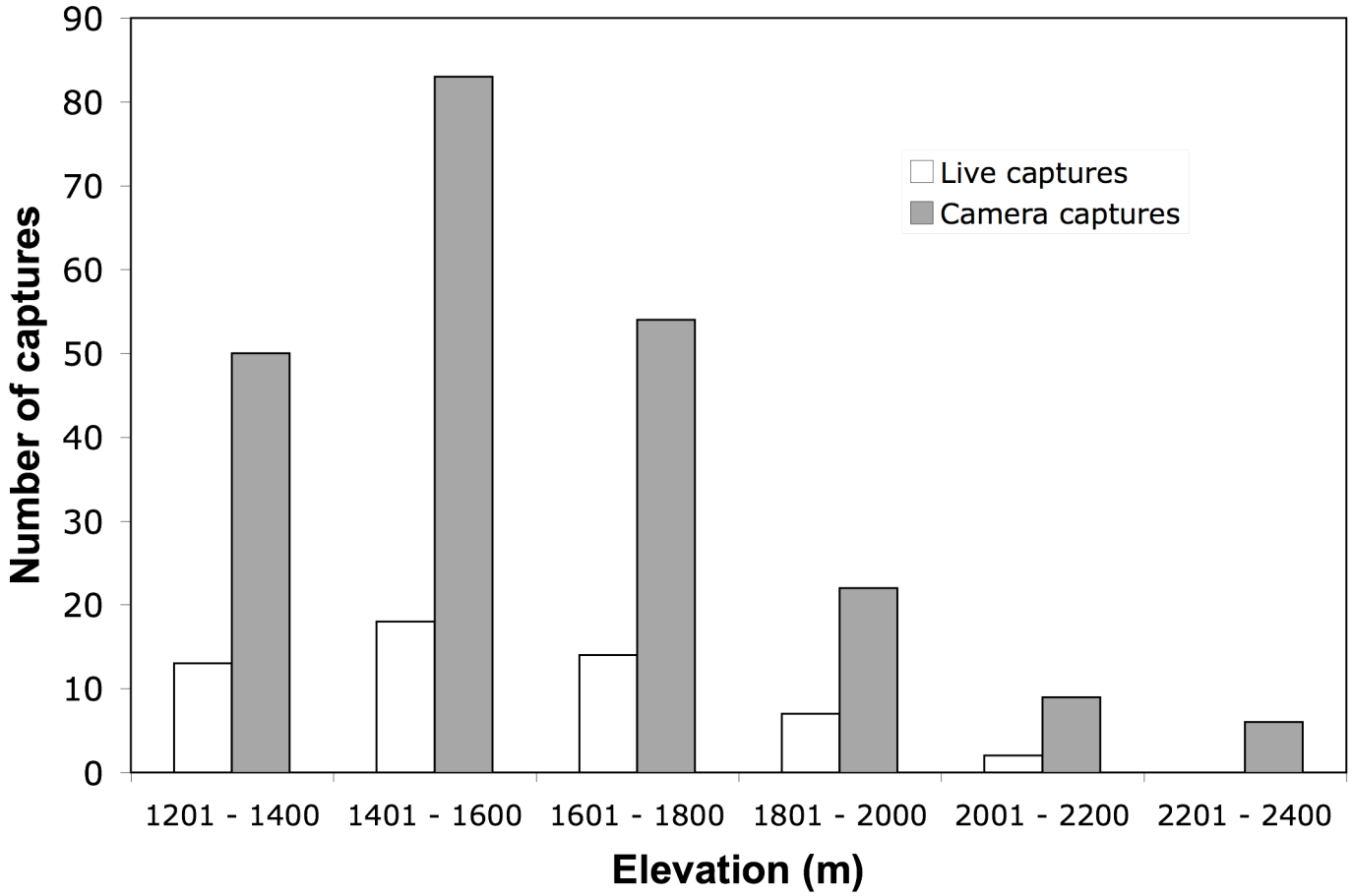


Fig. 1-3. Elevations of fisher captures in live and camera traps in the Sierra National Forest, Fresno County, California, USA. Data are combined for surveys conducted from 2002 to 2004. Live capture data for 2000 and 2001 are not presented.

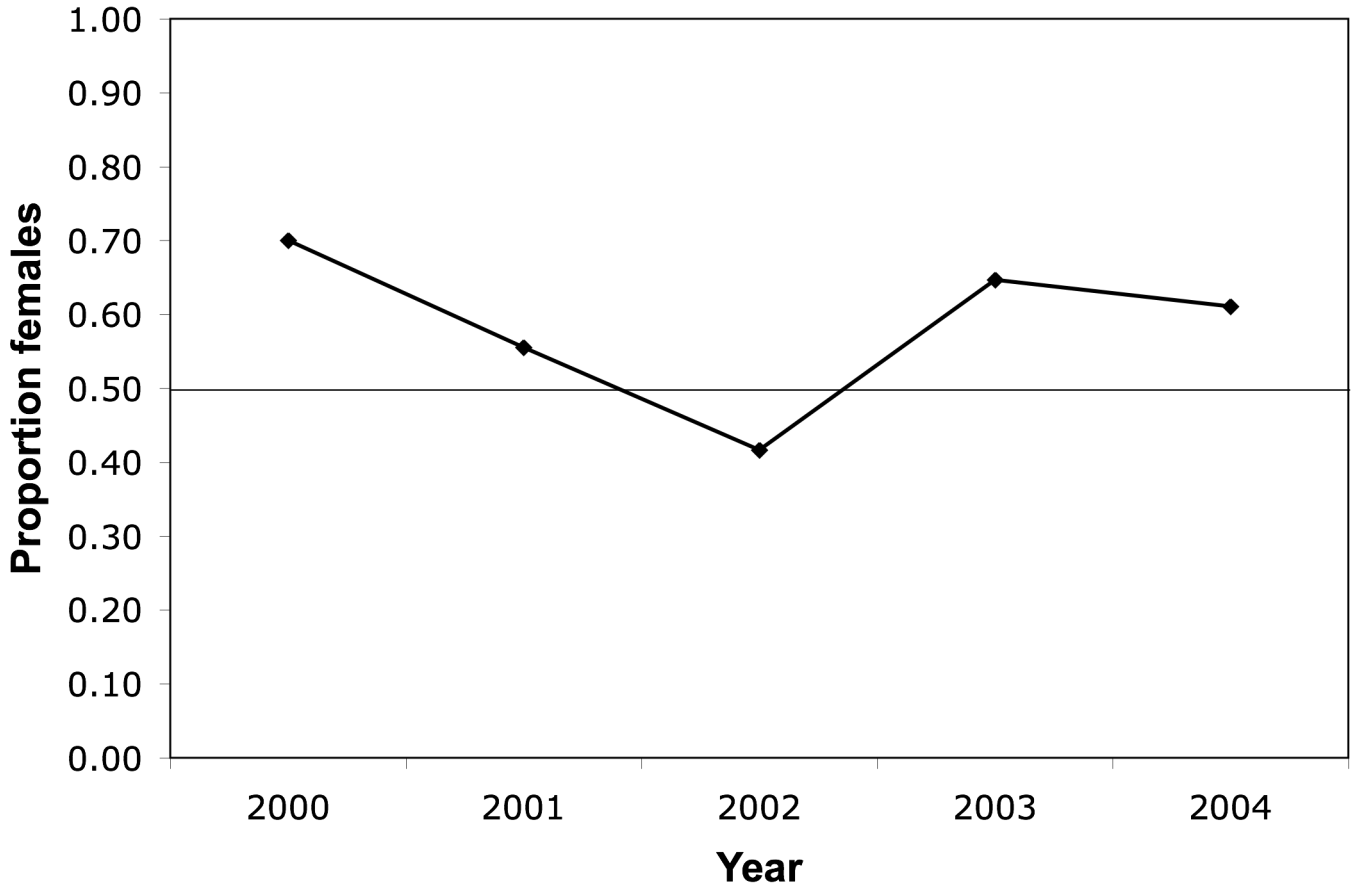


Fig. 1-4. Proportion of captures that were females during a live trapping study of fishers conducted in the Sierra National Forest, Fresno County, California, USA from 2000 to 2004.

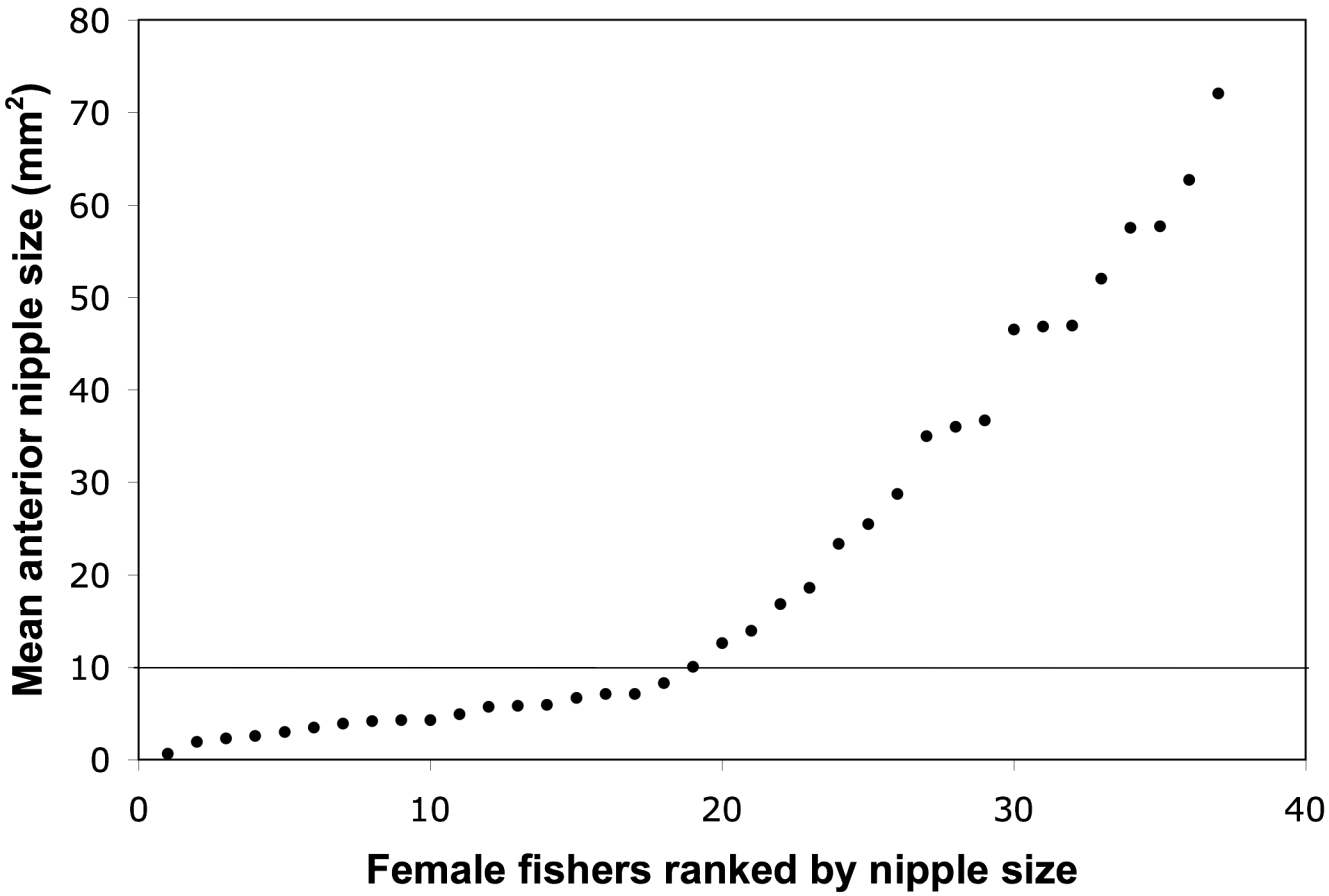


Fig. 1-5. Mean anterior nipple size (width × height) of female fishers caught in live traps in the Sierra National Forest, Fresno County, California, USA between 2000 and 2004. The fishers can be subdivided into 2 groups, above and below the point where the inflection of the curve changes. Females above this point, at 10 $mm^2$, have likely reproduced previously, though not necessarily in the same year.

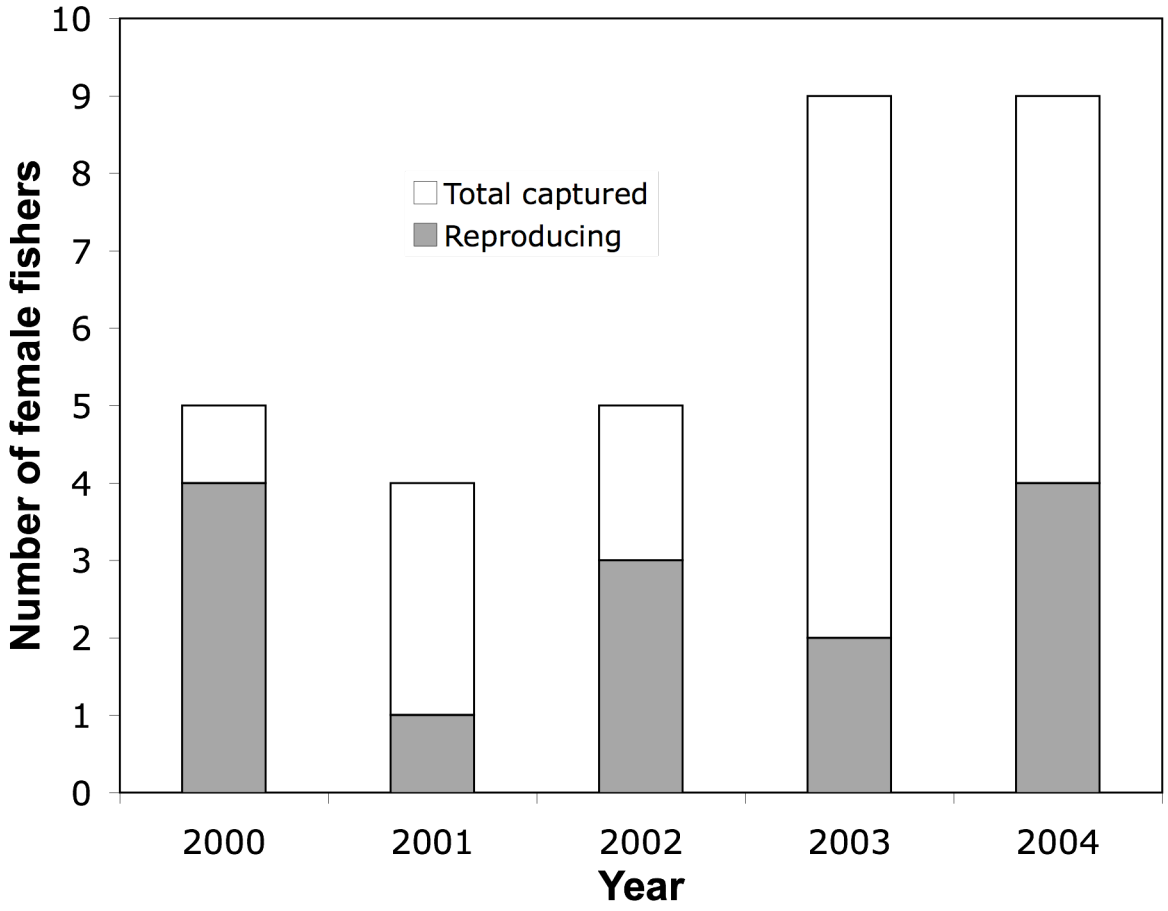


Fig. 1-6. Number of distinct females captured in live traps in the Sierra National Forest, Fresno County, California, USA between 2000 and 2004 and the number of them that had reproduced the previous spring. This latter number is the count of all live-trapped females that had an average anterior nipple size (width × height) > 20 $mm^2$.

Table 1-1. Parameter values simulated to determine the bias in an abundance estimate using camera traps (Bowden and Kufeld 1995) for a population of fishers in the Sierra National Forest, Fresno County, California, USA studied from 2002-2004. One simulation determined the impact of fishers that could not be identified as marked or unmarked when caught by a camera trap. The other simulation examined the impact of fishers losing their ear tags between live capture and camera resighting.

| Parameter | Low | High | Step |
|---|---|---|---|
| Abundance | 30 | 70 | 10 |
| Marking probability[a] | 0.30 | 0.50 | 0.10 |
| Resight probability[b] | 0.15 | 0.30 | 0.05 |
| Proportion unidentifiable[c] | 0.06 | 0.12 | 0.03 |
| Number of fishers losing tags[d] | 1 | 4 | 1 |

[a] Probability that each individual is live-trapped and marked
[b] Probability that an individual is captured during resighting
[c] Unidentifiable simulation only, set to 0 otherwise
[d] Tag loss simulation only, set to 0 otherwise

Table 1-2. Summary information for live and camera traps used to monitor a population of fishers in the Sierra National Forest, Fresno County, California, USA from 2000-2004.

| Year | Trap type | Number of traps | Elevation range of traps (m) | Active trap nights[a] | Lost trap nights (% of total) |
|---|---|---|---|---|---|
| 2000 | Live | 52 | 959-2006 | 554 | 49 (8.1%) |
| 2001 | Live | 145 | 870-2450 | 1066 | 122 (10.3%) |
| 2002 | Live | 159 | 1130-2244 | 1004 | 264 (20.8%) |
| | Camera | 109 | 1165-2282 | 1223 | 83 (6.4%) |
| 2003 | Live | 159 | 1130-2244 | 1118 | 154 (12.1%) |
| | Camera | 157 | 1110-2282 | 1628 | 232 (12.5%) |
| 2004 | Live | 158 | 1130-2244 | 1048 | 211 (16.8%) |
| | Camera | 156 | 1110-2282 | 1597 | 223 (12.3%) |
| TOTALS | Live | 673 | 870-2450 | 4790 | 800 (14.3%) |
| | Camera | 422 | 1110-228 | 4448 | 538 (10.8%) |

[a] Number of trap nights not lost to bear damage or other cause

Table 1-3. Mammal species caught in live or camera traps during a fisher population monitoring study on the Sierra National Forest, Fresno County, California, USA from 2000-2004.

| Species | | Live | Camera |
|---|---|---|---|
| Didelphimorphia | | | |
| Didelphidae | | | |
| Virginia opossum | *Didelphis virginiana* | | X |
| Rodentia | | | |
| Sciuridae | | | |
| Chipmunk | *Tamias sp.* | | X |
| California Ground squirrel | *Spermophilus beecheyi* | X | X |
| Western Gray squirrel | *Sciurus griseus* | X | |
| Douglas' squirrel | *Tamiasciurus douglasii* | X | X |
| Northern Flying squirrel | *Glaucomys sabrinus* | X | X |
| Muridae | | | |
| Deer mouse | *Peromyscus maniculatus* | | X |
| Dusky-footed woodrat | *Neotoma fuscipes* | | X |
| Carnivora | | | |
| Canidae | | | |
| Gray fox | *Urocyon cinereoargenteus* | X | X |
| Coyote | *Canis latrans* | | X |
| Domestic dog | *Canis familiaris* | | X |
| Ursidae | | | |
| Black bear | *Ursus americanus* | X | X |
| Procyonidae | | | |
| Ringtail | *Bassariscus astutus* | X | X |
| Raccoon | *Procyon lotor* | X | X |
| Mustelidae | | | |
| American marten | *Martes americana* | X | |
| Fisher | *Martes pennanti* | X | |
| Weasel | *Mustela sp.* | | X |
| Short-tailed weasel | *Mustela erminea* | X | |
| Long-tailed weasel | *Mustela frenata* | X | X |
| Western Spotted skunk | *Spilogale gracilis* | X | X |
| Striped skunk | *Mephitis mephitis* | | X |
| Felidae | | | |
| Bobcat | *Lynx rufus* | X | X |
| Artiodactyla | | | |
| Bovidae | | | |
| Cow | *Bos taurus* | | X |

Table 1-4. Live and camera capture data for fishers in the Sierra National Forest, Fresno County, California, USA from 2000 to 2004.

| | | All species | | | Fishers | | |
|---|---|---|---|---|---|---|---|
| Year | Trap type | Captures[a] | Capture rate (per active trap night) | Latency[b] | Captures[a] | Capture rate (per active trap night) | Latency[b] |
| 2000 | Live | 31 | 5.6% | 4.88 | 13 | 2.3% | 5.08 |
| 2001 | Live | 42 | 3.9% | 3.45 | 11 | 1.0% | 2.80 |
| 2002 | Live | 37 | 3.7% | 3.28 | 13 | 1.3% | 3.38 |
| | Camera | 381 | 31.2% | 2.64 | 90 | 7.4% | 4.35 |
| 2003 | Live | 36 | 3.2% | 4.64 | 20 | 1.8% | 5.11 |
| | Camera | 300 | 18.4% | 3.75 | 75 | 4.6% | 4.79 |
| 2004 | Live | 48 | 4.6% | 4.40 | 21 | 2.0% | 5.00 |
| | Camera | 393 | 24.6% | 3.61 | 62 | 3.9% | 5.03 |
| TOTALS | Live | 194 | 4.1% | 4.56 | 78 | 1.6% | 4.99 |
| | Camera | 1074 | 24.1% | 3.34 | 227 | 5.1% | 4.67 |

[a] Live capture or photograph.
[b] Average number of trap nights before a capture occurred. Does not include traps with no captures.

Table 1-5. Number of captures and capture rate per active trap night of non-fisher carnivores (including a carnivorous marsupial) in live traps in the Sierra National Forest, Fresno County, California, USA from 2000 to 2004. Species are ordered by number of captures.

| Species | 2000 | 2001 | 2002 | 2003 | 2004 |
|---|---|---|---|---|---|
| Ringtail | 8 (1.4%) | 17 (1.6%) | 9 (0.9%) | 6 (0.5%) | 11 (1.0%) |
| Grey fox | 3 (0.5%) | 5 (0.5%) | 4 (0.4%) | 3 (0.3%) | 1 (0.1%) |
| American marten | 0 | 3 (0.3%) | 0 | 2 (0.2%) | 9 (0.9%) |
| Spotted skunk | 1 (0.2%) | 0 | 0 | 2 (0.2%) | 0 |
| Virginia opossum | 1 (0.2%) | 0 | 0 | 0 | 0 |
| Raccoon | 0 | 0 | 1 (0.1%) | 0 | 0 |
| Bobcat | 0 | 0 | 0 | 1 (0.1%) | 0 |
| Ermine | 0 | 0 | 0 | 0 | 1 (0.1%) |
| Black bear | 0 | 0 | 0 | 0 | 1 (0.1%) |

Table 1-6. Number of captures and capture rate per active trap night of non-fisher carnivores (including a carnivorous marsupial) in camera traps in the Sierra National Forest, Fresno County, California, USA from 2002 to 2004. Species are ordered by number of captures.

| Species | 2002 | 2003 | 2004 |
|---|---|---|---|
| Black bear | 46 (3.76%) | 92 (5.65%) | 102 (6.39%) |
| Spotted skunk | 13 (1.06%) | 29 (1.78%) | 49 (3.07%) |
| Ringtail | 19 (1.55%) | 23 (1.41%) | 34 (2.13%) |
| Grey fox | 28 (2.29%) | 7 (0.43%) | 13 (0.81%) |
| America marten | 4 (0.33%) | 14 (0.86%) | 21 (1.31%) |
| Long-tailed weasel | 2 (0.16%) | 2 (0.12%) | 2 (0.13%) |
| Bobcat | 3 (0.25%) | 1 (0.06%) | 0 |
| Domestic dog | 1 (0.08%) | 2 (0.12%) | 1 (0.06%) |
| Virginia opossum | 0 | 0 | 3 (0.19%) |
| *Mustela sp.*[a] | 0 | 0 | 1 (0.06%) |
| Raccoon | 0 | 0 | 1 (0.06%) |

[a] Unidentifiable member of genus *Mustela* (long-tailed weasel or ermine), counted separately from long-tailed weasel.

Table 1-7. Model selection results for survival and recapture rate estimation from a combination of live and camera trapping in the Sierra National Forest, Fresno County, California, USA from 2000 to 2004.

| Model | $QAIC_c$ | $\Delta QAIC_c$ | $QAIC_c$ weights | Number of parameters | QDeviance |
|---|---|---|---|---|---|
| $\phi$. p. | 99.137 | 0.00 | 0.478 | 2 | 39.882 |
| $\phi$. $p_s$ | 101.280 | 2.14 | 0.164 | 3 | 39.802 |
| $\phi_s$ p. | 101.359 | 2.22 | 0.157 | 3 | 39.881 |
| $\phi$. $p_t$ | 102.947 | 3.81 | 0.071 | 5 | 36.774 |
| $\phi_s$ $p_s$ | 103.503 | 4.37 | 0.054 | 4 | 39.721 |
| $\phi_t$ p. | 104.329 | 5.19 | 0.036 | 5 | 38.155 |
| $\phi_s$ $p_t$ | 105.429 | 6.29 | 0.021 | 6 | 36.772 |
| $\phi_t$ $p_s$ | 106.809 | 7.67 | 0.010 | 6 | 38.152 |
| $\phi_t$ $p_t$ | 107.407 | 8.27 | 0.008 | 7 | 36.169 |
| $\phi_{s*t}$ p. | 111.406 | 12.27 | 0.001 | 9 | 34.691 |
| $\phi_{s*t}$ $p_s$ | 113.616 | 14.48 | 0.000 | 10 | 33.992 |
| $\phi_{s*t}$ $p_t$ | 115.211 | 16.07 | 0.000 | 11 | 32.552 |

$\phi$ survival rate; p capture rate

parameter varies by: $_s$ sex, $_t$ time, . parameter constant

Table 1-8. Survival and recapture rate estimates from model averaging of models combing live and camera trapping data from fishers in the Sierra National Forest, Fresno County, California, USA from 2000-2004.

| | | Survival | | | Recapture | | |
|---|---|---|---|---|---|---|---|
| Time[a] | Sex | Estimate | SE | 95% CI | Estimate | SE | 95% CI |
| 2000-2001 | F | 0.87 | 0.095 | 0.52-0.98 | 0.47 | 0.11 | 0.24-0.71 |
| | M | 0.87 | 0.10 | 0.47-0.98 | 0.48 | 0.12 | 0.23-0.74 |
| 2001-2002 | F | 0.89 | 0.093 | 0.54-0.98 | 0.51 | 0.11 | 0.28-0.73 |
| | M | 0.88 | 0.10 | 0.47-0.98 | 0.52 | 0.12 | 0.28-0.75 |
| 2002-2003 | F | 0.89 | 0.094 | 0.52-0.98 | 0.50 | 0.11 | 0.29-0.70 |
| | M | 0.88 | 0.10 | 0.46-0.98 | 0.51 | 0.11 | 0.29-0.72 |
| OVERALL[b] | F | 0.88 | 0.089 | 0.59-0.97 | 0.49 | 0.10 | 0.30-0.68 |
| | M | 0.88 | 0.099 | 0.54-0.98 | 0.50 | 0.10 | 0.30-0.70 |

[a] Apparent survival rate between first and second listed time. Survival and recapture rates for 2003-2004 are combined as a single parameter in the Cormack-Jolly-Seber model and are not separately estimable (Lebreton et al. 1992).

[b] Overall values are for the top 3 models only, which vary survival and recapture rate by sex and do not include a separate parameter estimate for each year of the study. These 3 models accounted for 80% of the $QAIC_c$ weights in the candidate model set.

# Chapter 2

## Development of 22 new microsatellite loci for fishers (*Martes pennanti*) with variability results from across their range[1]

Fishers (*Martes pennanti*) are carnivorous mammals found throughout forested regions of temperate North America. Recent declines in their distribution have prompted concern for their conservation, particularly in the western United States (Zielinski et al. 2005). However, relatively little is known about patterns of population structure and demography of fishers in this region. To better understand the ecology of this species, I developed microsatellite loci from a population in the Kings River region of the southern Sierra Nevada Mountains in California. I also screened the loci I developed in four other populations from across the fisher's range in the contiguous United States. These additional populations were in Idaho, Minnesota, Vermont, and a second population in California from the North Coast Range. Here I report on the development of the 22 loci that were variable in at least one of each of these populations.

[1] This chapter has been published with slight modifications as: Jordan, M. J., J. M. Higley, S. M. Matthews, O. E. Rhodes, M. K. Schwartz, R. H. Barrett, and P. J. Palsbøll. 2007. Development of 22 new microsatellite loci for fishers (*Martes pennanti*) with variability results from across their range. Molecular Ecology Notes 7:in press.

I isolated microsatellite loci following the protocol of Glenn and Schable (2005). Genomic DNA was obtained from tissue samples taken by ear punch from live-caught animals and stored in a saturated NaCl solution (6 M) containing 25% dimethyl sulfoxide. Extractions were performed using DNEasy extraction kits following manufacturer's instructions (QIAGEN Inc.). I digested 4 extracts with *Rsa I* (New England Biolabs) and then ligated the digested DNA to universal SNX linkers SuperSNX24F and SuperSNX24+4P (Hamilton et al. 1999). I enriched linker-ligated DNA by hybridization to biotinylated oligos using three separate oligo mixes: mix 1: $(AT)_4$ and $(GT)_{11}$; mix 2: $(TG)_{12}$, $(AG)_{12}$, $(AAG)_8$, $(ATC)_8$, $(AAC)_8$, $(AAT)_8$, and $(ACT)_8$; mix 3: $(AAAC)_6$, $(AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACCT)_6$, $(ACAG)_6$, $(ACTC)_6$, and $(ACTG)_6$. Enriched DNA was recovered using Dynabeads (Dynal Inc.). Recovered, enriched DNA was ligated into pCR®2.1-TOPO® plasmids (Invitrogen Inc.) and transformed into TOP 10 cells (Invitrogen Inc.).

I compiled a library of 288 recombinant clones after screening by α-complementation with X-gal (Invitrogen Inc.; Sambrook and Russell 2001). I lysed 179 colonies by boiling then amplified cloned inserts by PCR with M13 primers in a Dyad thermal cycler (MJ Research Inc.). Each 25 μL reaction contained approximately 10 ng of plasmid DNA, 20 mM Tris-HCl (pH 8.8), 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2.0 mM $MgSO_4$, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 625 μg Bovine Serum Albumin, 0.12 mM each dNTP, 0.5 U *Taq* DNA polymerase (New England Biolabs Inc.), and 250 nM of each primer. Reactions were run with an initial denaturing step of 95° for 3 min and then amplified for 28 cycles of 20 s at 95°, 20 s at 50°, and 1 min 30 s at 72°, followed by a 5 min extension step at 72°. Amplified

products were sequenced with M13 primers following a standard cycle sequencing reaction (Big Dye v 3.1, Applied Biosystems Inc.).

Out of 179 sequenced clones, I observed 152 sequences (85%) containing microsatellite DNA. Of these, 43 were dinucleotide repeats, 3 were trinucleotide repeats, 64 were tetranucleotide repeats, and 42 were compound or interrupted repeats. I designed primers for 50 of these microsatellites using PRIMER 3 (Rozen and Shaletsky 2000). To the 5'-end of each forward primer, I added a universal M13 tag (5'-TGTAAAACGACGGCCAGT-3').

I screened the 50 loci by amplifying genomic DNA isolated from tissue in an optimized PCR reaction and measuring the fragment lengths. I fluorescently labeled amplification products with a 6FAM-labeled M13 oligonucleotide primer using one of two methods. In the first method (Schuelke 2000), I set up a 10 μL reaction containing approximately 5 ng DNA template, a PCR cocktail mix [containing 67 mM Tris-HCl (pH 8.8), 2mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, 10 mM β-mercaptoethanol, 0.2 mM of each dNTP, 0.4 U *Taq* DNA Polymerase (New England Biolabs Inc.)], 800 nM reverse primer, 800 nM M13fwd-FAM primer, and 200 nM forward primer. This reaction was run for 2 min at 94°, followed by 22-23 cycles of 30 s at 94°, 30 s at an optimal annealing temperature (Table 2-1), and 30 s at 72° and subsequently for 8-9 cycles of 30 s at 94°, 30 s at 53°, and 30 s at 72°, followed by 10 min at 72°. Some loci would not amplify with this method, so I used a second protocol modified from Guo and Milewicz (2003). In this method, I set up a 10 μL reaction containing approximately 5 ng DNA template, the same PCR cocktail mix as above, and 1.0 mM each of the forward and reverse primers. I ran this reaction for 15 min at 94° followed

by 20 cycles of 30 s at 94°, 30 s at an optimal annealing temperature (Table 2-1), and 30 s at 72°, followed by 10 min at 72°. Amplification product from this step was diluted 1000X, and 1.0 μL of this dilution was then used as template in a second PCR reaction that used the same reagents except that I replaced the forward primer with an M13fwd-FAM primer. This reaction was run for 15 min at 94°, 28-30 cycles of 30 s at 94°, 30 s at 53°, and 30 s at 72°, followed by 10 min at 72°. I tested for variability with 32 samples from the Kings River population, 11 samples from the North Coast population, and 10 samples each from Idaho, Minnesota, and Vermont. Fragment lengths of PCR products were determined with an ABI 3730 sequencer using LIZ 500 size standard (Applied Biosystems) and analyzed using GENOTYPER 3.7 software (Applied Biosystems).

Out of the 50 loci I screened, I was unable to amplify 10. The number of polymorphic loci of the remaining 40 varied by population and in total, 22 were polymorphic in at least one population (6-21 loci; Table 2-2). The average number of alleles per locus among variable loci ranged from 2.6 to 3.2 depending on the population. One locus (MP0085) was variable only in the North Coast population, while all other variable loci were polymorphic in more than one population.

To determine if a locus was in Hardy-Weinberg equilibrium in a given population, I calculated observed ($H_O$) and expected ($H_E$) heterozygosities using an unpublished ANSI-C (P. Palsbøll, pers. comm.). The probability of obtaining $H_O$ by chance (assuming panmixia) was calculated as the proportion of 10,000 simulations of randomized genotypes based on the observed allele frequencies that yield a similar or more extreme estimate of $H_O$, after correcting for multiple tests (Table 2-2; Rice

1989). The locus MP0120 in the Vermont population and the locus MP0200 in the Idaho population both exhibited lower heterozygosity than expected assuming random mating. The probability of identity for each locus ranged from 0.083 to 0.84 across populations.

I used GENEPOP (version 3.4; Raymond and Rousset 1995) to test for linkage among the loci in each population separately. One pair of loci was significantly linked in the Vermont population after correcting for multiple comparisons (MP0188 and MP0288; $p < 0.001$), and although the test was not significant in any other population, it did approach significance in the other three populations where these two markers were variable, suggesting that these two loci may in fact be linked. In the Kings River population, I detected significant linkage between MP0059 and MP0144 ($p < 0.006$), however this relationship was not significant in any other population (*p*-value range: 0.33-0.72).

These 22 polymorphic loci will be useful in population genetic studies of this species across its range. The low number of variable loci within the Kings River population corroborates previous findings that fishers in the southern Sierra Nevada have very low genetic diversity relative to their counterparts in other populations (Wisely et al. 2004). This discovery underscores the importance these loci will have in studies of the ecology of this imperiled species.

Table 2-1. Microsatellite loci for *Martes pennanti* including locus name, forward and reverse primer sequences, PCR reaction conditions, and GenBank accession numbers for the clone sequences from which the markers were derived.

| Locus | Repeat unit | Primer sequences | $T_a$ | Cycles | GenBank Accession no. |
|---|---|---|---|---|---|
| MP0018 | $(GT)_{13}$ | F:ATTTGGGGCAACTGTCTTTG<br>R:CTGTGCTTTTGTGGGGATTT | 58/53[a] | 22/8 | EF042875 |
| MP0055 | $(GC)_4(CA)_{13}$ | F:GCCCCATGCCTGGTTTAT<br>R:GCTGGTCTAGAACCACCACAC | 59/53[a] | 22/8 | EF042876 |
| MP0059 | $(CA)_{19}$ | F:CCTCTCCCCTCAAACCTTCT<br>R:TTTCGGTATAAACTCCAACTACGA | 56/53[a] | 22/8 | EF042877 |
| MP0084 | $(GT)_5GC(GT)_{14}$ | F:GCTGGACCTGATGCTTGTAGA<br>R:GAATCCAAAACCAACGTGCT | 59/53[a] | 22/8 | EF042878 |
| MP0085 | $(TG)_{12}$ | F:AGGGAGTCTCTGCAGGTGAA<br>R:ACAATTGGTGTCAGGCAATG | 59/53[a] | 22/8 | EF042879 |
| MP0100 | $(CATT)_5$ | F:CTGGGACAACTGAACAACCA<br>R:ATCTTATCAGGGGCCCATTC | 60/53[a] | 22/8 | EF042880 |
| MP0114 | $(TTTC)_{11}TT(TTCC)_8$ | F:ATGACACGTTCCCCATTAGC<br>R:TCAGAGAGGCTCAAGAAGAGAAA | 60/53[a] | 22/8 | EF042881 |
| MP0120 | $(GTCT)_5(CT)_7(CA)_6$ | F:TCAGTGGATCTCTTGCTTGC<br>R:ATAGAAAAGTCGAGGAAGGAAATG | 58/53[a] | 22/8 | EF042882 |
| MP0144 | $(AGAA)_{16}$ | F:CCATCCCCTTTGGAAGAAA<br>R:GTGAGTTCAAGCCCCATGTT | 57/53[b] | 20/30 | EF042883 |
| MP0175 | $(CTTT)_{11}(CCTT)_3$ | F:CAGACCAAATGGACCCAATC<br>R:TTCTACATTCATACGTGAGTAAAAGC | 56/53[a] | 22/8 | EF042884 |
| MP0182 | $(GAAA)_4GAGA(GAAA)_{14}$ | F:TTTGCTGTATGGGATGTTGC<br>R:GAACTGACCCTATAAACCTAACAGGA | 59/53[b] | 20/30 | EF042885 |
| MP0188 | $(GATT)_7$ | F:GCAGAGCCAATCAGAGTTCC<br>R:GGACCTACAGCTCCATCCAA | 59/53[a] | 23/8 | EF042886 |

Table 2-1. *Continued*

| Locus | Repeat unit | Primer sequences | $T_a$ | Cycles | GenBank Accession no. |
|---|---|---|---|---|---|
| MP0190 | $(AGAC)_3(AG)_{12}$ | F:CTTCCAACCCTGGGTCAGT<br>R:GAACCCGATTAACTGCCAGA | 58/53[b] | 20/26 | EF042887 |
| MP0197 | $(TTTC)_{10}TCTC(TTTC)_2$ | F:GCTCAGCCAAAACTAAATCCA<br>R:CCACTTGATCACAATGTATGATCTTT | 59/53[a] | 23/8 | EF042888 |
| MP0200 | $(GAAA)_{16}$ | F:GCCAATTAAAACCACCAGGA<br>R:CCTGAGGGTTCCATTTCTCC | 58/53[a] | 22/8 | EF042889 |
| MP0227 | $(AC)_3AG(AC)_3AG(AC)_4AG$ $(AC)_4AG(AC)_9AG(AC)_2$ | F:TTGCCCAATGTTGAGACATC<br>R:CCACAGGAAACATAGATAAAATATGA | 58/53[a] | 22/8 | EF042890 |
| MP0234 | $(TGTT)_7$ | F:CAACATGCAAAGGTGATGCT<br>R:TTTTCCATTGCACTCAGGAA | 59/53[a] | 23/8 | EF042891 |
| MP0243 | $(TGTT)_7...(TGTT)_4$ | F:GAGGGTTCTGCAGGGGATA<br>R:CCACGGCATATTCTAGAGCAG | 56/53[b] | 20/29 | EF042892 |
| MP0247 | $(GAAA)_3(GA)_8(GAAA)_{11}$ | F:GCATTGTGCACCAGCATAAC<br>R:TTCCTTGCCTTTGCCTCA | 59/53[b] | 20/28 | EF042893 |
| MP0263 | $(TGTC)_8TGCC(TC)_4$ $(TTTC)_3$ | F:GAGTGTCCTCCTCAGGGCTA<br>R:AAACAGAACCTTAAGAGAGATAGGAA | 58/53[a] | 22/8 | EF042894 |
| MP0282 | $(TGTC)_8TGCC(TC)_4$ $(TTTC)_3$ | F:AGGCAGAGTGTCCTCCTCAG<br>R:TAAACAGAACCTTAAGAGAGATAGGAA | 58/53[a] | 22/8 | EF042896 |
| MP0288 | $(ATCA)_7$ | F:GGACCTACAGCTCCATCCAA<br>R:GCAGAGCCAATCAGAGTTCC | 58/53[a] | 22/8 | EF042897 |

[a]M13 labeling using first program described in text, adapted from Schuelke (2000)
[b]M13 labeling using second program described in text, adapted from Guo and Milewicz (2003)

Table 2-2. Microsatellite loci screening results showing the population(s) in which the locus is variable, the number of individuals sampled (*N*), the number of samples successfully genotyped, the number of alleles, allele size range, observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), *P* value for the probability of $H_O$, and probability of identity (*I*).

| Locus | Population | *N* | *N* genotyped | No. of alleles | Size range (bp) | $H_O$ | $H_E$ | *P* value | *I* |
|---|---|---|---|---|---|---|---|---|---|
| MP0018 | MN | 10 | 10 | 2 | 197-199 | 0.10 | 0.10 | <1.00 | 0.82 |
| | VT | 10 | 10 | 3 | 197-201 | 0.30 | 0.52 | <0.059 | 0.29 |
| MP0055 | NC | 11 | 11 | 2 | 133-137 | 0.27 | 0.24 | <1.00 | 0.61 |
| | ID | 10 | 7 | 3 | 133-137 | 0.57 | 0.50 | <0.66 | 0.32 |
| | MN | 10 | 10 | 4 | 133-139 | 0.70 | 0.66 | <0.67 | 0.19 |
| | VT | 10 | 10 | 4 | 133-139 | 0.40 | 0.53 | <0.14 | 0.26 |
| MP0059 | KR | 53 | 32 | 2 | 166-170 | 0.28 | 0.46 | <0.033 | 0.40 |
| | NC | 11 | 11 | 4 | 166-176 | 0.45 | 0.48 | <0.50 | 0.32 |
| | ID | 10 | 10 | 5 | 166-174 | 0.40 | 0.72 | <0.012 | 0.13 |
| | MN | 10 | 10 | 4 | 166-174 | 0.50 | 0.59 | <0.26 | 0.21 |
| | VT | 10 | 10 | 4 | 168-176 | 0.70 | 0.54 | <1.00 | 0.25 |
| MP0084 | ID | 10 | 9 | 3 | 152-156 | 0.56 | 0.51 | <0.71 | 0.32 |
| | MN | 10 | 10 | 5 | 144-158 | 0.90 | 0.74 | <0.93 | 0.12 |
| | VT | 10 | 10 | 4 | 144-154 | 0.90 | 0.72 | <0.95 | 0.13 |
| MP0085 | NC | 11 | 11 | 2 | 132-134 | 0.64 | 0.48 | <0.94 | 0.38 |
| MP0100 | NC | 11 | 11 | 2 | 216-222 | 0.64 | 0.43 | <1.00 | 0.41 |
| | MN | 10 | 10 | 3 | 212-220 | 0.70 | 0.54 | <0.89 | 0.29 |
| | VT | 10 | 10 | 3 | 212-220 | 0.30 | 0.49 | <0.14 | 0.34 |
| MP0114 | NC | 11 | 11 | 2 | 206-218 | 0.55 | 0.50 | <0.82 | 0.38 |
| | ID | 10 | 9 | 3 | 206-214 | 0.44 | 0.48 | <0.53 | 0.35 |
| | MN | 10 | 10 | 4 | 206-218 | 0.80 | 0.67 | <0.84 | 0.17 |
| | VT | 10 | 10 | 2 | 206-214 | 0.20 | 0.18 | <1.00 | 0.69 |

Table 2-2. *Continued*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MP0120 | NC | 11 | 10 | 2 | 112-116 | 0.70 | 0.46 | <1.00 | 0.40 |
| | ID | 10 | 10 | 3 | 112-120 | 0.20 | 0.54 | <0.013 | 0.29 |
| | MN | 10 | 10 | 4 | 108-120 | 0.20 | 0.55 | <0.0050 | 0.28 |
| | VT | 10 | 9 | 3 | 112-120 | 0.11 | 0.55 | **<0.0010** | 0.30 |
| MP0144 | KR | 53 | 28 | 4 | 206-218 | 0.29 | 0.31 | <0.39 | 0.50 |
| | NC | 11 | 11 | 3 | 198-214 | 0.55 | 0.64 | <0.29 | 0.20 |
| | ID | 10 | 9 | 5 | 198-226 | 0.44 | 0.38 | <1.00 | 0.40 |
| | MN | 10 | 10 | 5 | 186-210 | 0.70 | 0.74 | <0.42 | 0.12 |
| | VT | 10 | 10 | 4 | 194-206 | 0.70 | 0.73 | <0.41 | 0.12 |
| MP0175 | KR | 53 | 31 | 3 | 174-182 | 0.61 | 0.51 | <0.90 | 0.35 |
| | NC | 11 | 11 | 5 | 186-206 | 0.73 | 0.65 | <0.76 | 0.17 |
| | ID | 10 | 5 | 4 | 174-186 | 0.40 | 0.70 | <0.057 | 0.14 |
| | MN | 10 | 10 | 5 | 170-190 | 0.70 | 0.78 | <0.25 | 0.09 |
| | VT | 10 | 10 | 4 | 174-186 | 0.70 | 0.74 | <0.39 | 0.12 |
| MP0182 | NC | 11 | 11 | 3 | 204-216 | 0.45 | 0.62 | <0.14 | 0.22 |
| | ID | 10 | 10 | 2 | 216-220 | 0.30 | 0.46 | <0.30 | 0.40 |
| | MN | 10 | 10 | 5 | 204-220 | 0.50 | 0.55 | <0.34 | 0.24 |
| | VT | 10 | 10 | 4 | 204-216 | 0.70 | 0.57 | <0.88 | 0.24 |
| MP0188 | NC | 11 | 11 | 2 | 129-133 | 0.36 | 0.30 | <1.00 | 0.54 |
| | ID | 10 | 10 | 2 | 129-133 | 0.50 | 0.46 | <0.80 | 0.40 |
| | MN | 10 | 10 | 2 | 129-133 | 0.40 | 0.48 | <0.49 | 0.39 |
| | VT | 10 | 10 | 2 | 129-133 | 0.40 | 0.42 | <0.64 | 0.42 |
| MP0190 | NC | 11 | 11 | 2 | 197-199 | 0.45 | 0.48 | <0.61 | 0.38 |
| | ID | 10 | 5 | 2 | 197-199 | 0.20 | 0.50 | <0.24 | 0.38 |
| | MN | 10 | 10 | 2 | 197-199 | 0.30 | 0.38 | <0.50 | 0.46 |

Table 2-2. *Continued*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MP0197 | KR | 52 | 31 | 3 | 232-240 | 0.65 | 0.55 | <0.90 | 0.29 |
| | NC | 11 | 11 | 2 | 232-236 | 0.64 | 0.48 | <0.94 | 0.38 |
| | ID | 10 | 10 | 3 | 232-240 | 0.30 | 0.27 | <1.00 | 0.56 |
| | MN | 10 | 10 | 2 | 232-236 | 0.20 | 0.18 | <1.00 | 0.69 |
| | VT | 10 | 10 | 3 | 232-240 | 0.50 | 0.59 | <0.34 | 0.26 |
| MP0200 | KR | 52 | 26 | 2 | 173-177 | 0.23 | 0.20 | <1.00 | 0.65 |
| | ID | 10 | 9 | 6 | 161-181 | 0.22 | 0.77 | **<0.0010** | 0.09 |
| | MN | 10 | 10 | 4 | 153-173 | 0.30 | 0.60 | <0.023 | 0.23 |
| | VT | 10 | 10 | 3 | 161-169 | 0.40 | 0.34 | <1.00 | 0.47 |
| MP0227 | NC | 11 | 11 | 2 | 148-152 | 0.18 | 0.17 | <1.00 | 0.71 |
| | ID | 10 | 9 | 4 | 138-154 | 0.67 | 0.69 | <0.45 | 0.15 |
| | MN | 10 | 10 | 3 | 148-158 | 0.30 | 0.56 | <0.020 | 0.26 |
| | VT | 10 | 10 | 6 | 138-158 | 0.50 | 0.78 | <0.016 | 0.08 |
| MP0234 | ID | 10 | 10 | 3 | 133-141 | 0.80 | 0.64 | <0.89 | 0.21 |
| | MN | 10 | 10 | 2 | 133-137 | 0.60 | 0.48 | <0.89 | 0.39 |
| | VT | 10 | 10 | 3 | 133-141 | 0.50 | 0.51 | <0.57 | 0.31 |
| MP0243 | ID | 10 | 10 | 2 | 214-218 | 0.50 | 0.50 | <0.68 | 0.38 |
| | MN | 10 | 10 | 2 | 214-218 | 0.60 | 0.48 | <0.90 | 0.39 |
| | VT | 10 | 10 | 4 | 206-218 | 0.70 | 0.59 | <0.82 | 0.26 |
| MP0247 | KR | 53 | 28 | 4 | 139-159 | 0.68 | 0.63 | <0.75 | 0.20 |
| | NC | 11 | 11 | 4 | 139-159 | 0.73 | 0.67 | <0.71 | 0.17 |
| | ID | 10 | 4 | 4 | 139-155 | 0.75 | 0.66 | <0.78 | 0.17 |
| | MN | 10 | 10 | 4 | 147-163 | 0.60 | 0.66 | <0.40 | 0.18 |
| | VT | 10 | 10 | 3 | 139-155 | 0.50 | 0.59 | <0.31 | 0.24 |
| MP0263 | NC | 11 | 11 | 2 | 125-129 | 0.09 | 0.09 | <1.00 | 0.84 |
| | ID | 10 | 10 | 2 | 125-129 | 0.00 | 0.32 | <0.0060 | 0.51 |
| | MN | 10 | 10 | 2 | 125-129 | 0.70 | 0.50 | <0.96 | 0.38 |
| | VT | 10 | 10 | 2 | 125-129 | 0.20 | 0.42 | <0.14 | 0.42 |

Table 2-2. *Continued*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MP0282 | NC | 11 | 11 | 2 | 131-135 | 0.09 | 0.09 | <1.00 | 0.84 |
| | ID | 10 | 10 | 2 | 131-135 | 0.10 | 0.10 | <1.00 | 0.82 |
| | MN | 10 | 10 | 2 | 131-135 | 0.40 | 0.48 | <0.48 | 0.39 |
| | VT | 10 | 10 | 2 | 131-135 | 0.10 | 0.46 | <0.021 | 0.40 |
| MP0288 | NC | 11 | 11 | 3 | 126-134 | 0.27 | 0.38 | <0.20 | 0.42 |
| | ID | 10 | 7 | 2 | 126-130 | 0.57 | 0.49 | <0.86 | 0.38 |
| | MN | 10 | 10 | 2 | 126-130 | 0.40 | 0.48 | <0.45 | 0.39 |
| | VT | 10 | 10 | 2 | 126-130 | 0.40 | 0.42 | <0.65 | 0.42 |

Populations are abbreviated as follows: Kings River (KR), North Coast California (NC), Idaho (ID), Minnesota (MN), and Vermont (VT). Only populations that were polymorphic are displayed for each locus. Locus-population combinations that were out of Hardy-Weinberg equilibrium are indicated with *P* values in boldface

# Chapter 3

## A comparison of camera traps to hair snares and genetic tagging for obtaining population estimates of fishers (*Martes pennanti*)

Surveys designed to estimate demographic parameters for species of conservation concern often rely on capture of individual animals. Such studies can be logistically difficult, especially for wide-ranging species such as carnivores. Additionally, capture and handling disturbs the species under study, which can be of particular concern with politically sensitive species. Surveys relying on intensive capture and handling are also time and resource intensive.

Recently, researchers have shown increased interest in using noninvasive techniques to study animal populations, minimizing the degree of imposition by the researcher on the animals (Wilson and Delahay 2001, Waits and Paetkau 2005). These techniques hold promise as research tools because capture and handling of animals are not required, reducing both the logistical difficulty of the project and the risk of harm to the species studied. These methods are also frequently more cost effective than live trapping or telemetry-based techniques (Wilson and Delahay 2001, Waits and Paetkau 2005).

Camera traps are a common method for noninvasively identifying individuals and estimating demographic parameters (Mace et al. 1994, Karanth et al. 2006).

However, for many species lacking pelage patterns that can be used for individual identification, these methods still require capture and handling of the species for initial placement of a visible mark. Such was the case with my use of camera trapping for fishers (*Martes pennanti*) described in Chapter 1.

There has been a recent surge of interest in using genetic information for obtaining demographic parameter estimates (Waits and Paetkau 2005, Schwartz et al. 2007). Genotypes can be used to identify individuals in a capture-recapture context, serving the same role as tags in traditional capture-recapture studies (Palsbøll et al. 1997, Mowat and Strobeck 2000, Flagstad et al. 2004, Triant et al. 2004, Prugh et al. 2005, Piggott et al. 2006). Genetic information can be collected noninvasively from samples such as hair (Woods et al. 1999, Walker et al. 2006) or feces (Taberlet et al. 1997, Kohn et al. 1999, Bergl and Vigilant 2007). Hair samples contain enough DNA in their follicles for generating these data (Foran et al. 1997). These samples can be collected at snares specifically designed to snag hair, and the hair snares can be treated like traps in a conventional capture-recapture study.

The relative merits of different techniques for monitoring wildlife can vary by species (Gompper et al. 2006), so assessing these methods for a particular species requires controlled studies. I compared the efficacy of hair snares with genetic tagging to camera traps for obtaining density and survival estimates for fishers in the southern Sierra Nevada. These sampling methods can both be incorporated into a device currently used for surveying mesocarnivores in California (Zielinski et al. 2005), and therefore both methods can potentially be implemented throughout the

state. However, they have not been directly compared for obtaining demographic parameter estimates for fishers.

Camera traps and hair snares were combined in the same device. Camera recaptures consisted of photos of animals marked with uniquely colored ear tags. This method was not completely noninvasive because the animals were first caught in live traps and marked. Hair snaring was completely noninvasive because each animal's microsatellite genotype served as a tag, so I did not need to physically mark them. My goal was to determine which of these methods would be most effective at providing demographic parameter estimates for fishers.

## STUDY AREA

The study was conducted on the west slope of the southern Sierra Nevada mountains in a 317 $km^2$ area that is described in greater detail in the Introduction. I divided the study area into a trapping grid composed of 1 km × 1 km cells. With the exception of 3 cells that were not used for logistical reasons (Chapter 1), I placed a live trap or noninvasive bait station (described below) into each of the remaining cells.

## METHODS

I conducted this study from 2002 to 2004. Live trapping took place each year in July and August, which was followed by trapping with noninvasive bait stations in September and October. Live traps were placed in every other cell of the trapping grid, and the trapping procedure is described in Chapter 1. Noninvasive bait stations were placed in cells adjacent to those used for live trapping.

Noninvasive bait stations consisted of 2 separate capture devices, the first of which was a camera trap described in Chapter 1. The second device was a hair snare positioned across the entrance to the camera trap box. I constructed the snare from 3 strands of barbed wire that spanned the width of the box entrance in 3 horizontal rows (Fig. 3-1).

As with live traps, the station was baited with raw chicken and a commercial lure. The stations were deployed for 12 days and were checked every other day. I collected hair samples in 1 of 2 ways. In most cases, I removed the hairs from the barbs with forceps and placed them in paper envelopes [5.7 × 8.9 cm (2¼" × 3½")]. These envelopes were placed in plastic bags with silica gel desiccant beads (3-8 mesh, W. R. Grace & Co., Columbia, MD, USA) and stored at -80 °C. If there were very few hairs or they were difficult to remove without damaging the sample, I cut the barb from the strand of wire and placed the entire barb in an empty 35-mm film canister with 20-30 desiccant beads. If more than 1 barb contained a hair sample, I collected the hairs from each barb as a separate sample.

In 2003 and 2004 I wired a bristled pet brush purchased from a pet supply store to a log or tree near 30 camera traps. At each of these stations, I also I placed a small amount of fisher-specific lure (Hawbaker's Fisher Lure, Minnesota Trapline Products, Pennock, MN, USA) near the brush to see if fishers would rub against them. I collected hairs from the brushes with forceps and stored them in paper envelopes as above.

Hereafter, "trap night" refers to a 24 h period that a trap was open, while a "visit" refers to a researcher checking a trap and a "capture visit" refers to the first

researcher visit following an animal being photographed or leaving a hair sample. I determined the time that a camera trap was triggered by a time/date stamp on each photographic print. However, for hair samples, I could only determine that the capture occurred sometime between 2 particular visits. Therefore, all comparisons of camera trapping and hair snaring are based on capture visits rather than the total number of captures.

When an animal entered a baited station, 1 of 4 possible outcomes occurred depending on if the animal was photographed or not and whether or not it left a hair sample. I was unable to determine the number of times an animal came but did not leave any type of sample. To compare the ability of the 2 trap types to sample fishers, I used captures from camera traps to calculate snare effectiveness, defined as the percentage of times when an animal was photographed at a station between investigator visits and I collected a hair sample (Zielinski et al. 2006a).

**DNA extraction**

As many hairs as possible from each envelope or barb were used in each extraction, as the amount of template has a large impact on reducing genotyping error rates (Goossens et al. 1998). I extracted DNA from hair samples by boiling them in 25 μL 1× TE solution at 100 °C for 10 min. After boiling, I spun down the tubes, added 25 additional μL of 1× TE, and heated the samples at 100 °C for an another 10 min. I stored extracted DNA in TE solution at -20 °C.

**Species identification**

To determine if a hair sample was left by a fisher, I used a series of 3 restriction digests (Riddle et al. 2003). Extraction samples were first amplified at the cytochrome b locus in 20 μL reactions containing 2 μL DNA template, a PCR cocktail mix [containing 67 mM Tris-HCl (pH 8.8), 2mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, 10 mM β-mercaptoethanol, 0.2 mM of each dNTP, 0.4 U *Taq* DNA Polymerase (New England Biolabs Inc., Ipswich, MA, USA)], 1.0 mM primer H15149 (5'-AAACTGC-AGCCCCTCAGAATGATATTTGTCCTCA-3'; Kocher et al. 1989), and 1.0 mM primer CanidL1 (5'-AATGACCAACATTCGAAA-3'; Paxinos et al. 1997). Amplification was verified by running 4.0 μL PCR product on a 1.7% agarose gel for 20 min then staining the gel for 10-15 min in ethidium bromide. If a sample did not amplify, I attempted a second amplification.

Species identification proceeded with the following stepwise process. Samples amplified at cytochrome b with a visible band on agarose were first digested with the restriction enzyme *Hinf I*, which generates products of 212, 132, and 98 base pairs (bp) in fishers (Riddle et al. 2003). If a sample yielded these 3 products, I digested another aliquot of the cytochrome b PCR product with *Hae III*. For fishers, this enzyme cuts cytochrome b into pieces of 259 and 183 bp (Riddle et al. 2003). If this digest also yielded the expected fragment sizes for fishers, I used another aliquot of the PCR product in a digest with *Mbo I*, which does not cut cytochrome b in fishers (Riddle et al. 2003). All restriction digests had a 10.0 μL volume and contained 5.0 μL cytochrome b amplification product and 1.67 U enzyme. The *Hinf I* and *Hae III* digests also contained 5.0 mM NaCl, 1.0 mM Tris-HCl, 1.0 mM $MgCl_2$, and 0.1 mM

dithiotreitol, while the *Mbo I* digests contained 10.0 mM NaCl, 5.0 mM Tris-HCl, 1.0 mM $MgCl_2$, and 0.1 mM dithiotreitol. I ran all digest products for 45 min on a 3.0% agarose gel in batches of 8 alongside a negative and a positive control, then visualized the fragments by staining in ethidium bromide for 10-15 min. I used a low molecular weight DNA ladder (New England Biolabs Inc.) to measure fragment sizes.

To determine if the 3 above digests generated a set of fragments unique to fishers, I downloaded cytochrome b sequences from GenBank for all species that had been caught with the camera traps. Using the program BIOEDIT (v.7.05, ©1997-2005, Tom Hall, Ibis Therapeutics, Carlsbad, CA, USA), I generated restriction maps for each these sequences, which showed that only fishers have this combination of fragment lengths.

**Genotyping**

One complication associated with using genetic samples for a capture-recapture study is the possibility that a given genotype will be possessed by 2 or more different individuals, particularly in a population with low genetic variability. This "shadow effect" obscures some individuals and leads to an underestimation of abundance (Mills et al. 2000). The best way to decrease this effect is to increase the number of microsatellite loci used. An appropriately stringent analytical method for determining if any given genotype in a set of samples represents only 1 individual is to calculate the probability that any pair of siblings in the population will have an identical genotype (Probability of Identity for siblings: $PI_{sibs}$; Donnelly 1995). This number is multiplied across loci to determine the $PI_{sibs}$ for the population given the set

of loci used. While a threshold for this parameter will vary depending on the goals of the project, $PI_{sibs} < 0.05$ is a commonly used cut-off for noninvasive, capture-recapture studies (Woods et al. 1999, Waits and Paetkau 2005).

I genotyped 46 ear tissue samples collected from live-caught fishers between 2000 and 2004 to calculate $PI_{sibs}$ and determine an appropriate suite of microsatellite loci that ensured a low probability of overlapping genotypes. These samples were genotyped at 14 loci following the protocols outlined in Chapters 2 and 4. For these samples, I estimated an overall $PI_{sibs}$ of 0.019 for the following 7 loci: Ggu101B, Lut733, MP0059, MP0144, MP0175, MP0197, and MP0247 (Table 3-1). I chose these loci because they were highly variable in this population, with an overall estimate of $PI_{sibs} < 0.05$, and they each produced consistent results when genotyping ear tissue samples.

I amplified samples at all loci with optimized PCR reactions. I attached an 18 bp M13 tag (5'-TGTAAAACGACGGCCAGT-3') to the 5' end of each forward primer. Different labels were chosen to optimize the number of loci that could be simultaneously run in a given reaction plate. The PCR reactions conditions are described in Chapters 2 and 4. I amplified and genotyped each extracted hair sample 3 times to enable the detection of genotyping errors.

Fragment lengths were determined with an ABI 3730 sequencer using LIZ 500 size standard (Applied Biosystems, Foster City, CA, USA) and analyzed using GENOTYPER 3.7 software (Applied Biosystems). I used the program MICROCHECKER (van Oosterhout et al. 2004) to assess the rate of genotyping error.

## RESULTS

### Comparison of overall capture rates

Active trap nights were those that were not lost to some form of disturbance, the most common of which were bear damage and camera malfunction. Over the 3 years of the study, I had 4986 camera trap nights, 538 (10.8%) of which were lost to disturbance (Table 3-2). During the same time period, 434 out of 5024 (8.6%) hair snare trap nights were lost to disturbance. More trap nights were lost for cameras than for hair snares (Fisher's exact test: $P < 0.001$).

I used 2 h as the minimum interval between photographs to count 2 photos of the same species as different captures (Chapter 1). With this interval, I recorded 1074 camera captures from 2002-2004, with a capture rate of 0.24 per active trap night (Table 3-3). The number of captures between visits ranged from 1-16 ($\overline{X}$ = 1.9, SE = 1.7), with at least 1 capture recorded on 771 different visits.

I collected hair on 290 different visits to hair snares. The number of hair samples collected at each visit ranged from 1-4, with most (282) yielding only 1 sample. A total of 301 hair samples were collected from 2002-2004 (Table 3-3). In addition to these samples, 14 were collected from the hair brushes in 2003 and 2004 (Table 3-3). Overall, hair samples were collected at a rate of 0.07 and 0.02 per trap night for hair snares and hair brushes respectively (Table 3-3).

Of the visits where a capture occurred, there were more camera capture visits than hair snare capture visits (89% to 33%), and only 22% of visits yielded both capture types (Table 3-4). The capture visit rate for hair snares was less than that for camera traps (Fig. 3-2; Fisher's exact test: $P < 0.001$).

**Captures of fishers and individual identification**

There were 227 camera captures of fishers, with a capture on 5.1% of trap nights (Table 3-3). These captures occurred on 205 different visits. The captures represented at least 28 different individual fishers, with unmarked animals accounting for 51% of captures.

Snares had an overall effectiveness of 46%, and an effectiveness of 44% for fishers (Table 3-5). During the 2002 trapping season, 12 of the noninvasive bait stations were replaced by a device that tested the efficacy of different hair snare types. These stations used track plates instead of photographs to indicate presence inside a trap box, and yielded an effectiveness of 90% (Zielinski et al. 2006a).

Throughout the process of genetic species identification of hair samples, there was a decline in the number of useable samples. Some samples were of insufficient quality to attempt DNA extraction, so I only extracted DNA from 269 of the 301 hair snare samples, and 13 of the 14 hair brush samples. Many of these extracts did not have sufficient DNA to successfully amplify at cytochrome b and perform species identification. Ultimately, I was able to perform species identification on 97 hair snare samples and 4 samples from hair brushes. Fishers accounted for a relatively small proportion of the samples on which I performed the species identification: only 14 of the hair snare samples, and none of the hair brush samples had a restriction digest pattern consistent with fishers. Capture rates of fishers were higher at camera traps than at hair snares (Fisher's exact test: $P < 0.001$).

I obtained microsatellite genotypes of 13 fisher hair samples, and each of these samples was genotyped at between 5 and 7 loci (median = 6), with between 2 and 6

alleles at each locus. Most hair genotypes were constructed from electropherograms from 2 or 3 successful amplifications, and none of the scans yielded contradictory results for the same fisher and locus. None of them yielded a sample with more than 2 alleles, which would suggest mixing of samples. In only 6 cases was a genotype at a particular locus based on only 1 scan: sample 25.8 at MP0059, sample 28.10 at MP0059 and MP0197, sample 32.8 at MP0197, and sample 34.2 at MP0059. Using samples from 46 live-caught fishers, Ggu101B and MP0059 showed an excess of homozygotes, suggesting the presence of null alleles at these loci. None of the hair genotypes matched any of the 46 fishers caught in live traps, whether or not these 2 loci were included.

Eleven of the fisher hair samples had corresponding photo captures, of which 8 included photographs of fishers (Table 3-6). In 5 cases, there was more than 1 photo capture preceding a hair capture, and 2 visits yielded photographs of more than 1 fisher. Hair sample genotypes differed from their corresponding photograph at between 2 and 4 loci, or between 1 and 3 loci when Ggu101B and MP0059 were ignored (Table 3-6).

## DISCUSSION

Capture visit rates for all species, and for fishers alone, were higher for camera traps than for hair snares. The camera traps also yielded estimates of density and survival for fishers in the Kings River population (Chapter 1), which was not possible for hair snares because of the low sample size and questionable genotypes obtained from hair samples. A number of factors contributed to the lower success of the hair

snares, some of which were related to the device used in the field and others to the laboratory analyses.

**Field methods**

Fewer than half of the capture visits of fishers yielded a hair sample. I also measured effectiveness from a small subset of stations with a track plate inside the box instead of with a camera trap. This method yielded a much higher effectiveness for capture of hair samples. The higher effectiveness measured by track plates than by camera traps suggests that some animals were detected with the camera, but did not enter the bait station. One explanation for the higher number of camera captures than hair captures is that some animals avoided the bait station after being startled by the camera flash. Because of the placement of the infrared trigger (Fig. 1-2), it was possible for an animal to trigger the camera trap without entering the box. However, it is also possible that fishers are generally wary of entering the trap box, and it is impossible to tell if it was the flash or their reluctance to enter the box that prevented many fishers from doing so. Using a camera with an infrared flash that would not be perceived by the animal could address this question.

An additional snare design issue was the potential for more than 1 animal to enter the snare device between visits by researchers, and I feel that this is 1 of the more significant shortcomings of my hair snare's design. In cases where I had camera captures to compare to hair captures of fishers, there were often other species – and in 2 cases another fisher – visiting the station (Table 3-6). Although I tried to separate the samples by analyzing clumps of hair collected from different barbs separately,

there was still the potential for mixing of samples, leading to erroneous species identifications or genotypes (Roon et al. 2005a). Although none of the fisher genotypes had more than 2 alleles at each locus, this is not a guarantee that samples were not mixed, especially considering the low number of alleles at each of the loci used. A solution to this problem would be to use a hair snare that becomes disabled after a single use (e.g. Belant 2003, Beier et al. 2005).

**Laboratory methods**

The duration that samples are stored can significantly affect the quality of DNA contained in them, particularly when these samples have low quality DNA to begin with (Roon et al. 2003). I postponed extraction of the hair samples until after developing a genotyping method, so the samples were stored at -80 °C for between 2 and 4 years. This may have contributed to the substantial drop-off between the number of samples extracted and the number that I identified to species. Future studies of this nature should extract the DNA as soon as possible after the samples are brought in from the field, even if further laboratory analysis will not be completed immediately.

Genotyping errors that occur during amplification of DNA are a common problem associated with using noninvasively-collected samples in a capture-recapture context (Paetkau 2003, McKelvey and Schwartz 2004, Roon et al. 2005b). Two of the more common methods of addressing this problem require either genotyping each sample multiple times to obtain a consensus genotype (Taberlet et al. 1996, Valière et al. 2002) or using likelihood-based methods or deviations from Hardy-Weinberg

equilibrium to identify samples that may have incorrect genotypes (Miller et al. 2002, van Oosterhout et al. 2004, McKelvey and Schwartz 2005, Roon et al. 2005b). Due to budget and time limitations, I was unable to employ fully either of these methods, although in most cases, I had at least 2 consensus scans for each genotype.

**Comparison of camera trapping and hair snares**

Setting aside the difference in capture efficiency of the 2 methods explored in this chapter, it is worth addressing the different forms of data that are obtained. Each of the 2 trapping types generates data that can be used in a context other than a capture-recapture study. With camera traps, the composition of the mesocarnivore community can be analyzed from photographs. While it is also possible with genetic data, species identification is much easier to accomplish from a photograph. Also, because of the time/date stamp that appears on each photograph, one can learn more about the activity times of animals captured. Combining this information with the species composition information can be used to explore questions of resource partitioning among the members of the local wildlife community (Schoener 1974, Kronfeld-Schor and Dayan 2003). With hair snares, genotypic data can be used for any of a number of population genetic analyses, including studies of relatedness (Blouin 2003), estimation of effective population size (Waples 2005), and movements of individuals between populations (Wilson and Rannala 2003).

Both devices also have potential drawbacks. The challenges associated with using a hair snaring device have been described above. One of the more significant difficulties of using camera traps is that they are a mechanical device that can

malfunction. The higher number of lost trap nights for cameras represents the addition of nights lost to malfunctions and running out of film.

As mentioned above, another drawback to using camera traps, at least with a species like the fisher, is that live trapping is required prior to camera recapture. Although camera traps can be used in a capture-recapture context with species that have distinct pelage (Karanth et al. 2006), physical capture and marking animals is required for a species like the fisher. If the goal is to have a completely noninvasive study design, camera trapping is not a practical option for these species. The requirement for live-trapping also increases the cost of camera trapping because of the labor costs associated with the additional trapping.

## MANAGEMENT IMPLICATIONS

This study contrasted 2 methods for noninvasively monitoring a population of fishers in the southern Sierra Nevada. Several logistical limitations were observed for the hair snaring method, most notably the snare design that allows for multiple hair samples to be captured between visits, the long duration between hair snaring and DNA extraction, and resource limitations that prevented the use of a best-practices methodology for genotyping.

Hair snares with genetic tagging have been used successfully for monitoring other species of mesocarnivores (e.g. Mowat and Paetkau 2002), and none of challenges described here are insurmountable obstacles to developing an efficient method for monitoring fishers in this manner. Given the greater costs associated with

camera trapping as well as the disturbance of live-trapping, hair snaring may prove a better method in certain circumstances if these shortcomings can be overcome.

However, based on the results of this study, at present camera trapping is a more appropriate method for noninvasively obtaining demographic information for fishers. Particularly if live-trapping is already being used, such as during a telemetry study, camera trapping can be an effective method for noninvasively collecting demographic information from fishers.

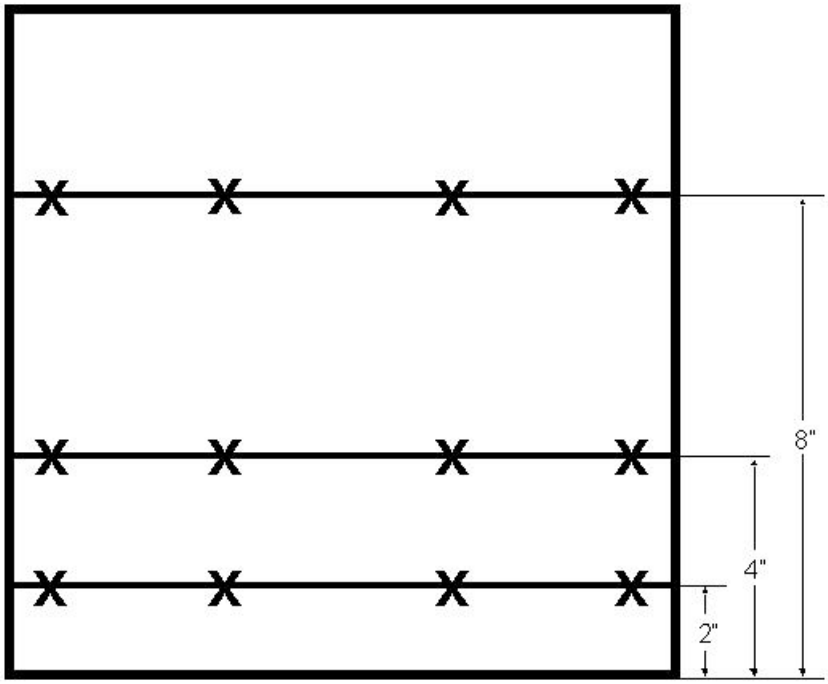


Fig. 3-1. Front view of hair snare showing the arrangement of the barbs used to collect samples from fishers in the Sierra National Forest, Fresno County, California, USA from 2002 to 2004.

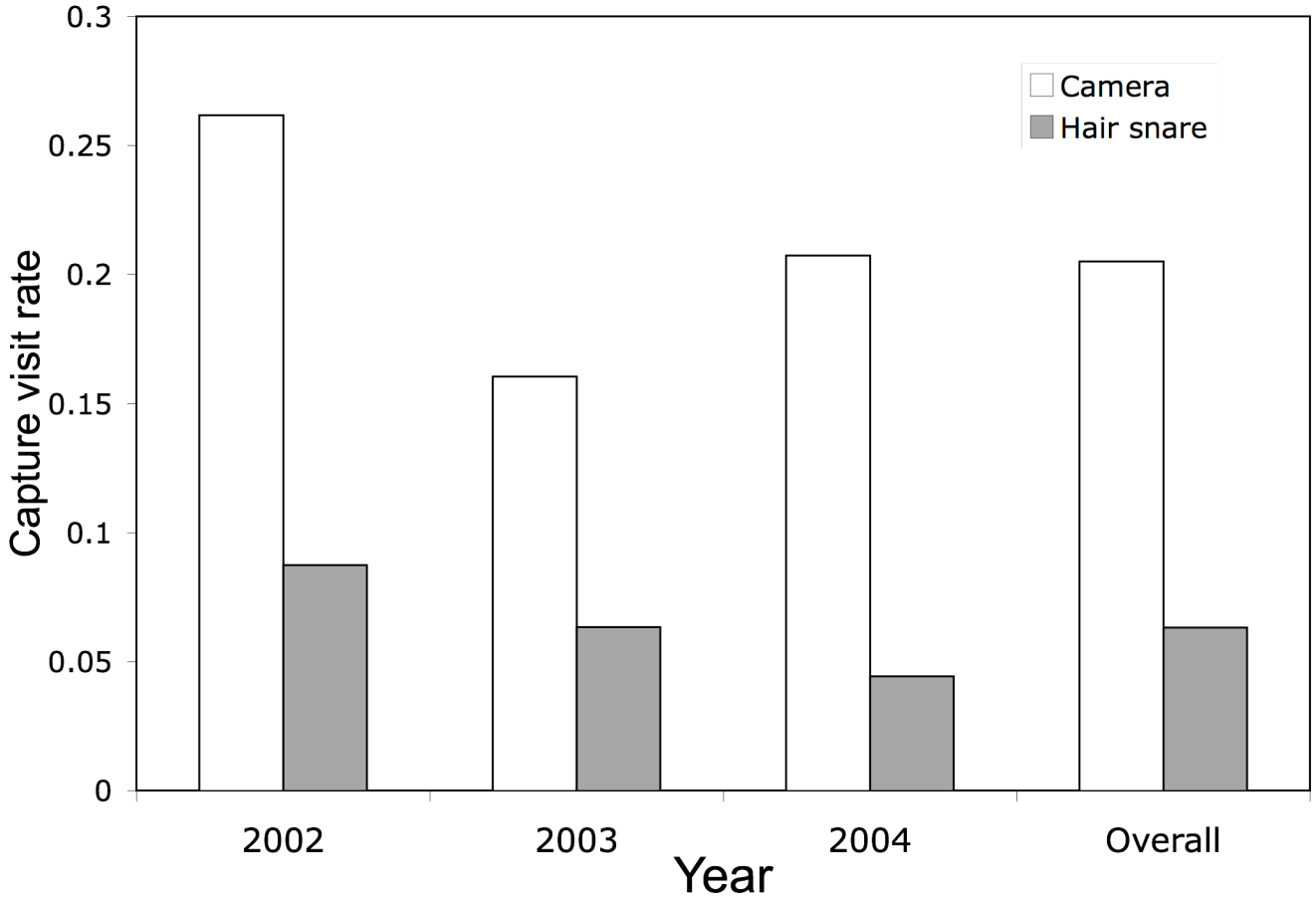


Fig. 3-2. Capture visit rates of all species combined for camera traps and hair snares from the Sierra National Forest, Fresno County, California, USA for 2002-2004. The capture rate was calculated as the number of capture visits per active trap night (all trap nights less those lost to bear damage or device malfunction), with a capture visit defined as a researcher visit when an animal had entered the station and been photographed or left a hair sample since the previous visit.

Table 3-1. Microsatellite loci and reaction conditions used to genotype fisher hair samples collected from hair snares in the Sierra National Forest, Fresno County, California, USA from 2002-2004.

| Locus | Annealing temp. | Cycles | GenBank Accession no. | Reference |
|---|---|---|---|---|
| Ggu101B | 52/53[b] | 20/30 | AF014840 | (Duffy et al. 1998) |
| Lut733 | 55/53[a] | 23/8 | Y16293 | (Dallas and Piertney 1998) |
| MP0059 | 56/53[a] | 22/8 | EF042877 | (Jordan et al. 2007) |
| MP0144 | 57/53[b] | 20/30 | EF042883 | (Jordan et al. 2007) |
| MP0175 | 56/53[a] | 22/8 | EF042884 | (Jordan et al. 2007) |
| MP0197 | 59/53[a] | 23/8 | EF042888 | (Jordan et al. 2007) |
| MP0247 | 59/53[b] | 20/28 | EF042893 | (Jordan et al. 2007) |

[a] Fluorescent labeling with M13 using first program described in Chapter 2, adapted from Schuelke (2000).

[b] Fluorescent labeling with M13 using second program described in Chapter 2, adapted from Guo & Milewicz (2003).

Table 3-2. Summary information for camera traps and hair snares used to monitor a population of fishers in the Sierra National Forest, Fresno County, California, USA for 2002-2004.

| Year | Trap type | No. of traps | Elevation range of traps (m) | Active trap nights[a] | Lost trap nights (% of total) |
|---|---|---|---|---|---|
| 2002 | Camera | 109 | 1165-2282 | 1223 | 83 (6.4%) |
| | Snare | 113 | 1110-2282 | 1282 | 62 (4.6%) |
| 2003 | Camera | 157 | 1110-2282 | 1628 | 232 (12.5%) |
| | Snare | 157 | 1110-2282 | 1660 | 200 (10.8%) |
| | Brush | 30 | 1236-2282 | 357 | 3 (0.8%) |
| 2004 | Camera | 156 | 1110-2282 | 1597 | 223 (12.3%) |
| | Snare | 156 | 1110-2282 | 1648 | 172 (9.5%) |
| | Brush | 30 | 1236-2243 | 347 | 1 (0.3%) |
| TOTALS | Camera | 422 | 1110-2282 | 4448 | 538 (10.8%) |
| | Snare | 426 | 1110-2282 | 4590 | 434 (8.6%) |
| | Brush | 60 | 1236-2282 | 704 | 4 (0.6%) |

[a] Number of trap nights not lost to bear damage or other cause.

Table 3-3. Camera trap and hair snare capture results for all species and fishers specifically in the Sierra National Forest, Fresno County, California, USA for 2002-2004.

| | | All species | | | Fishers | | |
|---|---|---|---|---|---|---|---|
| Year | Trap type | Captures[a] | Capture rate (per active trap night) | Latency[b] | Captures[a] | Capture rate (per active trap night) | Latency[b] |
| 2002 | Camera | 381 | 31.2% | 2.64 | 90 | 7.4% | 4.35 |
| | Snare | 112 | 8.7% | 2.58 | 7 | 0.5% | 3.43 |
| 2003 | Camera | 300 | 18.4% | 3.75 | 75 | 4.6% | 4.79 |
| | Snare | 105 | 6.3% | 3.00 | 6 | 0.4% | 4.17 |
| | Brush | 11 | 3.1% | 2.50 | 0 | 0 | n/a |
| 2004 | Camera | 393 | 24.6% | 3.61 | 62 | 3.9% | 5.03 |
| | Snare | 84 | 5.1% | 3.45 | 1 | 0.1% | 4.00 |
| | Brush | 3 | 0.9% | 3.67 | 0 | 0 | n/a |
| TOTALS | Camera | 1074 | 24.1% | 3.34 | 227 | 5.1% | 4.67 |
| | Snare | 301 | 6.6% | 3.02 | 14 | 0.3% | 3.79 |
| | Brush | 14 | 2.0% | 2.89 | 0 | 0 | n/a |

[a] Photograph or hair sample collected.

[b] Average number of trap nights before a capture occurred. Does not include traps with no captures.

Table 3-4. Comparison of capture visits at camera traps and hair snares from the Sierra National Forest, Fresno County, California, USA for 2002-2004. A capture visit was defined as a researcher visit when an animal had entered the station and been photographed or left a hair sample since the previous visit.

| | Photo | No photo | TOTAL |
|---|---|---|---|
| Hair | 193 (22%) | 97 (11%) | 290 (33%) |
| No hair | 578 (67%) | n/a[a] | 578 (67%) |
| TOTAL | 771 (89%) | 97 (11%) | 868 |

[a] Impossible to count.

Table 3-5. Hair snare effectiveness measured by the collection of hair samples when a camera trap was triggered by a particular species in the Sierra National Forest, Fresno County, California, USA for 2002-2004.

| Species | Camera capture visits | Hair samples | |
|---|---|---|---|
| | | Number | Proportion |
| Aves | 1 | 0 | 0 |
| *Bassariscus astutus* | 66 | 9 | 0.14 |
| *Bos taurus* | 1 | 1 | 1.00 |
| *Canis familiaris* | 4 | 2 | 0.50 |
| *Canis latrans* | 1 | 1 | 1.00 |
| *Didelphis virginiana* | 3 | 1 | 0.33 |
| *Glaucomys sabrinus* | 11 | 0 | 0 |
| *Lynx rufus* | 4 | 1 | 0.25 |
| *Martes americana* | 34 | 4 | 0.12 |
| *Martes pennanti* | 185 | 77 | 0.42 |
| *Mephitis mephitis* | 2 | 0 | 0 |
| *Mustela frenata* | 6 | 1 | 0.17 |
| *Mustela sp.* | 1 | 1 | 1.00 |
| *Neotoma fuscipes* | 2 | 0 | 0 |
| *Peromyscus maniculatus* | 10 | 0 | 0 |
| *Procyon lotor* | 1 | 0 | 0 |
| *Spermophilus beecheyi* | 65 | 5 | 0.08 |
| *Spilogale gracilis* | 68 | 12 | 0.18 |
| *Tamias sp.* | 13 | 2 | 0.15 |
| *Tamiasciurus douglasii* | 114 | 15 | 0.13 |
| Unknown | 6 | 1 | 0.17 |
| *Urocyon cinereoargenteus* | 44 | 16 | 0.36 |
| *Ursus americanus* | 230 | 71 | 0.31 |

Table 3-6. Correspondence of fisher hair captures and photographs from the same visit at camera traps in the Sierra National Forest, Fresno County, California, USA for 2002-2004.

| Fisher sample id | Species | Fisher id from photo | Number of different loci[a] | Comparison out of # loci[b] |
|---|---|---|---|---|
| 22.5 | *Spermophilus beecheyi* | | | |
| | *Spermophilus beecheyi* | | | |
| 24.4 | *Martes pennanti* | 5272720553 | 2 (1) | 7 (5) |
| 25.8 | *Bassariscus astutus* | | | |
| | *Martes pennanti* | UNMARKED | | |
| | *Tamiasciurus douglasii* | | | |
| 26.7 | *Tamiasciurus douglasii* | | | |
| 27.14 | *Martes pennanti* | UNMARKED | | |
| 28.10 | *Martes pennanti* | 42302B5F6B | 4 (3) | 5 (4) |
| | *Tamiasciurus douglasii* | | | |
| 29.2 | *Martes pennanti* | UNMARKED | | |
| 30.1 | *Ursus americanus* | | | |
| 32.5 | *Martes pennanti* | 422F526067 | 4 (2) | 6 (4) |
| 34.2 | *Martes pennanti* | UNMARKED | | |
| | *Martes pennanti* | MARKED[c] | | |
| 40.11 | *Martes pennanti* | 4230542C4A | 4 (3) | 6 (4) |
| | *Martes pennanti* | 527A47624D | 3 (2) | 6 (4) |

[a] Number of loci where the hair sample and corresponding tissue sample differ. The number in parentheses is the number of loci where the 2 differ after removing the 2 loci that showed evidence of null alleles (Ggu101B, MP0059).

[b] Number of loci where both hair and corresponding tissue sample had complete genotypes. The number in parentheses is the number of loci where the 2 differ after removing the 2 loci that showed evidence of null alleles (Ggu101B, MP0059).

[c] Ear tag visible, but not possible to identify.

# Chapter 4

## Using genetic estimates of relatedness to examine sex-based differences in dispersal in fishers (*Martes pennanti*)

### INTRODUCTION

Dispersal is a fundamental process in the life-history of many species. In mammals, dispersal is often sex-biased, with greater frequency and distances for males (Greenwood 1980, Handley and Perrin 2007). Two commonly-cited justifications for sex-biased dispersal are inbreeding avoidance (Wolff 1994, Perrin and Mazalov 1999) or a kin-selected reduction in local competition for resources or mates (Greenwood 1980, Dobson 1982).

Traditionally, dispersal has been studied by following marked animals, for example those with radio transmitters (White and Garrott 1990). Molecular methods can complement these data because researchers can use the genetic structure of populations to indirectly study the dispersal process (Prugnolle and de Meeus 2002, Handley and Perrin 2007). One method for examining genetic structure is to look at the relationship of distances between individuals and their pairwise relatedness (Knight et al. 1999, Matocq and Lacey 2004). The slope of a regression between these

2 estimates can describe the pattern of dispersal, with more negative slopes implying greater philopatry because close relatives are living nearer each other.

I investigated sex-biased dispersal in a population of fishers (*Martes pennanti*), a solitary carnivore. Fishers exhibit intrasexual territoriality, where individuals defend a home range against members of the same sex, but there is considerable overlap between sexes (Johnson et al. 2000). These territories are maintained year-round except during the breeding season when males trespass on each other's territories while they search for receptive females (Leonard 1986, Arthur et al. 1989b). Both males and females disperse as juveniles, however males tend to disperse greater distances (Arthur et al. 1993, Aubry et al. 2004).

My goal in this study was to investigate the pattern of dispersal in a population of fishers in the southern Sierra Nevada. I determined spatial relationships of fishers in the population using captures from live and camera traps. I then determined pairwise relatedness among all the captured individuals to explore the relationship between spatial distribution and genetic similarity. I hypothesized that there would be a sex-based difference in the relationship between relatedness and geographic distance. Additionally, I hypothesized that this relationship would decrease more significantly with distance in females than in males because females are more philopatric. The results of this study will yield insights into a poorly understood aspect of the ecology of fishers in the southern Sierra Nevada.

## METHODS

The research took place in the southern Sierra Nevada mountains in Fresno County, California, USA. The study area, which I describe in detail in the Introduction, was divided into a 317 $km^2$ grid where I trapped fishers with live and camera traps. I explain the live and camera trapping protocols in Chapter 1. I recorded UTM coordinates (NAD 1927, Zone 11) at each trap site. Live-trapping was conducted in 2000-2004, while camera trapping took place in 2002-2004. I collected small pieces of tissue from ear punches while implanting ear tags. These samples were stored in a saturated NaCl solution (6 M) containing 25% dimethyl sulfoxide at -80 °C.

For the comparison of geographic distance to relatedness, I calculated pairwise distances between animal locations. To determine locations for fishers that were captured more than 1 time, I calculated a centroid of 100% minimum convex polygons (MCP) made up of all of the live and camera capture locations for each animal. MCP's were described using the aspace (v. 1.0) package in R (v. 2.4.1; The R Foundation for Statistical Computing), and their centroids were calculated using the PBSmapping (v. 2.09) package. Because I was interested in studying the geographic and genetic patterns resulting from juvenile dispersal, I excluded captures of pre-dispersal juveniles from the analysis.

I extracted DNA from ear tissue samples using DNEasy extraction kits following manufacturer's instructions (QIAGEN Inc.). I amplified DNA from tissue at 14 microsatellite loci (Table 4-1) in an optimized polymerase chain reaction (PCR) and obtained genotypes by measuring fragment lengths. Depending on the locus, I

used 1 of 3 sets of reaction conditions and labeling methods (Table 4-1). In the first method, I set up a 10 μL reaction containing approximately 5 ng DNA template, a PCR cocktail mix [containing 67 mM Tris-HCl (pH 8.8), 2mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, 10 mM β-mercaptoethanol, 0.2 mM of each dNTP, 0.4 U *Taq* DNA Polymerase (New England Biolabs Inc., Ipswich, MA, USA)], and 1.0 μM each of forward and reverse primers. I attached a fluorescent label to the 5' end of each forward primer (HEX; Integrated DNA Technologies, Coralville, IA, USA). This reaction was run for 2 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at an optimal annealing temperature (Table 4-1), and 10 min at 72 °C.

In the other 2 PCR methods, I added a universal M13 tag (5'-TGTAAAACG-ACGGCCAGT-3') to the 5' end of each forward primer. Amplifications were conducted with 1 of 2 methods (Schuelke 2000, Guo and Milewicz 2003), using 1 of 4 fluorescent labels: 6-FAM, HEX (Integrated DNA Technologies), NED, PET (Applied Biosystems, Foster City, CA, USA). Different labels were chosen to optimize the number of loci that could be simultaneously run in a given reaction plate. The PCR reactions conditions for the 2 M13-primer methods are described in Chapter 2.

For all amplification methods, fragment lengths were determined with an ABI 3730 sequencer using LIZ 500 size standard (Applied Biosystems) and analyzed using GENOTYPER 3.7 software (Applied Biosystems). I used the program MICROCHECKER (van Oosterhout et al. 2004) to assess genotyping error rates.

I calculated pairwise relatedness (*r*) between each possible combination of individuals using a method of moments estimator developed by Wang (2002). Pairwise distances between post-dispersal individuals were calculated using either the

capture location for animals only caught once or centroids of all capture locations for animals caught more than once. To test the significance of the relationship between *r* and Euclidean distance between pairs of individuals, I used a Mantel test (Mantel 1967). Calculations of *r* and its comparison to geographic distance were conducted with the program SPAGeDi (Hardy and Vekemans 2002).

## RESULTS

From 2000 to 2004 I captured 46 different fishers in live or camera traps. These fishers were caught between 1 and 9 times each ($\overline{X}$ = 3.3, SE = 2.4). I documented 2 dispersal events in the study area based on locations that were outliers among inter-trap distances for each animal. I included only the post-dispersal locations for these animals in subsequent analyses. For fishers that were caught more than once, capture locations were an average of 1.26 km from the estimated centroids (SE = 784 m; Table 4-2). This value was less than the average radius of home ranges for males in the study area of 2.64 km based on 100% MCP's of radiotelemetry data (Mazzoni 2002). Pre-dispersal locations were >10 km from other capture locations for the 2 dispersing animals.

Pairwise distances between locations for females ($\overline{X}$ = 10.0 km, SE = 5.9 km) did not differ from pairwise distances between all pairs of males ($\overline{X}$ = 9.1 km, SE = 5.1 km; $t_{513}$ = 1.76, $P$ = 0.080). Among all fishers, there was an average pairwise distance between individuals of 9.5 km (SE = 5.5 km).

I genotyped each of the 46 samples at between 8 and 14 loci ($\overline{X}$ = 12.5, SE = 1.5). The loci Ggu101B and MP0059 showed an excess of homozygotes, suggesting the presence of null alleles. These loci were not included in subsequent analyses.

The average pairwise relatedness among all individuals was 0.017 (SE = 0.34). There was no difference in average pairwise relatedness between females ($\overline{X}$ = -0.0013, SE = 0.33) and males ($\overline{X}$ = 0.048, SE = 0.35; $t_{513}$ = 1.61, $P$ = 0.11).

Overall, there was a slight negative relationship between relatedness and geographic distance (slope = $-6.50 \times 10^{-3}$ km$^{-1}$, $R^2$ = 0.011, $P$ = 0.046; Fig. 4-1). Results for female fishers were comparable, although non-significant (slope = $-6.62 \times 10^{-3}$ km$^{-1}$, $R^2$ = 0.014, $P$ = 0.069; Fig. 4-2). There was also a negative relationship between relatedness and geographic distance for all pairs of males, which approached significance and had a higher magnitude than for either females or all fishers combined (slope = $-4.96 \times 10^{-2}$ km$^{-1}$, $R^2$ = 0.036, $P$ = 0.051; Fig. 4-3).

## DISCUSSION

Overall, there was a slightly negative correlation between the 2 for all fishers. Males did, however, show a slightly, though not significantly, different pattern than that found in the population as a whole. There was a greater correlation between relatedness and distance among males, and the absolute value of the slope of this relationship was nearly an order of magnitude greater than for females. Contrary to my expectations, these results suggest that males are slightly more philopatric than females.

Although the slope of the relationship between relatedness and distance was significantly different from 0 when males and females were analyzed together, its magnitude was close to 0. This suggests that dispersal distance is random because there is not a strong relationship between it and relatedness, and that other factors like habitat quality and availability of territories play a more important role than inbreeding avoidance in dispersal in this species. Additionally, the samples collected in this study represented multiple generations, so there was potentially mixing among generations that would obscure any relationship between close relatives and their geographic location.

The lack of a strong relationship between the geographic distance and relatedness as well as the spread of the data can be also be partially explained by the fact that the geographic scope of the study was small relative to the home range size and potential dispersal distance of fishers. The greatest intra-trap distance was ~34 km. Two other studies in the southern Sierra Nevada have estimated 100% MCP home ranges sizes between 21.9 $km^2$ (Mazzoni 2002) in the Kings River population and 30.0 $km^2$ in the Sequoia National Forest (Zielinski et al. 2004a). In Maine, dispersal distances ranged from 4 to 23 km with males tending to disperse farther than females (Arthur et al. 1993). These short dispersal distances (relative to the size of an adult home range) were probably due to the fact that the study population was trapped, leading to more territorial vacancies. In contrast, 1 male dispersed approximately 100 km in a study in Massachusetts (York 1996).

To adequately address questions of dispersal in fishers, one would ideally need to study fisher populations on a greater geographic scale. The southern Sierra Nevada

fisher population can be divided into approximately 5 subpopulations based on regions of available habitat separated by major river drainages (Truex, pers. comm.). This study sampled intensively within 1 of these subpopulations.

Using samples from related individuals has been proposed as a method for identifying dispersers between populations (Palsbøll 1999), which can then be used to estimate migration rates in a manner similar to that used with an assignment test (Wilson and Rannala 2003). Sampling adjacent populations to the north and south of my study area and analyzing the data in this way would allow for a clearer understanding of long-distance dispersal in this species. These data are critical to developing a more comprehensive understanding of the connectivity of fisher populations in the region, and the role of life-history in the demographic connectivity of this species.

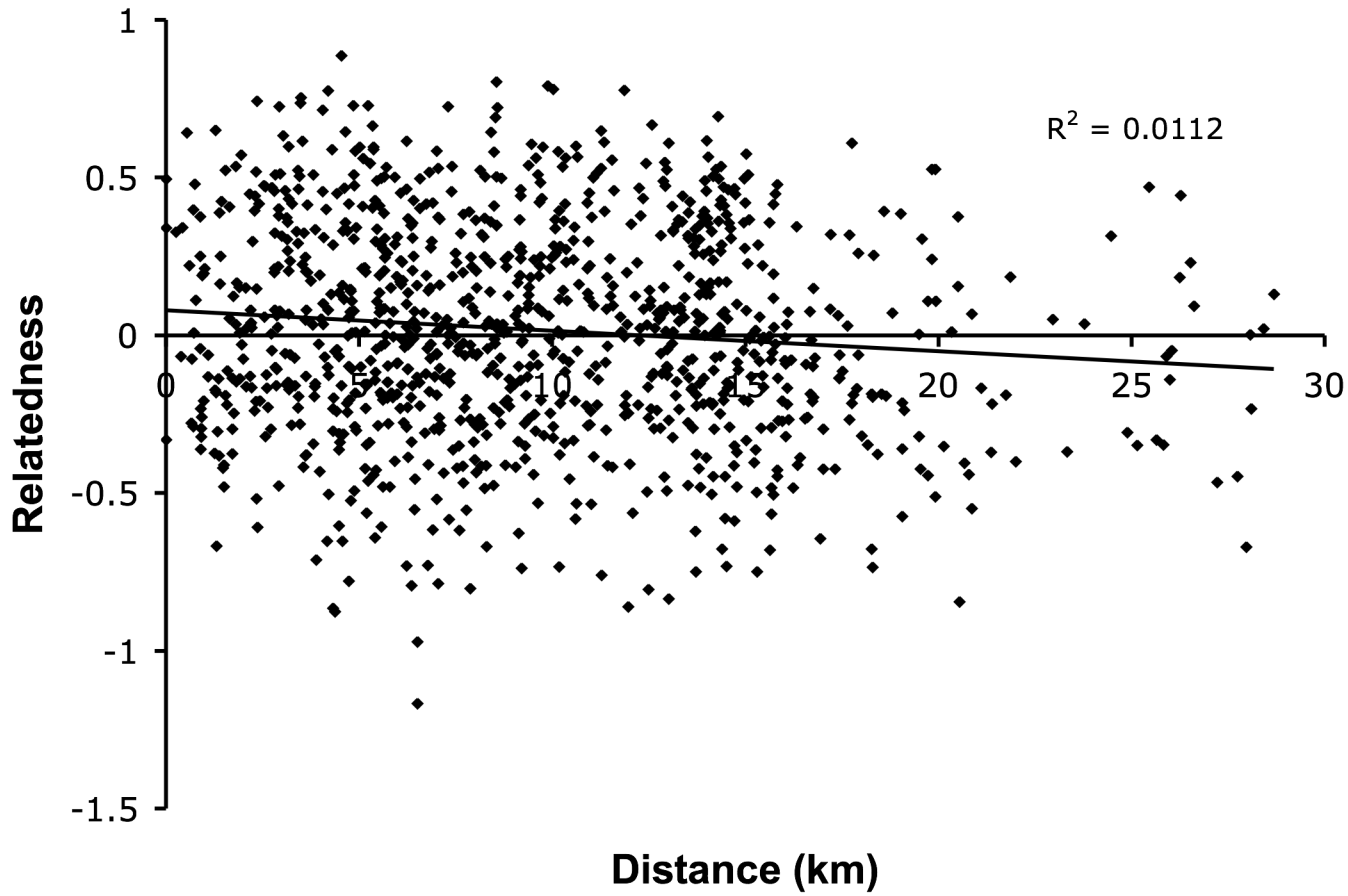


Fig. 4-1. Relationship between pairwise relatedness (Wang 2002) and geographic distance between all pairs of fishers caught in live and camera traps in the Sierra National Forest, Fresno County, California, USA, 2000-2004.

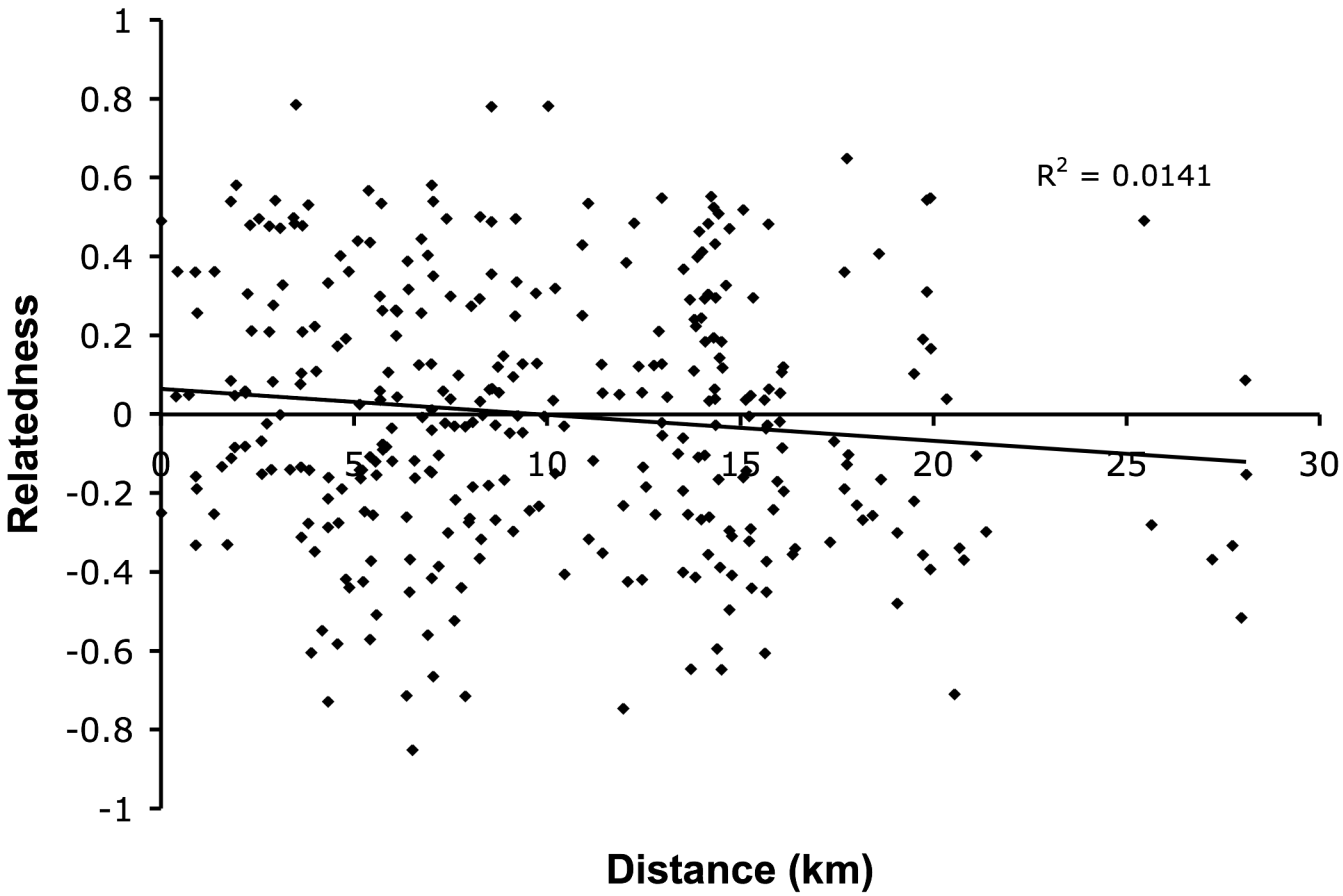


Fig. 4-2. Relationship between pairwise relatedness (Wang 2002) and geographic distance between all pairs of female fishers caught in live and camera traps in the Sierra National Forest, Fresno County, California, USA, 2000-2004.

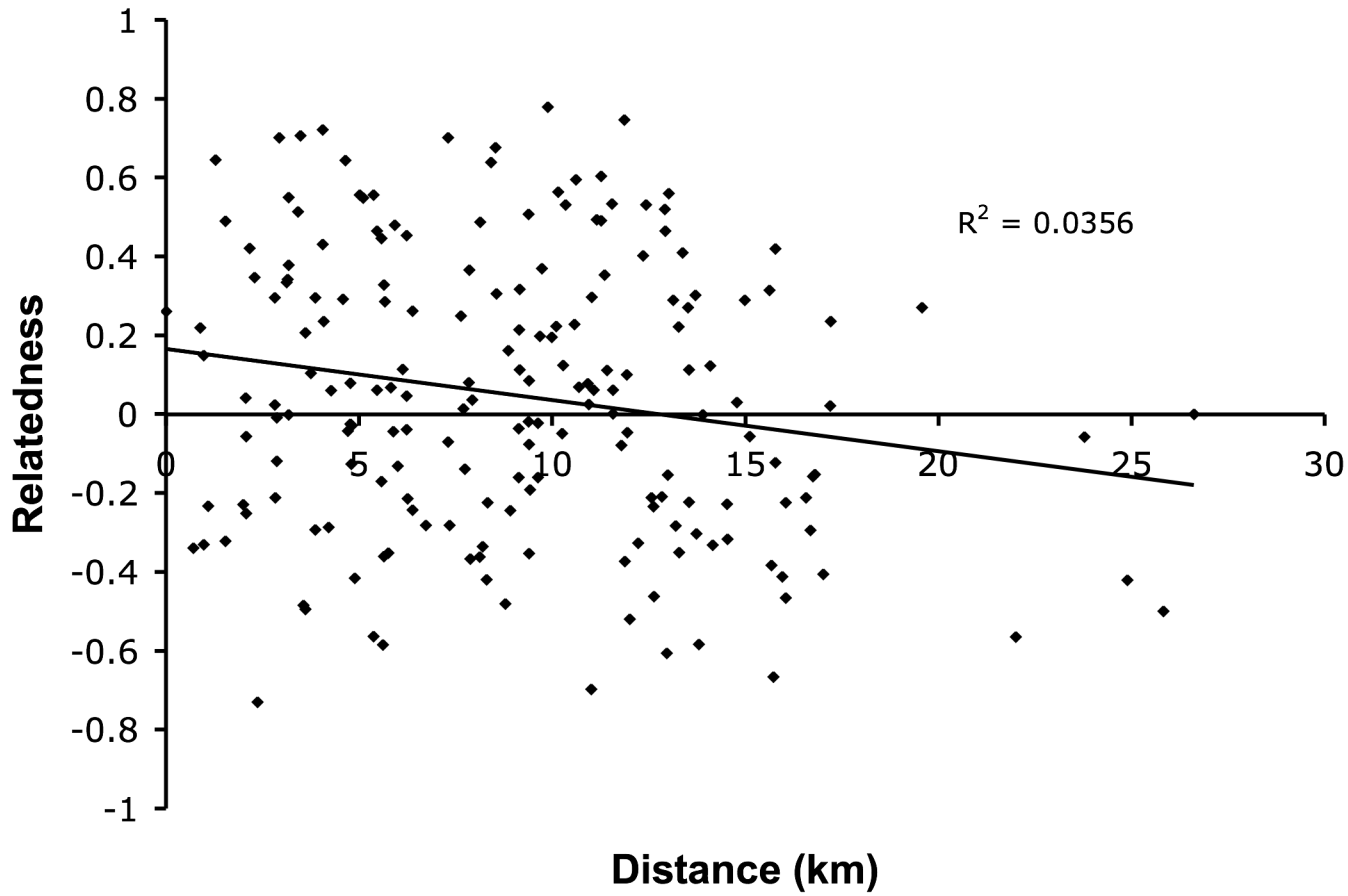


Fig. 4-3. Relationship between pairwise relatedness (Wang 2002) and geographic distance between all pairs of male fishers caught in live and camera traps in the Sierra National Forest, Fresno County, California, USA, 2000-2004.

Table 4-1. Microsatellite loci and reaction conditions used to genotype ear tissue samples collected from fishers in the Sierra National Forest, Fresno County, California, USA, 2000-2004.

| Locus | Annealing temp. | Cycles | GenBank Accession no. | Reference |
|---|---|---|---|---|
| Ggu101B | 52/53[c] | 20/30 | AF014840 | (Duffy et al. 1998) |
| Ggu216 | 53/53[c] | 20/30 | AF014841 | (Duffy et al. 1998) |
| Lut604 | 58/53[b] | 23/8 | Y16300 | (Dallas and Piertney 1998) |
| Lut733 | 55/53[b] | 23/8 | Y16293 | (Dallas and Piertney 1998) |
| MA-1 | 60[a] | 30 | AF075137 | (Davis and Strobeck 1998) |
| Mer009 | 50/53[c,d] | 22/32 | AF132108 | (Fleming et al. 1999) |
| Mer022 | 58/53[b] | 23/8 | AF132109 | (Fleming et al. 1999) |
| MP0059 | 56/53[b] | 22/8 | EF042877 | (Jordan et al. 2007) |
| MP0144 | 57/53[c] | 20/30 | EF042883 | (Jordan et al. 2007) |
| MP0175 | 56/53[b] | 22/8 | EF042884 | (Jordan et al. 2007) |
| MP0197 | 59/53[b] | 23/8 | EF042888 | (Jordan et al. 2007) |
| MP0200 | 58/53[b] | 22/8 | EF042889 | (Jordan et al. 2007) |
| MP0247 | 59/53[c] | 20/28 | EF042893 | (Jordan et al. 2007) |
| Mvis002 | 55[a] | 30 | AF132100 | (Fleming et al. 1999) |

[a] Fluorescent labeling with label attached to forward primer.

[b] Fluorescent labeling with M13 using first program described in Chapter 2, adapted from Schuelke (2000).

[c] Fluorescent labeling with M13 using second program described in Chapter 2, adapted from Guo & Milewicz (2003).

[d] Template diluted 200× (instead of 1000×) between first and second amplification steps.

Table 4-2. Capture locations of fishers from live and camera traps in the Sierra National Forest, Fresno County, California, USA from 2000-2004. UTM coordinates are for Zone 11, NAD 1927. For animals that were caught more than once, centroids are the geographic center of all capture locations. For animals caught only once, this number is the capture location. Capture locations that are pre-dispersal are indicated in ***bold italic*** type. These locations were not included when determining that animal's centroid.

| | Capture locations | | Centroid locations | | Distance to centroid | Average distance to centroid | SE of distance to centroid |
|---|---|---|---|---|---|---|---|
| Fisher ID | UTM E | UTM N | UTM E | UTM N | (m) | (m) | (m) |
| 421C1B707D | 305819 | 4092693 | 305732 | 4092972 | 292 | 293 | 0.67 |
| | 305645 | 4093252 | | | 293 | | |
| 421C3A7773 | 296670 | 4105941 | 296670 | 4105941 | 0 | 0 | 0 |
| 422F280952 | 296230 | 4104662 | 297076 | 4105500 | 1191 | 1272 | 302.50 |
| | 296589 | 4106472 | | | 1087 | | |
| | 297028 | 4104017 | | | 1484 | | |
| | 297381 | 4107145 | | | 1673 | | |
| | 297961 | 4105227 | | | 926 | | |
| 422F2B403D | 302805 | 4107921 | 302983 | 4106823 | 1112 | 1112 | 0.69 |
| | 303161 | 4105726 | | | 1111 | | |
| 422F346543 | 302636 | 4101003 | 302903 | 4101630 | 681 | 682 | 0.65 |
| | 303170 | 4102258 | | | 682 | | |
| 422F36764D | 296113 | 4103371 | 296113 | 4103371 | 0 | 0 | 0 |
| 422F3D7164 | 300299 | 4106758 | 300835 | 4106284 | 716 | 694 | 189.79 |
| | 301049 | 4105438 | | | 873 | | |
| | 301159 | 4106658 | | | 495 | | |
| 422F3F060A | 312733 | 4093192 | 312733 | 4093192 | 0 | 0 | 0 |
| 422F45284B | 319585 | 4088825 | 319947 | 4089188 | 513 | 513 | 0.50 |
| | 320309 | 4089552 | | | 513 | | |

Table 4-2. *Continued*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 422F455530 | 308687 | 4095203 | 309612 | 4095373 | 940 | 975 | 119.09 |
| | 309684 | 4096247 | | | 877 | | |
| | 310467 | 4094669 | | | 1108 | | |
| 422F4B404B | 308761 | 4098129 | 307472 | 4097513 | 1429 | 1444 | 590.95 |
| | 305246 | 4097774 | | | 2241 | | |
| | 308239 | 4097810 | | | 822 | | |
| | 308410 | 4096636 | | | 1284 | | |
| 422F526067 | 303091 | 4098878 | 304274 | 4099059 | 1197 | 1197 | 0.10 |
| | 305457 | 4099241 | | | 1197 | | |
| | ***295192*** | ***4102190*** | | | ***9607*** | | |
| 42302B5F6B | 310708 | 4107078 | 310708 | 4107078 | 0 | 0 | 0 |
| | ***300704*** | ***4107728*** | | | ***10025*** | | |
| | ***298940*** | ***4108601*** | | | ***11866*** | | |
| | ***297811*** | ***4109401*** | | | ***13105*** | | |
| 42302C5752 | 309774 | 4104235 | 309774 | 4104235 | 0 | | |
| 42302E0D40 | 304664 | 4097577 | 304664 | 4097577 | 0 | | |
| 4230312C24 | 308670 | 4106790 | 309222 | 4105512 | 1392 | 1392 | 0.64 |
| | 309774 | 4104235 | | | 1391 | | |
| 4230344717 | 299850 | 4111406 | 301407 | 4110716 | 1703 | 2311 | 901.05 |
| | 300267 | 4113431 | | | 2945 | | |
| | 302482 | 4107692 | | | 3209 | | |
| | 302792 | 4110719 | | | 1385 | | |
| 423035600D | 312677 | 4097966 | 312677 | 4097966 | 0 | 0 | 0 |
| 423048243B | 305819 | 4092693 | 306523 | 4094486 | 1926 | 1541 | 1005.81 |
| | 306238 | 4094206 | | | 400 | | |
| | 307513 | 4096559 | | | 2297 | | |

Table 4-2. *Continued*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 42304E7457 | 304664 | 4097577 | 304664 | 4097577 | 0 | 0 | 0 |
| 4230542C4A | 294646 | 4102913 | 295153 | 4102123 | 939 | 932 | 312.02 |
| | 295925 | 4101177 | | | 1221 | | |
| | 294888 | 4101702 | | | 497 | | |
| | 294888 | 4103162 | | | 1072 | | |
| 4310476479 | 307369 | 4095551 | 309081 | 4095782 | 1728 | 1207 | 487.27 |
| | 309684 | 4096247 | | | 761 | | |
| | 310190 | 4095549 | | | 1133 | | |
| 43104C6669 | 319531 | 4092324 | 319531 | 4092324 | 0 | 0 | 0 |
| 43104C7D46 | 297912 | 4104233 | 297946 | 4105212 | 980 | 655 | 549.22 |
| | 297961 | 4105227 | | | 21 | | |
| | 297967 | 4106177 | | | 965 | | |
| 4310500533 | 302482 | 4107692 | 302482 | 4107692 | 0 | 0 | 0 |
| 4310570156 | 313358 | 4092380 | 313314 | 4092519 | 146 | 729 | 510.38 |
| | 313042 | 4093578 | | | 1093 | | |
| | 313544 | 4091599 | | | 948 | | |
| 43105A4805 | 312733 | 4093192 | 312733 | 4093192 | 0 | 0 | 0 |
| 43106D0610 | 311136 | 4098699 | 311136 | 4098699 | 0 | | |
| 43112C4B07 | 309684 | 4096247 | 309928 | 4099425 | 3187 | 2784 | 608.22 |
| | 310370 | 4102656 | | | 3261 | | |
| | 310410 | 4096715 | | | 2753 | | |
| | 309256 | 4101241 | | | 1936 | | |
| 4313071F5E | 305645 | 4093252 | 305732 | 4092972 | 293 | 293 | 0.67 |
| | 305819 | 4092693 | | | 292 | | |

Table 4-2. *Continued*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 435F603224 | 304227 | 4101358 | 304488 | 4099204 | 2170 | 1447 | 834.32 |
| | 304573 | 4098677 | | | 534 | | |
| | 304664 | 4097577 | | | 1636 | | |
| 435F7B3676 | 304664 | 4097577 | 304109 | 4098377 | 974 | 887 | 300.56 |
| | 303091 | 4098878 | | | 1135 | | |
| | 304573 | 4098677 | | | 553 | | |
| 524E723719 | 308410 | 4096636 | 308410 | 4096636 | 0 | 0 | 0 |
| 524F582E41 | 304032 | 4104446 | 304363 | 4103794 | 731 | 731 | 0.63 |
| | 304694 | 4103143 | | | 730 | | |
| 5250310545 | 307085 | 4094155 | 307085 | 4094155 | 0 | 0 | 0 |
| 5257661328 | 297381 | 4107145 | 299796 | 4105592 | 2871 | 2638 | 611.88 |
| | 298542 | 4103542 | | | 2403 | | |
| | 301159 | 4106658 | | | 1730 | | |
| | 302587 | 4105969 | | | 2816 | | |
| | 303161 | 4105726 | | | 3368 | | |
| 525B467D7B | 303625 | 4103718 | 303625 | 4103718 | 0 | 0 | 0 |
| 5272491F00 | 309912 | 4097890 | 310537 | 4098311 | 754 | 754 | 0.58 |
| | 311163 | 4098732 | | | 754 | | |
| 5272720553 | 310213 | 4099585 | 310295 | 4097463 | 2124 | 1485 | 787.35 |
| | 312264 | 4096169 | | | 2356 | | |
| | 311046 | 4097602 | | | 764 | | |
| | 308410 | 4096636 | | | 2058 | | |
| | 310716 | 4097774 | | | 523 | | |
| | 310927 | 4098346 | | | 1086 | | |

Table 4-2. *Continued*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5273124122 | 306238 | 4094206 | 307397 | 4094588 | 1220 | 1028 | 407.77 |
| | 307192 | 4093440 | | | 1166 | | |
| | 307369 | 4095551 | | | 963 | | |
| | 307375 | 4094227 | | | 362 | | |
| | 308687 | 4095203 | | | 1429 | | |
| 52731A7E43 | 310410 | 4096715 | 310094 | 4096170 | 630 | 559 | 122.40 |
| | 309684 | 4096247 | | | 417 | | |
| | 310190 | 4095549 | | | 628 | | |
| 527321265D | 296113 | 4103371 | 295724 | 4103368 | 389 | 808 | 500.32 |
| | 296086 | 4103338 | | | 363 | | |
| | 295192 | 4102190 | | | 1293 | | |
| | 295869 | 4104545 | | | 1186 | | |
| 5274201930 | 308761 | 4098129 | 309347 | 4099022 | 1068 | 1068 | 0 |
| | 309933 | 4099915 | | | 1068 | | |
| 52760E754A | 309684 | 4096247 | 309684 | 4096247 | 0 | 0 | |
| 5278696C79 | 307375 | 4094227 | 307800 | 4095963 | 1787 | 1258 | 548.15 |
| | 307694 | 4097254 | | | 1295 | | |
| | 308331 | 4096408 | | | 693 | | |
| 527A47624D | 297332 | 4100708 | 296233 | 4101080 | 1160 | 1708 | 648.64 |
| | 294646 | 4102913 | | | 2425 | | |
| | 296722 | 4099619 | | | 1541 | | |

# Chapter 5

## Conclusions and management recommendations

### CAMERA TRAPPING

Camera trapping was useful for obtaining demographic data from fisher populations. I successfully obtained estimates of both density and survival for fishers in the Kings River Project area. Additionally, I estimated the reproductive rate of females that were live-caught in conjunction with marking for camera trapping.

The density estimates I obtained over the 3 years of this study are lower than most estimates reported for fishers. The relatively low density of fishers suggests that habitat quality is not as high in this area as it is elsewhere. One substantial difference between my study area and many of the other places where these estimates were obtained is the scarcity of porcupines. Where they are found, porcupines are a significant contributor to the energetic budget of fishers (Powell 1979), so it is reasonable to assume that their absence is of great consequence to fishers in the southern Sierra Nevada. Because fishers in this region eat a more general diet composed of lower energy foods (Zielinski et al. 1999), a given area may not be able to support as many animals.

By contrast, adult survival rates were roughly comparable to those observed in other populations. However, because of the short duration of the study and low sample size, the capture-recapture estimates of survival had very wide confidence

intervals. This uncertainty in the parameter estimate makes projections of future trends difficult.

## HAIR SNARING AND GENETIC TAGGING

Unlike camera traps, hair snares were ineffective for monitoring fishers during this study. A number of factors potentially contributed to this difference in efficacy. The snare itself may have been ineffective at collecting samples, either because of the orientation of the barbs or the barbs themselves. A more substantial concern with the design of the hair snare was that more than 1 individual was able to come to the station between investigator visits, leading to the possibility of samples from multiple animals being collected together. If these samples were mixed and analyzed as if they had come from 1 animal, the result would be spurious genotypes that would either not match any animal in the population or incorrectly match the wrong individual.

Hair samples collected in the field have low levels of DNA, so proper preservation and laboratory methods are crucial for successfully obtaining genotypes from them. One potential cause of the low number of extracted samples with sufficient DNA for species identification was sample degradation during storage time. Even for samples that had sufficient DNA, I did not have enough resources to use best-practices techniques for mitigating genotyping errors.

Although the genetic tagging portion of this study fell below expectations, the microsatellite markers developed in the process will be very useful for fisher research in the future. Excluding the Kings River population, at least 17 markers were

polymorphic in each population from which I had samples. These new markers will greatly enhance our ability to conduct ecological studies on fishers.

Genetic sampling still holds promise for improving our understanding of fisher ecology and for developing effective management plans. I recommend additional effort at developing and improving a hair snaring device for fishers (e.g. Zielinski et al. 2006a). Further, other methods for genetic sampling that were not explored in my study can also be used. One potentially effective method is the use of trained dogs to detect scat samples (Smith et al. 2001, Smith et al. 2005). This method has already been employed succesfully to collect samples from the Kings River population (K. Purcell, pers. comm.).

## DISPERSAL

This study showed a possible difference in dispersal pattern between the sexes, at least within the confines of the study area. In general, relatedness declined slightly with increasing geographic distance. However this pattern was more pronounced (by almost an order of magnitude) in males, which contradicts theoretical predictions of greater female philopatry in mammals. This is an intriguing finding and suggests a fruitful area for future study, particularly at larger geographic scales than the one investigated here. Expanding the geographic scope of the present study may lead to results more in keeping with theoretical expectations.

## MANAGEMENT RECOMMENDATIONS

This study has shown the potential for intensive monitoring of fisher populations to obtain population parameter estimates and to understand the process of dispersal. I recommend continuing research in this vein to better understand the relationship between established regional surveys and local demography. Of particular interest is comparing density estimates to the number of detections at sample stations for regional surveys (Zielinski and Stauffer 1996). Other studies in the region in and adjacent to Sequoia Kings Canyon National Park would be interesting from this standpoint because there have been more fisher detections in this area (Zielinski et al. 2005).

More research is needed to understand the impact of forest management activities on fisher habitat and population trajectory. Recent challenges to Forest Service management actions on the Sequoia National Monument and in the Kings River Project area stress the need for clearer understanding of how these activities affect population persistence for fishers. What are the short-term effects of fuels-reduction treatments? Do these balance the presumed long-term benefits of management activities designed to reduce the risk of catastrophic wildfire?

Obtaining precise parameter estimates, understanding critical life-history processes like reproduction, mortality, and dispersal, and predicting population trend and responses to management activities all require large amounts of data collected over multiple years and at landscape scales. Future research on fishers should be conducted with a view toward long-term studies and placed in an adaptive management framework where management activities are the experimental treatments

(Marzluff et al. 2000, Nichols and Williams 2006). I recommend intensive studies of habitat use by telemetry of marked fishers. These could then be coupled with capture-recapture studies using camera traps like those described in this dissertation to obtain population parameter estimates.

One major limitation of this study was its geographic scope. To understand the population dynamics of an animal as wide-ranging and vagile as a fisher, one must design a study that covers a large geographic area. Radiotelemetry is a vital tool for observing the movements of individuals, particularly to gain an understanding of how they might use dispersal habitat. I have shown that genetic methods can also be employed to study this process in fishers by documenting the movement of genes in related individuals. However, my study was limited by its geographic scope and by the fact that dispersal was common within the population, potentially obscuring any pattern because the samples were drawn from overlapping generations.

Future research on dispersal in fishers should take a similar approach as in my study, but look at movements over a wider geographic scale. Because dispersal events are presumably more rare between than within subpopulations in the southern Sierra Nevada, there would be more signal in the genetic data. Using samples collected from the studies I describe above, researchers could determine if the pattern I observed in male fishers (that they are slightly more philopatric than females) holds at larger geographic scales. It could also directly estimate migration rates between populations, which would enhance our understanding of the metapopulation biology of fishers in the southern Sierra Nevada.

Understanding the metapopulation dynamics of fishers, as well as their responses to forest management, will provide valuable insights into how to manage for this species' continued persistence in the southern Sierra Nevada. However, the ultimate goal for fisher management in California should not be harm reduction, but enhancement of the current population. Ultimately, the primary objective of fisher management in the state should be to recover the species in the central and northern Sierra Nevada, either through natural expansion of the current range or translocations of animals from areas where fishers are found in high densities. A better understanding of the pattern of dispersal in fishers could yield insights into this species' ability to recolonize the area from which it has been extirpated, particularly when these data are coupled with an enhanced understanding of the fisher's habitat requirements.

There is a great need to proactively manage populations of fishers in California because of their dependence on late successional habitats and a substantial reduction in their distribution over the past century. The management situation for this species has become more constrained with the pending Endangered Species Act listing and challenges to Forest Service management activities. However, this is also a great opportunity to engage in further research on the responses of threatened wildlife to the forest management activities that can most directly impact their populations. This dissertation has presented an assessment of different monitoring methods as well as a preliminary analysis of dispersal in fishers using genetic data. Further, I have provided vital information about the status of the Kings River population of fishers.

This is a critical time for management of this species, and the methods I have described can enhance our ability to study and manage it.

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