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## **Developing a DNA-based Population Estimator for Wolves in Southeast Alaska**

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This is a progress report on continuing research. Information may be refined at a later date.

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## RESEARCH PERFORMANCE REPORT APPENDIX

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**STUDY TITLE:** Developing a DNA-based Population Estimator for Wolves in Southeast Alaska

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### SUMMARY

The objective of our research during this reporting period was to produce a detailed proposal for the development of a method to estimate populations of wolves in Southeast Alaska. We reviewed a number of different methods including aerial counts, track surveys, the use of biomarkers, and DNA-based techniques. We chose to experiment with a DNA-based mark-resight method because we believe that it may be the most feasible and cost-effective of the methods that we explored. We also are able to test the key assumptions associated with the method. We propose to extract the DNA of wolves from their feces and identify individuals using DNA fingerprinting techniques. These data will be analyzed using a Bayesian mark-resight population estimation model. We will start by conducting a small pilot study on a wolf population of known size on Heceta Island. We will verify that individuals can be successfully identified from scats and that the Bayesian model functions properly. We will also test how quickly DNA in scats is degraded under the wet conditions encountered in Southeast Alaska. If we are successful with the pilot study, we propose to test the method on a portion of Prince of Wales Island. We will collect scats from a series of transects established along roads and trails. Transects will be located within an area used for a concurrent study of wolves and deer. A population estimate derived from the direct observations of radio-collared wolves will be compared to the DNA-based estimate. We will consider the method to be successful if the DNA-based estimate is within 10% of the estimate derived from radiocollared wolves, and if the 95% confidence interval is <25% of the estimate.

**Key words:** *Canis lupus ligoni*, DNA fingerprinting, mark-resight, microsatellite DNA, population estimation, Southeast Alaska, wolves.

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## INTRODUCTION

Studies investigating the ecology of the wolf-deer-human system in southeastern Alaska are hampered by the lack of extensive data on the demographics of wolves (*Canis lupus ligoni*) and deer (*Odocoileus hemionus sitkensis*). Of paramount importance are reasonably precise estimates of the size of wolf populations occupying different island clusters and the ability to track changes in populations over time. Wildlife managers are particularly interested in these data. For example, in game management unit 2 (Prince of Wales and the immediately adjacent islands), the annual harvest of wolves is capped at 25% of the population to reduce the risk of overharvesting. The most current quantitative estimate of the wolf population in GMU 2 is based on radio-telemetry data obtained in 1995. The objective of our research during the current reporting period was to prepare a detailed proposal for developing a method to estimate wolf populations in Southeast Alaska.

## BACKGROUND

Enumerating populations of wolves in Southeast Alaska is difficult because of the forested habitat and variable weather conditions. Techniques that are used elsewhere in Alaska such as direct counts from the air and track surveys are not likely to be feasible. The following list describes different methods for estimating wolf populations that we explored:

1. *Use of radio-telemetered wolves to find and count wolves* -- this method requires catching one or more wolves in each or most of the packs occupying the area of interest. Relocating the packs from the air enables an observer to eventually count the number of wolves in each pack (Person

estimates for areas where intensive studies of wolves are taking place, it is unlikely that the method would be feasible or cost effective if used strictly to obtain wolf population estimates.

2. *Howling surveys*-- systematic howling surveys have been used to document the presence or absence of packs in relatively small geographic areas (Harrington and Mech 1978). Unfortunately no estimates of the number of wolves can be made. Further, howling surveys are only feasible where roads exist or boat access is convenient. Surveys must be repeated several times to assure that all wolf packs in the area responded. The method does not account for dispersers.

3. *Track surveys and scent posts*-- track surveys rely on snow conditions, which are highly variable in Southeast Alaska. In some years little or no snow may accumulate making aerial track surveys unreliable or impossible. Scent post surveys have been used to track fox and coyote populations; however, like track surveys, they are only useful for monitoring trends and cannot be used to estimate populations. Further, weather conditions and the scarcity of sandy or silt type soils in Southeast Alaska make scent posts impractical.

4. *Harvest data and trapper surveys*-- although trapper surveys and harvest data are useful, they are inadequate to estimate populations. Assuming that harvest information is accurately reported, the annual kill in a particular area may reflect access and trapper effort rather than the level of a wolf population (Person et al. 1996). At best, harvest data would indicate trends in wolf populations not absolute numbers. Information from surveys of trappers can be misleading and unreliable. Wolf packs in Southeast Alaska often do not travel together as a single pack, rather they split up into smaller hunting groups that may be separated for several days or even weeks (Person *in prep.*). Consequently, trappers and hunters that encounter several small groups of wolves within a particular area often mistakenly believe that more than one pack exists in the area, when in fact the groups observed are part of the same pack. As a result, trappers and hunters frequently overestimate the number of wolves.

5. *Biomarkers*-- marking animals by allowing them to consume biomarker-containing bait or by injecting the marker by darting has been used successfully on black bears. Garshelis and Visser (1997) marked black bears by supplying them with baits containing tetracycline. Tetracycline, which is an antibiotic, is deposited in newly formed bone and fluoresces when viewed under ultraviolet light. The examination under UV light of carcasses or teeth from animals killed during the hunting season constituted the recapture phase of the method. Garshelis and Visser assumed that different bears consumed each bait and, therefore, the number of animals marked equaled the number of baits removed. Knowing the number of baits consumed, the number of bears killed during the hunting season, and the number of harvested bears that were marked, they were able to estimate the bear population using a simple Lincoln-Peterson estimator.

The application of this method to wolves in Southeast Alaska would likely be confounded by a number of problems. It is unlikely that the number of individual wolves consuming bait at a bait station could be known with accuracy. The distribution of wolves is highly clumped because they occur in packs and the home ranges of packs are very large. Bait stations would have to be spaced very far apart to reduce the probability that a single wolf would consume bait at more

than one station. The spacing would be so far apart that very few wolves within a particular geographic region actually would be marked. Nontarget animals such as bears also would consume bait, forcing us to use the method only when bears are in dens. If darting were used to administer the marker, the number of wolves marked would be known but the effort to tag enough animals for statistical reliability would be immense. Juvenile wolves constitute 40–50% of the annual wolf harvest in Southeast Alaska. Rapid bone development in pups during growth may obscure the deposition of tetracycline. Consequently, marked pups may be undetected in the sample of harvested wolves.

6. *DNA-based estimator*-- DNA extracted from hair or feces has been used successfully to identify individuals within some wild mammal populations (Hoss et al. 1992, Kohn et al. 1995, Woods et al. 1996, Foran et al. 1997, Kohn and Wayne 1997). Epithelial cells from the intestines that are sloughed off during defecation and deposited on feces (scats) can be used as a source of DNA. The DNA can be amplified using the polymerase chain reaction (PCR) (Mullis and Faloona 1987) and subjected to DNA fingerprinting techniques based on microsatellite DNA analysis. Scats are collected on the basis of a random sampling design and individual animals are identified. These animals become the marked population. The population is resampled at some later date and individuals that have been previously identified constitute the resighted population. Mark-resight estimation techniques (White 1996) can be applied to the data to produce a population estimate.

There are a number of difficulties that need to be overcome before a DNA-based approach is likely to work well in Southeast Alaska. Rainy weather may degrade the DNA beyond usefulness in a very short time after the scat is deposited. Although scats from resident wolves may be deposited in relatively obvious places such as trails and roads, dispersing wolves tend to defecate in obscure places and may be under-represented in a sample of scats. It may be necessary to examine a relatively large suite of DNA loci to observe sufficient genetic variability to identify individuals because wolf packs are generally composed of close siblings. Finally, any program based on DNA from scats will require the analysis of many samples. This will require the services of a genetic laboratory capable of processing samples on a production basis, while still maintaining a high level of quality control to avoid sample contamination.

## **OBJECTIVES**

We propose a research program, conducted at multiple geographic scales, with the aim of developing a quantitative method to estimate wolf populations in at least portions of southeastern Alaska. We seek to develop techniques that are economical and cost-efficient with respect to personnel and time. We will experiment with a DNA-based mark-resight procedure to estimate wolf populations. We will initially test and verify the technique on Heceta Island, which has a known population of wolves. We will then use the method to estimate the wolf population on a portion of Prince of Wales Island that coincides with the area used in the study of wolf demographics (Person 1999). Radio-collared wolves from that study will provide an independent way to estimate wolf numbers (Person et al. 1996) within the study area. Ultimately, we will apply the method generally to Prince of Wales Island and other areas.

If the method proves to be feasible and cost-effective, we propose that the DNA-based population estimator be used every 3 years in a particular area. Areas that are selected for surveying could be those in which overharvesting of wolves may be suspected or where there are concerns about the effects of wolf predation on ungulate populations. Population modeling (Person and Bowyer 1997) could be used to track wolf populations between the years that estimates are made. Parameterization of the models will be based on demographic data from radio-collared wolves on Prince of Wales Island, Heceta Island, the Cleveland Peninsula, and possibly other areas. Additional data on wolf mortality will come from information on the harvesting of wolves collected by the Alaska Department of Fish and Game. Our methodology will combine information from radio-tagged wolves, population modeling, and periodic population estimates.

This approach has several advantages. Reasonably accurate and precise mark-resight population estimates every 3 years should be sufficient to satisfy the needs of wildlife managers who must evaluate the effects of harvesting and habitat change on wolf populations. Periodic population estimates also will serve as benchmarks with which to calibrate and refine our population model. Modeling will enable us explore hypotheses concerning the effects of habitat changes, hunting and trapping seasons, and island biogeography on wolf populations. This will be important with respect to interpreting trends that become apparent in the wolf population estimates. Thus our proposed methodology will serve to monitor wolf populations and to provide a hypothesis framing and testing tool.

## METHODS

We will modify and test a DNA-based mark-resight method to estimate wolf populations on Heceta Island (Fig. 1). Fresh wolf feces will be collected along roads and trails in August 1999. Most of the roads on Heceta Island are driven at least every other day as part of an ongoing deer-wolf study begun in 1996 (Farmer et al. 1998). As a result, locating and collecting fresh scats is relatively easy and efficient. Scats will be stored in plastic bags containing an equal volume of silica desiccant and sent to the University of Alaska Fairbanks (UAF) for analysis of microsatellite nuclear DNA. Tissue samples from wolves (live captured or collected from trappers) and their principle prey (deer and beaver) will be sent to UAF for species identification. This tissue will serve as a reference collection with which to compare DNA isolated from wolf scats.

Individual wolves will be identified from epithelial cells that coat the surface of the scats. These animals will serve as the “marked population.” We will use a Bayesian population estimator (Gazey and Staley 1986, Underhill and Fraser 1989) that treats the mark-resight experiment as a counting process. It solves for the probability that a population is of size  $N$  given that the  $i^{\text{th}}$  scat collected is from a new individual or one that has been previously identified. First, to provide an upper limit for the probability distribution of potential population sizes we guess what the maximum population ( $N_{max}$ ) could be for a given area. Let  $p_i(N)$  = the probability of population size  $N$  after the  $i^{\text{th}}$  scat is collected and identified,  $m/N$  = the probability that the  $i^{\text{th}}$  scat is from a previously identified wolf, and  $(N-m)/N$  = the probability that the scat is from a new individual. If the  $i^{\text{th}}$  scat is from a new individual then it follows from Bayes' Theorem that

$$p_i(N) = \frac{\frac{N-m}{N} p_{i-1}}{\sum_{N=m}^{N_{\max}} \frac{N-m}{N} p_{i-1}}$$

where,  $m$  = the number of scats that have been previously identified. If the  $i^{\text{th}}$  scat is from an animal that has already been identified then,

$$p_i(N) = \frac{\frac{m}{N} p_{i-1}}{\sum_{N=m}^{N_{\max}} \frac{m}{N} p_{i-1}}$$

Initially  $p_0(N)$  can be set to  $1/N_{\max}$  (a noninformative prior probability) or to some other value that reflects other information about the population. For example, if data from telemetry studies or trapper harvests suggest the limits of the population distribution, this information can be integrated into the prior probability. The selection of a prior probability does not influence the eventual population estimate, only the efficiency in terms of the number of scats needed to arrive at that estimate with adequate accuracy and precision (Tables 1 and 2).

We conducted simulations using a uniform prior probability to demonstrate the behavior of the estimator when little prior information about wolf numbers is available. We repeated the simulations with a normal prior distribution to show how the estimator behaves if substantial prior information is available (Tables 1 and 2, and Fig. 2). Simulations of the estimator for conditions likely to be encountered on Heceta Island suggest that a random sample of 20-25 scats should be sufficient to accurately estimate the population with a 95% confidence interval less than 25% of the mean. The current population is 8 wolves based on direct observations of the pack. Simulations involving a larger population of wolves suggest that 50 scats may be sufficient to accurately estimate a population of 70 wolves with similar precision provided all individual wolves are equally likely to be enumerated (Fig. 3).

Some key assumptions and conditions must be tested before our proposed DNA-based mark-resight method can be considered to be reliable:

1. It must be possible to unambiguously identify individual wolves from their scats. To do this we must derive the primers necessary to sample a number of DNA base pairs sufficient to reliably fingerprint individual wolves. Staff at the University of Alaska Fairbanks (UAF) molecular biology core facility will do this work in cooperation with the Alaska Cooperative Fish and Wildlife Research Unit.
2. We must determine how long scats may remain in the wet environment of Southeast Alaska before the DNA is degraded to a point that it is no longer useful. To determine this we will allow fresh scats to remain exposed to the environment for 1, 2, 4, 7, and 10 days before placing them in bags containing silica desiccant and sending them to UAF. Ten scats will be tested within each time category.

3. Scat samples must be representative of the local wolf population. Radio-telemetry data from wolves on Prince of Wales Island suggest that dispersing and extra-territorial wolves are more frequently in the vicinity of roads than resident wolves ( $\chi^2 = 20.85$ , exact  $p = 0.0000$ , Person et al. *in prep*). Nevertheless, dispersing wolves may not deposit scats in obvious places such as roads because they risk detection by resident wolves (Rothman and Mech 1979). We will perform exhaustive searches along roads and trails to maximize the probability that scats are collected from all classes of wolves. In addition, because we know the number of wolves on Heceta Island and their social status, we will be able to determine the probabilities that specific individuals are detected, and relate those probabilities of detection to the animal's status as a resident pack member or disperser. Combining this knowledge with computer simulation should give us a good understanding of the effects of unequal detectability and enable us to mitigate these effects by modifying our search protocol and incorporating appropriate prior probabilities in our Bayesian population estimator.

4. The population estimator assumes that the population is closed. We will attempt to collect scats over the shortest period possible (<1 month) to reduce the probability that wolves immigrated, emigrated, or died during the survey period. We will also conduct simulations of the estimator that violate the assumptions of closure to determine how sensitive the model is to these factors.

The Heceta Island experiment will determine if individual wolves can be reliably identified from their scats and if the mark-resight estimator works for a small, discrete population. By starting small with a known population we reduce the risk of wasting time and resources on a technique that is unfeasible. If we are successful, the next step is to use the method to estimate wolf numbers within a portion of our study area on Prince of Wales Island. Locating packs containing radio-collared individuals will enable us to estimate the wolf population in the study area (Person et al. 1996). This value will serve as an independent estimate that will be compared to a DNA-based estimate. We will establish a series of transects that will be located along roads and trails that are distributed throughout a portion of the study area that overlaps the home ranges of at least 4 wolf packs. The area will encompass about 1000 km<sup>2</sup> because the average home range is about 250-280 km<sup>2</sup> (Person et al. 1996). Transects will be at least 3 kilometers in length and we will target areas that are likely to have wolf activity (e.g, below 250 meters elevation, along creeks, rivers, shorelines and other natural corridors [Person et al. *in prep*.]). Each transect will be traversed 6 times over a two-week period in late July or early August. All fresh scats will be collected and placed in plastic bags with a silica desiccant. Scats will be sent to the University of Alaska Fairbanks for DNA analysis. We will consider our experiment a success if we can produce an estimated mean population of wolves within 10% of our independent population estimate and a 95% confidence interval <25% of the mean.

Our proposed research comprises the first two steps in developing a DNA-based population estimator for wolves; namely the initial trial on Heceta Island and a follow-up experiment applying the technique at a larger geographic scale. If we are successful with the first two phases, the next step will be to apply the method to an area the size of Prince of Wales Island. A number of additional technical problems will have to be overcome before we can do this. An area the size of POW will be difficult to survey. We probably would have to estimate the number of wolves at



different sampling locations on the island and extrapolate from these samples. This requires that we define discrete areas that are sampled so that the number of wolves estimated at each sampling location can be converted to a density estimate. This problem will require extensive simulation work and a logistic effort beyond the scope of the current proposal. If our initial experiments are successful, we will submit a follow-up proposal to estimate wolf populations at a large geographic scale.

## **PERSONNEL AND BUDGET**

The principal investigator on this project will be Dave Person. A graduate student currently studying wolves and deer on Heceta Island and an ADF&G seasonal technician will assist with the work. The estimated total budget for the 3-year project is \$42,300. This budget includes \$20,000 for a contract with Dr. Pamela Groves of the University of Alaska Fairbanks Molecular Biology Core Laboratory to perform the DNA analysis, \$4,000 for supplies, \$15,600 for salary for the PI (3 months), and \$2,700 for salary for the seasonal technician (0.75 months). In addition, the Forest Service will contribute housing space in the Thorne Bay bunkhouse for project personnel and supply occasional aircraft and vehicle support.

ADFG proposed budget (in thousands of dollars).

	<u>2000</u>	<u>2001</u>	<u>2002</u>	<u>Total</u>
Supplies	2.0	2.0	0.0	4.0
DNA Analysis	10.0	10.0	0.0	20.0
Salary for Tech. III	0.9	1.8	0.0	2.7
Salary for P.I	5.2	5.2	5.2	15.6
Totals	18.1	19.0	5.2	42.3

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Table 1 Results from a simulation of the Bayesian population estimator using a uniform prior probability. The true population ( $\mathbf{N}$ ) = 8.

# scats collected ( $i$ )	5	10	15	20	25
$m$	4	6	8	8	8
$N$	$p_5(N)$	$p_{10}(N)$	$p_{15}(N)$	$p_{20}(N)$	$p_{25}(N)$
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0.043	0	0	0	0
5	0.070	0	0	0	0
6	0.085	0.090	0	0	0
7	0.092	0.140	0	0	0
8	0.094	0.150	0.110	0.340	0.560
9	0.094	0.014	0.180	0.290	0.270
10	0.093	0.012	0.180	0.180	0.100
11	0.091	0.010	0.160	0.090	0.030
12	0.088	0.080	0.130	0.050	0.010
13	0.085	0.070	0.100	0.030	0.004
14	0.082	0.060	0.080	0.010	0.001
15	0.080	0.050	0.060	0.008	0
Mean	9.7	9.7	10.9	9.4	8.6
95% CI	4-15	6-14	8-15	8-12	8-11
Median	9	9	10	9	8

Table 2 Results from a simulation of the Bayesian population estimator using a normal probability,  $N(8,2)$ . The true population (N) = 8.

# scats collected ( <i>i</i> )	5	10	15	20	25
<i>m</i>	3	7	7	8	8
<i>N</i>	$p_5(N)$	$p_{10}(N)$	$p_{15}(N)$	$p_{20}(N)$	$p_{25}(N)$
1	0	0	0	0	0
2	0	0	0	0	0
3	0.020	0	0	0	0
4	0.060	0	0	0	0
5	0.110	0	0	0	0
6	0.170	0	0	0	0
7	0.200	0.090	0.280	0	0
8	0.180	0.220	0.340	0.460	0.650
9	0.130	0.270	0.230	0.340	0.270
10	0.080	0.220	0.110	0.140	0.070
11	0.030	0.120	0.040	0.040	0.012
12	0.010	0.050	0.010	0.009	0.001
13	0.004	0.020	0.002	0.002	0
14	0.001	0.004	0	0	0
15	0	0.001	0	0	0
Mean	7.3	9.3	8.3	8.8	8.4
95% CI	3-11	7-12	7-11	8-11	8-10
Median	7	9	8	8	8