ALASKA DEPARTMENT OF FISH AND GAME
DIVISION OF WILDLIFE CONSERVATION
PO Box 115526
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Alaska Department of Fish and Game
Wildlife Restoration

Grant Number: **W-33** **Segment Number: 8**

Project Number: 4.37

Project Title: Non-invasive sampling of brown bears

Project Duration: July 1, 2007 – June 30, 2010

Report Due to HQ: September 1, 2010

Principle Investigator: Sean Farley

Project Location: Region II, Alaska

Cooperators: USGS

I. PROBLEM OR NEED THAT PROMPTED THIS RESEARCH

Managing brown bear populations for near-simultaneous use in tourism (through bear viewing) and in hunting is difficult and fraught with deep political and biological divisions. For example, recently, the number of bears recorded using the McNeil River Game Sanctuary has declined, and nearby hunting pressure as well as decreased salmon numbers have been suggested as possible explanations. This project will develop a sampling procedure that will enable researchers to address the question of hunting pressure as a contributing factor to the decline in McNeil River bear numbers. The procedure to be developed will non-invasively identify bears using the McNeil River falls and Mikfik River area. These identifications will then be matched against identifications of bears harvested from nearby regions. This project, when fully developed, will provide the means of quantifying the influence of hunting on the number of bears using the McNeil River area as well as provide an example of the application of genetics to a critical management question.

Before this project began management identified additional needs for collecting and storing genetic samples from sealed bears, particularly from the Kenai Peninsula, and from region 16 areas soon to be managed under predator control guidelines. Additional interest was expressed to examine samples for stable isotope signatures in the hope of determining diet preferences of bears in Unit 16.

II. REVIEW OF PRIOR RESEARCH AND STUDIES IN PROGRESS ON THE PROBLEM OR NEED

Previous ADFG work at McNeil falls has focused on stream use by McNeil bears and conducting general bear counts. In 1997 hair was collected opportunistically in the McNeil area, however no analyses were conducted. It has not been possible to reliably determine which, if any, bears using the McNeil falls were harvested.

Genetic samples from Kenai Peninsula bears have been collected and used to test hypotheses of relatedness and genetic structure for several years (Jackson et al. 2008), however until recently it was

not possible to compare the findings to information on nearby Anchorage bears, thus findings were subject to error from isolation by distance.

No sample or additional work had been conducted on Unit 16 bears.

III. APPROACHES USED AND FINDINGS RELATED TO THE OBJECTIVES AND TO PROBLEM OR NEED

Objective 1: Develop methodology to non-invasively collect biological materials from bears using the McNeil River area.

Job/Activity 1a: Building upon efforts of previous McNeil sanctuary staff, barbed wire wrapped posts will be placed at key points of the sanctuary routinely accessed by staff and bears. Staff will be trained to collect hair from posts. Equipment (barbed wire, posts, and collection materials) will be purchased and supplied to staff. Procedures will be established for the timely submission of samples.

Turnover in staff prevented this activity from occurring. No work was conducted on this job.

Job/Activity 1b: Previous researchers have reported success at utilizing fresh feces for both mitochondrial and nuclear DNA extractions. Staff will be trained to collect small samples of fresh feces during their daily travels. Collection materials will be purchased and provided to staff. Procedures will be established for the timely submission of samples.

Turnover in staff prevented this activity from occurring. No work was conducted on this job

Objective 2: Collect biological samples from bears that have been harvested

Job/Activity 2a : ADFG staff will be provided materials and training sufficient to enable them to collect small amounts of biological material from bears during the course of sealing. Collection materials will be purchased, and along with written materials, provided to staff. Procedures will be established for the timely submission of samples.

Kits were sent to all Region II area offices and additional materials supplied on demand.

Objective 3: Clean, curate, and archive samples

Job/Activity 3a : Samples collected during objectives 1 and 2 will be cleaned in the laboratory and sorted according to potential for DNA extraction. Archival sub-samples will be appropriately processed and retained at -84C. Laboratory supplies will be purchased. A -84C freezer will be purchased.

Tissue swab samples (1024) were collected region-wide. Two hundred seventy one (271) black bear samples, 503 brown bear samples, and 428 yet to be identified samples were collected from sealed bears. Samples have been processed in the Molecular Ecology Laboratory in Anchorage, Alaska.

Objective 4: Determine the effectiveness of sample collection procedures.

Job/Activity 4a : A random subset of all samples will have mitochondrial and nuclear DNA extracted and amplified. The sex, species, and individual identification of each sample will be determined. The effectiveness of the sampling methods will be determined. QIAamp DNA stool mini kits, QIAamp DNA mini kits, various chemicals, primers, and polymerases will be purchased. An agreement with the Molecular Ecology Laboratory (MEL) of the USGS in Anchorage will be established for their assistance with final laboratory analysis.

A small subset (n =67) was pulled for extraction testing. The first run showed promise with exceptionally large DNA yields, however proofing of the gels raised suspicion that the extraction protocol was flawed. Samples were re-extracted and extra care was taken with the preparation. DNA yield was significantly lower; however gels produced were consistent with clean DNA and thus useable. The sampling technique relies upon moistening a sterile cotton swab in Longmire buffer, then wiping it on the dried skin/skull of the sealed animal. Those samples containing small bits of tissue gathered during the wipe yielded more DNA and could be multiplexed, whereas poorer quality samples could not be multiplexed.

Samples that must be analyzed individually will have approximately 9 and 3 times higher costs in chemical and labor, respectively, than samples that can be multiplexed. The variable nature of sample quality (sealed bear hides and skulls) indicates that samples are best collected with heavy wiping action to collect tissue with the swab. While retaining a large piece of tissue for DNA would seem preferable, our experience has shown that often too large a sample is collected and the material rapidly decays.

Samples from the Kenai peninsula bears were collected as blood or tissue and not just cotton wipes, and thus DNA yield was significant. The utility of testing hypotheses regarding wildlife populations was demonstrated with the Kenai samples as it was shown that the Kenai brown bear population experiences little gene flow on/off the Peninsula (see appendix).

Objective 5: Collect biological samples of bone, muscle, and hair from all bears harvested in Unit 16.

Job/Activity 5a: Samples of bone, hair, and muscle will need to be collected from every bear harvested within Unit 16, regardless of method of take, gender, or age class. The variance estimates determined from analysis of a small subset of samples will be used to determine the final sample size required to establish a reasonable statistical precision. All sample selection in the laboratory will be conducted as a single blind.

In order to avoid any bias or skew in the data samples of bone, hair, and muscle must be collected from as many bears as possible, regardless of harvest method, location of harvest, gender, or age of the bear. This includes cubs and subadults.
Sample kits will be assembled and provided to the Palmer, Anchorage, and Soldotna Fish and Game offices, with instructions to sample all unit 16 bears. Additional kits will be provided to selected ADFG designated bear sealers personal contact will be made to provide instruction on sample collection and storage.

Preliminary analysis of sealing records indicated that close to 70% of all samples would be handled by 3 ADFG offices and one private sealing station (fig. 1). However regulations on bear hunting, bear baiting, and bear snaring in Unit 16 were changed multiple times during the course of this study, and bears were sealed throughout the region. While bear harvest was high in Unit 16, sealing occurred at locations and times not expected and thus fewer samples were collected than anticipated. In regulatory year 2008 approximately 645 bears (both black and brown) were harvested in unit 16, of which approximately 40% were sealed by ADFG personnel.

Objective 6: Perform analysis on biological samples of bone, muscle, and hair collected from all bears harvested in Unit 16.

Job/Activity 6a:
Samples will be processed in the ADFG laboratory and then shipped to the USGS isotope lab at the Denver Federal Center, Colorado for determination of carbon/nitrogen/ and sulfur isotope ratios and for mercury content. Samples will also be sent to the USGS Molecular Ecology Lab in Anchorage, Alaska for determination of allele frequency of Major Histocompatability Complex, μsat, SSP sequencing for individual identification, determination of relatedness, assessment of population health, and haplotypic diversity.

Samples collected have been freezedried, but no further work was accomplished.

IV. MANAGEMENT IMPLICATIONS

Genetic information would be acutely important in addressing wildlife management questions, ranging from which, if any, bears from McNeil falls are harvested each year to the characterization of relatedness amongst unit 16 animals. Stable isotope analyses could provide useful insight to the diet of a bear population (and individual bears) implicated in affecting ungulate numbers. However methods of incorporating these techniques into established funding and management strategies still need to be developed.

Sample preservation for genetic work is a continually moving target. Initial work had suggested alcohol storage was preferable; however issues with hazmat regulations and storage space are rapidly causing researchers to look at other methods. Sterile cotton swabs and Longmire buffer will collect and store useful material; however the quantity may be so low as to preclude multiplexing samples, which will drive up costs considerably.

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APPENDIX

Application of genetics to Region II brown bear management

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Genetic research (Jackson et al. 2008) on the Kenai brown bear population did not find evidence of significant inbreeding, and only one of three algorithms used to test for the presence of a genetic bottleneck had positive results (infinite alleles versus step-wise mutation and two-phase). However the level of mtDNA haplotypic diversity is remarkably low for Kenai brown bears, even when compared to a true island brown bear population such as Kodiak Island brown bears. In addition, Jackson et al. (2008) did not find evidence of population structure across the Kenai Peninsula.

Recently, nuclear microsatellite fragment and mitochondrial DNA control region nucleotide sequence data obtained from south central and southwestern Alaskan brown bear (*Ursus arctos*) tissue samples were used to estimate genetic parameters of the Kenai Peninsula brown bear population. Similar to brown bear populations found in the Bristol Bay and Yukon-Kuskokwim regions, Kenai Peninsula brown bears show a weak signature of a recent demographic bottleneck. Expanded analyses based on traditional and Bayesian analyses of population differentiation show that these brown bear populations are characterized by substantial interpopulational structuring. Thus, we reject the null hypothesis that the Kenai Peninsula and Anchorage brown bears are panmictic (nuclear DNA: $\chi^2_{nuc} = \infty$, $\theta_{ST} = 0.113$; mitochondrial DNA: $\chi^2_{mit} = \infty$; $\Phi_{ST} = 0.8$; $P < 0.05$ for all tests; Bonferroni correction applied for microsatellite data). In other words, the Kenai Peninsula brown bear population is significantly differentiated from the nearby Anchorage brown bear population, as well as from the more distant southwestern Alaskan populations, based on both microsatellite loci and mitochondrial DNA control region data. Thus, while previous research demonstrated that the Kenai brown bear population *is not* structured across the Kenai Peninsula proper, Kenai brown bears *are* genetically isolated from close mainland populations.

Identification of maternity and dispersal distances of female offspring are being compared to known reproductive histories and home ranges in order to determine if there is differential reproductive success among Kenai brown bears.

Management of Kenai brown bears will benefit from expanding bear sample collection across the only geographic corridor to the Kenai Peninsula (Placer and Twenty mile rivers) and augmenting datasets from additional south central Alaskan populations (i.e., upper Eagle River, Girdwood, and western Prince William Sound). Management will also benefit from assessing the genetic diversity found in the major histocompatability complex (MHC) of Kenai brown bears. Expanded sample collections will establish if the most logical land route is a travel corridor connecting the Kenai Peninsula, and the variation of the MHC will assess the biological relevance of the Kenai brown bears genetic isolation.

Samples collected on Elmendorf Air Base, Ft. Richardson Army Post, and Bicentennial Park in Anchorage have been used to determine the minimum number of bears present over a 2 year period. Additional samples from clothing of Anchorage-area mauling victims were used to identify bears responsible for the attacks.

Jackson, J., S. Talbot, and S. Farley. 2008. Genetic characterization of Kenai brown bears (*Ursus arctos*): microsatellite and mitochondrial DNA control region variation in brown bears of the Kenai Peninsula, south central Alaska. Canadian Journal Zoology. 86:756-764.

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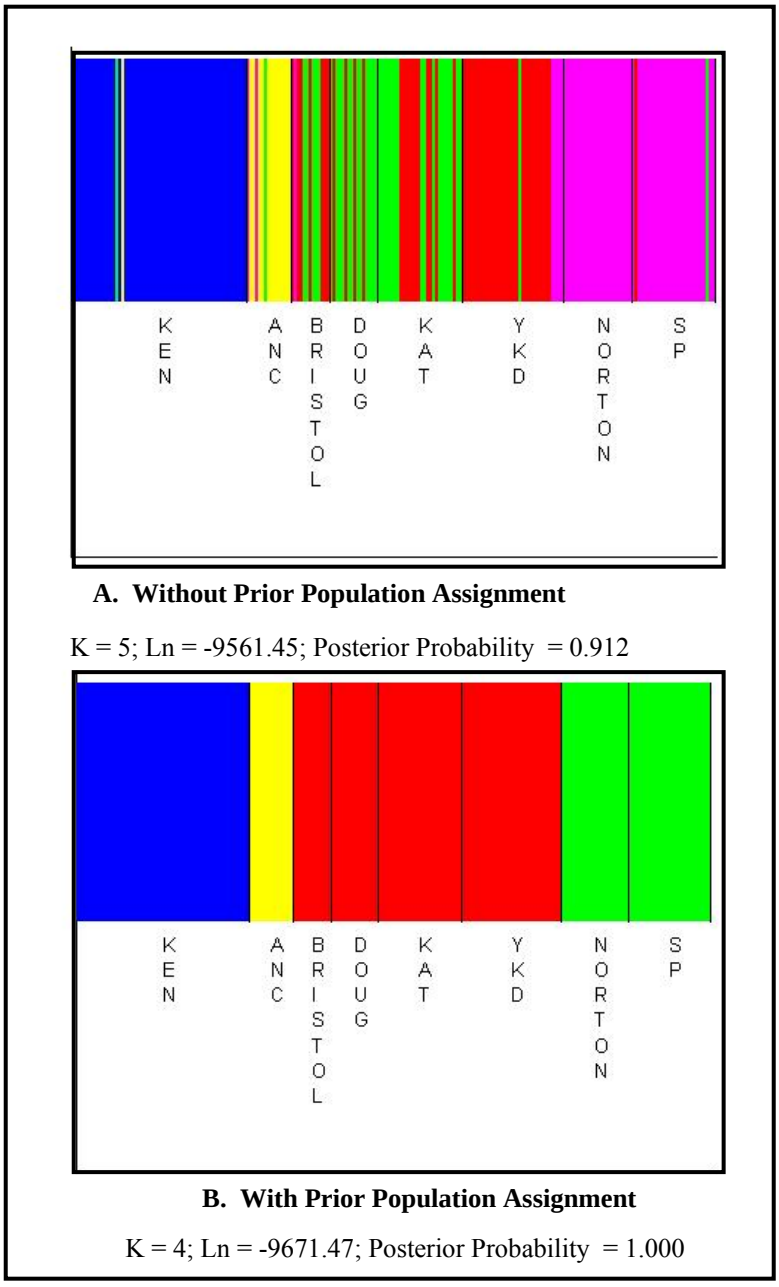

Figure 1. Genetic structure among south central and southwestern Alaskan brown bear populations, estimated by Bayesian analyses using 14 microsatellite loci. Analyses were conducted using BAPS 5.1 (available at http://www.abo.fi/mnf/mate/jc/smack_index_eng.html). Among the 8 populations assayed, BAPS5.1 identified 5 (without priors, Figure 1A) or 4 (with priors, Figure 1B) clusters (populations). In both cases, Kenai and Anchorage brown bears were assigned to different populations.

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Figure 1. SEALING LOCATIONS UNIT 16 BEARS KILLED FALL 2006 TO FALL 2008