**PROJECT TITLE:** Non-invasive sampling of brown bears

**PRINCIPAL INVESTIGATOR:** Sean Farley

FEDERAL AID GRANT PROGRAM: Wildlife Restoration

**GRANT AND SEGMENT NO.** W-33-7

**PROJECT NO.** 4.37

WORK LOCATION: Region 2

**STATE:** Alaska

**PERIOD:** July 1, 2008 – June 30, 2010

# I. PROGRESS ON PROJECT OBJECTIVES SINCE PROJECT INCEPTION

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

Equipment was purchased to collect fecal samples in the summer of 2006.

OBJECTIVE 2: Collect biological samples from bears that have been harvested.

Over 2,500 sampling kits were distributed to all Region II area offices. Each kit contained sterile buccal swabs and Eppendorf microcentrifuge tubes filled with "Longmire" buffer. Most area biologists submitted samples from bears they sealed.

OBJECTIVE 3: Clean, curate, and archive samples.

Samples are being processed.

OBJECTIVE 4. Determine the effectiveness of sample collection procedures.

A subset of samples is being analyzed.

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

Objective added to project statement on May 2009.

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

Objective added to project statement on May 2009.

# II. SUMMARY OF WORK COMPLETED ON JOBS IDENTIFIED IN ANNUAL PLAN THIS PERIOD

JOB/ACTIVITY 1A: <u>Building upon efforts of previous McNeil sanctuary staff</u>, 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.

No work was completed on this job.

JOB/ACTIVITY 1B: <u>Staff will be trained to collect small samples of fresh feces during their</u> daily travels. Collection materials will be purchased and provided to staff. Procedures will be established for the timely submission of samples.

No work was completed on this job.

JOB/ACTIVITY 2A: <u>ADFG staff will be provided materials and training sufficient to enable</u> 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.

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

Two hundred seventy one (271) black bear samples, 503 brown bear samples, and 428 yet to be identified samples were collected from sealed bears.

JOB/ACTIVITY 4A: <u>A random subset of all samples will have mitochondrial and nuclear</u> 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 MEL will be established for their assistance with final laboratory analysis.

Fifty six (56) brown bear samples have been extracted are awaiting polymerase chain reaction (PCR). Most were extracted with phenol:chloroform, two samples were extracted with MZV salt extraction. All but the MZV extracted samples and three of the phenol:chlororm samples produced Fluorometer readings in excess of 400 ng/ul samples. Samples are being processed in the Molecular Ecology Laboratory (MEL) of the USGS.

JOB/ACTIVITY 5A: <u>Samples of bone, hair, and muscle will need to be collected from every</u> <u>bear harvested within Unit 16, regardless of method of take, gender, or age class. The</u> <u>variance estimates determined from analysis of a small subset of samples will be used to</u> <u>determine the final sample size required to establish a reasonable statistical precision. All</u> <u>sample selection in the laboratory will be conducted as a single blind. Sample kits will be</u> <u>assembled and provided to the Palmer, Anchorage, and Soldotna Fish and Game offices,</u> 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.

Kits were assembled and sent to the Soldotna and Palmer area offices, and placed in the Anchorage office.

JOB/ACTIVITY 6A: <u>Samples will be processed in the ADFG laboratory and then shipped to</u> 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 <u>sent to the MEL</u>, <u>Alaska for determination of allele frequency of Major</u> <u>Histocompatability Complex</u>, <u>µsat</u>, <u>SSP sequencing for individual identification</u>, determination of relatedness, assessment of population health, and haplotypic diversity</u>.

Twenty seven (27) kits have been collected at this point. Additional kits are anticipated from Palmer and Kenai. Samples from the 27 kits were separated and bone and tissue samples freeze dried. Further analysis will proceed when all completed kits have been collected.

# III. ADDITIONAL FEDERAL AID-FUNDED WORK NOT DESCRIBED ABOVE THAT WAS ACCOMPLISHED ON THIS PROJECT DURING THIS SEGMENT PERIOD

None.

#### **IV. PUBLICATIONS**

None at this time.

## V. RECOMMENDATIONS FOR THIS PROJECT

None.

## VI. APPENDIX

None.

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APPROVAL DATE: \_\_\_\_\_