Alaska Department of Fish and Game
Research Grant

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Project Title: Estimating wolf populations in Southeast Alaska using noninvasive DNA sampling

Project Duration: 1 July 2009–30 June 2018

Report Due Date: 1 September 2014

Principal Investigators: Rodney Flynn, Gretchen Roffler, and Kris Larson

Cooperators: USFS Tongass National Forest, Rocky Mountain Genetics Laboratory

Work Location: Prince of Wales Island and Ketchikan, Alaska

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I.   PROGRESS ON PROJECT OBJECTIVES DURING LAST SEGMENT

OBJECTIVE 1: Our objective is to devise a protocol that enables us to estimate wolf numbers in Game Management Unit (GMU) 2.

Due to difficulties collecting a sufficient sample of wolf (*Canis lupus*) scat to estimate wolf abundance within our study area, we substantially modified this project. Instead of using scats as the source for DNA samples used to measure wolf population abundance, we collected hair. During autumn 2012 and 2013, we deployed several hundred hair traps (Ausband 2011) at scent stations systematically located throughout our study area on Prince of Wales Island (POW). Each scent station was marked with an abundance of lure intended to induce wolves to roll on the hair traps and leave hair. Hair was collected and used for DNA extraction and analyses. We will use a spatial explicit capture recapture (SECR) method to analyze the data.

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

JOB/ACTIVITY 1: Collection of wolf hair and tissue

Between 21 October 2013 and 27 December 2013, we conducted the second noninvasive wolf-sampling event. Using the same design as 2012, we deployed 180 hair traps distributed among 36 sampling nodes; the mean distance between the nodes was 3.9 km. Because of road construction, we did not activate one node (node 53). We generally had 2 teams checking nodes, and they could usually do a complete survey of nodes within 3–4 days. We attempted to check each node weekly, but it was not possible with the staffing allocated to the project. Another change we made in 2013 was the wiring of the boards to nearby trees. Wiring the boards resulted in no stolen/missing boards. In 2012, we had many of our hair boards disappear (often taken by wolves, other animals, or

people). Also, we tried vertical hanging of some of the hair boards. Vertically mounted boards were readily hit and collected large amounts of hair.

Heavy snow during the 16 December checking event caused us to miss a few of the hair boards (3 of the 180 deployed boards). The snow buried the flagged trees that the boards were wired to.

Wolves visited and left hair at 16 (38%) of the 36 nodes. Eight nodes had repeat hits (total of 29 node hit events) and 86 hair samples were collected from the nodes (69 of which were wolf). Some nodes and boards were visited multiple times. We also collected one scat adjacent to a hair trap. Photos at nodes showed wolves investigating hair traps and rubbing and rolling on the boards. They also showed some hair traps were rubbed by several wolves during a single visit, thus some traps hadhad hair from >1 wolf. We also observed several wolves investigating traps but not rubbing.

JOB/ACTIVITY 2: Extracting DNA from hair and tissue and genotyping wolves

On 7 February 2014, we sent 93 hair samples collected from hair snares targeting wolves to USFS Rocky Mountain Research Station in Missoula, MT for genotyping. We also sent a blood and hair sample from a study animal (Wolf_JM435). Samples were collected between October and December 2013. The objectives of sample analyses were to genetically identify unique wolves from the hair and blood samples, compare these individuals to those identified from hair samples collected in 2012, and compare individuals identified from hair samples to those harvested from the area (as determined from DNA collected in tissue samples).

DNA extractions were performed on the majority of non-invasively collected hair samples (80) using our standard protocols (targeting 10 good hairs with follicles for the extraction). However, 13 hair samples were identified as having more than one individual on the rub board from cameras set up nearby. In order to detect multiple individuals from these samples and eliminate the chance for mixed hair samples, we performed DNA extractions on single hairs from these 13 sample envelopes. We chose the 4 best hairs from each of these envelopes (from different locations in the clump of hairs as much as possible) for DNA extraction and analysis for a total of 52 single-hair extractions (these samples are labeled with the sample ID and "A-D"). While notes for sample 20133301 indicate 2 wolves were observed on photos from the deployed camera, this sample was not highlighted; hence a standard DNA extraction was performed.

We tested the 80 hair samples performed from regular DNA extractions for species using the control region of mitochondrial DNA. This analysis does not distinguish between wolves and dogs (*Canis familiaris*), and, therefore, samples with this DNA type are written as wolf/dog. Seventy-three samples (91%) were successfully identified to species: 54 were from wolf/dog, 18 from black bear (*Ursus americanus*), and one hair sample was from marten (*Martes americana*).

We analyzed DNA for individuals using a panel of ten variable microsatellite loci. These loci provide an acceptable cumulative PID= 5.35×10^{-7} (1 in 2,298,317.73 chance that we

are calling two samples the same when they are actually from different individuals). We analyzed 54 hair samples identified as wolf/dog from species identification analysis plus the 52 single-hair extractions, for a total of 106 non-invasive samples tested. Blood sample JM435 was also tested.

We obtained DNA for individual identification from 45 of the 54 standard-hair extraction samples (83%). Thirty-nine samples amplified with alleles consistent for Alaska wolves, representing 18 unique individuals. Six samples (representing 4 individuals) were listed as suspected dog based on the photos obtained from the cameras at these stations. While we don't have domestic dogs from Alaska in the dataset, these samples contain alleles across five of the microsatellite loci that we did not observe in harvested wolf samples from Alaska. Based on microsatellites, the putative dogs cluster together apart from the known harvested wolf tissue and the wolf hair samples. This analysis, along with evidence from the photos, suggests these 4 individuals are dogs and not wolves.

We analyzed DNA for individual identification from 52 single-hair DNA extractions. Twelve hair samples (23%) contained DNA for this analysis, representing 7 individuals. Four of these individuals were also represented in the standard-hair extractions while 3 individuals were not.

Twenty-one unique wolves were identified from 2013 non-invasive hair samples. Blood sample from Wolf_JM435 represents a unique wolf, for a total of 22 wolves. Individual wolves were tested for sex (we identified 11 females and 11 males, including Wolf_JM435, in these samples).

On 28 April 2014, we sent 49 tissue samples from harvested wolves (2013-2014 seasons) collected in the same area as the hair samples. Wolves identified from hair samples from 2012 (5 that were not harvested in the 2012–2013 season) and 21 wolves identified from 2013 samples were compared to the harvested tissues from 2013–2014. Ten wolves identified from 2013 hair samples were harvested.

JOB/ACTIVITY 3: Analysis and population estimation

Using only the DNA results, we counted a minimum of 21 wolves in our study during 2013–2014. We recorded 10 harvested wolves, whose hair was captured in the hair traps.

We began to explore using a spatial explicit capture recapture (SECR) method to analyze the data. We completed no additional analyses during this report period.

JOB/ACTIVITY 4: Publication and report writing

We completed this annual progress report. No other publications were completed during this reporting period.

III. Not applicable.

IV.   SIGNIFICANT DEVIATIONS AND/OR ADDITIONAL FEDERAL AID-FUNDED WORK NOT DESCRIBED ABOVE THAT WAS ACCOMPLISHED ON THIS PROJECT DURING THIS SEGMENT PERIOD

None.

V.   PUBLICATIONS RECOMMENDATIONS FOR THIS PROJECT This project was redesigned and is beginning a new phase of sample collections and analyses. The work will occur in conjunction with a separate wolf radio-collaring effort that will compliment the DNA analyses with respect to providing an independent means of estimating wolf abundance for the study area.

Literature Cited

Ausband, D. E. 2011. Hair of the dog: obtaining samples from coyotes and wolves noninvasively. Wildlife Society Bulletin 35:105–111.

Prepared by: Rodney Flynn**,** Gretchen Roffler, and Kris Larson

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