### Alaska Department of Fish and Game Wildlife Restoration Grant

GRANT NUMBER: AKW-B-R1-2020 Amendment #1 SFY2021

**PROJECT NUMBER:** 14.32

PROJECT TITLE: Predation patterns and foraging ecology of wolves in Southeast Alaska

PERIOD OF PERFORMANCE: July 1, 2020 - June 30, 2021

**REPORT DUE DATE:** Submit to FAC August 28, 2021

PRINCIPAL INVESTIGATOR: Gretchen Roffler

**COOPERATORS:** National Genomics Center for Wildlife and Fish Conservation, Dr. Taal Levi (Oregon State University)

#### I. PROGRESS ON PROJECT OBJECTIVES DURING PERIOD OF PERFORMANCE

**Strategy: Research, Survey, Data Collection and Analysis** Objective 1: Conduct 1 investigation by 06-30-2021

Activity 1A: Capture a sample (1-3) of wolves in each study area using foot-hold traps or aerial chemical immobilization. Chemically immobilize, and instrument wolves with GPS radio collars. Collect biological samples for stable isotope analyses (wolf diets) and individual identification from DNA.

ACCOMPLISHMENTS: During FY 2021 we captured and instrumented 9 wolves using foot hold traps. We captured 2 female adults and 1 female pup in Gustavus during early fall 2020, and 2 female adults during late spring 2021. We captured 2 female adults and 1 female pup (although the pup was not large enough to retain a GPS collar) on Pleasant Island during fall 2020, and 1 adult female during late spring 2021. We also captured 1 adult female on Revilla Island during early summer 2021. We also weighed and took standard morphometric measurements from each animal and collected biological samples (blood, tissue) for stable isotope analysis and individual identification.

Activity 1B: Track radiocollared wolves before and during kill rate estimation periods, and additionally 4-6 times per year.

Accomplishments: We radiotracked collared wolves from aircraft as time and weather allowed during kill rate estimation periods and 4-6 times during the remaining 10 months of each year.

Activity 1C: Remotely download GPS data every 2 days.

Accomplishments: We remotely downloaded GPS locations from collared wolves every two days and stored data in a database.

Activity 1D: Assess spatial and temporal distribution of GPS radio collar locations to identify clusters.

Accomplishments: We regularly assessed GPS location clusters from individual collared wolves during kill rate estimation periods. Doing this is essential to locate potential kill sites.

**OBJECTIVE 2:** Investigate kill sites.

Activity 2A: Locate GPS clusters and investigate to identify kill sites.

ACCOMPLISHMENTS: We located and visited all GPS point clusters deemed to be potential kill sites and searched for prey remains.

Activity 2B: Visually identify prey species and distinguish kill sites from scavenging sites and resting sites based on prey remains, sign, and other characteristics.

ACCOMPLISHMENTS: We distinguished kill, scavenging, and resting sites, identified prey species from remains, and determined if the animal had been killed by wolves or scavenged.

Activity 2C: Collect ungulate prey mandibles and teeth for cementum age determination.

ACCOMPLISHMENTS: At each site with ungulate prey remains we collect mandibles with teeth for cementum aging.

Activity 2D: Collect ungulate long bones (e.g., femurs) for characterization of nutritional condition from the percentage of bone marrow fat.

ACCOMPLISHMENTS: At each site with ungulate prey remains we collected a long bone to evaluate the nutritional condition of the prey animal.

Activity 2E: Deploy and monitor motion-detecting trail cameras at a subset of the kill sites to characterize use over time.

ACCOMPLISHMENTS: At a subset of kill sites we placed motion-detecting trail cameras to learn whether wolves returned to the site.

Activity 2F: Collect wolf scats to identify prey species genetically (See Objective 6).

ACCOMPLISHMENTS: At each site we searched for and collected wolf scats to evaluate prey species through DNA metabarcoding techniques.

Activity 2G: Collect biological samples from prey species (e.g., tissues, swabs) to identify prey species and sex of prey molecularly.

ACCOMPLISHMENTS: At each site we collected tissue samples to help confirm species and sex of prey animal.

ACCOMPLISHMENTS SUMMARY FOR ACTIVITIES 2A-2G: We visited clusters of GPS wolf locations during late summer (15 August-15 September) 2020 in Berners Bay, Revilla Island, and Gustavus, and late winter (15 February-15 March) 2021 in Gustavus. Berners Bay wolf 201802 was discovered shot but not recovered by hunters in October 2020; Berners Bay wolf 202001 disappeared during this same time and the last known locations of 202001 and 201802 were at the mouth of the Antler River, thus it is suspected that 202001 was also killed by people but not reported. Due to these wolf mortalities, we were able to conduct GPS cluster investigations during the late summer period, but not the late winter period for this wolf pack. Shelter Cove wolf 201902 was trapped in early November 2020, therefore we were able to conduct GPS cluster investigations during the late summer period, but not the late winter period for this wolf pack. The GPS collar of wolf 202002 of the Ketchikan pack failed during early January 2021, and wolf 202003 dispersed from the Ketchikan pack towards Misty Fjords and was killed by other wolves 14 February 2021. Therefore, we were able to conduct GPS cluster investigations during the late summer period, but not the late winter period for the Ketchikan wolf pack. Pleasant Island wolf 202006 died of natural causes in November 2020, and wolf 202008 was snared in February 2021, thus we were not able to investigate wolf GPS clusters on Pleasant Island. Wolf 202004 was captured in Gustavus on September 5, allowing us to collect data at GPS clusters during the last part of the late summer period.

We visited 30 of the 59 clusters in the Berners Bay study area during the late summer 2020 predation rate investigation period. Three of the Berners Bay wolf clusters had evidence of predation and were classified as kill sites (1 moose adult female [collar#78] and 2 mountain goat adult males). The Berners Bay wolves also had 4 scavenging sites consisting of 2 adult moose previously killed by this wolf pack and 2 adult moose killed recently by brown bears. All transportation to the sites was conducted by Hughes 500 helicopter. We visited 26 of the 70 clusters in the Shelter Cove study area on Revilla Island during the late summer 2020 predation rate investigation period. Eleven of the clusters had evidence of predation and were classified as kills sites (8 deer [3 adults, 1 yearling, 3 fawns, and 1 undetermined age], 2 black bears [1 adult and 1 cub], and 1 site with salmon carcasses and a sea otter pup carcass). The Shelter Cove wolves also had 2 scavenging sites including 1 human-killed deer and 1 site with black bear and deer remains. We visited 65 of the 126 clusters in the Ketchikan study area on Revilla Island during the late summer 2020 predation rate investigation period. Fourteen of the clusters had evidence of predation and were classified as kills sites. Wolf prey at the kill sites included mountain goats (2 adults and 3 kids), deer (4 adults, 1 yearling, 2 fawns and 1 undetermined age) and geese. The Ketchikan wolves also had one scavenging site where we found the remains of a mountain goat. Transportation to the sites on Revilla Island was conducted by Hughes 500 helicopter and by foot from the road system. We visited 7 of the 17 clusters in the Gustavus study area, one of which was a kill site (an adult male moose). Transportation to the sites was by foot from the road system.

We visited 62 of 78 clusters in the Gustavus study area during the late winter 2021 predation rate investigation period. We found evidence of predation at 8 of the clusters,

thus these were classified as kill sites. The prey species at the kill sites consisted of 3 moose (2 cows and 1 calf), 2 deer (both adults), 2 sea otters (both were at the same GPS cluster), 1 goose and 1 unknown bird. We also found evidence of scavenging at 6 of the clusters. We found remains of sea otters at 4 of the scavenging sites (2 of the sites had 2 individual sea otters and 2 of the sites had 1 sea otter each, thus 6 sea otters scavenged total). The other 2 scavenging sites had remains of moose and an unknown species of bird. In addition, we encountered 2 kill sites randomly while conducting cluster investigations (1 adult deer and 1 sea otter) and investigated a radiocollared moose in the study area that had been killed by wolves. Transportation to the sites was mostly by foot from the road system, but a few remote sites were access using a Hughes 269 helicopter.

OBJECTIVE 3: Determine predation patterns during late summer and late winter. Activity 3A: Calculate kill rate as the number of prey killed/wolf/day and the biomass killed/wolf/day.

Activity 3B: In study areas with ungulate abundance information, estimate predation rate as the number of prey killed/prey abundance for each study area.

Activity 3C: Examine the influence of the following factors on kill rate and prey selection: 1) wolf pack size, 2) season (late summer vs, late winter), 3) prey diversity, 4) indices of winter severity.

ACCOMPLISHMENTS: No work was planned or completed on Objective 3 during the reporting period. This objective will be accomplished at the end of the project once all field data are collected.

OBJECTIVE 4: Determine minimum counts of wolves. Activity 4A: Obtain wolf numbers from trail cameras established in the study areas.

ACCOMPLISHMENTS: We installed trail cameras in the territory of each monitored pack to determine number of pack members.

Activity 4B: Count wolf pack members visually when possible, during radiotracking flights.

ACCOMPLISHMENTS: We made visual counts of monitored wolf packs when they were visible during radiotracking flights.

Activity 4C: Collect snagged wolf hair opportunistically to genetically identify and enumerate individuals in the wolf pack.

ACCOMPLISHMENTS: Whenever possible we collected wolf hair at kill sites to genetically identify and enumerate pack members.

ACCOMPLISHMENTS SUMMARY: We conducted periodic radiotelemetry flights to track the collared wolves. We have reduced the frequency of radiotelemetry flights due to the marginal success of aerial observations of wolves in dense forest cover. Using the successful aerial observations, ground observations and camera data, we determined the minimum count of the wolf packs in our study areas. The minimum count of the Berners

Bay pack during summer 2020 was 5 (1 adult collared female, 1 adult collared male, 1 uncollared adult, and 3 pups). The minimum count of the Shelter Cove pack during summer 2020 was 7 (1 collared adult, 1 uncollared adult, and 5 pups), and the Ketchikan wolf pack minimum count was 5 (1 adult collared female, 1 adult collared male, and 3 uncollared adults). During June 2021 the minimum count of the Ketchikan wolf pack was 6 (1 collared breeding female and 5 pups). The minimum count of the Gustavus wolf pack during summer 2020–spring 2021 was 5 wolves.

OBJECTIVE 5: Assess spatial distribution and movement patterns of radiocollared wolves.

Activity 5A: Analyze GPS location data to assess seasonal home range size, distribution, and geographic extent of wolf packs.

ACCOMPLISHMENTS: We used GPS location data to assess seasonal home ranges of packs with collared wolves.

Activity 5B: Characterize GPS locations and wolf movement patterns associated with kill sites of large-and small-bodied prey.

ACCOMPLISHMENTS: We use GPS location data to characterize wolf movement patterns in relation to small and large-bodied prey.

ACCOMPLISHMENTS SUMMARY: We downloaded GPS locations of wolves and archived the data in a data base. We evaluated wolf home range distribution and movement patterns to determine pack home range boundaries, potential denning locations, and dispersal events of GPS collared wolves.

OBJECTIVE 6: Characterize variation in Southeast Alaskan wolf diets.

Activity 6A: Collect georeferenced and date-specific wolf scats.

ACCOMPLISHMENTS: We recorded GPS location and date for each wolf scat collected.

Activity 6B: Prepare collected scats for lab analysis, and record collection information in a database.

ACCOMPLISHMENTS: We prepared all scats for analysis and recorded data in a database.

Activity 6C: Identify prey species consumed by wolves using DNA metabarcoding.

ACCOMPLISHMENTS: We shipped scats to a cooperating lab for DNA metabarcoding identification of prey species.

Activity 6D: Quantify the relative proportion of each prey species consumed using PCR with species-specific primers.

ACCOMPLISHMENTS: We worked with a cooperating lab to use PCR to evaluate the relative quantity of each prey species in each scat.

Activity 6E: Collect wolf hair and muscle tissue samples for stable isotope analysis.

ACCOMPLISHMENTS: We collected wolf hair and muscle tissue samples to evaluate wolf food habits using stable isotope analysis.

Activity 6F: Collect muscle tissue samples from putative prey species to obtain sufficient resolution to distinguish different prey species from each other in the stable isotope analyses.

ACCOMPLISHMENTS: We also collected tissue samples from likely prey species to gather data of the unique signature of each species so they could be distinguished using stable isotope analysis.

ACCOMPLISHMENTS SUMMARY: During the reporting period we collected 731 scats from GMUs 1A, 1C, 2, 3, and 4 (Pleasant Island). Samples were stored frozen until shipment to Oregon State University for analysis.

We received results of metabarcoding of prey species from fecal DNA from 342 wolf scats collected during April–October 2020. Of the samples analyzed, 324 provided successful DNA amplification results of prey taxa (94.7%). One of the scat samples presumed to originate from wolves were identified molecularly as originating from black bear, 2 scats from brown bear, and 3 scats from marmot.

We shipped 16 wolf and 11 sea otter samples (hair muscle tissue, and vibrissae) from GMUs 1C, and 4 (Pleasant Island) for stable isotope analyses to the Alaska Stable Isotope Facility and received  $\delta 13$  C and  $\delta 15$  N results from these samples during spring 2020. We collected samples from wolves harvested during the 2020–2021 season and have begun processing them for lab analysis; we expect to complete sample processing and shipment by December 2021.

OBJECTIVE 7: Describe genetic relationships and structure of Southeast Alaskan wolves.

Activity 7A: Collect hair and muscle tissue samples from harvested captured, or road-killed wolves from throughout Southeast Alaska and neighboring regions.

ACCOMPLISHMENTS: We collected hair and tissue samples from wolves throughout Southeast Alaska and adjacent regions.

Activity 7B: Genotype individual wolves using microsatellite and/or single nucleotide polymorphisms markers (SNP).

ACCOMPLISHMENTS: We worked with a cooperating lab to genotype individual wolves.

ACCOMPLISHMENTS SUMMARY: We developed and used a custom hybridization-based capture to generate 37,082 neutral, genome-wide single nucleotide polymorphism (SNP) genotypes and over 500,000 SNP genotype likelihoods. Currently, DNA from 443 muscle tissue samples and 174 hair samples from wolves from across Southeast Alaska and the Yukon Territory has been extracted and sequenced at the 37,082 SNPs. We are awaiting progress from the National Genomics Center for Wildlife and Fish Conservation on library preparation and bioinformatics to prepare the genomic data for regional structure analyses.

**OBJECTIVE 8:** Data synthesis and preparation of publications

Activity 8A: Analyze and synthesize data.

ACCOMPLISHMENTS: We continued to analyze and synthesize data toward fulfilling goal of this project.

Activity 8B: Prepare final reports and publications.

ACCOMPLISHMENTS: We prepared reports and publications as detailed below.

Activity 8C: Archive data, metadata, and biological samples.

ACCOMPLISHMENTS: We archived all samples using appropriate techniques and archived all data and metadata on professionally managed databases.

ACCOMPLISHMENTS: We published 2 manuscripts describing 1.) analyses of regional differences in wolf diets using metabarcoding of fecal DNA, and 2.) comparison of identifying wolf prey species using mechanical sorting and metabarcoding (see section IV). We prepared and submitted a manuscript describing use of keratinized tissues for measuring reproductive and stress-related steroid hormones in wolves. We conducted data analyses and preparation of a manuscript describing wolf populations and the influence of marine subsidies predator-prey dynamics on islands.

### II. SUMMARY OF WORK COMPLETED ON PROJECT TO DATE.

FY2021 was year 3 in a 5-year project. Since initiation of this project during Spring 2018, 18 wolves from 5 packs have been instrumented with GPS collars and fieldwork for 6 predation rate estimation periods have been completed. We have investigated 562 wolf GPS clusters and identified 91 kill sites and 39 scavenging sites. We have collected data and biological samples to identify the prey species, sex, age, and body condition. Prey composition varied greatly by study site. In study areas where alpine habitat was present, mountain goats made up a substantial portion of the ungulate prey and was nearly equivalent to the proportion of assumed primary ungulate prey (either deer in Ketchikan, or moose in Berners Bay). Alternate prey also varied by study site with beaver prevalent in Berners Bay, and salmon, black bears, and bird species in Gustavus, Ketchikan and Shelter Cove. During the winter predation rate fieldwork in Gustavus we documented 3 deer kills, considered to be a novel prey in this moose-dominant area, and 2 sea otter kills and 6 scavenged sea otters. Sea otters have become a prevalent alternate prey in Gustavus and primary prey on adjacent Pleasant Island indicating the dynamic nature of this rapidly changing landscape. We also documented many instances of wolf competition with brown bears including kleptoparasitism in both directions (wolves stealing prey killed by bears,

and wolves displaced from their kill sites by bears) alluding to the complex relationship between these 2 carnivore species.

### III. SIGNIFICANT DEVELOPMENT REPORTS AND/OR AMENDMENTS.

There are no significant developments to report.

### IV. PUBLICATIONS

- Roffler, G. H., J. M. Allen, A. Massey, and T. Levi. 2021. Metabarcoding of fecal DNA shows dietary diversification in wolves substitutes for ungulates in an island archipelago. Ecosphere 12:e03297.
- Roffler, G. H., J. M. Allen, A. Massey, and T. Levi. 2021. Wolf Dietary Diversity in an Island Archipelago. Bulletin of the Ecological Society of America 102:1–6.
- Massey, A., G. H. Roffler, T. Vermeul, M. Allen, Jennifer, and T. Levi. 2021. Comparison of mechanical sorting and DNA metabarcoding for diet analysis with degraded wolf scats. Ecosphere 12:e03557.

A media piece (website) was released on 20 and 29 of April 2021 describing results from this project:

https://www.hakaimagazine.com/news/alaskas-coastal-wolves-are-not-picky-eaters/

https://www.krbd.org/2021/04/29/southeast-alaskas-wolves-are-not-picky-eaters-study-finds/

### V. RECOMMENDATIONS FOR THIS PROJECT

This project has provided valuable results for understanding variation in wolf diets and seasonal predation patterns. This project should continue for a minimum of 2 years as planned in order to achieve sufficient data for analyses.

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**Date:** August 6, 2021