# Alaska Department of Fish and Game Wildlife Restoration Grant

**GRANT NUMBER:** AKW-20 1.68

**PROJECT TITLE:** Moose Diet FY2017: Factors affecting moose forage quality and subsequent reproductive success

PROJECT DURATION: 1 July 2016 to 30 June 2020

**REPORT DUE DATE:** 1 September 2017

PRINCIPAL INVESTIGATOR: William B. Collins

**COOPERATORS:** Don Spalinger, University of Alaska Anchorage; Lindsey Solden, and Kelly Wrighton, The Ohio State University

**WORK LOCATION:** Matanuska Research Farm, Togiak Valley, Colville River, Nelchina Basin, Game Management Units 15, 17

## I. PROGRESS ON PROJECT OBJECTIVES DURING LAST SEGMENT

OBJECTIVE 1: Availability of digestible nitrogen to moose across a spectrum of marginally to highly productive moose ranges in Alaska.

We have completed analysis of our Nelchina, Placer, and Denali samples, and most of our Colville samples. We have completed approximately 1/3 of our Togiak and Goodnews samples. Inability to commit fulltime to laboratory work has slowed our progress. We are also encumbered by a much larger number of samples than we originally intended to collect. We are currently exploring options for increasing the rate of processing the remaining samples.

Our ultimate goal is to compare the quality of diets across a broad spectrum of ranges to better explain variations in forage quality and related herd productivities. Our intermediate goal is to have completed analyses of all the samples we currently have by the end of 2018.

OBJECTIVE 2: Effects of climate and utilization on browse quality.

No further work was done regarding this objective, as most of the Salix pulchra plants in our transplant garden died back during winter 2015/2016. We do not know why this happened, but we suspect it had to do with an unusual winter having very little snow and repeated freeze-thaw cycles, combined with the fact that our transplant garden is

directly in the path of the Matanuska wind and was readily swept clear of what little snow there was. Plants in the mountains and in low elevations elsewhere did not experience the same lack of snow and die-back.

OBJECTIVE 3: Microbiological link between diet quality and reproductive performance of moose.

Ruminants and their gastrointestinal (GI) microbial communities have coevolved, with the latter providing host energy by digesting plant materials in the rumen. Our objective is to define microbial contributions to forage digestion in moose in attempt to understand factors potentially limiting to their condition and reproductive potential. Assessment of rumen flora responses to variations in diet quality has required use of rumen fistulated moose. Thus, our initial approach has been to focus on microbial responses to variations in quality of diets selected by individuals by season, rather than to assess response differences across geographic gradients. Microbial communities of digestive tracts respond to specific variations in diet quality similarly, whether they are of seasonal or geographic origin. Identification of GI microorganisms and their function in the various aspects of forage digestion, and comparisons of those organisms across moose populations will help us better understand the linkage between forage quality and animal performance.

To uncover lignocellulose degrading taxa and establish an ecosystems biological ruminant model, we sampled rumen fluid from moose consuming native Alaskan plants in Winter when lignin and cellulose were 2.5X higher than herbaceous diets in Spring. Metagenomic reconstruction yielded 207 genomes, 82 of which were unique and complete to near complete. Thirty-three of the unique genomes provided first genomic representatives for a novel TM7 class, two orders previously identified in rumen (RF9 and RFP12 in the Tenericutes and Lentisphaera, respectively), and prevalent rumen Bacteroidetes families (BS11 gut group) and genera (RC9). In contrast to other studies that suggested Firmicutes cellulosomes mediate rumen carbon cycling, our metaproteomics data detected 91 polysaccharide utilization loci (PUL) predicted to degrade starch, pectin, cellulose, hemicellulose, and mucin substrates. These represent new metabolic activities for uncultivated Bacteroidetes.

Our study provides the first 6 genomes from the RC9 genus revealing extensive complex carbon degradation capacities, including up to 20 PUL systems per genome and pathways for lignin degradation. We also sampled the first four genomes from the BS11 family within Bacteroidetes, which were inferred via proteomics to be involved in hemicellulosic monomer fermentation. Additionally, we identified 1,497 viral contigs, which represented an estimated 110 new viral genera and included 93 closed genomes and 457 prophages. Of the non-prophages, 48 were active as inferred from representation in metaproteomes and 69 were linked to microbial hosts, including active carbon degraders, suggesting viral predation is active and of paramount ecosystem importance in the rumen. These findings illuminate the cross-kingdom taxonomic and metabolic novelty and activity in the rumen ecosystem, functional roles and metabolic handoffs in the rumen ecosystem.

OBJECTIVE 4: Hormonal link between diet quality and reproductive performance of moose.

We examined patterns of nutritional hormones (leptin, ghrelin, Insulin-like growth factor-1) in captive, non-pregnant female moose fed a maintenance diet from November to August. Over the 10-month period, plasma concentrations for leptin, ghrelin, and IGF-1 averaged  $1.36 \pm 0.81$  ng/ml,  $0.229 \pm 0.110$  ng/ml, and  $114.0 \pm 30.5$  ng/ml, respectively. Leptin and IGF-1 did not respond to changes in season while ghrelin did. Plasma ghrelin concentrations were significantly elevated during the winter months (P = 0.009), suggesting that ghrelin may be sensitive to changes in season and maybe indicative of the animal's nutritional status. This objective was completed, and a manuscript covering the objective was prepared and accepted for publication in Alces.

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

**Job/Activity 1-a:** Forage N and Protein-binding Capacities of Forages across a Spectrum of Ranges.

**Accomplishments:** We completed analysis of our Nelchina, Placer, and Denali samples, most of our Colville samples, and approximately 1/3 of our Togiak and Good News samples.

**Job/Activity 1-b:** Forage Selection and Food Habits of moose in Colville Range and Habitats Adjacent to Togiak Valley Based on Alkane Analysis

**Accomplishments**: We have compared data from an alkane-based method to those obtained by microhistology and by direct observation and have begun using the method to compound forage samples for nutritional analysis of diets in Colville and Togiak ranges.

**Job/Activity 2:** Effects of Climate and Utilization on Digestible N, Tannins, Digestibility, and Palatability in Potted Willows

Accomplishments: No work was accomplished on this job, due to dieback of potted plants during an atypically warm and snow-free winter.

Job/Activity 3: Moose Microbiome responses to diet quality

Accomplishments: Considerable work and major findings regarding moose microbiome responses to diet quality were accomplished this year. They are described in manuscripts accepted for publication in Microbial Ecology Journal, Nature Communications, and American Society for Microbiology

Job/Activity 4: Hormonal Link between Diet Quality and Reproduction

Accomplishments: A manuscript concluding this job was revised and accepted for publication in <u>Alces.</u>

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

#### **IV. PUBLICATIONS**

Carnahan, A., D. Spalinger, and W. Collins. 2017. Alkane and long-chain alcohol recovery in moose (*Alces alces*), a browsing herbivore. Can. J. Zoology.

Stantorf, C. J., L. C. Buck, D. H Keisler, W. B. Collins, and D. E. Spalinger. 2017. Seasonal variation of nutritional hormones in captive female moose. Alces.

Solden, L. M., D. W. Hoyt, W. B. Collins, J. E. Plank, and R. A. Daly E. Hildebrand, T. J. Beavers, R. Wolfe, C. D. Nicora, S. O. Purvine, M. Carstensen, M. A. Lipton, D. E. Spalinger, J. L. Firkins, B. A. and Wolfe, K. C. Wrighton. In press. First genomic insight into the enigmatic BS11 gut group reveals roles in hemicellulose degradation. Nature Communications.

Solden, L. M., D. W. Hoyt, W. B. Collins, J. E. Plank, R. A. Daly, E. Hildebrand, T. J. Beavers, R. Wolfe, C. D. Nicora, S. O Purvine, M. Carstensen, M. A. Lipton, D. E. Spalinger, J. L Firkins, B. A. Wolfe, and K. C. Wrighton. 2016. New roles in rumen hemicellosic sugar fermentation for the uncultivated Bacteroidetes family BS11. Int. Soc. Microbial Ecology Journal 1-13.

Solden, L. M., A. Naas, S. Roux, R. A. Daly, W. B. Collins, R. A. Wolfe, C. D. Nicora,
S. O. Purvine, D. E. Spalinger, M. S Lipton, M. S. Sullivan, P. Pope, and K. C. Wrighton.
2017. Microbial dark matter in the rumen ecosystem: Assigning the function of
uncultivated Bacteroidetes and novel viruses in anoxic carbon transformations. Abstract.
American Society for Microbiology.

## V. RECOMMENDATIONS FOR THIS PROJECT

Prepared by: William B. Collins

Date: September 1, 2017