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FISH 541 – Draft

**Correlation Between Commensal Bacteria and Hormone Levels in the Alberta Oil Sands Caribou**

**Abstract**

Gut microflora in mammals play important roles in digestive and immune system processes. Microbes abundance and richness might also be a good indicator of psychological and dietary stress. We investigated possible correlations between dietary stress measured through hormone levels and abundance of four genera of commensal bacteria commonly found in mammals. We focused on a Woodland caribou (Rangifer tarandus caribou) population that has been affected by oil sand operations in Canada. We hypothesized that individuals with low glucocorticoid might also have compromised microflora with lower abundance or richness. This study aims at improving noninvasive methodologies to test wildlife health and physiological responses to environmental changes. Information provided by gut microbes can possibly corroborate hormone studies given the different response time to changes in physiology.

**Introduction**

Wildlife in the Alberta oil sands region of Western Canada has been subject to unprecedented anthropogenic pressure caused by surveying, construction, and oil extraction activities (Bradshaw 1997). These activities are not only heavily modifying the landscape by fragmenting and reducing wildlife habitat but are also imposing behavioral changes in mammal community dynamics (Dyer 2001). Populations of moose, dear, and caribou foraging patterns have been impacted and individuals are forced to gravitate towards subprime foraging grounds (Wasser 2011). According to Wasser et al. in periods of intense human activities caribou are avoiding areas containing high density of lichens which is their preferred and most nutritious food source. This was determined by observing a correlation between glucocorticoid (GC) concentration in feces and human presence. GC levels were the highest near primary roads and oil expiration roads when humans were most active. This correlation suggested that caribou preferred areas that offered more security at the cost of a compromised diet. GC levels can also be related to psychological stress thus it is unclear what other consequences different type of stress can have on the physiology of the population.

Another important measure of an organism health is gut microbes presence and abundance. A wide range of commensal bacteria have been found in mammalian species which played an important evolutionary role (Ley 2008). These microbial communities can regulate the immune system (Kelly 2005), attenuate inflammation (Kelly 2003), and have recently been associated with psychological stress response (Bailey 2010). We wanted to investigate if (i) it is possible to reliably measure bacteria presence and abundance from caribou feces and (ii) there is a correlation between GC levels and bacteria. We hypothesize that individuals with higher GC might have compromised microflora as a result of higher psychological and dietary stress. Thus, we want to compare bacteria presence and abundance in fecal samples of caribou with different GC levels. We selected three genera of commensal bacteria *Lactobacillus, Bacteroides,* and *Clostridium* that are commonly present in mammals and ruminants in particular (Endo 2010, Nelson 2003, and Sundset 2007). In addition to *Akkermansia muciniphila* a single species genus that has been associated with mucosal inflammation and immune response (Derrien 2011).

**Study area and methods**

The study area is situated on the east side of the Athabasca River in Alberta Canada. The caribou range partially overlaps with the oil sands operations south of Fort McMurray between highway 63 and 881 (56.0˚N, 111.3˚W).

Fecal samples were collected by Wasser et. al in the winter of 2013 with the use of scat detection dogs. Samples were immediately frozen upon collection; in addition, air temperatures below freezing reduced degradation of non-fresh samples. DNA was extracted according to the fecal swab DNA extraction protocol: 96-well Plate Format with homemade Celite plates and DNeasy 96 Blood and Tissue Kit Reagents (appendix I). Samples were genotyped and GC concentrations measured.

We selected 16 DNA samples from all different male adults. We chose male samples to reduce the amount of variability involved as GC is also affected by pregnancy. Further, we only selected adults because microflora might still be developing in calves. Table I shows the location and GC levels of the samples. Of all the 100 samples available we eliminated the ones from the same individuals and picked the ones with extreme GC concentrations and, when possible, collected at different times and not spatially clustered. We assume that individuals with low GC spent more time in highly nutritious area.



Table I

To measure abundance and presence of bacteria we utilized conventional Polymerase Chain Reaction (PCR) and agarose gel. We selected primers developed in other studies for the detection of bacteria from fecal samples (Rinttilä 2011) Table II. The most common technique to quantify bacteria from feces uses 16S-RNA targeted primers (Walter 2001) and many studies rely on real-time PCR. PCR reaction was 25 µL composed of 12.5 µL of GoTaq Green buffer, 1 µL of forward primer, 1µL of reverse primer, 2 µL of DNA template, and 8.5 µL of nuclease free water. The cycles were run as follows: Initial Denaturation at 95° for 5 min, then, [denaturation at 95° for 30 seconds, annealing at the temperature prescribed for each primer between 55° and 63° for 30 seconds, extension at 72° for 90 seconds ] (40 cycles). A final extension step at 72° for 3 min. was performed

The PCR products were then run in agarose gel with TAE buffer and 7 µL 100bp ladder with 20 µL of PCR sample, and run at 100v for one hour.

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| A.muciniphila\_16S\_rRNA\_F | CAGCACGTGAAGGTGGGGAC |
| A.muciniphila\_16S\_rRNA\_R | CCTTGCGGTTGGCTTCAGAT |
| Lactobacillus\_spp\_16s\_rRNA\_F | AGCAGTAGGGAATCTTCCA |
| Lactobacillus\_spp\_16s\_rRNA\_R | CACCGCTACACATGGAG |
| Bacteroides-Prevotella-Porphyromonas\_spp\_16s\_rRNA\_F | GGTGTCGGCTTAAGTGCCAT |
| Bacteroides-Prevotella-Porphyromonas\_spp\_16s\_rRNA\_R | CGGAYGTAAGGGCCGTGC |
| Clostridium-Eubacterium-Ruminoc\_spp\_16s\_rRNA\_F | CGGTACCTGACTAAGAAGC |
| Clostridium-Eubacterium-Ruminoc\_spp\_16s\_rRNA\_R | AGTTTYATTCTTGCGAACG |

Table II

PICTURE OF GEL

**Expected Results**

We expect all samples to show presence of all the four genera of bacteria because they should be present in every individual. The gel should be able to tell the relative abundance among samples of each genera. In this study we are not interested in overall abundance.

**Conclusions**

**Sources**

Bailey, Michael T., et al. "Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by Citrobacter rodentium." *Infection and immunity* 78.4 (2010): 1509-1519.

Bradshaw, Corey JA, Stan Boutin, and Daryll M. Hebert. "Effects of petroleum exploration on woodland caribou in northeastern Alberta." *The Journal of wildlife management* (1997): 1127-1133.

Derrien, Muriel, et al. "Modulation of mucosal immune response, tolerance, and proliferation in mice colonized by the mucin-degrader Akkermansia muciniphila." Frontiers in microbiology 2 (2011).

Dyer, Simon J., et al. "Avoidance of industrial development by woodland caribou." *The Journal of wildlife management* (2001): 531-542.

Endo, Akihito, Yuka Futagawa-Endo, and Leon MT Dicks. "Diversity of< i> Lactobacillus</i> and< i> Bifidobacterium</i> in feces of herbivores, omnivores and carnivores." Anaerobe 16.6 (2010): 590-596.

Kelly, Denise, et al. "Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-γ and RelA." *Nature immunology* 5.1 (2003): 104-112.

Kelly, Denise, Shaun Conway, and Rustam Aminov. "Commensal gut bacteria: mechanisms of immune modulation." Trends in immunology 26.6 (2005): 326-333.

Ley, Ruth E., et al. "Evolution of mammals and their gut microbes." Science 320.5883 (2008): 1647-1651.

Nelson, Karen E., et al. "Phylogenetic analysis of the microbial populations in the wild herbivore gastrointestinal tract: insights into an unexplored niche." Environmental microbiology 5.11 (2003): 1212-1220.

Rinttilä, T., et al. "Development of an extensive set of 16S rDNA‐targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real‐time PCR." Journal of applied microbiology 97.6 (2004): 1166-1177.

Sundset, Monica A., et al. "Novel rumen bacterial diversity in two geographically separated sub-species of reindeer." Microbial ecology 54.3 (2007): 424-438.

Walter, Jens, et al. "Detection of Lactobacillus, Pediococcus, Leuconostoc, and Weissella species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis." Applied and Environmental Microbiology 67.6 (2001): 2578-2585.

Wasser, Samuel K., et al. "The influences of wolf predation, habitat loss, and human activity on caribou and moose in the Alberta oil sands." *Frontiers in Ecology and the Environment* 9.10 (2011): 546-551.