Backcountry Recreation Modeling and the Extent of Human and Canine Fecal Markers in Subalpine Lakes of Mount Rainier National Park

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Abstract

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Alpine lakes are some of the most fragile ecosystems in our national parks and forests and a focal point of backcountry visitation. As a result of climate change, access to these ecosystems is increasing due to annual reductions in snow cover. This projected extension of shoulder seasons from warming and the increased public interest in national parks and forests creates a confluence of conditions creating concern among land managers. Because of this concern evaluating tools capable of quantifying basin-level visitation and capturing its direct consequence has garnered [more recent] interest. One impact of concern is the infiltration of fecal matter into aquatic systems from unburied waste and leaking privies, which conflicts with principles outlined in the Organic Act of 1916 and the Wilderness Act of 1964. In this study, we sought to evaluate the plausibility of models, parameterized on existing data from Mount Baker-Snoqualmie National Forest (MBS), in estimating basin-level visitation in Mount Rainier National Park (MORA) and, as a consequence of visitation, detect putative human and canine fecal markers in subalpine lakes using microbial source tracking (MST) methods. In MORA, we examined the transferability of a visitation model developed by Wood et al., 2020, and employed MST methods to detect the possible presence of the human-specific and canine-specific *Bacteroides* (*Bacteroidales*) markers in 11 subalpine lakes. Here we show that visitation models derived from data outside of MORA lacked transferability compared to the original Wood et al., 2020 study and did not explain enough variation in weekly on-site visitation for accurate estimation in MORA. Though no human-specific marker was detected during the sampling period, these findings do not preclude their presence in the subalpine lakes. Finally, the presence of a canine-specific marker in Upper Deadwood Lake suggests further application of MST methods has value in understanding the pervasion of fecal markers in protected alpine waterways

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Chapter 1. THESIS INTRODUCTION

For the thousands of visitors who recreate on public lands, subalpine and alpine lakes are a focal point of attraction due to their beauty and as a plentiful water source for backcountry recreation. In these locations, the traditional relationship between recreationists and aquatic ecosystems is becoming complicated by climate change. Temperature and visitation analysis across all national parks suggests warming will augment both visitation numbers and extend periods of patronage at high-latitude -elevation parks by extending access at shoulder seasons (Fisichelli et al., 2015). These factors create an imperative for managers to find reliable ways of understanding the nature of human contact with alpine and subalpine lakes using basin-level visitation numbers. Basin-level visitation counts, using social media data and other predictors, could identify recreation hotspots, generating targeted and efficient MST sampling campaigns. This will be especially valuable as climatic change proceeds, extending visitation periods in the cryosphere.

National Parks and National Forests do not keep data on waste flux, but it can be assumed there's a robust correlation to visitation. The Enchantments and Robin Lakes within the Alpine Lakes Wilderness in the Cascade Mountains of Washington are a highly visited backpacking destination. Permitted users in the Enchantments increased from 2012 to 2019 -- over a ten-fold increase (National Forest Service, 2021). On the first Saturday in August of 2020, rangers counted 999 people at Colchuck Lake and buried 200 piles of feces despite the presence of four privies (Stinchcombe, 2020a). Dogs are also a constant presence along the lake's edge, a direct violation of wilderness regulations, and provide another putative source of exogenous fecal contamination. Like the Enchantments, Robin Lakes are also carved out of granitic substrate, creating limited options for waste disposal, as the rocky surface does not allow for sinking pit toilets or catholes.

The impervious catchments that cradle these alpine lakes also assist in the transportation of enteric bacteria during heightened precipitation (McDonald and Kay, 1981). With no permit system, overnight visitation is unchecked, and the lack of privies and compostable toilets stresses the limited resources of national forest staff. To attenuate the tide of fecal waste, rangers make the 15-mile round-trip to Robin Lakes with garbage bags and metal shovels to remove fecal deposits. But for the obvious signs of contamination available to the senses, there is an unknown extent to which this waste infiltrates alpine and subalpine aquatic ecosystems creating potential water degradation issues that conflict with the core, preservative tenants of the Organic Act of 1916, U.S.C., title 16, sec. 1 (Organic Act, 1916) and the Wilderness Act of 1964, Public Law 88-577 (Wilderness Act, 1964). Work done in the Sierra Nevada Mountains has quantified contamination of backcountry waterways by measuring total fecal indicator bacteria (Clow et al., 2013; Davies-Colley et al., 2004; Derlet et al., 2008; Forrester et al., 2017) but has not tied this contamination to species-specific contributors, leaving a gap in the understanding of how unmitigated human waste pervades aquatic alpine environments.

The growth in the patronage of high-elevation wilderness areas, the tide of collaterally generated fecal waste, and its pervasion of aquatic environments requires further scrutiny. Land managers need an updated suite of tools to locate recreational hotspots efficiently and evaluate in-place waste mitigation practices. In this thesis, I assess models developed by Wood et al., 2020 in enumerating absolute, basin-level visitation as well as the feasibility of enlisting molecular DNA methods in detecting fecal contamination. To parse human fecal contribution from other warm-blooded animals, I specifically employ microbial source tracking (MST) methods, designed to detect warm-blooded, species-specific markers on the 16s rRNA gene of the gut bacteria *Bacteroides spp*. (Bernhard and Field, 2000b; Kildare et al., 2007; Layton et al., 2006; Seurinck et al., 2005). To our knowledge, this is the first implementation of MST methods in understanding species-specific degradation of alpine water bodies in wilderness areas.

Chapter 2. PAPER

2.1 INTRODUCTION

The Western United States contains the country's highest concentration of alpine and subalpine lakes. From 2010 to 2020 this region saw above-average population growth and increasing use of public lands that contain these high-elevation water bodies. Of the eleven states that comprise the western U.S., eight saw population growth above the national average (>7.4%), with five of those eight growing around double the national average (14.9%) (United States Census Bureau, 2021). Washington, home to three National Parks (North Cascades, Olympic, and Mount Rainier) with vast alpine ecosystems, added one million people during the same period (Bureau, 2021). Backcountry recreation in those three national parks swelled by 85% from 2011 to 2021 (National Park Service, 2022), putting heavy stress on their respective backcountry waste mitigation infrastructure (Chapman, 2018; Stinchcombe, 2020b; Stringer, 2020). These remote locations have been hit especially hard as human waste removal or sequestration in these areas can be very cost-prohibitive due to remoteness and other factors. Further complicating these efforts is the extension of the recreation season due to warming, which is especially evident on public lands saddled with extensive snow and ice.

The dynamic confluence of climate change and elevated park recreation are conspicuous in the PNW of the United States. Temperature and visitation analysis across all national parks suggests warming will augment both visitation numbers and extend periods of patronage at high-latitude -elevation parks by extending access at shoulder seasons (Fisichelli et al., 2015). Glaciers in North Cascades National Park have receded 56% since 1900, and spring snowpack has declined by 30% since 1955 (Roop and Krosby, 2020). Visitation to public lands in the area is also increasing, and the diminishing cryosphere expands the potential for longer recreational periods. In Mount Rainier National Park (MORA), visitation increased by 30% from 2008 to 2018 with further growth expected (Bergstrom et al., 2020; Fisichelli et al., 2015). In MORA, one documented effect of heightened visitation is visible toilet paper and unburied feces. Increased precipitation falling as rain, due to climate change, could provide a conduit, especially on impervious surfaces, for feces to wash into alpine lakes (McDonald and Kay, 1981). However, more needs to be learned about the presence of fecal contamination in these systems and who is contributing.

Because recreative impacts on wilderness waters often have a localized effect with minimal importance on the landscape level (Cole, 2008) they have been studied little when compared to other ecological impacts (Marion et al., 2016). However, a facet that has garnered some interest, is how human use of alpine lakes and their surrounding landscape contributes to the introduction of exogenous, enteric, gut bacteria (Clow et al., 2013; Derlet et al., 2008; Ursem et al., 2009).

Total coliforms and *Escherichia coli* have been established by the Environmental Protection Agency (EPA) as benchmarks for fecal-based contamination of freshwater (Environmental Protection Agency, 2003, 2016). Over the last decade or so, these indicator bacteria have also been used outside of their usual urban and sub-urban waterways to investigate recreation impacts in national parks. These studies found locations frequented by hikers, backpackers, and stock animals in Yosemite, Kings Canyon, and Great Smoky Mountains

National Parks were correlated with the presence of fecal coliforms and *E. coli* (Derlet et al., 2008; Forrester et al., 2017; Reed and Rasnake, 2016; Ursem et al., 2009). However, the culture techniques implemented in these studies cannot identify species-specific fecal contributors and, therefore, cannot link human activity to contamination.

Over the last 20 years, microbial source tracking (MST) methods have been utilized in municipal, recreational, and marine waters to track both human- and canine-specific fecal contamination by targeting the hypervariable V2 region of the 16S rRNA gene of Bacteroides spp. (order Bacteroidales) (Bernhard and Field, 2000a; Kildare et al., 2007). Located in the lower intestines of warm-blooded animals, Bacteroides species comprises 30% colonic bacteria (Bennett et al., 2020). Though an obligate anaerobe, once shed from the body in feces, the identifying genetic markers can remain detectable in freshwater. Seurinck et al. 2005 found markers present for 24 days at 4°C and ca. 8d at 24°C, while Bell et al. found 6d at 5°C to 1d at 25°C. The most significant factor affecting longevity was biological (grazing by other bacteria, bacteriophage infection, cell death) followed by temperature (Bell et al., 2009). For humans, the HF183 cluster has been established as the preeminent genetic marker for identifying human fecal contamination (Green et al., 2014a; Layton et al., 2006) and has been validated in a multi-laboratory study (Boehm et al., 2013) resulting in EPA Method 1696: Characterization of Human Fecal Pollution in Water by HF183/BacR287 TaqMan quantitative PCR (qPCR). Similarly, the BacCan-UCD assay provides an enumeration of dog-specific, fecal bacteria markers (Kildare et al., 2007) and has also been vetted in a multi-lab comparison study (Schriewer et al., 2013). These established MST methodologies could provide insight into human- and dog-specific fecal contamination of alpine and subalpine lakes during climate-driven visitation and connect human activity to water degradation that conflicts with principles outlined in the Organic Act of 1916, U.S.C., title 16, (Organic Act, 1916) whose proclamation “is to conserve the scenery and the natural and historic objects and the wild life therein and to provide for the enjoyment of the same in such manner and by such means as will leave them unimpaired for the enjoyment of future generations” (sec. 1) and the Wilderness Act of 1964, Public Law 88-577 (Wilderness Act, 1964) which states the lands are, “for the use and enjoyment of the American people in such manner as will leave them unimpaired for future use and enjoyment as wilderness ” (sec. 2a) and “as an area where the earth and its community of life are untrammeled by man… . with the imprint of man's work substantially unnoticeable” (sec. 2c).

Lack of controlled access in the backcountry makes visitation difficult to enumerate (Leggett, 2015), but geotagged social media posts – automatically stamped by phone GPS or designated by user – have shown promise as a predictor for visitation. Foundational work in visitation modeling has revealed the number of people visiting parks is generally reflected on social media platforms (Heikinheimo et al., 2017; Mancini et al., 2018; Sessions et al., 2016; Tenkanen et al., 2017; Walden-Schreiner et al., 2018). Evidence is growing that geotagged social media data can provide absolute visitation numbers for specific locations at different times (Fisher et al., 2018; Toivonen et al., 2019). Wood et al. (2020) showed that a model based on social media and other predictors, parameterized in Mount Baker-Snoqualmie National Forest (MBS) in Washington State, predicted visitation in the National Forests, Parks, and Monuments of Valles Caldera, Jemez, Kasha Katuwe Tent Rocks, and Bandelier in the state of New Mexico.

To address basin level visitation in MORA and the direct impacts this visitation can have on aquatic environments we sought to investigate (1) if the model developed by Wood et al. provides meaningful visitation predictions in the backcountry of MORA and (2) can human and canine fecal markers, if present, be detected in the subalpine lakes of MORA using MST methods.

2.2 MATERIALS AND METHODS

2.2.1 Visitation modeling

2.2.1.1 Study area

Mount Rainier National Park (MORA) and Mount Baker-Snoqualmie National Forest (MBS) are adjacent public lands in the Cascade Mountains of Washington (Figure 1). MORA abuts the southern boundary of MBS. The two units have similar proximity to urban areas, topography, ecology, and backcountry use. Though both management areas are sought for their remote subalpine and alpine destinations they are operated by different agencies and mandates: Mount Baker-Snoqualmie National Forest and Mount Rainier National Park are managed by the National Forest Service and the National Park Service, respectively.

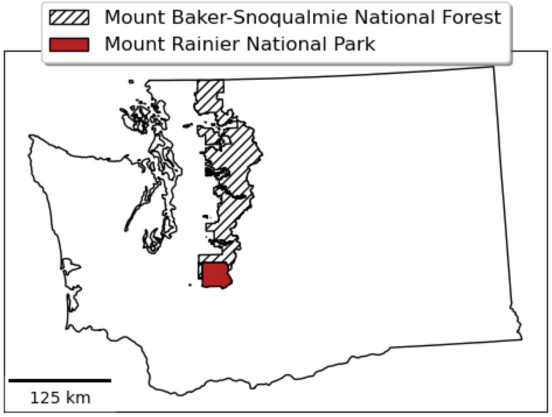


Figure 1. The location of Mount Baker-Snoqualmie National Forest and Mount Rainier National Park in Washington State

2.2.1.2 Site selection

The Eleven study sites designated for this study were the Green Lake Trail, Eunice Lake and Tolmie Peak Trail (combined as a site), Lake Eleanor Trail, Palisades Lake Trail and Sunrise Lake Spur (combined as a site), Glacier Basin Trail, Shriner Peak Trail, Pinnacle Peak Trail, Van Trump Trail, Rampart Ridge Trail, and Lake George Trail and Gobbler's Knob Trail (combined as a site) (Figure 2). These sites were selected based on four primary conditions; (1) the trails were monitored with a passive infrared (IR) pedestrian counters (TRAFx Research Ltd.) that captured all hiking traffic, (2) were 'out-and-back' where hikers returned on the same path, (3) could be visited in one day, and (4) counter data was collected within the last six years (2015-2020). To reflect the areas that would capture all visitors logged by an IR, we consulted with land managers to accurately draw polygons using geographical information system (GIS) software. These polygons served to represent the extent to which hiking traffic was likely contained by ridges and natural features. Those social media posts that fall within the polygon's coordinate system are combined with other predictors to enumerate visitation.

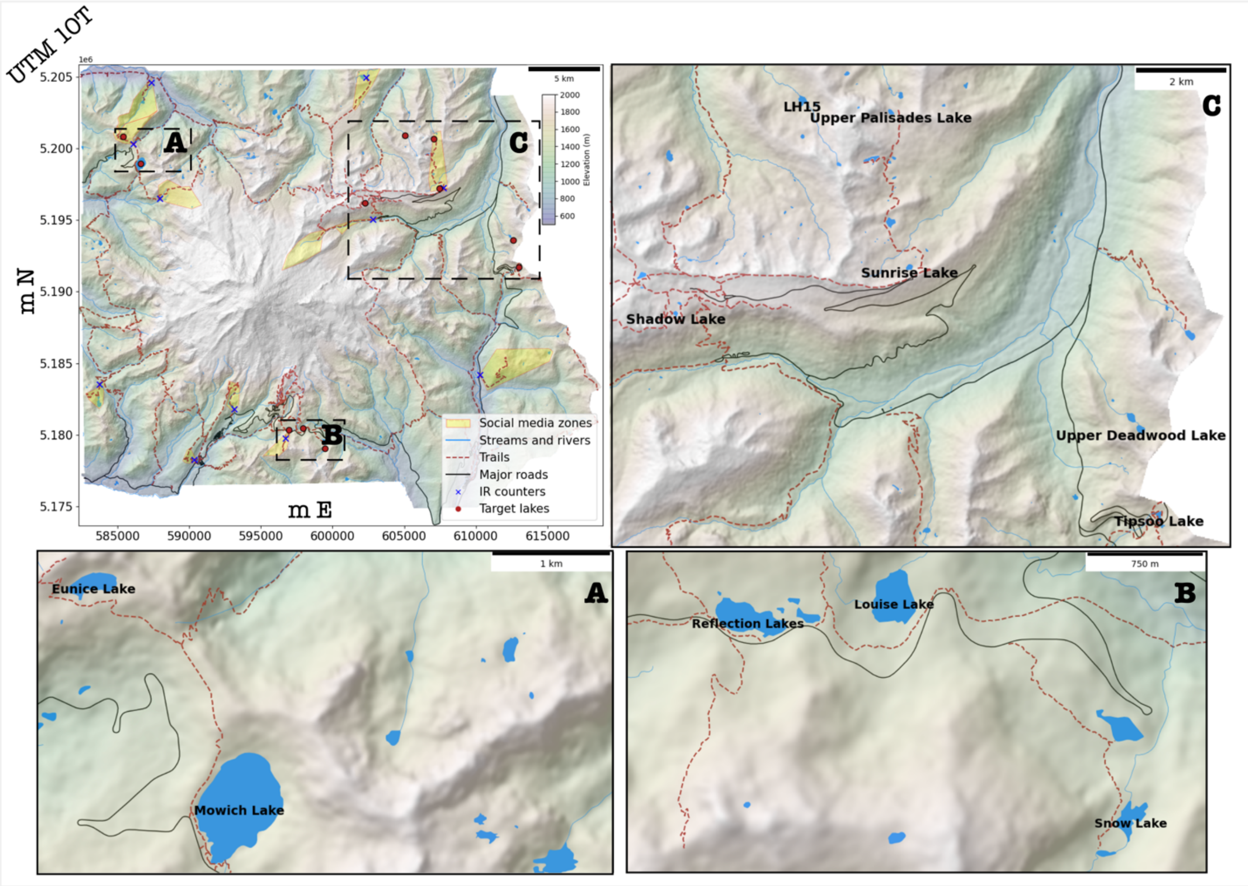


Figure 2. (Top left) Map of MORA depicting polygons from which geotagged social media data were pulled and the location of IR counters used to capture on-site estimates, both of which were used for visitation modeling. Insets A (bottom left), B (bottom right), and C (top right) show target lakes where MST methods were leveraged. Figure generated using Python v 3.8 and code from Geospatial Analysis in Python (Shean, 2020).

2.2.1.3 Data sources

2.2.1.3.1 MORA Infrared sensors and on-site estimates

IR were installed and maintained by MORA, and we were given access to raw count data for further analysis. Raw data from 2015-2020 was limited to May 1st until October 31st of each year, to exclude winter months when IR sensors were not maintained trails were inaccessible due to snow. Raw count data was aggregated into daily, then weekly counts to produce "weekly on-site counts". Those weeks missing data were removed from analysis. Despite inherent sources of error common with IR, these are the most robust and accurate visitation counts at each site.

2.2.1.3.2 MORA Social media estimates and other predictors

Methods for collecting public, geotagged social media posts and other predictors were strictly followed from Wood et al., 2020 and provided by the Outdoor Recreation & Data Lab. These social media data and predictors were used to estimate visitation at study sites: Flickr, Twitter, Instagram, and AllTrails posts. These platforms were chosen because of their ability to generate posts with GPS coordinates, consistent use by the public, and relative ease in accessibility to volunteered data. Flickr is a photograph sharing platform and its application programming interface was used to access public geotagged posts. Tweets were gathered using Twitter's "statuses/filter", again using their application programming interface. Instagram posts were pulled from sites related to target locations and then coordinates. AllTrails, a running and hiking app, similarly had posts during the time period of interest accessed through its application programming interface. Posts from these platforms that fell within the delineated study site polygons in MORA, during 2015-2020, were aggregated through official application programming interfaces (API) or acquired manually through internet searches based on study site name or location (Wood et al., 2020). Additional predictors of precipitation, week of year, and holidays were also aggregated by ORDL to more accurately compensate for seasonal fluctuations in backcountry recreation (Wood et al., 2020).

2.2.1.3.3 MBS on-site and social media estimates, and predictors

MBS on-site estimate data was collected in the same fashion as was done in MORA following the steps outlined in the above section 2.2.1.3.1. Similarly, social media estimates and other predictors for MBS were gathered in the same methods described in the above section 2.2.1.3.2.

2.2.1.3.4 MORA and MBS Data manipulation

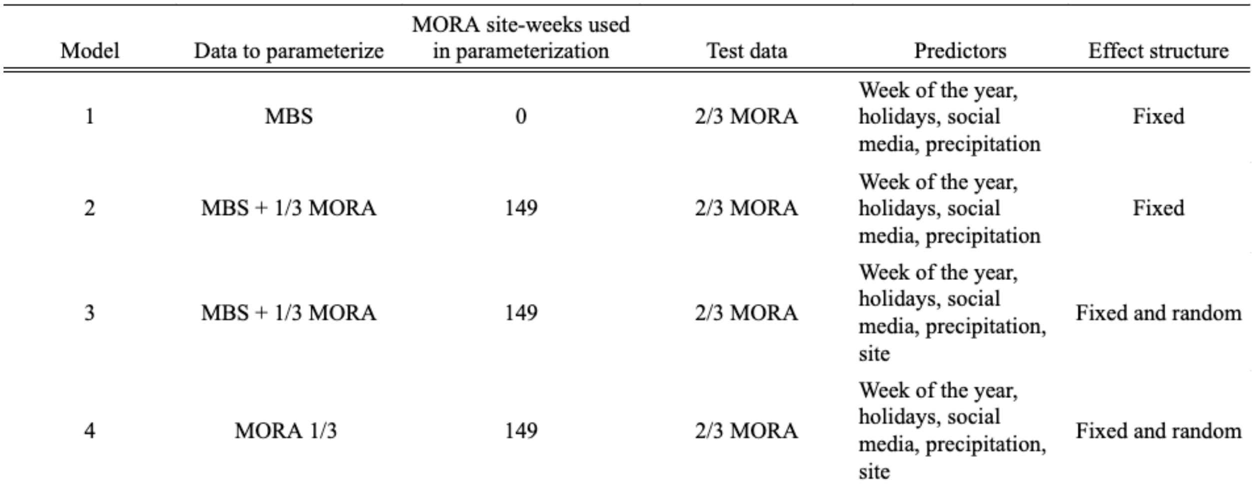
All data manipulation followed the methods in Wood et al. (2020) exactly, unless noted. All on-site counts and predictor data were aggregated to the week scale. Weeks started on Thursday and ended on Wednesday to include complete weekends. Those weeks that were incomplete due to malfunctioning IR's or park closures were removed from the data set. This resulted in site-week counts of 414 for MORA and 2,115 for MBS.

Weekly on-site counts, weekly user-day counts for social media platforms, and precipitation were log-transformed to meet the assumption of normality for a simple linear regression. A quadratic term for week of the year was added to allow the model to fit higher visitation during the summer months.

2.2.1.3.5 Models

Testing methods and linear models come directly from Wood et al., 2020 and procedures were followed exactly unless otherwise noted. Four linear models were tested in their ability to estimate visitation in MORA by making predictions to the eleven study sites and comparing those predictions to on-site counts captured by IR. Model 1 (Table 1) was parameterized with only MBS data and quantified how much variability in visitation can be explained by data exogenous to MORA and represents the pure transferability of the MBS model. Model 2 was parameterized with all of the MBS and 1/3 of MORA data to reflect scenarios where land managers have access to some on-site IR counts to use to (re)parameterize the model for their specific locale. Models 1 and 2 are fixed-effects models, equation (1) from Wood et al. (2020), where Y_{it} is the visitation observed at site i during week t, $\boldsymbol{x}_{\boldsymbol{it}}^{\boldsymbol{T}} = (x_{it1}, x_{it2}, \ldots, x_{itp})$ are the predictors (p) at site i during week t, are a vector of coefficients. These models were fit using the `lm` function (Bates et al., 2015) in R v 1.4.1106.

Table 1. Parameterization, test data, predictors, and effects structure for models 1-4. Models were parameterized with only MBS, all MBS + 1/3 MORA, or only MORA data, but all were tested against a randomized 2/3 of MORA data. The random effect of 'site' was added to mixed-effect (fixed and random) models.



Model	Data to parameterize	MORA site-weeks used in parameterization	Test data	Predictors	Effect structure
1	MBS	0	2/3 MORA	Week of the year, holidays, social media, precipitation	Fixed
2	MBS + 1/3 MORA	149	2/3 MORA	Week of the year, holidays, social media, precipitation	Fixed
3	MBS + 1/3 MORA	149	2/3 MORA	Week of the year, holidays, social media, precipitation, site	Fixed and random
4	MORA 1/3	149	2/3 MORA	Week of the year, holidays, social media, precipitation, site	Fixed and random

Because of the variation between sites and to allow each site to have an unique relationship with predictors the random effect of site was added to Model 3 and Model 4 are mixed-effects models. They retain the same fixed-effects as Models 1 and 2, but also include the random effect of site, i, which is equation (2) from Wood et al., 2020. These models were parameterized in the same fashion as model 2. Mixed-effects models were fit using the lme4 (Bates et al., 2015) package in R version 1.4.1106.

2.2.1.3.6 Model testing

Model testing followed methods outlined in Wood et al (2020) exactly, except where noted and used out-of-sample predictive power to assess model performance. For each of the four models, we randomly sampled 2/3 of the MORA data to serve as the out-sample, reserved the remaining data for model training (Model 1 only used MBS data for training), made predictions to the out-of-sample data set, and calculated the mean square prediction error (MSPE) and the Pearson's correlation coefficient (r) between the week-site predictions and the week-site observations. MSPE was not calculated in Wood et al., but utilized here because of its ability to measure out-of-sample prediction error in mixed models. This process and calculations were conducted 500 times, from which a mean MSPE and mean correlation for each model was computed.

2.2.2 Microbial Source Tracking

2.2.2.1 Lake sampling and site selection

Reflection Lakes, Louise, Snow, Mowich, Eunice, Shadow, Sunrise, Tipsoo, Upper Palisades, Upper Deadwood, and LH15 (control) (Figure 2) were selected by MORA staff to capture a range of visitor use and amenities (mean elevation of 1612 meters, SD ± 45 meters). Sampling was divided into two campaigns (permit and study number MORA-2020-SCI-0021, MORA-00279). The first campaign was scheduled and conducted July 13, 2021 – July 15, 2021, to obtain samples influenced by surface run-off from snowmelt. Due to depleted resources during the first campaign, a single sampling day occurred August 5, 2021 to collect water samples from Eunice and Mowich Lakes. Though not sampled with the first campaign, these lakes are included in molecular analysis for campaign 1. The second sampling campaign was completed September 7, 2021 – September 10, 2021 to capture the onset of the rainy seasons and identify additional impacts from a season of recreational use. To increase signal capture, 250 mL aliquots of lake water were collected from equidistant locations around the lake's edge and combined into 1L composite sample. Samples were kept cold with ice packs in a cooler and transported back to the lab within 12 hours of collection. For canine-marker detection, Reflection, Louise, Tipsoo, and Upper Deadwood Lakes were sub-selected because of observed dog activity and proximity to the heavily traveled Nisqually and highway 410 corridor. Green Lake, an urban lake in Seattle, WA, with heavy human and canine activity, was also sampled as a likely positive control with which to compare to subalpine lakes of MORA.

2.2.2.2 Filtration

The filtration and extraction methods followed EPA Method 1696 (Environmental Protection Agency, 2019). However, modification of the method was needed in certain circumstances, and specific changes are noted below. Sample bottles were shaken to distribute bacteria uniformly, and a 100 mL sample aliquot was filtered through a 250 mL 47 mm, 0.2 µm, single-use analytical cellulose nitrate filter funnels from Thermo Scientific™ Nalgene™ (Thermo Scientific, Waltham, Massachusetts). This filter selection deviates from EPA Method 1696, but was chosen because of the resource-heavy nature of remote, lake sampling and the desire to maximize, even if marginally, the probability of capturing enteric bacteria by using a smaller filter aperture (Boström et al., 2004; Oyafuso et al., 2015). An additional 100 mL aliquot was filtered using the empty disposable filter funnel and a 47mm, 0.4 µm, Whatman® Nuclepore™ track-etched membrane (Whatman, Buckinghamshire, United Kingdom) to compare filtration efficiency between filter pore sizes.

2.2.2.3 Human and Canine positive control

A single 0.21 g human fecal aliquot was mixed with 100 mL of LH15 (control) water and filtered using the previously mentioned protocol. Each filter was folded using forceps sterilized in 70% isopropanol and flame, then placed in a 2 mL screwcap tube. Each tube was promptly placed in a -80°C freezer until extraction. gBlocks gene fragments (Integrated DNA Technologies™) designed using GenBank ascension number AY597171.1 (Layton et al., 2006) were used as a positive control for the BacCan-UCD ddPCR assay (Table 2). This was due to an inability to characterize dog microbiome in a national park whose visiting canine population is arguable national and global.

2.2.2.4 DNA Extraction

Extraction for the first campaign was conducted at King County Environmental Lab (KCEL), Seattle, Washington. Sample filters were suspended in 590 µL of AE buffer spiked with deoxyribonucleic acid sodium salt from salmon testes (salmon DNA), as a sample processing control (SPC) following section 7.18 of EPA Method 1696, then bead beaten using 212-300 µm acid-washed glass beads in a BioSpec Mini-Beadbeater-24 (BioSpec, Bartlesville, OK). The extracted DNA was removed and placed in a 2 mL tube and stored at -20°C.

Extraction for the second campaign was conducted at the School of Environmental and Forest Sciences Genetics Lab at the University of Washington. Filters were again spiked with salmon DNA, and Qiagen™ PowerWater kits (Qiagen GmbH, Hilden, Germany) were used which is a different extraction method than described in EPA Method 1696. This was done because the 47 mm, 0.2 µm, single-use analytical cellulose nitrate filters (Thermo Scientific) were deemed too bulky for bead beating in 2 mL tubes. The extractions were stored at -80°C.

2.2.2.5 BacCan-UCD validation

Performance characteristics of gene markers can vary geographically (Ahmed et al., 2019; Boehm et al., 2013), so validation of performance characteristics is important (Ahmed et al., 2019; Harwood et al., 2014). Though this guidance was practical, the conditions of this study were made it impossible to characterize dog microbiome that has national and global distribution. Because MORA attracts visitors from across the US and the world, empirically characterizing the canine microbiome found in the park was deemed outside the scope of possibility, as visitors and their dogs would be coming from far-flung regions. Review of BacCan-UCD assay validations that were conducted during the last 15 years in Australia (Ahmed et al., 2019; Gyawali et al., 2020; Yasar et al., 2021), California (Kildare et al., 2007; Schriewer et al., 2013), Singapore (Nshimyimana et al., 2017), Texas (Hinojosa et al., 2020), and Finland (Rytkönen et al., 2021) showed all canine microbiomes from these regions produced positive results after few localized fecal samples were combined as a positive control. This led the authors of this study to make two assumptions: (1) the assay would be functional in MORA and (2) the seemingly global presence of the BacCan-UCD marker would overlap with the microbiome of canines visiting MORA. In the unlikely event that the BacCan-UCD marker was present, the high sensitivity (1.00-0.76) and specificity (0.87-0.71) observed in these studies of domestic dogs and would offer probabilistic confidence that canine-specific markers would be detected. Empirical, cross-reactivity assessments of BacCan-UCD with other fecal sources seemed improbable because of the high number of elusive, warm-blooded animals in these subalpine ecosystems with cryptic latrine behaviors. Because all subalpine lakes in this study come from the same ecosystem and have the same warm-blooded species richness, ad hoc analysis of any positive results in the LH15 (control lake) or lakes other than those where dog activity has been witnessed (n=4) would suggest cross-reactivity of BacCan-UCD.

2.2.2.6 qPCR and digital PCR assays

Table 2. Droplet digital PCR assays employed to detect human and canine Bacteroidales 16s rRNA genes as well as inhibitory control assay (Sketa22). A gBlock fragment (BacCan gBlock) was used as a positive control for the BacCan-UCD assay and was generated using a canine-specific Bacteroidales 16s rRNA gene sequence from GenBank (ascension number AY597171.1)

Assay	Forward		Reverse		Probe		References
HF183	HF183	ATCATGAGTTCACATGT	BacR287	CTTCCTCTCAGAACCCCTATC	BacP234MGB	[6-FAM]-5'-CTAATGGAACGCATCCC-MGB	EPA Method 1696, 2019
Sketa22	SketaF2	GGTTTCCGCAGCTGGG	SketaR2	CCGAGCCGTCCTGGTC	SketaP2	5[6-FAM]-5'-AGTCGCAGG/ZEN/CGGCCACCGT-3'-[IABkFQ]	EPA Method 1696, 2019
BacCan-UCD	acCan-545f1	GGAGCGCAGACGGGTT	BacUni-690r1	CAATCGGAGTTCTTCGTGATA	BacUni-656p	[6-FAM]-5'-TGGTGTAGCGGTGAAA-MGB	Kildare, 2007

BacCan-UCD Positive Control	Sequence	References
Canine *Bacteroidales* 16s rRNA	GGAGCGCAGACGGGTTTTTAAGTCAGCTGTGAAAGTTTGGGGCTCAACCTTAAAATTGCAGTTGATACTGGAGACCTTGAGTGCAGTTGAGGCAGGCGGAATTCG TGGTGTAGCGGTGAAATGCTTAGATATCACGAAGAACTCCGATTG	Layton et al., 2006
BacCan gBlock	CGATCGATGCGGAGCGCAGACGGGTTTTTAAGTCAGCTGTGAAAGTTTGGGGCTCAACCTTAAAATTGCAGTTGATACTGGAGACCTTGAGTGCAGTTGAGGCAG GCGGAATTCGTGGTGTAGCGGTGAAATGCTTAGATATCACGAAGAACTCCGATTGCGTCAGTAGT	This study

HF183 and BacCan-UCD assays (Table 2) were used to identify the presence of human- and canine-sourced fecal contamination. To establish a cross-laboratory comparison and validate the techniques and results, the HF183 marker was analyzed at King County Environmental Lab using qPCR. Subsequent analysis for both campaigns, using all assays, was done at School of Environmental and Forest Sciences Genetics Lab using droplet digital PCR (ddPCR) system QX200 (Bio-Rad, Hercules, CA, USA). After an initial screening with ddPCR, samples generating positive droplets with relative fluorescence units equal to the positive control were classified as possible positive samples for gene markers. Each potential positive sample was rerun at 2x, 1x, and 0.5x concentrations to check for linearity in copies/uL. If linearity existed, the mean of both 1x-reactions (n=2) was reported as the positive result.

For qPCR, each 25 µL reaction contained reagent volumes as described in EPA Method 1696, without the internal amplification control and 5 µL of sample DNA. qPCR was conducted on a Thermo Fisher/ABI Step One Plus using the following conditions: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C, with a C_T "cutoff" of 35.

22 µL reactions were used for ddPCR, which contained 1 x ddPCR Supermix for Probes (no dUTP), 900 nmol l^{-1} of each primer, 250 nmol l^{-1} of the probe, and 5 µL of sample DNA. 20 µL of each reaction was mixed with 70 µL of droplet generation oil for probes (Bio-Rad) using a QX200™ Droplet Generator (Bio-Rad). Newly formed droplets were transferred to a 96-well plate and sealed with foil using a PX1 PCR plate sealer (Bio-Rad) and placed in a C100 96-well thermocycler (Bio-Rad). Thermocycling conditions described in Cao et al. 2015 were used for the HF183 assay. The Sketa22 inhibition control assay used the Bio-Rad probe chemistry conditions: 10 min at 95°C, followed by 40 cycles of 30 sec at 94°C and 1 min at 60°C with a ramp rate of 2.0°C, and 10 minutes at 98°C for signal stabilization. The BacCan-UCD assay followed the same conditions as Sketa22 except the annealing temperature was changed to 57°C.

2.2.2.7 Quality control

Filtration, extraction, and reagent negative controls were used to detect possible cross-contamination between samples. School of Environmental and Forest Sciences Genetics Lab filtration, extraction, and ddPCR procedures were conducted in different locations. No contamination in negative controls was detected.

2.2.2.8 Specimen processing and inhibition control

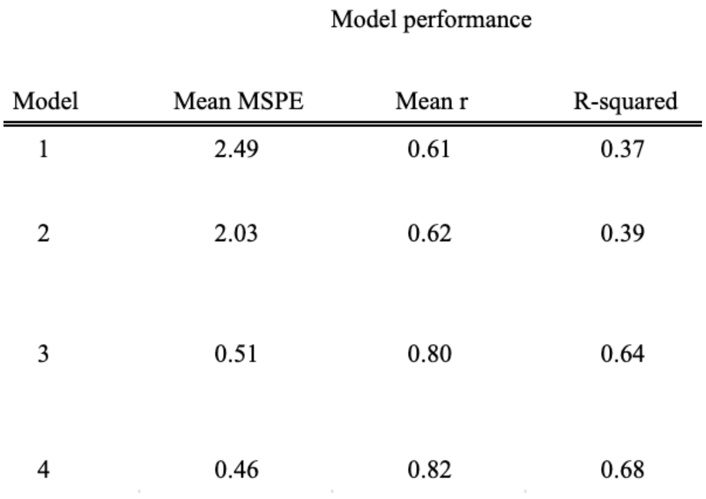
Sample loss due to filtration and extraction inefficiencies, as well as PCR inhibition from ions and organic acids, can produce artificially low quantification of gene targets. The Sketa22 assay (Table 2) and conditions from EPA Method 1696 were implemented as sample processing controls (SPC) to monitor sample loss and flag any possible inhibition. Though no inhibition was detected, a spill during the extraction of the second campaign created some sample loss, which was reflected in the results of the Sketa22 assay.

2.3 RESULTS

2.3.1 Results

2.3.1.1 MBS model transferability and predictive power

Table 3. 297 site-weeks of MORA data served as an out-sample for model testing. Mean square prediction error (MSPE) and Pearson's correlation (r) were calculated during each of the 500 iterations, then averaged to demonstrate the effectiveness of visitation modeling. Variance found in observed, weekly on-site counts explained by model predictions was captured by the coefficient of determination (R^2), which was calculated by squaring r.



Model performance

Model	Mean MSPE	Mean r	R-squared
1	2.49	0.61	0.37
2	2.03	0.62	0.39
3	0.51	0.80	0.64
4	0.46	0.82	0.68

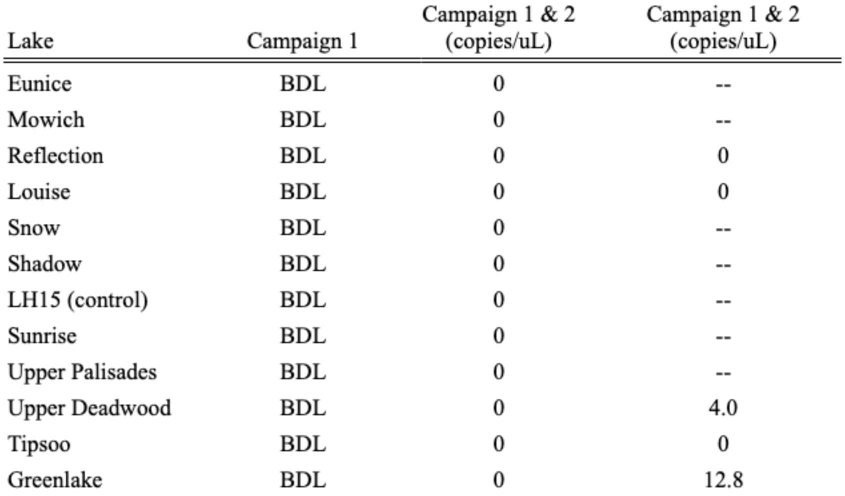
Out-of-sample testing of the four visitation models, across the eleven study sites in MORA, showed variation in predictive performance (Table 3). The addition of site-level random effects and 149 site-weeks (random ⅓ of MORA data) of MORA data, seen in Model 3, saw the largest jump in model performance explaining 64% of the variation seen in weekly on-site observations (MSPE = 0.0017, r = 0.80, R^2 = 0.64). Model 4, which excluded MBS data entirely and used only 149 site-weeks of MORA data to parameterize and 297 site-weeks to cross-validate, showed marginal, improvement over model 3, accounting for 68% of the variability in observed visitation to MORA sites (MSPE = 2.49, r = 0.82, R^2 = 0.68). Model 1 was parameterized only with MBS data and performed the poorest of all models tested, capturing 37% of the variation in observed weekly visitation (MSPE = 0.0083, r = 0.61, R^2 = 0.37). The addition of 149 site-weeks of MORA data (Model 2) to parameterization yielded only scant improvements to predictive power (MSPE = 0.0068, r = 0.62, R^2 = 0.39).

2.3.1.2 HF183 and BacCan-UCD counts

No human-specific markers were detected in either campaign using qPCR and ddPCR. All human gene marker targeted samples tested by KCEL using qPCR exceeded the cycle threshold cutoff of 35 cycles and zero positive events were detected using ddPCR methods for all assays and campaigns (Table 4).

The BacCan-UCD canine-marker returned positive events for the Upper Deadwood Lake (UDL) and Green Lake (GL), both from sampling campaign 1. UDL is a subalpine lake on the eastern border of MORA and GL is an urban lake in Seattle, WA. The average between two ddPCR plates for UDL was 4.0 copies/µL and 12.8 copies/µL GL.

Table 4. Copy number of human and canine markers found in each lake, during sampling campaigns 1 and 2 using qPCR and ddPCR. Below detectable limit for qPCR is > cycle threshold of 35. For ddPCR is no copies were detected with 0 per µL. A subset of four lakes were selected for interrogation of canine markers. Canine marker was detected in Upper Deadwood Lake and Green Lake (Seattle).



Lake	Campaign 1	Campaign 1 & 2 (copies/uL)	Campaign 1 & 2 (copies/uL)
Eunice	BDL	0	--
Mowich	BDL	0	--
Reflection	BDL	0	0
Louise	BDL	0	0
Snow	BDL	0	--
Shadow	BDL	0	--
LH15 (control)	BDL	0	--
Sunrise	BDL	0	--
Upper Palisades	BDL	0	--
Upper Deadwood	BDL	0	4.0
Tipsoo	BDL	0	0
Greenlake	BDL	0	12.8

2.3.2 3. DISCUSSION

2.3.2.1 Visitation modeling

In this study, we sought to estimate weekly, basin-level visitation in MORA with visitation models parameterized with social media data and other predictors that are widely available. We tested a variety of approaches parameterized with on-site counts of visitors taken from the focal sites within MORA and from the adjacent MBS National Forest. In the cross-validation of our four models, it was found that using only MBS data to parameterize the model

(Model 1) produced the poorest predictive ability. The addition of MORA data in parameterization and the inclusion of the random effect of site (Model 3) saw the largest increase in predictive power. Model 4 was marginally the best-performing model in this study, but used only MORA data for parameterization and testing, eschewing the benefits of using exogenous training data. Models 3 and 4 from this study underperformed in comparison to the Wood et al. (2020) paper.

In an effort to boost the predictability of Model 1, I conducted post-hoc subanaylsis to test they hypothesis that visitors to sites in MORA are more like visitors to sites with similar characteristics in MBS. I created and tested Model 3 (best performing model) for estimating visitation at this narrower site-type, with only those sites in MBS that fell within 1 SD of the average distance and elevation gain per mile found at MORA. This newly curated MBS training data provided did not increase in predictive ability ($r = 0.80$, $R^2 = 0.64$) and thus failed to support my hypothesis.

The introduction of the random effect of site in Model 3 saw the greatest increase in predictive power with an $R^2 = 0.64$. This was an increase of 25 percentage points over the fixed-effects in Model 2, which had an $R^2 = 0.39$. The Wood et al. study mirrored the same trend when the random effect of site was added, however, the magnitude of the predictive increase in the Wood et al. study was 27 percentage points higher ($R^2 = 0.91$) than in this study ($R^2 = 0.64$). The most obvious supposition for this disparity is relationship visitation has with predictor variables in MORA is less constant than in New Mexico. Further work to better characterize variation, possibly through year-to-year differences in social-media-platform use, could aid in increasing predictive ability.

Model 4 parameterization using only MORA data and random effects saw the highest visitation prediction in this study ($R^2 = 0.68$), which was expected. Interestingly, Model 3 ($R^2 = 0.64$), which used MBS data in parameterization, explained only 4 percentage point less error than Model 4. This implies that MBS training data hinders prediction to a small degree.

One of the values in investigating the transferability of models parameterized with MBS is to lighten the burden of on-site data collection by managers or at least attenuate the amount of data required to build a successful backcountry visitation model. For those parks and public lands that collect on-site visitation, it would be plausible to investigate the potential Model 4 would have in better understanding visitation to remote locations. Those management areas with no on-site visitation data would be completely reliant on the transferability of models parameterized on pre-established data sets.

There were a couple of limitations in the modeling section of this study that merit discussion. Limiting the analysis to MORA and prevented the opportunity for meaningful comparison between national parks. The three national parks in Washington (MORA, Olympic National Park, and North Cascades National Park) share the same urban areas, rugged topography, and similar ecology. Vetting model performance in other National Parks besides MORA could be helpful for understanding the generality of this visitation model and the utility of this approach to provide visitation estimates that function as a management tool. Also, it should be noted that social media data access changes over time, as seen in 2016 when Instagram stopped sharing the geocoordinates attached to users' posts (Martí et al., 2019). This model relies on access to geotagged social media posts (Wood et al., 2020), and limited access in the future could affect its predictive power and feasibility as a management tool.

2.3.2.2 Microbial source tracking

An additional aim of this study was to detect putative human and canine fecal markers as a consequence of basin-level visitation in MORA. To our knowledge, this is the first study to use MST methods to examine the extent to which human and canine fecal waste has infiltrated waterways protected by the Organic Act of 1916, U.S.C., title 16, sec. 1 and the Wilderness Act of 1964, Public Law 88-577. We found no evidence of the human-specific HF183 gene marker in any of the lakes sampled during either campaign using qPCR or ddPCR. However, low copy numbers of canine-associated *Bacteroides* 16S rRNA gene markers were possibly detected in Upper Deadwood Lake and Green Lake. Though this study could provide a baseline for lakes in MORA with respect to HF183 markers and highlight the potential dispersion of canine-associated markers, more context is required to guide effective next steps.

Hydrological activity, impervious catchment-surface percentage, and vegetated lake margins have shown influence on fecal bacteria transport and capture. Because increased terrestrial water flow is correlated with the transportation of enteric bacteria (McDonald and Kay, 1981) and the percentage of impervious surface in catchment areas are positively correlated with higher gene marker capture (Ahmed et al., 2019; Oyafuso et al., 2015), it was the goal of this study to sample true alpine lakes (impervious adjacent surfaces) during seasonal spring snowmelt and the beginning of fall precipitation. However, more permeable catchment soils surrounding the subalpine-sample lakes, record spring temperatures leading to early snowmelt, and minimal fall rains (Figure 3) prevented terrestrial runoff and relegated planned sampling

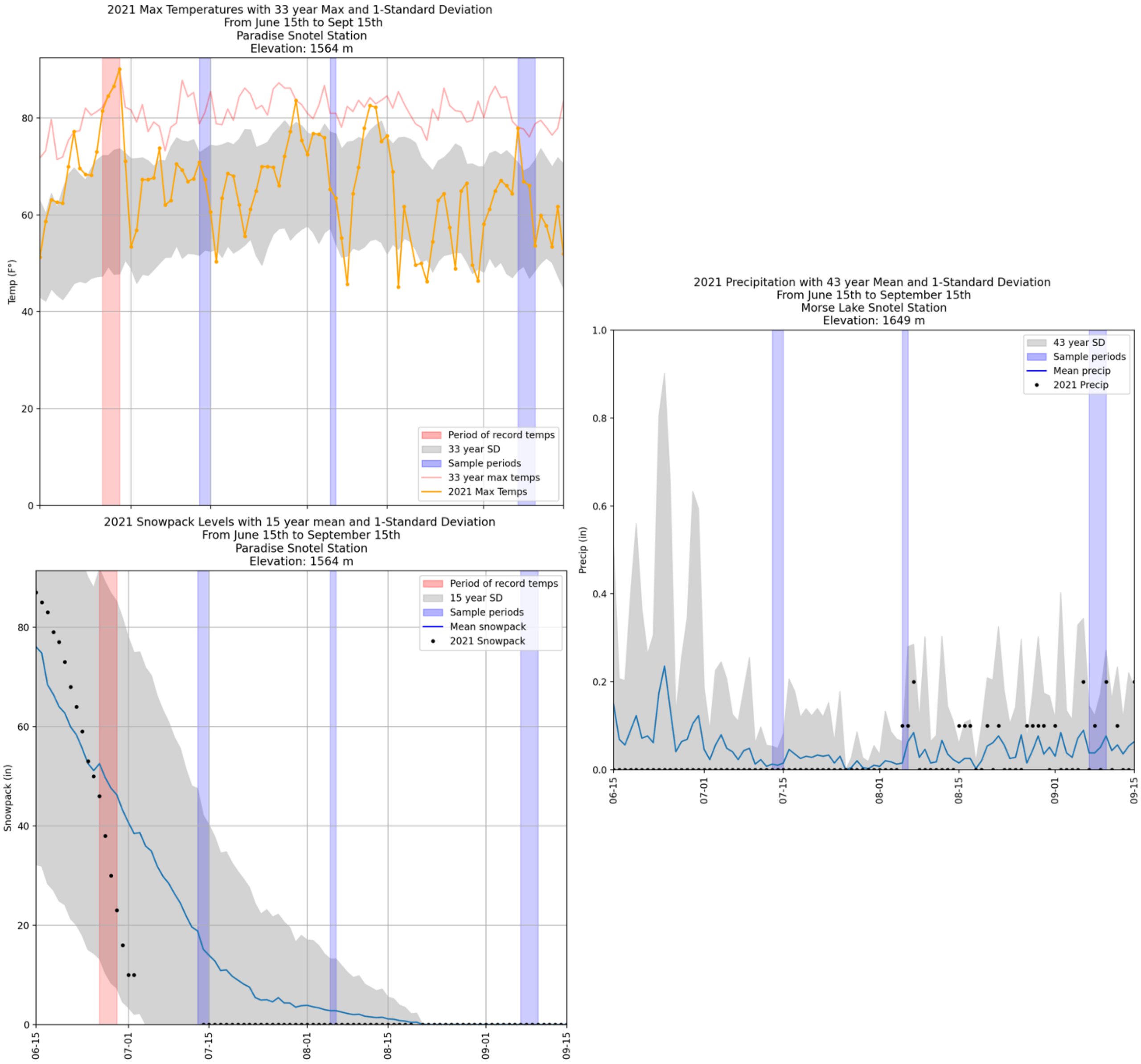


Figure 3. Max temperature (top left) and snowpack (bottom left) data from the Paradise SNOTEL site on Mount Rainier and daily precipitation data (right) from Morse Lake SNOTEL site just outside the E park boundary. Blue ribbons show lake sampling periods, while the red ribbon shows a period of record maximum temperatures. During the period of maximum temperatures, it can be seen the rate of snowmelt drastically increases and prevents any collection during a melting period. Precipitation data reveals no rainfall > 0.2 in during the sampling period, with zero precipitation from June 15th to August 8th Figure created using Python v3.8 and code from Geospatial Programming (Shean, 2020).

campaigns to periods with no surface-water flow. Also common to these subalpine lakes is a vegetated boundary that Tate et al., 2004 has shown limits the passage of enteric organisms. Though it is unknown to what extent maximizing the aforementioned factors would elevate gene marker capture in alpine or subalpine lakes, sampling during periods of heightened, terrestrial water flow would be fundamentally important to increasing the probability of marker capture.

The lack of HF183 markers in this study does not preclude their existence in the MORA or in other backcountry locations across the Mountain West. A discerning expansion of sampling to include high-visitation alpine lakes, in basins with a high percentage of impervious surfaces, across the Western cryosphere is required to fully understand the potential distribution of human waste markers in protected waterways. An interdisciplinary approach using visitation modeling, hydrology, and remote sensing would help identify those locations likely to harbor human markers, transport, and retain them. For instance, ordinal visitation modeling (low, medium, and high visitation classifications) could provide investigators with locations across national parks and forests where human activity in the backcountry is high, indicating the likelihood of large amounts of fecal deposits. To sketch their ability to retain and transport fecal contamination these target catchments would undergo further remote sensing analysis using digital elevation models, Landsat imagery and spectral unmixing (Powell et al., 2008) to classify alpine catchment surface composition. In order to expand our knowledge to which lake flushing at these sites was due to surface or groundwater, simple conductivity and pH measurements would be taken. Furthermore, synchronization of lake sampling with the onset of snow-contaminant release during spring melt, would be achieved with the use of synthetic aperture radar on Sentinel-1 (Marin et al., 2020). This would provide investigators localized notice of ideal collection events. These remote sensing and chemistry analyses would efficiently expand the number of sample sites viable for human marker analysis and simultaneously generate alpine catchment profiles that could be compared in their ability to retain and condense human fecal markers in alpine lakes.

The presence of canine markers in UDL is a novel result and, to our knowledge, the first subalpine lake in which dog-specific fecal contamination markers have been detected. UDL is located on the eastern boundary of MORA and is accessed from outside the park boundary, on a ca. 1.5-mile path that is a spur off the Pacific Crest Trail, which sees heavy through-hiking traffic. Investigators in this study witnessed an off-leash dog swimming in the lake during sampling and, through casual conversation with the dog's owner, were notified this was common. The only other lake that reported canine markers was GL, which is an urban lake outside of MORA, in Seattle, WA, where dog walking and off-leash behavior is common.

It is possible that these canine-marker counts and prevalence among sampled lakes are artificially low due to the use of a single assay. Combining BacCan-UCD with other high-performing assays like DogBact from Schriewer et al., 2013 (Gyawali et al., 2020) DG3 and DG37 (Green et al., 2014b) would help ensure broad signal capture of canine-specific markers, especially those coming from a geographically expansive canine microbiome.

There are a few limitations in the MST section of this study that warrant additional discussion. Low sample size and sampling frequency possibly constrained the potential to capture human- and canine-specific markers, if present. In these remote locations, it assumed marker concentration was relatively low, especially in comparison with the routine use of MST methods applied in urban environments. The results from the HF183 assay especially can only speak to the presence of markers at the point in time campaign one, and campaign two were conducted. Contamination that occurred not long before, after, or in between campaigns would not be reflected in these results. Degradation of DNA from warming lake waters and exposure to sunlight could also attenuate marker signals and contribute to false-negative results.

In future studies, many of these limitations could possibly be minimized by filtering samples on-site and placing filters in lightproof containers with 200-proof molecular-grade ethanol (Laramie et al., 2015). This would better preserve DNA against temperature changes during transport and prevent further photodegradation. This simple shift in sampling protocol would theoretically allow for geographical expansion of study sites and sampling frequency as transportation times could be increased due to newfound DNA stability. In theory, in-kind support from public land managers and staff could also be folded into the logistics of sample collection. Filters could be stored in freezers at parks and national forests allowing for the inclusion of sample sites far from analysis laboratories. Further testing on *Bacteroides spp.* DNA degradation rates in these specific conditions are needed, but the likelihood of diminishing some of the limitations seen in this study is probable.

2.3.3 Conclusion

In this study, we sought to evaluate the plausibility of linear fixed- and mixed-effects models, parameterized on existing data from MBS, in estimating basin level visitation in MORA and, as a consequence of visitation, detect putative human and canine fecal markers in subalpine lakes using MST methods. The best performance in explaining weekly on-site could be utilized by mangers to predict ordinal and enumerate ordinal use in remote areas. This would be sufficient to locate those areas seeing the highest visitation, but might lack the predicative power for absolute visitation. There would likely be added benefit to characterize more variation in the model through random effects and postulate additional fixed effects that could help explain error.

To better understand the model performance in MORA more testing in National Parks in Washington and in other states is needed. This could provide insight into the visitation habits of recreationists to MORA or their part of a larger trend seen in National Park visitation.

Climatic shifts to spring snowmelt behavior and fecal deposits frozen in snow and ice could have a dynamic effect on the transportation of unburied fecal waste. There is evidence from Denali National Park and Preserve that fecal bacteria remain persistent in the cryosphere for long periods of time and are detectable in seasonal melt (Goodwin et al., 2012). This suggests in MORA and other glaciated public lands with high snowpack the potential to archive fecal deposits from previous end-of-season and winter recreation is highly likely and especially problematic as visitation increases. More frequent rain-on-snow events due to climate change could also generate massive seasonal flushing events capable of transporting large stores of preserved fecal waste into alpine waters. To maximize the evaluation of human marker pervasion future investigators should remain cognizant of the preservation potential of fecal deposits in the cryosphere and the probable increase of large flushing events.

I would also like to add that MST work on those public lands with large amounts of impervious surface, like those found in the canyonlands of the American Southwest could provide insight to those land mangers struggling with high recreation during all times of the year. Slot canyons condense recreation activity and thus fecal waste deposits creating ample contamination opportunities. The ebb and flow created by flash flooding and ephemeral lotic systems likely allow buildup of surficial fecal deposits and create concentrated contamination pulses. Further work in these systems could reveal surprising fecal loading and flushing events. Additional investigative work using MST methods is warranted.

Canine-specific markers were detected by the BacCan-UCD assay in one subalpine study lake that had off-leash dog activity. Though detection of canine-marker is novel, analysis with other canine-specific assays and increased sampling frequency is required to understand the level of pervasion in subalpine waterways. This paper has three conclusions: (1) further model refinement is necessary before backcountry visitation in MORA can be reliably estimated, (2) there was no detectable HF183 marker present during sampling campaigns, and (3) the presence of canine-specific markers in UDL suggests further application of MST methods has value in understanding the pervasion of fecal markers in protected alpine waterways.

Chapter 3. THESIS CONCLUSION

The exploration of visitation modeling and MST methods as tools for managers to enumerate visitation and track fecal contamination produced mixed results. The model from this this study could not explain variation in on-site visitation in MORA to the same degree as found in the Wood et al. (2020) study. Next steps for modeling refinement could include expansion to other national parks or the discovery of additional factors that can further explaining variation in on-site visitation. Human fecal marker was not detected during sampling periods, but its presence outside of these periods and in different lakes remains possible. However, copies of canine-marker were detected in UDL where off-leash dog behavior was observed. This finding sets the stage for the expanded use of MST methods in national parks and forests to quantify direct impacts to protected waterways. Adjustments to both modeling and MST methods will benefit their application and position them as valuable tools for managers hoping to better understand changing human-ecosystem interactions.

The high performance found in the Wood et al., 2020 study ($R^2 = 0.91$) did not transfer well to MORA ($R^2 = 0.64$). Though efforts to refine the model through changes in training data proved insufficient in explaining more variation, it is possible lowering the fidelity of predictions could prove actionable. By shifting from absolute visitation estimations to low, medium, or high ordinal estimations, managers could identify backcountry locations of concern with a relative amount of certainty – especially when the recreation levels could be corroborated by qualitative observations. As iterative testing continues for the model at other national parks and forests discovery of additional fixed effects could provide the additional explanation of error needed to make accurate predictions in MORA.

Future studies intending to apply MST techniques in the alpine would benefit from capitalizing on factors that increase the likelihood of detecting species-specific markers. For capturing human markers, site selection and timing will be integral in increasing the probability of detection. Target lakes should be highly visited, sampled during or after high terrestrial water flow (McDonald and Kay, 1981), have a high percentage of impervious surfaces (Ahmed et al., 2019; Oyafuso et al., 2015), and minimal vegetative barriers (Tate et al., 2004). Using molecular-grade alcohol to store filters will increase DNA stability in remote locations and facilitate greater sample collection. Unforeseen and confounding cross-reactivity issues may be ameliorated by multiplexing different assays that target the same species, like BacCan-UCD and DogBact (Gyawali et al., 2020). Inclusion of multiple assays could, in that same reaction, flag possible off-target issues that would be difficult to assess in areas with high warm-blooded species richness. These next steps in the application of MST methods will assist in increasing the possibility of capturing fecal markers exogenous to alpine lakes, expanding sampling capacity, and improving diagnostic accuracy.

Enumerating backcountry visitation and its direct consequence on water bodies has yet to be fully realized and necessitates further experimentation. As summer recreation periods in the cryosphere extend into shoulder seasons due to retreating snowpack from climate change (Fisichelli et al., 2015), alpine water bodies will be exposed to more direct and indirect anthropomorphic pressures. Understanding the dynamic confluence of these processes and how they generate conflicts with the Organic Act of 1916 and the Wilderness Act of 1964 will further underline the reach of human activity in our most sensitive ecosystems. This work, along with others tracking less noticed alpine, aquatic contamination will hopefully mobilize policy apparatus to keep up with a quickly changing cryosphere.

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