TECHNICAL REPORT

An Interpretation of the North Coast Regional Water Quality Control Board Narrative Natural Background Water Quality Objective for Bacteria as Applied in Freshwater Streams of the Coast Range Ecoregion

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1. Introduction

The North Coast Regional Water Quality Control Board (Region 1) *Water Quality Control Plan for the North Coast Region* (Basin Plan) establishes both numeric and narrative bacterial water quality objectives (Objectives) for the protection of the beneficial uses and high quality waters of inland surface waters, enclosed bays, and estuaries in the North Coast Region (North Coast Regional Water Quality Control Board, 2018). One of the numeric Objectives included in the Basin Plan is for the protection of water contact recreation (REC-1), and the other numeric Objective is for the protection of shellfish harvesting (SHELL). These two numeric Objectives are derived from the Bacteria Provisions of the Water Quality Control Plan for Inland Surface Waters, Enclosed Bays, and Estuaries of California (ISWEBE Plan) (State Water Resources Control Board, 2019). The narrative objective states that "the bacteriological quality of waters of the North Coast Region shall not be degraded beyond natural background levels (North Coast Regional Water Quality Control Board, 2018)." The narrative objective is designed to protect high quality waters from degradation, as is required by the antidegradation policy.

A Reference Stream Study was initiated in 2016 to characterize *Escherichia coli* (*E. coli*) and enterococcus concentrations (see Section 1.1) in streams identified as minimally disturbed (see Section 1.2) to develop an approach for interpreting the narrative natural background objective. The purpose of this report is to present the findings of the Reference Stream Study and to propose an evaluation approach to determine if a water of interest[1] is consistent with the narrative natural background objective. The approach utilizes hypothesis testing, comparing data from minimally disturbed waters to data from a stream of interest using a statistical test. The data collected under the Reference Stream Study allows for the evaluation of freshwater minimally disturbed streams in the United States Environmental Protection Agency (USEPA) Level III Coast Range Ecoregion (see Section 1.3). The evaluation approach also provides a framework for collecting bacteria data in minimally disturbed waters of other Level III ecoregions in the North Coast Region.

Specifically, this technical report describes 1) the general hypothesis-based evaluation process developed to assess whether a freshwater, or saline, water of interest in a particular ecoregion is consistent with the narrative natural background Objective, and 2) the application of the evaluation process to waters of interest in the Coast Range Ecoregion using the data collected as part of the 2016 Reference Study.

This assessment process is not recommended for the purpose of impairment identification, as being inconsistent with the Basin Plan natural background objective for bacteria is not synonymous with beneficial use impairment. None the less, a finding that a water of interest is inconsistent with the natural background objective for bacteria may mean that water is inconsistent with the antidegradation policy and requires increased

1 A water of interest may be any water, whether associated with urban, rural, managed or wild landscapes.

investigation, source control, or other actions as necessary to identify and reduce pathogen discharges to a given waterbody. This assessment process can be used to support total maximum daily load (TMDL) projects, permit development and compliance work, and as part of other watershed assessment efforts. As with all environmental assessment, evaluating multiple lines of evidence helps to strengthen conclusions.

1.1. Fecal Indicator Bacteria

The fecal indicator bacteria *E. coli* and enterococci were selected for evaluation since they are commonly used types of fecal indicator bacteria when measuring the bacteriological quality of water. They also form the basis for the statewide bacteria objective for the protection of REC-1 contained in the Inland Surface Waters, Enclosed Bays, and Estuaries of California (ISWEBE) Plan and incorporated into the Basin Plan (State Water Resources Control Board, 2019). The ISWEBE Plan establishes REC-1 objectives for *E. coli* in fresh waters and enterococci in saline waters (State Water Resources Control Board, 2018). Therefore, the analytic approach described in this report is based on the assessment of *E. coli* in freshwater and enterococci in saline water.

1.2. Minimally Disturbed Condition

The U.S. Environmental Protection Agency (USEPA) defines a "natural background condition" as "characteristics of a waterbody in the absence of any pollutants or other anthropogenic stressors (United States Environmental Protection Agency, 2021b)." Anthropogenic stressors are environmental stressors associated with human activities. All water bodies are assumed to have some anthropogenic stresses (Stoddard et al., 2006). Therefore, a "natural background condition" is interpreted as the condition of a water body in the absence of "significant" human disturbance or alteration, also known as a "minimally disturbed condition" (Ode & Schiff, 2009; Stoddard et al., 2006).

1.2.1. USEPA Level III Ecoregions

Bacteria, and therefore fecal indicator bacteria (FIB) concentrations in surface waters, are impacted by several external abiotic and biotic factors that can vary by location or geographic region. These factors include land cover, land use, wildlife diversity and density, population density, topography, geology, hydrology, water quality, chemistry, flow, and meteorological factors (Herrig et al., 2019; Korajkic et al., 2019; Lipp et al., 2001; Majedul Islam et al., 2017; United States Environmental Protection Agency, 2010). With a large geographic spread, Region 1 displays significant heterogeneity with respect to these factors. This region-wide heterogeneity could result in significant spatial variability of the FIB concentrations representative of minimally disturbed conditions in different parts of the region.

When selecting minimally disturbed sites to sample, the California Department of Fish and Wildlife, Southern California Coastal Water Research Project, United States Geological Survey, and the San Francisco Bay Regional Water Quality Control Board recommend balancing two goals – 1) selected sites should "uniformly represent the least disturbed conditions throughout the region(s) of interest, minimizing the effects of

anthropogenic stress on the indicator of interest", and 2) "sites should represent the full range of environmental settings in the region in sufficient numbers to adequately characterize natural variability in the indicator(s) of interest (Ode et al., 2016)."

Ecoregions have been designed by USEPA to serve as "a spatial framework for the research, assessment, and monitoring of ecosystems and ecosystem components" and "denote geographic areas of general similarity with respect to biotic, abiotic, terrestrial, and aquatic ecosystem components" (United States Environmental Protection Agency, 2021a). Therefore, staff used the ecoregion approach for this study in order to select minimally disturbed freshwater streams that represent natural background conditions, but also account for the variability in natural background FIB concentrations as a result of environmental variability within the North Coast Region.

The North Coast Region is comprised of five Level III ecoregions. To reasonably interpret the natural background objective, data from minimally disturbed waters within each Level III ecoregion will need to be collected and evaluated to produce a unique dataset for each ecoregion. The analytic approach presented in this report includes the results of the 2016 Reference Study which was conducted in the Coast Range Ecoregion of the North Coast Region, and provides a framework for collecting bacteria data in minimally disturbed waters of other Level III ecoregions in the North Coast Region.

2. General Evaluation Process

This section summarizes the general evaluation process to determine whether the bacteriological water quality of a freshwater or saline water of interest particular ecoregion is consistent with the narrative natural background Objective for that ecoregion.

The assessment process will be performed for a specific ecoregion and sampling period (dry, wet, or year-round), with the data collected from the freshwater or saline water of interest being compared to the corresponding minimally disturbed streams dataset. In addition to a year-round assessment, a wet, or dry sampling period assessment will be performed to account for the seasonal fluctuation of *E. coli* (or enterococci) concentrations due to precipitation and runoff.

For a particular ecoregion and sampling period, the *E. coli* (or enterococci) concentrations measured in a freshwater (or saline) water of interest will be compared to a dataset of *E. coli* (or enterococci) concentrations measured in minimally disturbed freshwater (or saline) stream samples for that particular ecoregion and assessment period using hypothesis testing. A statistical test will be used to evaluate the hypotheses.

Hypothesis testing involves the evaluation of statistical hypotheses (null and alternative hypotheses), which are statements “that may be supported or rejected by examining relevant data (United States Environmental Protection Agency, 2002).” A null hypothesis (H_0), is any assumption to be tested that is “set up to be rejected (United States Environmental Protection Agency, 2002).” The null hypothesis states the results of the hypothesis test are due to chance, and are not statistically significant, and therefore do not support the statement being tested. An alternative hypothesis (H_A) is “the logical opposite of the null hypothesis” and is “usually logically the same as the investigator’s research hypothesis (United States Environmental Protection Agency, 2002).” The alternative hypothesis is “the conclusion we accept if we find sufficient evidence to reject the null hypothesis (United States Environmental Protection Agency, 2002).” For this type of hypothesis testing, “the data should provide statistically significant evidence that the null hypothesis is false”; that is, for this particular assessment process, the *E. coli* (or enterococci) concentration measured in the freshwater (or saline) water of interest exceeds the minimally disturbed freshwater (or saline) stream *E. coli* (or enterococci) concentration (United States Environmental Protection Agency, 2002). The null and alternative hypotheses that will be tested for this assessment process, for a particular ecoregion and assessment period, are described below.

The null hypothesis (H_0): The median *E.coli* (or enterococci) concentration in samples collected from a freshwater (or saline) water of interest in a particular ecoregion is less than or equal to the median *E. coli* (or enterococci) concentration in the minimally disturbed freshwater (or saline) streams dataset for that ecoregion.

The alternative hypothesis (H_A): The median *E.coli* (or enterococci) concentration in samples collected from a freshwater (or saline) water of interest in a particular ecoregion is greater than the median *E. coli* (or enterococci) concentration in the minimally disturbed freshwater (or saline) streams dataset for that ecoregion.

A statistical test is used to evaluate the null and alternative hypotheses using the data collected. A probability (*p*) value, calculated during the statistical test, describes the likelihood of the data having occurred by random chance (i.e. the null hypothesis is true). A *p* value is usually a number between 0 and 1, and the smaller the *p* value the stronger the evidence for rejecting the null hypothesis. To determine whether the results of the test are statistically significant, the *p* value is compared to a threshold known as the significance level (α) which is a fixed probability of wrongly rejecting the null hypothesis H_0 if it is in fact true. It is set by the investigator in relation to the consequences of such an error (United States Environmental Protection Agency, 2022). The commonly selected significance level is 5% (or 0.05) (United States Environmental Protection Agency, 2022). A *p* value less than the significance level (< 0.05) provides strong evidence against the null hypothesis since it means that there is less than a 5% probability that the null hypothesis is correct, and that the results obtained were due to chance. Therefore, a *p* value < 0.05 indicates that the findings are statistically significant allowing the investigator to reject the null hypothesis. A *p* value > 0.05 indicates that the findings are not statistically significant, and that the investigator should reject the alternative hypothesis.

The *E. coli* (or enterococci) concentration data collected from a freshwater (or saline) water of interest, and the *E. coli* (or enterococci) concentration data collected from minimally disturbed freshwater (or saline) streams, are both expected to follow a non-normal distribution, which is typical for environmental data (Helsel et al., 2020). Data that are not normally distributed are often assessed using non-parametric analysis methods since these analysis methods are free of assumptions about how the data are distributed and are minimally affected by extreme values. Therefore, a non-parametric statistical test will be used to evaluate the hypotheses. Specifically, the Wilcoxon Rank Sum Test will be used determine whether the median *E. coli* (or enterococci) concentration in a freshwater (or saline) water of interest is statistically significantly higher than the median *E. coli* (or enterococci) concentration of the minimally disturbed freshwater (or saline) streams dataset for a particular assessment period (dry, wet, or year-round) for a particular ecoregion. The year-round assessment will be conducted by analyzing the wet and dry sample data together. A *p* value < 0.05 will be considered statistically significant.

If the median *E. coli* (or enterococci) concentration measured in the freshwater (or saline) water of interest is found to be statistically significantly higher (*p* value < 0.05) than the median *E. coli* (or enterococci) concentration in the minimally disturbed freshwater (or saline) stream sample dataset then the null hypothesis can be rejected, and the alternative hypothesis is true. That is, the freshwater (or saline) water of interest

being assessed is not consistent with the narrative natural background Objective for *E.coli* (or enterococci), for a particular assessment period and ecoregion. However, it is important to note that a statistically significant result can still mean that there is a slight probability that the results occurred by chance, and the null hypothesis was mistakenly rejected. Therefore, it is recommended that the finding that a freshwater (or saline) water of interest is not consistent with the narrative natural background Objective should be considered together with other lines of evidence about the water quality of that freshwater (or saline) water of interest.

3. 2016 Reference Stream Study

A Reference Stream Study was initiated in 2016 to characterize *E. coli* and enterococcus concentrations in streams identified as minimally disturbed to develop an approach for interpreting the narrative natural background objective. All samples were collected in the Coast Range ecoregion of the North Coast region.

3.1. Sampling Site Selection

Minimally disturbed freshwater streams in the Coast Range Ecoregion of the North Coast region were identified by assessing land cover, and structure type and density, to ensure the selection of sampling locations without “significant human disturbance or alteration”. Sixteen sampling locations across seven coastal redwood parks within the Coast Range ecoregion were subsequently identified as satisfying minimally disturbed conditions for this study (Figure 1). The coastal redwood parks are in Del Norte, Humboldt, and Sonoma counties. All seven coastal redwood parks are either national parks, state parks, or regional parks, all of which are protected areas designated by national, state or local government agencies for conservation and recreation purposes (GreenInfo Network, 2018). Anthropogenic impact in these areas usually results from recreational activities (day-use, camping, etc.).

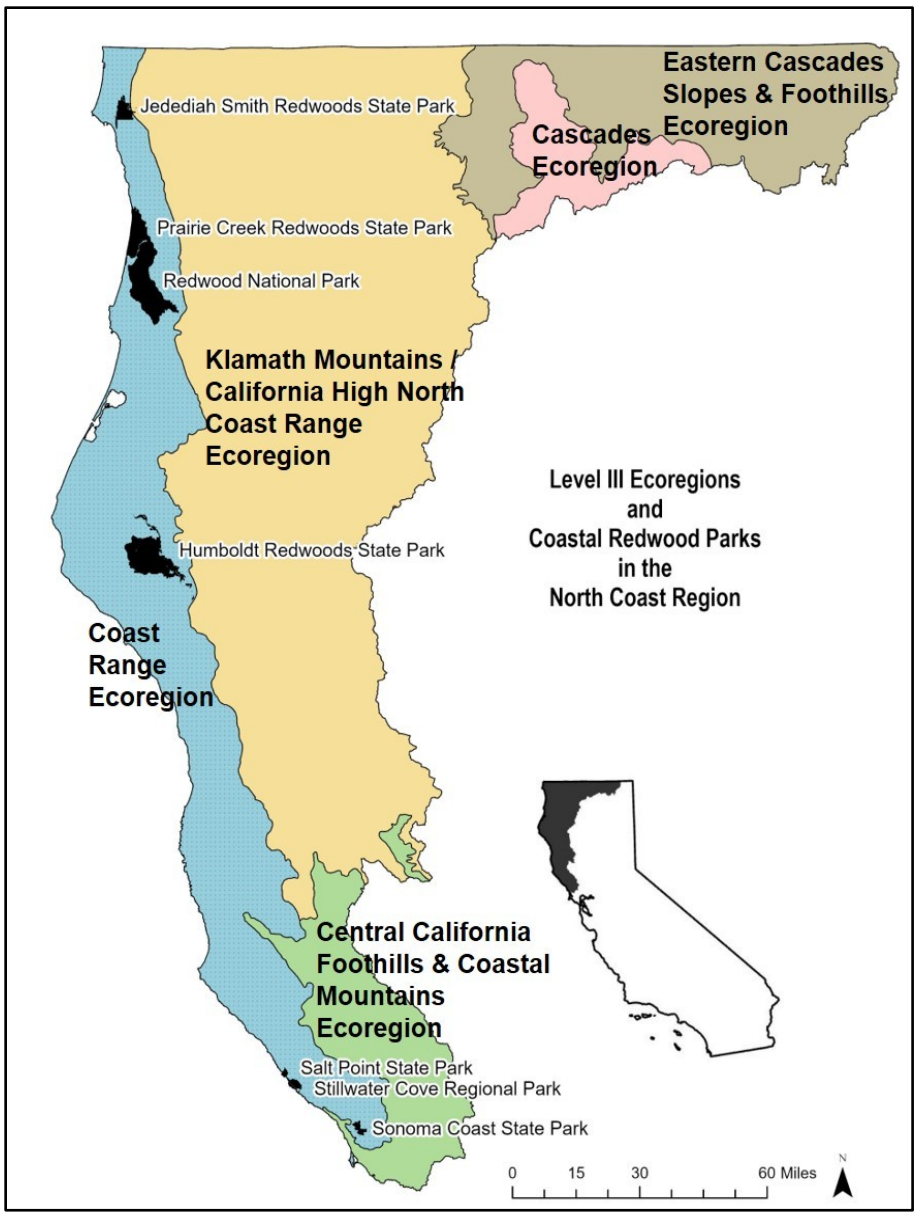

Figure 1 Level III Ecoregions in the North Coast Region, and Location of Coastal Redwood Parks Representing Minimally Disturbed Conditions

3.2. Sample Collection

Sample collection was performed as per the standard operating procedures included in the Quality Assurance Project Plan (QAPP) developed for this project (North Coast Regional Water Quality Control Board, 2015). A total of 95 grab samples of water (58 dry weather samples and 37 wet weather samples) were collected from 16 freshwater sampling locations across seven North Coast coastal redwood parks in the Coast Range ecoregion (Table 1).

Table 1 Sample Collection Details for Minimally Disturbed Freshwater Streams in the Coast Range Ecoregion

Coastal Redwood Park	Sampling Location Code[a]	Sampling Location Name	Number of Samples Collected	
			Dry Weather	Wet Weather
Jedediah Smith Redwoods State Park	103CD0679	Cedar Creek at Howard Hill Road	4	2
Jedediah Smith Redwoods State Park	103CK4061	Clarks Creek at Walker Road	3	3
Jedediah Smith Redwoods State Park	103ML0155	Mill Creek at Howard Hill Road	4	2
Prairie Creek Redwoods State Park	107PR7848	Prairie Creek at Drury Parkway	4	2
Redwood National Park	107LL0600	Little Lost Man Creek at Lost Man Creek Exit	4	2
Redwood National Park	107LM1856	Lost Man Creek at Lost Man Creek Exit	4	2
Humboldt Redwoods State Park	111AL1359	Albee Creek at Bull Creek Flats Road	4	2
Humboldt Redwoods State Park	111CF1805	Calf Creek at Bull Creek Flats Road	3	2
Humboldt Redwoods State Park	111CW0458	Cow Creek at Bull Creek Flats Road	4	2
Humboldt Redwoods State Park	111HR0606	Harper Creek at Bull Creek Flats Road	4	2
Humboldt Redwoods State Park	111LM0001	Little Mill Creek at Bull Creek Flats Road	4	2

Coastal Redwood Park	Sampling Location Code[a]	Sampling Location Name	Number of Samples Collected	
			Dry Weather	Wet Weather
Humboldt Redwoods State Park	111ML0252	Mill Creek at Bull Creek Flats Road	4	2
Salt Point State Park	113MR1171	Miller Creek at Highway 1	3	3
Salt Point State Park	113PG1586	Phillips Gulch at Highway 1	3	3
Stillwater Cove Regional Park	113ST0986	Stockhoff Creek at Highway 1	3	3
Sonoma Coast State Park	114FZ3710	Freezeout Creek at Freezeout Creek Road	3	3

[a]The sampling location code has been developed according to the Surface Water Ambient Monitoring Program (SWAMP) naming convention for sampling locations. SWAMP requires a numeric 3-digit code for the hydrologic unit of the stream being sampled followed by a random 6 digit code, which, in Region 1, consists of a 2-letter code for the stream name, and a 4-digit code signifying the distance of the sampling location from the mouth of the stream.

One sample was collected from each sampling location per sample collection event. However, at a small number of randomly selected locations, duplicate samples were also collected to serve as field replicates for quality assurance and control. For sampling locations where duplicate samples were collected, only the first replicate was included during data analysis.

Samples were collected during dry and wet weather periods from January to December 2016. The dry weather samples were collected in April, May, July, August, September, November, and December; and the wet weather samples were collected in January, February, October, and November. A dry weather sample was collected after 72 hours of dry weather, and a wet weather sample was collected during or following storm events that were predicted to generate 0.2 inches or greater of rainfall. Antecedent precipitation data and related information about precipitation stations were obtained from the California Department of Water Resources (DWR) California Data Exchange Center (CDEC) (California Department of Water Resources, 2023) for stations closest to each of the coastal redwood parks sampled.

Salinity data were not collected when samples were initially in 2016. Therefore, the salinity of each location was determined in 2022 using a desktop assessment process.

The desktop assessment was based on a combination of Region 1 staff's local knowledge of the streams along with the use of Geographic Information System (GIS software) by mapping all sampling locations using the "World Topographic Map" and "World Imagery" layers of ArcGIS (ESRI, 2018), and using United States Geological Survey (USGS) elevation data for each sampling location. An elevation cutoff of 50 feet above sea level was used to determine tidal influence. The desktop assessment found that all 16 sampling locations were freshwater 100% of the time. Further details about the salinity determination process can be found in the Technical Memorandum "2022 Salinity Study" (Tracy, 2022).

3.3. Sample Analysis

All samples collected from Jedediah Smith Redwoods State Park, Prairie Creek Redwoods State Park, and Redwood National Park were analyzed by the Humboldt County Public Health Laboratory. All samples collected from Humboldt Redwoods State Park, Salt Point State Park, Stillwater Cove Regional Park, and Sonoma Coast State Park were analyzed by the Sonoma County Public Health Laboratory. Sample analysis was conducted according to the standard operating procedures described in the QAPP for this project (North Coast Regional Water Quality Control Board, 2015). Sample analysis consisted of the detection and enumeration of *E. coli* and enterococcus in order to determine the presence and concentration of both FIB in each sample collected. FIB detection and enumeration was conducted using Environmental Laboratory Accreditation Program (ELAP) accredited methods. The Colilert and Enterolert tests were used for the detection and enumeration of *E. coli* and enterococci, respectively, according to the USEPA Standard Method 9223B (Enzyme Substrate Coliform Test) (Standard Methods, 2016).

Although samples were analyzed for the detection and enumeration of both *E. coli* and enterococcus, only *E. coli* data were used for the assessment of consistency of waters of interest with the natural background Objective since the minimally disturbed dataset is composed entirely of freshwater streams. A summary of E. coli and enterococcus concentrations measured in the dry and wet sampling periods of the minimally disturbed streams sampled are described in Table 2 below.

Table 2 Summary of FIB Concentrations Measured in Samples Collected from Minimally Disturbed Freshwater Streams in the Coast Range Ecoregion

FIB Analyte	Period	Number of Samples	Concentration (MPN/100 mL)[a]		
			Minimum	Median	Maximum
E. coli	Dry	58	1.0	4.1	195.6
E. coli	Wet	37	1.0	17.1	93.3
Enterococcus	Dry	58	1.0	4.1	488.4
Enterococcus	Wet	37	1.0	9.7	75.9

[a]MPN – Most Probable Number

4. Application of the Evaluation Process to the Coast Range Ecoregion

This section describes how the general evaluation process can be applied to evaluate freshwater waters of interest in the Coast Range Ecoregion. For the Coast Range Ecoregion the general evaluation has been adapted due to certain limiting characteristics of the current Coast Range ecoregion minimally disturbed freshwater stream dataset collected during the 2016 Reference Stream Study.

Currently, only freshwater waters of interest in the Coast Range Ecoregion can be evaluated for consistency with the narrative natural background Objective. This is because of the minimally disturbed streams dataset for the Coast Range Ecoregion which was collected during the 2016 Reference Study only contains samples collected from freshwater stream locations. Specifically, during minimally disturbed stream sample collection, all samples were collected from locations that were above tidal influence and above locations where salinity intrusion could potentially occur in a freshwater stream. Therefore, currently, saline waters of interest in the Coast Range ecoregion cannot be assessed for consistency with the narrative natural background Objective.

Furthermore, only the dry or wet weather assessment periods can currently be evaluated for the Coast Range ecoregion. A year-round assessment cannot be conducted due to the potential for incorrect interpretation of assessment results due to an overrepresentation of dry weather data in the Coast Range ecoregion minimally disturbed freshwater stream dataset – 58 dry weather samples were collected compared to 37 wet weather samples. This overrepresentation of dry weather data results in an inaccurate representation of year-round *E. coli* data characteristic of this ecoregion. Therefore, the general assessment process will be adapted for the Coast Range ecoregion in the following way – the *E. coli* concentrations measured in samples collected from a freshwater water of interest in the Coast Range ecoregion will be compared to the minimally disturbed freshwater stream *E. coli* concentrations dataset for the Coast Range ecoregion for the dry and wet assessment period.

The following hypotheses will be tested for a dry or wet assessment periods:

The null hypothesis (H_0): The median *E.coli* concentration in samples collected from a freshwater water of interest in the Coast Range ecoregion is less than or equal to the median *E. coli* concentration in the minimally disturbed freshwater streams dataset for the Coast Range ecoregion.

The alternative hypothesis (H_A): The median *E.coli* concentration in samples collected from a freshwater water of interest in the Coast Range ecoregion is greater than the median *E. coli* concentration in the minimally disturbed freshwater streams dataset for the Coast Range ecoregion.

These hypotheses will be tested using the Wilcoxon Rank Sum Test, for the dry or wet weather assessment period, and a *p* value < 0.05 will be assumed to be statistically significant. If the median *E. coli* concentration in samples collected from the Coast Range ecoregion freshwater water of interest being assessed is found to be statistically significantly higher (*p* value < 0.05) than the median *E. coli* concentration in the minimally disturbed freshwater streams dataset for the Coast Range ecoregion then the null hypothesis can be rejected, and the alternative hypothesis is true. This means that the freshwater water of interest being assessed can be considered not to be consistent with the narrative natural background Objective.

5. Future Studies

The data collection and assessment process documented in this report could potentially be replicated in other ecoregions across Region 1 in order to interpret the narrative natural background Objective in those ecoregions. If such data collection occurs, a monitoring plan should be developed which ensures that an adequate number of samples is collected in order to provide statistically meaningful results and accurately represent the variability of natural background bacteriological conditions within each ecoregion. A power analysis should be conducted in order to determine the minimum number of minimally disturbed stream samples that need to be collected in order to be able to perform a statistically meaningful evaluation. Efforts should also be made to obtain a balanced representation of dry and wet weather minimally disturbed stream samples in order to account for seasonal variability and to prevent the excessive influence of either weather condition on the minimally disturbed stream FIB dataset. Sampling should include auxiliary data collection (salinity, pH, temperature, precipitation, etc.). Data collection should be repeated periodically in order to update the dataset to account for changes in minimally disturbed conditions due to climatic and other natural cycles, and for changes in anthropogenic impact. Samples from additional minimally disturbed locations within the Coast Range ecoregion could also be included in future sampling efforts to make the dataset more representative and robust. A sufficient number of dry and wet weather samples should also be collected from the waters of interest being assessed in order to be able to conduct a meaningful comparison with the corresponding minimally disturbed stream dataset to prevent erroneous interpretations of the hypothesis evaluation results. If the number of dry or wet water of interest samples is insufficient, a caveat to the results should be provided, and additional lines of evidence should be evaluated.

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