AN ABSTRACT OF THE THESIS OF

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Traditional public health bacterial indicators of water quality and the Biolog® system were evaluated to compare their response to other indicators of stream condition with the state of Oregon and between ecoregions (Coast Range, Willamette Valley, Cascades, and Eastern Oregon). Forty-three randomly selected Oregon rivers were sampled during the summer low flow period in 1997 and 1998. Testing included heterotrophic plate counts (HPC), total coliforms, fecal coliforms, E. coli, and Biolog® GN plates. Statewide, HPC correlated strongly with physical habitat and chemistry indicators while fecal coliforms and E. coli were highly correlated only with the river chemistry indicators. Total coliform bacteria did not correlate with either of the above environmental indicators. Dividing the sites by ecoregion, Eastern Oregon was
characterized by high HPC, fecal coliforms, *E. coli*, nutrient loads, and indices of human disturbance, whereas the Cascades ecoregion had correspondingly low counts of these indicators. The Coast Range reflected statewide results and the Willamette Valley presented no consistent indicator pattern. Attempts to separate ecoregions with the Biolog system were not successful nor did a statistical pattern emerge between the first five principle components and the other environmental indicators. Our research has shown that traditional public health microbial indicators may, however, be useful in measuring the effects of anthropogenic stress over large spatial scales.
Evaluating Microbial Indicators of Environmental Condition
In Oregon Rivers

by

Alan Travis Pennington

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Alan Travis Pennington, Author
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Contribution of Authors

Dr. Charles Hendricks was involved in the design, analysis, and writing of the manuscript, as well as providing laboratory support and supervision. Dr. Anna Harding assisted with the writing and editing of the manuscript. Heidi Campbell performed some of the laboratory assays and also assisted in the interpretation of the data.
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EVALUATING MICROBIAL INDICATORS OF ENVIRONMENTAL CONDITION IN OREGON RIVERS

INTRODUCTION

It is estimated that there are approximately 3,500,000 miles of perennial and intermittent rivers and streams in the continental United States (Larsen, 1997). Within this boundary the demand for aquatic resources for domestic, agricultural, industrial, and recreational use are often in conflict with the needs of aquatic and terrestrial flora and fauna. With a growing human population and ever-increasing demands for available water resources, the need to assess the status of our waters to satisfy the needs of all parties becomes imperative. Since the rise of the environmental movement in the 1960s, the federal government has increased its efforts to protect water resources through numerous legislative acts. The creation of the United States Environmental Protection Agency (EPA) in 1970 and the subsequent Clean Water Act (1972 and revised in 1977, 1981 and 1987), the Safe Drinking Water Act (1974 and 1977), and the Water Quality Act (1987) have all played pivotal roles in increasing the protection of our aquatic heritage (Fisher, 1994; Larsen, 1997).

Despite notable success in decreasing contamination along our shores, lakes, and streams, reports of current or potential ecosystem problems continue to arise: declining fish harvests, toxic algal blooms, dying or diseased forests, cancerous fish and mammals, and declining biodiversity (Messer et al, 1991). It became obvious to many scientists and regulators that a critical lack of data regarding the state of the environment, particularly the aquatic
environment, was hampering decisions affecting both regulation and research (Larsen, 1997). To address this problem, the EPA developed the Environmental Monitoring and Assessment Program (EMAP) in 1988 to establish a baseline measurement of the nation's ecological resources so that present conditions and future environmental trends can be evaluated (Larsen et al, 1991). This program is also designed to aid the states in implementing status reports on the quality of surface waters as required by section 305b of the Clean Water Act.

Within the EMAP mandate is an inclusion for the selection, development and evaluation of indicators that can describe the overall status of the ecosystem biota, as well as identify pollution sources and ecological changes within a region over time. Along with indicators that monitor the physical habitat and water chemistry, 'sentinel' species, or bioindicators, whose presence or absence are particularly sensitive to pollution or are necessary to maintain ecosystem integrity, are paramount to the evaluation of stream condition (Messer et al, 1991). These indicators, when applied together, can be used in unbiased statistically-based sample surveys to make inferences about the condition of the aquatic region under study (Paulsen and Linthurst, 1994).

Despite the many years of broad use of microbial indicators to define the sanitary condition of water and foods, bacteria have only recently been seriously examined as to their value in evaluating ecological conditions (Lemke, et al, 1997). The relative abundance of known microorganisms that are pollution sensitive and the savings in time and money they offer make bacteria
attractive candidates in any biomonitoring of natural systems. The question then arises as to whether bacteria are sufficiently sensitive to serve as biological indicators of ecological condition and, if so, which microorganisms or assays will be most useful in these determinations.

**Research Approach**

Microbial indicators of the sanitary condition of water have been used in earnest since the beginning of this century with dramatic success in elevating the quality of drinking and recreational waters. Their applicability as bioindicators of stream condition, however, has not yet been determined. To evaluate their usefulness, investigators within the EMAP program began applying microbial assays to Oregon stream and river samples in 1996 (Hendricks et al, 1999). The current study, which continues this research, enumerated microbial populations from 43 Oregon rivers that were sampled during the summers of 1997 and 1998. Testing included heterotrophic plate counts, coliform bacteria, fecal coliforms, and *E. coli*. The streams, which were all described as boatable, were randomly selected using EMAP protocol and were subsequently divided into four ecoregions (Eastern Oregon, Willamette Valley, Coast Range, and Cascades). This research compares the variability between traditional public health microbial indicators and other indicators of stream condition both statewide and between ecoregions.

Another potential indicator of environmental condition, the Biolog system (Biolog, Inc. Hayward, CA), was originally developed as a tool for the
identification of isolated bacteria. Within the last ten years researchers have utilized this system to evaluate the functional diversity of microbial communities in a variety of ecosystems such as soil, rhizosphere, and waste water communities (Konopka et al, 1998) through the analysis of bacterial utilization of sole carbon sources; however, only recently has it been used to describe freshwater streams and evaluated as a potential indicator (Hendricks et al, 1999). Biolog GN plates were used during the 1997-1998 sampling period and this study investigated the ability of the Biolog system to distinguish between river ecoregions and their suitability as indicators of environmental condition.

Statement of Purpose

The purpose of this research was to: 1) evaluate the effectiveness of traditional public health indicators and Biolog GN microtiter plates in assessing the environmental condition of Oregon rivers, and 2) determine if ecoregion conditions are reflected in the microbial responses with other indicators of stream condition.

Research Questions and Study Objectives

The following research questions directed this study:

1. Are microbial public health indicators useful as indicators of environmental condition?
2. Are Biolog results useful as indicators of stream condition?
The objectives of this study were the following:

1. Determine whether traditional public health indicators and the Biolog system correlate with other indicators of stream condition in Oregon rivers.

2. Determine if ecoregion separation of microbial samples provides a meaningful method to analyze indicator results.
Definitions of Terms

Average Well Color Development (AWCD): the average standardized optical density of the substrate wells of a Biolog plate. Standardization is derived by subtracting the optical density of the control well (A1) from each of the substrate wells (A2-H12) and dividing the total by 95 (Garland and Mills, 1991).

Biolog System: computerized system for the identification of bacterial species based on the growth of isolates on a microtiter plate containing 96 unique carbon sources. Periodic optical density readings measure the growth of bacteria within the individual carbon wells that presents a metabolic pattern used by the computer to identify known bacteria.

Escherichia coli: a member of the bacterial family Enterobacteriaceae and a subset of the fecal coliform group. *E. coli* are identified through their production of the enzyme β-glucuronidase that then hydrolyzes 4-methylumbelliferone-β-D-glucuronide (MUG) to the fluorescent umbelliferone, which may then be observed with long-wave UV light (Clark, D.L. et al, 1991; Robison, 1984).

Ecoregions: homogenous geographical regions that are delineated due to similar physical and biological characteristics (Clarke, 1997).
Fecal Coliforms: a thermotolerant subset of the Enterobacteriaceae family. Members of this group are capable of fermenting lactose at 44-45°C and are comprised of certain bacteria from the genus Escherichia, Klebsiella, Citrobacter, and Enterobacter (APHA, 1995).

Heterotrophic Plate Count bacteria: a diverse group of aerobic gram-negative and gram-positive bacteria that have a broad range of metabolic capabilities and culture requirements (Lechevallier, 1985).

Total Coliform bacteria: bacterial members of the family Enterobacteriaceae and are defined as aerobic and facultative anaerobic gram-negative nonspore forming rods that ferment lactose and produce gas within 48 hours at 35°C (APHA, 1995). Many of the genera found within this family are normal inhabitants of the mammalian digestive tract and their presence in stream water may indicate fecal contamination.
LITERATURE REVIEW

Humans have historically congregated along rivers and lakes for cooking, drinking, bathing, and food gathering. Besides their obvious value to humans, riparian areas provide habitat and sustenance for countless aquatic and terrestrial organisms. However, this resource has been degraded as increases in agricultural and industrial development have taxed the ability of aquatic ecosystems to cope with the great quantities of chemical and biological effluents and habitat changes that have occurred. For example, the 1992 National Water Quality Inventory revealed that 43% of America's lakes contained toxic contamination (Fisher, 1994), whereas 13,892 miles of Oregon rivers, representing approximately 13% of the state's streams, are listed on the state's 1998 303(d) list of waterbodies that do not meet state standards (ODEQ, 1998).

To address this problem, the United States Environmental Protection Agency was established in 1970 to respond to the myriad problems created by water, air and land contamination. Since its creation, the levels of many pollutants in freshwater sources have dropped significantly. Much of the progress in reducing water pollution can be attributed to the passage of the Clean Water Act in 1972 and the Safe Drinking Water Act of 1974. These two acts, both subsequently amended, provide the legal authority for the
enactment of federal standards, permits, and enforcement of industrial and municipal discharges as well as regulations pertaining to public drinking water systems (Fisher, 1994).

Yet, despite the millions of dollars spent each year for environmental monitoring and research, in the 1980s it became apparent that additional environmental problems, such as lake acidification, existed but relatively little reliable data was available (Christensen, 1996). In an effort to learn how to assess the condition of the environment in the United States, the Environmental Protection Agency established the Environmental Monitoring and Assessment Program (EMAP) in 1989 (Messer et al., 1991). The stated goals of EMAP are to estimate, with known confidence, the status, extent, change, and trends in indicators of the ecological condition of natural resources. These resources encompass rivers, streams, lakes, wetlands, estuaries and forests (Larsen et al., 1995). Information from EMAP research is forwarded to the U.S. EPA Administrator, the Congress, and the public with statistical summaries and reports regarding current ecological conditions and trends (Larsen et al., 1991). This program is based upon the application of environmental indicators that describe the overall condition of the biota in an ecosystem, can diagnose probable causes of poor condition, identify pollution sources that contribute to these causes, and discern between natural and anthropogenic disturbances. These indicators should also ideally reflect the
ecological endpoints valued by society (recreational uses such as swimming or fishing) as well as allow comparisons to be made between regions over time (Messer et al, 1991).

**Biological Indicators**

The assessment of the ecological condition of the world's aquatic resources and the subsequent quantification of point source water pollution are relatively new scientific endeavors. For the last 30 years, technological advances in measurement sensitivity have allowed investigators to utilize chemical and physical indicators as primary tools for the protection of water resources (Karr, 1993). These sampling methods, however, when used alone, are problematic for the following two reasons:

1) Pollutant levels in natural waters vary with time due to season, stream runoff, depth, tides, and other factors.

2) While the contaminant may be present in the aquatic system, it may not be biologically available to sensitive biota (Phillips, 1980).

Chemical monitoring can miss many of the short and long term anthropogenic disturbances, such as habitat degradation, heated effluents, and flow alterations, that impair stream use (Karr and Dudley, 1981). The advantage, therefore, of using biological indicators, is that the researcher may measure both the direct biological availability and a time-averaged index of pollution availability (Phillips, 1980).
The practice of using biological indicators is not new—public health workers have successfully employed microbial indicators to monitor the status of drinking and bathing waters for many years (APHA, 1995). However, the resurgence of using biological indicators (or bioindicators) to assess the ecological integrity of the nation's waters only began in earnest in the 1980s (Karr, 1993). Since then, investigators have examined a variety of possible bioindicators that can measure a wide spectrum of aggregation. For example, population-level indicators may focus on sensitive species, whereas community-level measurements detect species diversity or richness (Fausch et al, 1990). The ecosystem-level effects may be reflected in the measurement of its production or nutrient spiraling (Odum, 1985). Frequently, wide arrays of terrestrial and aquatic species or assemblages are examined and evaluated for taxa richness, diversity, abundance, etc., using several different scales. These variables may then each be given a numerical score, indicative of some impact on condition, and combined into an overall biological index of integrity (IBI) that presents a composite of the state of aquatic biota (Karr, 1981).

The use of microbial bioindicators may serve to elucidate the status of aquatic ecosystems if they can be of practical use in measuring the effects of anthropogenic stress on those streams. Human disruption of aquatic ecosystems may be classified in four ways - physical restructuring, such as damming and dredging; overharvesting of biota; the addition of chemical or
biological wastes; and the introduction of exotic flora and fauna (Rapport et al., 1998). Biological wastes from point and non-point human or domestic animal sources represent important areas of anthropogenic stress that may be measured by bacteria (Niemi and Niemi, 1991; Allan, 1995).

Investigations into using bacterial indicators of aquatic condition have lagged despite the fact that microorganisms play important ecological roles in aquatic ecosystems (Ricklefs and Schulter, 1993; Garland and Mills, 1991). Microbes are key players in the nutrient cycling of minerals (Edwards, 1990), provide a food source for grazing protists (Carlough and Meyer, 1990) and are primarily responsible for the degradation and detoxification of many environmental contaminants (Zak, 1994). Their small size and rapid growth allow for complex community interactions to be studied much more easily than with plants and animals (Garland, 1997). Bacteria are ubiquitous in stream waters and their development as environmental indicators offer practical advantages- they may be more inexpensive and time effective to utilize than costly chemical or physical analyses (Lemke, 1997). Yet, much remains to be determined; which bacterial population or community best assesses stream ecological condition and are traditional public health indicators of drinking/bathing water useful in this assessment?
Public Health Indicators

*Bacterium coli*, described as early as the 19th century, was the first bioindicator of water pollution to be utilized (Pipes, 1982). Subsequent work, such as the attempt to correlate the presence of *Bacillus typhosis* in drinking water supplies to epidemics of typhoid fever (Fuertes, 1905), demonstrated the importance of microbes in respect to the sanitary condition of water. Since then investigators have developed various analyses which utilize bacterial populations or communities to assess the overall quality of water and ensure that public waters are free of pathogenic organisms associated with fecal contamination (Dufour, 1984). The following public health tests of sanitary water quality will be discussed: heterotrophic plate count, total coliforms, fecal coliforms and *Escherichia coli*.

The heterotrophic plate count (also known as the standard plate count) provides an estimation of the number of viable heterotrophic bacteria in water and can be useful when interpreting data from other microbial tests. Historically, HPC have been used to measure changes during treatment in water plants or swimming pools (APHA, 1995). The microorganisms captured in this test represent a diverse group of aerobic bacteria (both gram-negative and gram-positive) that have a broad range of metabolic capabilities and culture requirements (Lechevallier, 1985). The heterotrophic plate count (HPC) has no legal regulatory status because bacteria detected in this analysis do not
necessarily represent sanitary pollution (Clark, 1980; APHA, 1995); nonetheless, high densities raise concerns about the presence of possible opportunistic pathogens (Lye and Dufour, 1991). HPC numbers have been correlated to temperature, water level, channel substrate, and an array of chemical tests such as conductivity, chloride, nitrate and total phosphorus concentrations, but these relationships are not always consistent (Hendricks et al, 1999; Osgood and Boylen, 1990; Rattray and Logan, 1993).

The total coliform group comes from the family Enterobacteriaceae and are defined as aerobic and facultative anaerobic gram-negative nonspore forming rods that ferment lactose and produce gas within 48 hours at 35°C (APHA, 1995). Many of the genera found within this family are normal inhabitants of the mammalian digestive tract and their presence in stream water may indicate fecal contamination. Since the end of the 19th century, the total coliform group has been used by many states to assess water quality (Pipes, 1982) and gained wide acceptance as a standard assay for bathing waters after the American Public Health Association established a total coliform classification scheme in 1943 (Dufour, 1984). Many studies have since identified problems with using total coliform assays for water quality assessment. One of the serious obstacles for its continued use as an indicator of fecal pollution is that many species within the coliform group are found in waters where fecal wastes are not present, due to soil or plant sources (Hendrix, 1970). Another potential problem is that coliform detection may be
suppressed when heterotrophic plate counts exceeded 500 to 1000 colony
forming units per milliliter (LeChevallier, 1985). Nevertheless, theoretical
acceptance of no coliforms per 100 mL of water sample remains an important
assurance of drinking water quality (Hendrix, 1982; APHA, 1995).

The fecal coliform group is a thermotolerant subset of the
Enterobacteriaceae family. Members of this group are capable of fermenting
lactose at 44-45°C and are comprised of bacteria from the genus Escherichia,
Klebsiella, Citrobacter, and Enterobacter (APHA, 1995). While primarily fecal in
origin, pulp-mill processing effluents may significantly raise the level of fecal
coliform densities in stream waters due to Klebsiella and Enterobacter growth,
thus giving a false impression of sewage contamination (Hendry, 1982).
Besides seasonal variability, which may very well affect all bacterial indicators
to some extent, fecal coliforms display a diurnal fluctuation, with lower numbers
being present at night (Bahlaoui, 1998). Within the public health context,
numerous studies have reported conflicting results regarding the association
between fecal coliform levels and outbreaks of swimming-associated
gastroenteritis in either marine or freshwater areas (Drenchen, 1994; Dufour,
1984; Prüss, 1998).

*Escherichia coli* is the most common species within the fecal coliform
group and is only present in waterways due to animal or human waste (APHA,
1995). *E. coli* are typically identified through their production of the enyzme
β-glucuronidase. This enzyme hydrolyzes 4-methylumbelliferone-β-D-glucuronide (MUG) to the fluorescent umbelliferone, which may easily be observed with a long-wave (366-nm) UV light source (Clark, D.L. et al, 1991; Robison, 1984). The normal procedure for identifying *E. coli* from water samples requires incubation at 44.5°C for 22 hours (APHA, 1995), although the pathogenic strain O157:H7 does not grow above 41°C and may fail to be detected during routine screening of fecal coliforms (Raghubeer and Matches, 1990).

*E. coli* have been documented to grow and survive in surface waters (Flint, 1987) and studies show that their presence in freshwaters correlate strongly to attacks of swimming related gastroenteritis (Dufour, 1984). Enteropathogenic strains of this organism have also been identified in deadly outbreaks due to diarrhea or kidney failure (Raghubeer and Matches, 1990). Four major categories of diarrheagenic *E. coli* have been identified: enterotoxigenic *E. coli*, which results in traveler's and infant diarrhea in underdeveloped countries; enteroinvasive *E. coli*, which may cause dysentery; enteropathogenic *E. coli*, a significant contributor to diarrhea in daycare settings; and enterohemorrhagic *E. coli*, which may cause hemorrhagic colitis and hemolytic uremic syndrome (Levine, 1987). A member of this latter group, an *E. coli* O157:H7 strain, was identified as the probable cause of a waterborne outbreak that occurred in Cabool, Missouri, in 1989 and resulted in 240 illnesses and three deaths (Parmelee, 1990).
Each of the microbial tests discussed above have their respective advantages and disadvantages regarding their utility as public health indicators. Each has been rigorously tested and improved upon over the past century and has contributed greatly to our overall ability to monitor the status of our drinking and recreational waters. However, the usefulness of traditional public health indicators to assess the ecological condition of lakes and rivers and is not yet known. Heterotrophic plate count bacteria do not necessarily represent any known contaminant group of organisms, but preliminary studies show that their diversity might correlate well with other stream habitat and biotic indicators (Hendricks et al, in press). The total coliform group can signal both endogenous presence as well as fecal contamination, yet inhibition by other microbes might underestimate their numbers. The fecal coliforms and E. coli are well-studied indicators of mammalian fecal contamination; yet, when the bacterium is absent, interpretation of stream condition, other than pollution, is difficult.

**Biolog ® System**

The characterization and classification of heterotrophic bacterial communities has been hindered due to the small size and morphological similarity of constituent members. While a few highly structured communities with microorganisms of distinct morphological and/or nutritional strategies have
been reported, descriptions of heterotrophic aquatic communities are lacking (Garland and Mills, 1991). Traditional methods of isolating, cultivating and identifying bacteria are labor-intensive and time-consuming (Hans-Jürgen and Has-Jürgen, 1994). Isolation techniques may present a biased view of bacterial communities due to the selective nature of the media and the unculturability of certain microbes (Kersters et al, 1997). Modern molecular techniques, such as 16s RNA sequencing or DNA hybridization, are more rapid but require a pre-established data base for identification and contribute no information about the metabolic potential of the organism (Hans-Jürgen and Hans-Jürgen, 1994).

The Biolog system was originally developed to identify pure cultures of aerobic Gram positive or Gram negative bacteria in a hospital or clinical setting (Konopka, 1998). The system is based on the inoculation of 96 well microtiter plates with a pure culture of an isolated organism. Each well contains a tetrazolium dye and a unique carbon source that serves as a basal nutrient medium which may or may not support the growth of microbes. If growth occurs, the tetrazolium dye is reduced during the oxidation of the carbon source and the resulting color change can be measured with a spectrophotometer (Garland and Mills, 1991). A single control well contains the dye but no carbon source. In 1991, Garland and Mills introduced the concept of using the Biolog system to characterize the functional potential of microbial
communities. Using soil, rhizosphere, and water samples, they found that these disparate communities could be differentiated through their pattern of carbon source utilization, both between and among sample types. Since then, researchers have utilized the Biolog system to differentiate bacterial communities in a variety of soil and water ecosystems (Konopka, 1998) as well as such diverse environments as activated sludge (Knight et al., 1995), compost (Insam et al., 1996), and a wastewater treatment system (Schneider et al., 1998).

The difficulties of modern and traditional culture methods, as discussed above, make the simplicity and rapid processing of the Biolog system very attractive (Verschure et al., 1997). Low manpower requirements to operate the system enables intensive sampling across temporal and spatial scales. Multiple ecological effects such as microbial density, diversity, or similarity, may be examined through the analysis of the community-level response using both univariate and multivariate statistical techniques (Garland, 1997). And the application of this method may also allow the classification of bacterial communities based on their metabolic differences in utilizing the sole carbon sources (Garland and Mills, 1991).

Criticisms of the Biolog method have centered on questions regarding the inoculum density. Vershuere et al (1997) found that the community Biolog patterns were dominated by the fastest growing strain, even when that strain represented only 10% of the inoculated cells. They also noted that metabolic
activities of the slowest growing bacteria were partially masked by the faster ones. Differences among rapidly metabolized substrates dominate interpretive statistical techniques, such as principle component scores, generated after short incubation times, whereas their contribution to principal components decreases, relative to slower growing microbes after prolonged incubation (Lindstrom et al, 1997). The relative contribution of any substrate to a pattern of potential carbon source use characteristic depends on the time chosen to evaluate substrate use and original inoculum density (Lindstrom et al, 1997; Garland, 1997; Haack et al, 1995). Konopka et al (1998) argue that the inoculation of low cell densities into the substrate wells means that the technique is a culture-based method in which any enrichment culture may bias the test, causing the results to be unrepresentative of the original native flora. They also state that this approach to measure functional diversity is hindered due to the fact that the microtiter substrates do not reflect the type of substrates normally found in ecosystems.

To avoid problems associated with bias due to inoculum density differences, alternatives have been suggested to minimize density influence. Garland (1997) suggests that normalization of inoculum density can be achieved by standardizing the number of cells prior to inoculating the Biolog plates, although this procedure increases both the labor and time of the assay. Vershuere et al (1997) report that it is difficult, if not impossible, to inoculate the
microtiter plates at the densities recommended by the manufacturers as samples do not often reach such high levels or are loaded with organic material. However, some investigators have succeeded in using this method to classify and characterize communities (Haack et al., 1995; Staddon et al., 1996; Winding and Hendriksen, 1997).

Another suggested alternative is to standardize metabolic development through semicontinuous monitoring of the color change in the microtiter plates so that samples of equivalent average well color development (AWCD), and not incubation time, may be compared (Garland and Mills, 1991; Garland, 1997). This allows the investigator to select a location in the middle of the sigmoidal response function of AWCD versus time that would tend to maximize differences (Konopka, 1998). Studies utilizing this method have been able to differentiate diverse varieties of communities such as rhizospheres and rhizoplanes (Grayston and Campbell, 1995) and activated sludge habitats (Guckert et al., 1996).

Responding to the problem of uneven growth in the substrate wells, Guckert et al. (1996) assert that the rate and extent of utilization of any particular carbon source will be related to the original microbial community structure and metabolic capacity for that sample. Since each community will have a unique carbon source to utilize in each well, the microbial community will likely change independently in each of these wells; thus, the measured
Biolog response is still related to the functional potential of the original community. Smalla et al (1997) found that the patterns of substrate development in individual Biolog wells was different than those obtained for the inocula, indicating that changes in the structure of the community had occurred during the assay. They also found that occasionally the numerically dominant bacteria were not represented in the well profiles. This suggests that the populations that become dominant in various wells are versatile with respect to their ability to utilize carbon sources and are competitive under incubation conditions. Smalla et al (1997) argue that while utilization patterns may be habitat specific, the pattern of a community does not necessarily reflect the functional potential of the community at the time of inoculation.

Due to the enormous amount of data that may be generated, analysis of Biolog substrate utilization patterns is typically resolved through the use of multivariate techniques such as principle component analysis or canonical correlation analysis (Garland, 1997; Hackett and Griffiths, 1997). Analyses may be based on all 95 individual substrates or on subsets of similar carbon sources. Garland and Mills (1991) originally divided the carbon sources into 11 similar categories, while Zak et al (1994) condensed the classification into 6 groups. The obvious advantage of such a system is the flexibility afforded the investigator to examine both diverse and similar microbial communities in a variety of ecological settings. The ability to manipulate a wide range of variables allows one to experimentally identify important contributing
responses. Such a technique may provide insights into the utility of the Biolog system to characterize the ecological condition of freshwater streams.

**Statistical Sampling and Ecoregions**

The impossibility of sampling and evaluating the millions of miles of streams and lakes within the United States has led the Environmental Monitoring and Assessment Program to utilize random sampling techniques in their pilot surveys (Messer et al, 1991). Randomization of the sampling procedure is a fundamental aspect of these surveys because it prevents the introduction of intentional or unintentional biases into the selection of sample sites, allows each population a known chance of appearing in the sample, and spreads the sample to assure spatial representation (Larsen, 1997). Site specific sampling, while providing information about a specific locale, may skew interpretation toward less or greater ecological impairment if those results are used to assess conditions outside of the sampling area (White and Merritt, 1998).

Because each ecological resource type has unique features, each EMAP survey design must be adjusted to fit the needs of the area under study. Inclusion properties, or weightings, for each sample unit characterizes the proportion of the target population the sample unit represents. For example, stream sites selected with relatively high probability represent very few streams in the population and are subsequently weighted less than streams that had a
low probability of being selected. This approach minimizes selection biases from the stream selection process and provides a representative picture of aquatic condition, while sampling only a small fraction of the possible stream sites in the population (Peterson et al., 1996).

One method of defining ecological resource types is through delineating spatial boundaries drawn around relatively homogenous areas at a specific scale. These areas, known as ecoregions, possess similar geology, topography, soil, and vegetation that allow comparisons of streams of similar size within the region (Bryce and Clarke, 1996). Ecoregions have been defined at various levels of magnitude. Omernik (1987) originally divided the conterminous United States into three levels, but subsequent work has divided those areas into even smaller units (Bryce and Clarke, 1996). Ecoregions allow investigators much flexibility to choose the geographical scale that reflects the needs of their study and can be useful in extrapolating results to similar ecoregions (Hughes et al., 1994).
EVALUATING MICROBIAL INDICATORS
OF
ENVIRONMENTAL CONDITION IN OREGON RIVERS

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Introduction

Aquatic ecosystems provide habitat and sustenance for countless organisms. However, these systems have been degraded by landscape alterations and increases in agricultural and industrial development that have taxed their ability to cope with the great quantities of chemical and biological effluents to which they have been subjected (Allan and Flecker, 1993).

The impact of human disturbance on aquatic ecosystems and riparian zones, while intuitively apparent, is not necessarily easy to quantify. Point and non-point sources of pollution from agriculture, mining, and industry contribute a wide array of pathogens, pesticides, metals, and nutrients into our rivers and streams that result in myriad forms of stress on aquatic organisms (Fisher, 1994). Despite notable success over the last 30 years in decreasing contamination along our shores, lakes, and streams, reports of current or potential ecosystem problems continue to arise, such as declining fish harvests, toxic algal blooms, dying or diseased forests, cancerous fish and mammals, and declining biodiversity (Messer et al, 1991). It became obvious to many scientists and regulators that a critical lack of data regarding the state of the environment, particularly the aquatic environment, was hampering decisions affecting both regulation and research (Larsen, 1997).

Measuring and monitoring environmental trends depend upon the application of indicators that are sensitive to the differences between
stressors of natural and anthropogenic origin (Frost et al, 1992). Ideally, environmental indicators should possess the ability to describe the overall condition of the biota in an ecosystem, diagnose probable causes of poor condition, and identify pollution sources. These indicators should also reflect the ecological endpoints valued by society (recreational uses such as swimming or fishing) as well as allow comparisons to be made between regions over time (Messer et al, 1991).

Despite the many years of broad use of microbial indicators to define the sanitary condition of water and foods, bacteria have only recently been seriously examined as to their value in evaluating ecological conditions. The relative abundance of known microorganisms that are pollution sensitive and the savings in time and money they offer (Lemke et al, 1997) make bacteria attractive candidates in any biomonitoring of natural systems. The question then arises as to whether bacteria are sufficiently sensitive to serve as biological indicators of ecological condition and, if so, which microorganisms or assays will be most useful in assessing these conditions.

To evaluate their utility, investigators within the Environmental Monitoring and Assessment Program (EMAP) began applying microbial assays to Oregon stream and river samples in 1996 (Hendricks et al, 1999). The current study, which continues this research, enumerated microbial populations from 43 boatable (non-wadeable) Oregon rivers that were sampled during the
summers of 1997 and 1998. Testing included using traditional public health indicators such as heterotrophic plate counts, coliform bacteria, fecal coliforms, and *E. coli*, as well as the Biolog® system. This research provides a statewide and between-ecoregion comparison of the status and variability of microbial indicators and other indicators of environmental condition.

**Materials and Methods**

**Site Selection and Ecoregion Division**

Forty-three Oregon river sites (Figure 1) were randomly chosen (20 sites in 1997, 23 sites in 1998). Sites were chosen through a systematic design adopted by U.S. EPA's Environmental and Assessment Program (EMAP) that attempts to reflect the density and distribution of the river network while promoting spatial balance of possible sampling sites, thus allowing inferences to the entire river network from the sample data (Herlihy et al, in press). Only rivers that were considered "boatable" were included in this analysis. The blue line network from the digital version of the 1:100,000 scale United States Geological Survey topographic maps was used as the sample frame from which the sampling sites were selected (US EPA, 1998).

The ecoregions used in this study were aggregations of the eight level 3 ecoregions defined by Omernik (1987). The Eastern Oregon aggregate (number of sites = 9) included the Columbia Basin, the Blue Mountains, Eastern Cascades and Foothills, and the Snake River Basin ecoregions. The
Cascades ecoregion aggregate (n= 8) included the Western Cascades and two Eastern Cascade sites that more closely resembled the western sites than the eastern ones. The Coast Range aggregate (n= 12) was combined with 4 sites from the Klamath Mountain ecoregion. The Willamette Valley ecoregion (n= 14) was not joined with any other (Figure 3).

**Sampling and Analytical Methods**

Sampling was conducted during the summer low flow period during the months of July, August and September in 1997 and 1998. The global positioning satellite system was used to identify site latitude and longitude. Trained EMAP field crews were responsible for conducting on-site measurements and sampling for various chemical, physical and biological assays. One water sample was collected at each site for bacterial analysis. Bacterial samples were collected from the water column mid-stream as grab samples just below the surface of the water in sterile 125 ml glass bottles. The bottles were then placed in an ice chest and transported to the laboratory for analysis (US EPA, 1998). All of the samples were analyzed within 48 hours after collection except two samples that were processed 72 hours after collection.

Bacteria were enumerated for heterotrophic plate count (HPC) bacteria, total coliforms (TC), fecal coliforms (FC) and *E. coli* (EC) according to APHA Standard Methods (1995). Sample volumes of 0.01ml to 10 ml were filtered to
obtain desired colony counting densities. Analyses were performed using membrane-filtration on HA 45 (m filters which were then transferred to the appropriate media. HPC bacteria were enumerated after incubation on Bacto m-Plate Count Broth (Difco) for 24 ± 2 hours at 35 ± 0.5°C. Filtered total coliforms were placed onto Bacto MacConkey Agar (Difco) after filtration and incubated for 24 ± 2 hours at 35 ± 0.5°C, while fecal coliforms were placed onto Bacto m-FC broth base (Difco) and incubated for 24 (2 hours at 45.5 ± 0.5°C. To enumerate *E. coli*, sample filters that supported growth of fecal coliforms were transferred onto Bacto EC broth (Difco) containing MUG (4-methylumbelliferyl-(D-glucuronide) and incubated for an additional 24 ± 2 hours at 45.5 ± 0.5°C. Colonies that produced a blue fluorescence around the periphery of the colony when exposed to 366 nm ultraviolet light were considered *E. coli* colonies.

By convention (APHA, 1995), microbial counts for HPC were recorded as colony forming units (CFU) per 1 ml. TC, FC, and EC were recorded as CFUs per 100 ml. Counts were recorded for those samples that fell within the range of desired counting densities. If more than one plate fell within this range the results were averaged (APHA, 1995). Samples that produced a number of colonies below the acceptable range of bacteria were assigned the count from the lowest dilution.

The Biolog system is based on the inoculation of 96 well microtiter plates with a pure culture of an isolated organism. Each well contains a tetrazolium
dye and a unique carbon source that serves as a basal nutrient medium which may or may not support the growth of microbes. If growth occurs, the tetrazolium dye is reduced during the oxidation of the carbon source and the resulting color change can be measured with a spectrophotometer (Garland and Mills, 1991). A single control well contains the dye but no carbon source.

In 1991, Garland and Mills introduced the concept of using the Biolog system to characterize the functional potential of microbial communities. Using soil, rhizosphere, and water samples, they found that these disparate communities could be differentiated through their pattern of carbon source utilization, both between and among sample types. Since then, researchers have utilized the Biolog system to differentiate bacterial communities in a variety of soil and water ecosystems (Konopka, 1998) as well as such diverse environments as compost (Insam et al, 1996) and a wastewater treatment system (Schneider et al, 1998). To avoid problems associated with bias due to inoculum density differences, we standardized metabolic development through semicontinuous monitoring of the color change in the microtiter plates so that samples of equivalent average well color development (AWCD), and not incubation time, could be compared (Garland and Mills, 1991; Garland, 1997). This allows the investigator to select a location in the middle of the sigmoidal response function of AWCD versus time that would tend to maximize differences (Konopka, 1998).
Biolog GN microtiter plates were directly inoculated with 150 µl of river sample into each well with a calibrated 8-channel micro-pipette. Plates were incubated at 35 ± 0.5°C until they produced an average well color density (AWCD) of approximately 0.75 (± 0.14). Optical density of the wells was read at 590 nm during periodic readings with a Biolog Microstation System equipped with an EMAX (Precision Microplate Reader). The optical density (OD) of the 95 individual wells was standardized by subtracting the OD of the control well (A1) from each well. AWCD was calculated by summing the standardized values of the response wells and dividing by 95 (Garland and Mills, 1991). Because low bacterial counts signal a potential problem of an increased probability of uneven inoculation, five of the 43 samples were excluded due to low HPC counts (1.5 Log HPC). Five of the samples were excluded because their AWCD fell outside of the acceptable range (0.75 ± 0.14).

Statistical Analysis

Weighting factors were used during data analysis in order to make inferences regarding the population instead of the sample. Each site had a sample weight that was calculated as the inverse of the probability of being selected. The total river length (6182 km) of the study population can be calculated by summing the sample weight of each site. (Herlihy et al, in press).

Differences between ecoregions and other classification variables were analyzed using One-way ANOVA. Significant results were followed up with
Tukey-Kramer multiple comparison tests. Bacterial counts were transformed to common log 10 so that the data would closely approximate a normal distribution. Statistical analyses of HPC bacteria, TC, FC, EC and response variables were performed using Spearman's ranked correlations. To discover important chemical contributors to bacterial growth, ranked river chemistry variables and HPC bacteria were analyzed using stepwise multiple regression. For statistical purposes, samples of TC, FC, and EC that yielded zero CFUs were given a value of 5 CFU/100 ml (1/2 of their smallest possible value of one CFU per 10 ml). Alpha levels were set at 0.05. Biolog plates were analyzed using principle components analysis and were not weighted. JMP (version 3.2, SAS Institute, Cary, NC) statistical software was used for the analysis.

Results

Results of this study are divided into public health bacteria/environmental indicator correlations within the entire state of Oregon, between ecoregions, and with the Biolog system.

Statewide Indicator Results

Spearman's rank correlations revealed that heterotrophic plate count bacteria were strongly related to most of the other physical habitat indicator variables (Figure 2). Positive correlations resulted with indicators of human disturbance such as the agriculture, pavement, road, pasture, and total
disturbance indices. Negative correlations were recorded for areas of decreased vegetation along the bankside. These measurements include bankside canopy density, % of river canopy density, and the index of riparian complexity (a measure of multi-layered woody vegetation). HPC also correlated strongly with fecal coliform bacteria and *E. coli*. HPC bacteria were also strongly correlated with river chemistry measurements. Statistically significant positive correlations occurred with 8 of the 12 variables (NO$_3$-N, NH$_3$-N, and chlorine did not significantly correlate). The one significant negative correlation, dissolved oxygen, decreased as the number of HPC bacteria increased. Stepwise regression of the various chemistry responses showed that pH, dissolved oxygen, and total nitrogen are the best subset ($R^2=0.60$) of regressors for the whole-model test (Figure 3).

Total coliform counts correlated with few of the other environmental indicators. Fecal coliforms and *E. coli* correlated highly with chemical indicators but were poorly related with physical habitat indicators of anthropogenic stress. Many river samples (Table 1) resulted in zero counts (7 zero counts for TC; 9 zero counts for FC and EC).

**Ecoregions**

One-way ANOVA was applied to the microbial, chemical, and physical variables to examine differences by ecoregions. Eastern Oregon was significantly different ($p<0.05$) from the other ecoregion aggregates for many of
the environmental indicators (Table 2). Heterotrophic plate count bacteria, fecal coliforms, and *E. coli* were significantly higher in the eastern ecoregion. Other indicators that were significantly higher for the eastern region include: agriculture disturbance index, road disturbance index, pasture disturbance index, pH, acid neutralizing capacity, specific conductance, total nitrogen, total phosphorus, silicon dioxide, and sulfate. Indicators that were significantly lower in the eastern ecoregion were index of riparian complexity, bankside canopy density, % of canopy cover, and dissolved oxygen.

The Cascades ecoregion aggregate was significantly lower in nitrates and chlorides and averaged the lowest mean water temperature (12.7°C). The Willamette Valley had a significantly higher percentage of fish anomalies than either the Cascades or Eastern Oregon.

**Biolog**

Principle component analysis of Biolog microtiter plates was applied to 33 of the river samples and evaluated for their relationship with the response variables. To avoid confounding due to differences in inoculum density, normalization of the results was achieved through semicontinuous monitoring until the desired AWCD endpoint of 0.75 (± 0.14) was obtained (Garland, 1997). Neither of the first two principle components were correlated with AWCD.
The first five principle components extracted a substrate utilization pattern of 41.5% of the variability: principle component one explained 12.2%, principle component two explained 10.3%, and subsequent components explained less than 10%. Only the second PC was significantly correlated with any of the bacterial tests (Log HPC; $R^2=0.16$, $p<0.05$). Attempts to narrow the AWCD endpoint of $0.75 \pm 0.14$ by one-half to $0.75 \pm 0.07$ ($n=22$) did not result in an appreciable difference in explained variability by the first PC (12.8%). PCA did not discriminate Biolog samples between the ecoregion aggregates nor was separation by ecoregion apparent. However, sites in the eastern aggregate did cluster together (Figure 4).

Discussion

The use of microbial bioindicators may serve to elucidate the ecological condition of aquatic ecosystems if they can be of practical use in measuring the effects of anthropogenic stress. Rapport et al (1998) classified human disturbance in four ways - physical restructuring, such as damming and dredging; overharvesting of biota; the addition of chemical or biological wastes; and the introduction of exotic flora and fauna. Typically, biological wastes from point and non-point human or domestic animal sources represent important areas of anthropogenic stress that may be measured by bacteria.
(Niemi and Niemi, 1991; Allan, 1995). However, this work shows that even physical habitat parameters can be reflected in the number and type of bacteria present. Correlations between bacteria and environmental indicators of stream condition point to a strong relationship between heterotrophic plate count bacteria and water chemistry and physical habitat metrics.

Among the physical habitat responses, human disturbance may be reflected by measurements of streamside land use patterns such as the percentage of land dedicated to agriculture or paved for roads. Human activity near or within a watershed contributes greatly to non-point sources of pollution and increases the chemical/biological load on the stream (Allan and Flecker, 1993). The absence of riparian vegetation or canopy cover also signals human disturbance within the riparian zone and may indicate a lack of a vegetative buffer in areas of agriculture or animal grazing. In this study HPC bacteria strongly correlated to the physical habitat indicators in the expected manner—positive correlations resulted from measurements of increased land use patterns and negative correlations reflected decreases in riparian vegetative complexity. Total coliforms, fecal coliforms, and *E. coli* did not correlate well with these indicators, which may be partly explained by the high number of microbial samples from which these organisms were not recovered, thus decreasing the statistical sample size. The river chemistry data reveals strong correlations between HPC bacteria, fecal coliforms, and *E. coli*. Although many of these responses are statistically significant, they may not prove useful in
interpreting water column bacterial results. For example, silicon dioxide is a major component of igneous rock and arises mainly due to weathering of rocks and leaching of minerals. Silicon compounds are common constituents of aquatic plants and animals, particularly diatoms, yet play no direct role in heterotrophic bacteria presence or absence (Dojlido and Best, 1993). However, other chemical variables do intuitively make sense regarding their importance to microbial numbers. Nitrogen, carbon, and phosphorus are obvious nutritional elements that comprise a major portion of a bacterial cell and it is reasonable to assume that an increase in their availability is related to an increase in bacterial numbers.

A stepwise regression was performed with all of the chemistry parameters in an attempt to discover which combination of chemical indicators created the best model for predicting bacterial density. Dissolved oxygen, pH, and total nitrogen resulted in the highest correlation with HPC bacteria (Figure 2). Another model that included total phosphorus, dissolved organic carbon (DOC), NH$_3$-N, and dissolved oxygen resulted in a slightly lower $R^2$ of 0.57. Our results correspond with other studies that find that only a small subset of chemical indicators are important contributors to bacterial counts (Antonietti and Sartore, 1996; Espigares et al, 1996).

The advantage of stratifying the river sites into their respective ecoregions is threefold. First, an ecoregion classification scheme provides a
geographic framework to stratify variance by defining areas of similar geophysical characteristics. Second, ecoregions can be useful in designing research studies in areas where environmental conditions can be anticipated. Third, results may be more meaningful when extrapolated within an ecoregion (Hughes et al., 1994). Using ANOVA allowed us to contrast indicator differences by ecoregion and evaluate whether or not these differences made sense. For example, Eastern Oregon is a semi-arid region characterized by a continental climate, with broad expanses of mid-elevation shrubland interspersed by forested mountains. Cattle grazing has been a stressor of riparian cover and water quality in many parts of this region for over a century (Wissmar et al., 1994). Despite combining four ecoregions into one aggregate and the low number of sample sites (due to low water levels in the summer), the Eastern Oregon ecoregion presents a fairly homogenous picture for comparative purposes.

The ANOVA analyses used to characterize Eastern Oregon accentuate the effects of human activities. Anthropogenic stress indicators, such as indices of agriculture and pasture disturbance, correspond to the addition of aquatic nutrient loading. Increased amounts of dissolved organic carbon, phosphorus and nitrogen (Figure 2) may act to increase bacterial counts while lowering the available dissolved oxygen (Allan, 1995). Similarly, this region had a high negative correlation with indicators of bankside woody vegetation and canopy
cover, which are results that one might expect in an area highly impacted by domestic animal grazing and farming.

The Cascade aggregate was also well characterized by the various ecological and bacterial indicators. This region had the coldest water temperature (12°C), the fewest nutrients (N, DOC, NO₃-N, NH₃-N, SO₄), the fewest fish anomalies, and the least human disturbance (pipe, agriculture, pasture, total disturbance, bankside canopy density). Presented with the composite above one would predict that this region would also have the fewest bacterial counts. HPC bacteria, fecal coliforms, and E. coli numbers were, in fact, all lower in this ecoregion.

The other two ecoregions did not present such a homogeneous picture. The Willamette Valley is an important agricultural region that is characterized by a broad, lowland valley that contains both deciduous and coniferous woodlands. The valley is the most densely populated ecoregion in the state. No consistent patterns emerged between bacterial counts and the other environmental indicators in this region. The Coast Range ecoregion (which included 4 sites in the Klamath Mountain ecoregion) is noted for its high precipitation and low coniferous mountains. This region's forests are intensively managed and highly logged. Coast Range correlations between HPC bacteria and other environmental parameters basically reflected the statewide results in this study.
Dividing sampling sites by ecoregion may be most meaningful when one considers the anthropogenic effects within those regions. As stated previously, the bacterial counts and other environmental indicators reflected the generally high human disturbance of Eastern Oregon and the relatively low human impact in the Cascades. The Coast Range and Willamette Valley ecoregions, on the other hand, showed much greater variability. The rivers that flow through these latter two regions generally originate in the Cascades and are subject to varying levels of anthropogenic stress as they travel throughout the lower elevation ecoregions and empty into the Pacific Ocean. These results highlight the difficulty in using ecoregions to characterize differences in lotic systems due to the vast geographical areas in which they may drain. Future research conducted into ecoregional differences might consider smaller subregional scales (level 4) to reduce the variability in order to better characterize the environmental status of the aquatic networks (Bryce and Clarke, 1996).

The overall bacterial counts from this study were unexpectedly low. While low counts from the Cascade ecoregion were anticipated, the counts from the heavily impacted Eastern Oregon ecoregion were not as high as we expected. Several of the sites, particularly in the relatively pristine Cascades, recorded zero coliforms. None of the 43 sampling areas surpassed the Oregon state *E. coli* single sample standard of 406 organisms per 100 ml for
recreational waters (OAR, 1998). However, bacterial levels generally increase during wet weather events. Since precipitation is strongly seasonal in Oregon, bacterial levels tend to increase in the spring and fall and decrease during the drier summer months (ODEQ, 1995). Our sampling during the summer low river flow period may have reflected this phenomena.

Our intention was to evaluate the utility of the Biolog system in assessing stream condition by comparing Biolog GN results with other bacterial assays and environmental indicators in boatable rivers both throughout the state and between distinct ecoregions. Similar research of smaller, wadeable streams has shown that Biolog GN plates were useful in distinguishing microbial community differences between ecoregions (Campbell, 1998; Hendricks et al, 1999). However, our attempts in this study to separate ecoregions with the Biolog system were not successful nor did a coherent statewide correlational pattern emerge between the first five principle components present and the other environmental indicators.

Lack of resolution with the Biolog system could be attributed to the inoculation of samples with low cell densities (total HPC mean was 319 CFU/ml). When a sample with a low cell density, such as 40 CFU/ml (our lowest count), is inoculated into the 150 μl microtiter wells, the probability of at least one of the individual wells not receiving any cells is 21% (Poisson distribution). A minimum of 80 cells per ml is necessary for complete well
coverage (99.99%). But even this figure only supposes at least one cell per well, which is not a broad representation of the microbial population. Clearly, the low cell densities of pristine freshwater streams are problematic for the Biolog system and its utility in interpreting stream condition. The apparent solution to this dilemma is to enrich the samples before inoculating the Biolog plates, yet this procedure increases the time, costs, and labor of the assay. Konopka et al (1998) also argue that the inoculation of low cell densities into the substrate wells means that the technique is a culture-based method in which any enrichment culture may bias the test, causing the results to be unrepresentative of the original native flora. Nevertheless, future studies may find the Biolog system to be more practical in characterizing heavily polluted waters.

Our research has shown that traditional public health microbial indicators can be useful in measuring the effects of anthropogenic stress over large spatial scales. HPC bacteria correlate highly with other indicators of stream condition. The heterotrophic plate count, often used in regulatory analysis of drinking and recreational waters, has no legal regulatory status because bacteria detected in this analysis do not necessarily represent sanitary pollution (Clark, 1980; APHA, 1992). Nevertheless, high HPC densities raise concerns about the presence of possible opportunistic pathogens (Lye and Dufour, 1991).
The fecal coliform and E. coli results strongly reflect human disturbance by their presence as well as their absence. For example, the nine sampling sites with no detectable E. coli presence had significantly (t test; p<0.05) lower levels of DOC, total N, total P, agriculture and pastureland disturbance when compared to the entire statewide set. These zero E. coli counts might imply a lack of anthropogenic stress in the form of domestic waste on the aquatic system. E. coli, the most common species within the fecal coliform group, are only present in waterways due to animal or human feces (APHA, 1995) and their presence in freshwaters correlate strongly to attacks of swimming related gastroenteritis (Dufour, 1984). Therefore, they act as two kinds of indicators—one for public health regulation and another for measuring stress on lotic systems.

A suite of environmental indicators is necessary to ascertain the complex chemical, physical, and biological interactions within an aquatic ecosystem. Microbial organisms play a key ecological role in the stability of these systems and demonstrate great utility as bioindicators of stream condition. Further research is under way to explore the relationship between these various indicators in other aquatic systems and regions. Biological indicators, such as fish or invertebrate indices, are important components of long term measurements of ecosystem health (Karr, 1981) and should be included in these studies.
Conclusions

This study correlated traditional public health water quality bioindicators and Biolog system results with chemical and physical habitat data to evaluate their effectiveness as environmental indicators of stream condition. Comparisons were made both within the state of Oregon and between four ecoregions within the state. The following conclusions were produced:

1. Many traditional public health indicators may be useful in defining the environmental condition of rivers and streams. HPC bacteria significantly correlated with most of the chemical and physical habitat environmental indicators throughout the state of Oregon. Fecal coliform and *E. coli* significantly correlated with most of the chemical indicators of anthropogenic stress. Total coliforms were not sensitive to these types of comparisons.

2. Principle components failed to identify relationships between the Biolog system and all other indicators, possibly due to low inoculum density.

3. Ecoregion correlations with environmental indicators worked very well for the Eastern Oregon and the Cascades aggregates. The results were ambiguous for the Coast Range and the Willamette Valley. This was perhaps due to the extremes in human disturbance that the two former regions represented in this study, whereas the later two areas were much less uniform in their overall disturbance along their sampling sites.
Recommendations

1. Further investigation into the use of these microbial indicators to assess the environmental condition of streams and rivers should be explored in larger areas and with greater sample sizes throughout the United States. A larger sample size would improve statistical accuracy of the survey.

2. Other public health microbial indicators, such as members of the fecal streptococcus group, should be evaluated as possible bacterial indicators of environmental condition.

3. Environmental indicators of anthropogenic stress may be made more meaningful when sampling sites are divided by ecoregions (level 3). However, due to the broad areas that may drain into a river, smaller subregions (level 4) should be explored in order to reduce variability.

4. The Biolog system may prove unsuitable for evaluating the environmental condition of rivers and streams unless the problem of low inoculum densities is resolved.

References


Garland, J.L. and A.L. Mills. 1991. Classification and Characterization of Heterotrophic Microbial Communities on the Basis of Patterns of


Figure 1. 1997 and 1998 EMAP river sampling locations.
Correlations

Log HPC
Log TC
Log FC
Log EC
Fish Anomaly %

Physical Habitat Responses
Elevation
Temperature
Riparian Complexity Index
% Canopy Presence
Bankside Canopy Density
Agriculture Disturbance Index
Pavement Disturbance Index
Road Disturbance Index
Pipe Disturbance Index
Pasture Disturbance Index
Total Disturbance Index

River Chemistry
pH
Dissolved O2
Specific Conductance
Acid Neutralizing Capacity
Dissolved Organic Carbon
Total Nitrogen
Total Phosphorus
SiO2
NO3-N
NH3-N
SO4
Chloride

Figure 2. Spearman's Rank correlations of bacterial counts with physico-chemical habitat data in 43 Oregon rivers.
Figure 3. Stepwise regression of Log HPC with pH, dissolved oxygen, and total nitrogen. \( R^2 = 0.60 \). F ratio 19.5 (p < 0.0001). This subset of regressors was the best predictor of HPC bacteria.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Maximum</th>
<th>Minimum</th>
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</thead>
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<tr>
<td>HPC/1ml</td>
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<td>453</td>
<td>2600</td>
<td>2.2</td>
</tr>
<tr>
<td>TC/100ml</td>
<td>124</td>
<td>184</td>
<td>1100</td>
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<td>FC/100ml</td>
<td>38</td>
<td>61</td>
<td>310</td>
<td>0</td>
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<td>EC/100ml</td>
<td>31</td>
<td>44</td>
<td>200</td>
<td>0</td>
</tr>
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</table>

Table 1. Bacterial summary from 43 sampling sites of Oregon rivers in 1997 and 1998.
<table>
<thead>
<tr>
<th>INDICATOR</th>
<th>EAST</th>
<th>CASCADES</th>
<th>WILLAMETTE</th>
<th>COAST RANGE</th>
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</thead>
<tbody>
<tr>
<td>LOG HPC</td>
<td>37 (2.9) a</td>
<td>12 (3.8) b</td>
<td>15 (2.8) b</td>
<td>22 (3.9) b</td>
</tr>
<tr>
<td>LOG FC</td>
<td>35 (2.8) a</td>
<td>9.8 (3.7) c</td>
<td>28 (2.8) ab</td>
<td>19 (3.9) bc</td>
</tr>
<tr>
<td>LOG EC</td>
<td>35 (2.6) a</td>
<td>9.8 (3.5) c</td>
<td>26 (2.6) ab</td>
<td>17 (3.6) bc</td>
</tr>
<tr>
<td>Fish Anomalies</td>
<td>16 (2.6) b</td>
<td>8.6 (3.5) b</td>
<td>30 (2.6) a</td>
<td>18 (3.6) ab</td>
</tr>
<tr>
<td>Temperature (C)</td>
<td>19 (2.3) a</td>
<td>4.3 (3.1) b</td>
<td>24 (2.3) a</td>
<td>28 (3.2) a</td>
</tr>
<tr>
<td>Index of Riparian Complexity</td>
<td>5.9 (2.3) b</td>
<td>14 (3.0) b</td>
<td>35 (2.2) a</td>
<td>28 (3.1) a</td>
</tr>
<tr>
<td>% of Canopy Presence</td>
<td>4.4 (2.5) b</td>
<td>24 (3.3) a</td>
<td>25 (2.4) a</td>
<td>27 (3.4) a</td>
</tr>
<tr>
<td>Bankside Canopy Density</td>
<td>5.5 (2.6) b</td>
<td>24 (3.5) a</td>
<td>27 (2.6) a</td>
<td>22 (3.6) a</td>
</tr>
<tr>
<td>Agricultural Disturbance Index</td>
<td>30 (2.7) a</td>
<td>9.4 (3.5) b</td>
<td>17 (2.6) b</td>
<td>19 (3.6) ab</td>
</tr>
<tr>
<td>Road Disturbance Index</td>
<td>28 (3.1) a</td>
<td>18 (4.1) ab</td>
<td>8.9 (3.0) b</td>
<td>25 (4.2) a</td>
</tr>
<tr>
<td>Pasture Disturbance Index</td>
<td>32 (2.6) a</td>
<td>11 (3.4) b</td>
<td>17 (2.5) b</td>
<td>22 (3.6) ab</td>
</tr>
<tr>
<td>pH</td>
<td>33 (2.5) a</td>
<td>17 (3.3) bc</td>
<td>10 (2.4) c</td>
<td>26 (3.4) ab</td>
</tr>
<tr>
<td>Dissolved O\textsubscript{2}</td>
<td>6.6 (3.0) b</td>
<td>20 (4.0) ab</td>
<td>28 (3.0) a</td>
<td>22 (4.2) ab</td>
</tr>
<tr>
<td>Specific Conductance</td>
<td>39 (2.2) a</td>
<td>10 (3.0) bc</td>
<td>18 (2.2) bc</td>
<td>23 (3.1) b</td>
</tr>
<tr>
<td>Acid Neutralizing Capacity</td>
<td>32 (2.5) a</td>
<td>14 (3.3) b</td>
<td>14 (2.4) b</td>
<td>22 (3.7) ab</td>
</tr>
<tr>
<td>Dissolved Organic Carbon</td>
<td>40 (2.1) a</td>
<td>5 (2.7) c</td>
<td>24 (2.0) b</td>
<td>22 (2.8) b</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>34 (2.7) a</td>
<td>6.2 (3.6) b</td>
<td>25 (2.7) a</td>
<td>24 (3.8) a</td>
</tr>
<tr>
<td>Total Phosphorus</td>
<td>35 (3.3) a</td>
<td>24 (4.4) ab</td>
<td>21 (3.2) b</td>
<td>16 (4.5) b</td>
</tr>
<tr>
<td>SiO\textsubscript{2}</td>
<td>37 (2.4) a</td>
<td>33 (3.1) a</td>
<td>14 (2.3) b</td>
<td>15 (3.2) b</td>
</tr>
<tr>
<td>Chloride</td>
<td>21 (3.1) ab</td>
<td>12 (4.1) b</td>
<td>26 (3.0) a</td>
<td>30 (4.2) a</td>
</tr>
<tr>
<td>NO\textsubscript{3}N</td>
<td>25 (2.9) ab</td>
<td>13 (3.8) b</td>
<td>27 (2.8) a</td>
<td>25 (3.9) a</td>
</tr>
</tbody>
</table>

Table 2. Mean scores of ecoregions with a significant ANOVA for various environmental indicators (p<0.05). The standard error of the mean is in parenthesis. Significant differences between ecoregions are denoted by different letters (ex. a, b, or c).
Figure 4. Principle component analysis of Biolog patterns of four Oregon ecoregions at 0.75 average well color development (AWCD).
BIBLIOGRAPHY


