AN ABSTRACT OF THE THESIS OF

William James Kelch for the degree of Master of Science

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Title: DRUG RESISTANCE, SOURCE, AND ENVIRONMENTAL FACTORS THAT INFLUENCE FECAL COLIFORM LEVELS OF TILLAMOOK BAY

Abstract approved: Dr. Jong S. Lee

In order to determine the source of bacteria in Tillamook Bay, Oregon, water samples were collected monthly for six months during the rainy season from October 1975 through March 1976 from the bay and its tributaries, the Kilchis, Trask, Tillamook, and Wilson Rivers.

Fecal coliform levels of these samples were determined and the 1,917 bacteria isolated were tested for their resistance patterns to chloramphenicol (Cm), streptomycin (Sm), ampicillin (Am), tetracycline (Tc), chlortetracycline (Ct), oxytetracycline (Ot), neomycin (Nm), nitrofurazone (Ni), nalidixic acid (Na), sulfathiazole (Su), kanamycin (Km), and procaine penicillin G (Pe).

The fecal coliform count per 100 ml of bay water ranged from 3.6 to 42.0. The counts for Tillamook River ranged from 13.5 to 112.0, Trask River from 0.0 to 132.0, Wilson River from 8.5 to 105.0, and Kilchis River from 0.5 to 13.9. The rise and fall of fecal coliform levels were characteristic of the sampling date and each

sampling station showed its characteristic maximum and minimum levels.

The 1,917 fecal coliform isolates showed 176 different resistance patterns to the 12 antibiotics tested. None of the patterns, however, was characteristic of any specific sampling site.

The fecal coliform counts of the bay were statistically compared to 135 independent variables that included the fecal coliform counts of tributaries, temperature, river flow data, tide information, antibiotic use data, and the antibiotic resistance patterns.

Bay fecal coliform levels were highly correlated with the fecal coliform counts of tributaries especially those of the Trask and Wilson Rivers, degree of resistance to antibiotics, recreational activities, and precipitation. Negative correlation existed between bay fecal coliform count and the ambient temperature.

Two potentially useful linear regression models to predict bay fecal coliform level were developed using a computerized stepwise multiple linear regression program.

Drug Resistance, Source, and Environmental Factors That Influence Fecal Coliform Levels of Tillamook Bay

by

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INTRODUCTION

Fecal contamination of waterways is generally considered undesirable. It detracts from nature's aesthetic beauty, alters entire ecosystems by changing the types of fish and fauna, or, conceivably, eliminates fish and fauna altogether, and potentially provides a threat to the public health. The recent intensity of "environmental consciousness" in our society and the world has tasked the scientific community to provide more specific information regarding the effects of fecal contamination on our environment.

Notwithstanding this demand for information, our scientific knowledge of the effects of fecal contamination on our environment is woefully lacking. Given that a certain amount of fecal material from wildlife is ecologically "normal," just how much is too much and just how do we define "normal" vs. "abnormal" levels? Our ability to answer this question depends upon an accurate, easily-obtained, and efficient indicator of fecal contamination. Unfortunately, such an indicator system does not exist.

The literature review will present the myriad indicators of fecal contamination which have been proposed. This study will describe efforts to use a fecal coliform indicator system in describing fecal contamination in an Oregon estuary. Attempts to expand the use of this indicator system by determining the antibiotic resistance patterns

of these fecal coliform organisms will be described. Results will be analyzed statistically with attempts made to predict estuarine fecal coliform counts from other environmental and bacteriological information, the genetic nature of the antibiotic resistance will be explored, and the study's implications for the survival of fecal coliforms in an estuarine environment will be discussed. This study was a part of a six month water quality survey conducted in Tillamook, Oregon during the rainy season from October, 1975 through March, 1976.

LITERATURE REVIEW

Indicator Systems

Coliform Organisms

The coliform organisms have long been used as indicators of fecal contamination for fresh water, sea water, and wastewater effluents, and, in fact, have been incorporated into generally accepted standard procedures (2,3). The coliforms belong to the family Enterobacteriaceae and include all aerobic or facultative anaerobic, gram-negative, nonsporeforming rods which ferment lactose with gas formation within 48 hours at 35°C. The enumeration of these organisms by most probable number or membrane filter methods has been a commonly used indicator system for many years, but this "total coliform" method suffers from the inclusion of microorganisms of nonfecal origin. Consequently, efforts to refine this method to exclude nonfecal coliforms have been made. A new "fecal coliform" method has been introduced which, by elevating the incubation temperature to 44.5°C and using a specific selective medium, excludes many, but not all, nonfecal coliforms (11,40,51). This method also employs either a most probable number or Millipore filter technique (42,61) and has been adopted as a standard method (2,3). Many efforts have been made to evaluate and refine these methods, particularly the membrane filter techniques. These include testing the membrane filter method using Escherichia coli as a test organism (58), using a two-layer agar method to recover fecal coliforms (99), studying the characteristic fecal coliform recoveries using different membrane types and procedures (36,49,102,104,117), and refining the medium used with

membrane filter techniques (103). The influence of coliform source on membrane filter procedures has been discussed (19), and efforts to enumerate the component genera of coliforms recovered on membrane filters have been made (30). Efforts have also been made to relate the presence of coliform indicator organisms with the presence of pathogenic microorganisms (23,112,113).

Other methods for enumerating coliform organisms have been devised. These include a chromatographic method (89), a method using coliform-specific bacteriophages (62), and a radiometric method (102).

The wide use of coliform indicators as reasonably acceptable indicators of fecal pollution is reflected by the number of comprehensive studies which have been based wholly or in part upon coliform indicator systems (23,36,54,70,90,101,112,113,114). The relatively widespread use of coliform indicators has also prompted numerous studies of the persistence of this group of organisms in various environments including sediments (44,50), seawater (100,111), surface-drainage water (34), sewage (17,45), sewage sludge (32), fresh-water (13,27,85,121), and estuarine water (33,37,63,120). The role of predation in the persistence of coliform indicators has been studied (33,63), and other studies have discussed the effects of time, nutrient depletion, water temperature, dissolved oxygen, salinity, chemical pollutants, dissolved ions and suspended particulate matter on the survival of coliform bacteria (16,37,63,96,134). Both deterministic and statistical models using coliform indicators have been developed to predict changes in water quality (22,73) or to relate environmental factors to numbers of coliform organisms (16).

Fecal Streptococci

The fecal streptococci are the second most commonly used indicator of fecal water pollution. These organisms are defined as those which produce dark red to pink colonies on agar containing sodium azide and bromcresol purple indicator after 48 hour incubation at 35°C. They include: Streptococcus faecalis; S. faecalis var. liquefaciens; S. faecalis var. zymogens; S. durans; S. faecium; S. bovis; and S. equinus. The enumeration of fecal streptococci is included in standard methods (2). Fecal streptococci count data can be used alone as an indicator of fecal pollution, but they are more commonly used as an adjunct to the use of coliform indicators.

As with coliform indicators, fecal streptococci can be enumerated by either a most probable number or membrane filter technique. A direct plating technique has also been developed (93), an overlay technique has been studied (25), and the efficacy of various media has been compared (20,25,93). Efforts have been made to relate the presence of fecal streptoccoci to the presence of bacterial pathogens (23,112,113). In-depth studies of certain areas have been conducted using fecal streptococci as indicators (23,27,101), and efforts have been made to establish the effects of various environmental stresses upon the survival of fecal streptococci (13,16,27,85,119,120).

Clostridia

Numerous reports indicate the usefulness of clostridia as an indication of fecal contamination. A proportional relationship has been demonstrated between numbers of Clostridium perfringens in marine

sediments and the amount of fecal pollution (14,79). The use as a fecal pollution indicator of sulfite-reducing anaerobic sporeformers has been suggested because they are ultimately of fecal origin, cannot multiply in nature, and their numbers in sediments correlate well with distance from untreated sewage outlets (14,15). The clostridia flora of sewage effluent has been described (103) and it has been suggested that sewage effluent is a primary source of clostridia in marine sediments (77). Although "background" or "baseline" levels of clostridia exist in unpolluted waters, a close relationship appears to exist between the degree of fecal pollution and numbers of clostridia (76). The vegetative cells of C. perfringens die off rapidly below ambient temperatures. Since the water temperature off-shore is usually colder than that of inshore, the presence of C. perfringens there was considered to imply serious encrochment of fecal pollution (10). Clostridia indicators have not been widely accepted as fecal contamination indicators, but they may be very useful in certain well defined environments such as near-shore sediments.

Miscellaneous

Several other indicators of fecal pollution have been proposed although none have been widely accepted as yet. These include the biochemical measurement of fecal steriods (29), a nematode indicator (82), bacteriophage indicators (64), a Candida albicans indicator (60), bacterial spore indicators (94), and the measurement of serum antibody titers in bottom feeding catfish (118).

Point Source Indicators

The above indicators are all used to quantitatively determine

the extent of fecal contamination in a body of water, but none are proposed as guides to the actual point source of this contamination. Streams, rivers, lakes, estuaries, etc. receive fecal material from myriad sources including wildlife, public recreational activities, domestic animals, privately treated human waste, publicly treated human waste, industrial waste, commercial shipping, private boating, and others. The knowledge that a waterway is contaminated with fecal material has limited usefulness without the concurrent knowledge of the source of that contamination. Little success has been achieved in this area.

The conjunctive use of fecal coliform (FC) and fecal streptococci (FS) indicators has been proposed as a useful tool in distinguishing between human and non-human sources of fecal contamination (40,41,42). An FC:FS ratio less than 0.7 was shown to indicate fecal contamination by domestic farm animals while a ratio over 4.0 indicated human sources. A ratio between 0.7 and 4.0 was considered equivocal. The ratio, however, changes with time because the die-off rate of FC is faster than that of FS. Therefore the method is only proposed to be reliable within the first 24 hours subsequent to discharge of the bacteria into the water. This limits the usefulness of this indicator system since it is often difficult or impossible to ascertain the age of the bacteria being discharged. In fact this limitation has led some to conclude that this method is of little value in determining the source of domestic sewage (80). Others have suggested that this differential die-off characteristic strengthens rather than weakens the usefulness of this method (38). The FC:FS has been extensively used as a research tool

in numerous studies (23,101,112,113,132).

The usefulness of the FC:FS ratio is limited because it is not a direct indicator of the source. It suggests whether the fecal material is of human or non-human origin, but, nonetheless, it is still an indirect measure.

The O-antigen typing of Escherichia coli is a potentially useful tool in identifying the point source of fecal contamination. The presence of a particular O-antigen type in a contaminated waterway and the concommitant presence of that same O-antigen type in only one other location would suggest that the contamination source was at that location. A specific point source indicator system using O-antigen types has not been proposed, although several studies provide information that might aid in the development of such a system.

It has been suggested that "human" and "animal" E. coli are antigenically distinct (12). If so, a useful indicator system using O-antigen typing might be developed to distinguish "animal" and "human" sources, and, conceivably, even pinpoint a specific source location. However, other studies suggest that there is considerable overlap in O-antigen types between human and animal populations (53,56). Another study presents additional O-antigen typing data for cattle (57). It should be noted that this considerable overlap of O-antigen types between humans and animals is not necessarily fatal to the development of a point source indicator, since one absolutely unique animal antigen type and one similarly unique human antigen type could provide a reliable system.

Bacteriophage typing could be used in conjunction with antigen

typing or it might be used independently. No systematic effort, however, has been made in this regard.

Antibiotic resistance patterns of E. coli or other bacterial species might provide a useful point source indicator system. In the same fashion as O-antigen typing, the presence of an E. coli organism with a particular antibiotic resistance pattern in a contaminated waterway and the simultaneous presence of another E. coli organism with the same antibiotic resistance pattern in another location would suggest that the contamination source was at that location. This type of tracer technique has not been used in water quality studies, although this principle has been used in an epidemiological investigation of a salmonellosis outbreak (24).

Other workers have reported that the incidence of coliform organisms with transferable drug resistance (R + coliform organisms) was notably higher in hospital sewage than in city sewage (46). It was suggested that this might serve to differentiate contamination from hospital sources from that of city sewage sources. Similarly, a high incidence of coliform organisms resistant to both streptomycin and tetracycline was found in effluents from human and domestic animal sources (116). It was suggested that this might serve to differentiate domestic from non-domestic sources of fecal contamination.

Antibiotic Resistance

Some bacteria were antibiotic resistant prior to the emergence of widespread clinical and other uses of antibiotics. The presence of penicillinase producing bacteria was noted as early as 1940 (1). Other enzyme systems have been demonstrated to inactivate chloramphenicol

and aminoglycoside antibiotics (28) as well as adenylating enzymes which inactivate streptomycin and other aminoglycoside antibiotics (91). Although other antibiotic inactivation mechanisms may exist, it has been generally concluded that enzyme inactivation is the primary mechanism (95), and it has been presumed that this enzyme producing capacity is, in most cases, genetically determined (98,126).

The genetic determinant which enables a bacterium to produce these antibiotic inactivating enzymes may be contained in the bacterial chromosome or may be found in extrachromosomal genetic packages called R-factors which are specialized plasmids (48,75,107,123,124,125,126, 133). The origin of this genetic resistance remains unknown, but, presumably, the chromosomal resistance has arisen by mutation within the bacterial chromosome (26,48,107,126). The origin of R-factors is similarly obscure. Several hypotheses have been proposed.

It has been suggested that the autonomous extrachromosomal genetic material may have temporarily become integrated into the host cell chromosome, have associated itself with chromosomal genetic material which coded for antibiotic resistance, and then have permanently detached itself from the host cell chromosome carrying with it the genetic material which produces antibiotic resistance (123,127). Since R-factors have also been shown to combine with other episomal elements (a class of plasmids which are capable of combining with the host cell chromosome) (52,130), it is also possible that the extrachromosomal combination of genetic elements might have resulted in R-factor formation (127). Similarly, mutations have been observed in R-factors (84,123),

and the possibility has been presented that R-factors might have arisen by mutation of existing extrachromosomal genetic elements (127). The origin of episomes has also been likened to viruses (4), therefore the origin of R-factors could have been similar to that of viruses (59, 127). Regardless of origin, evidence strongly suggests that R-factors were present in the bacterial population before man harnessed antibiotic agents to his use (105). The origin of antibiotic resistance in bacteria has been the subject of in-depth review (98,127).

The transferable (infective) nature of bacterial antibiotic resistance was first demonstrated in the late 1950's and early 1960's in Japan. This early work was comprehensively reviewed by Watanabe (123) in 1963. Subsequently, numerous reports have been made of resistance transfer between different strains of the same bacterial species as well as between different bacterial species (7,8,9,26,31,39,46,66,69,75,81, 83,88,106,108,109,110,115,116,122,128,131). Organisms with infective antibiotic resistance have been isolated from various sources which include: fowl (69,106,108), man (9,21,26,69,75,88,106,108), swine (66,81,106,122), raw meat (9), fresh water (39,109,131), estuarine water (39), cattle (31,66,81,106,108,122), coastal beaches (110), sewage effluents (46,115,116), fish (7,105), and sheep (106). Bacterial genera which have demonstrably been involved in this resistance transfer include: Vibrio (7,8,131), Escherichia (7,8,9,31,39,46,66,69,75,81,83, 106,108,109,110,115,116,122,128,131), Citrobacter (115,131), Enterobacter (131), Aeromonas (8,131), Pseudomonas (131), Staphylococcus (21), Salmonella (26,31,39,75,88,109,128), Klebsiella (115), and Shigella

(39,128).

Three mechanisms account for the transfer of genetic material and the consequent transfer of antibiotic resistance between bacterial strains. Transformation is a process in which deoxyribonucleic acid (DNA) is excreted from one bacterial cell, is transported naked through an intervening medium, and is then incorporated into a recipient cell. This mode of transfer has been reviewed thoroughly by Hotchkiss and Gabor (55). This mode of transfer is probably of little importance in the natural environment since naked DNA is extremely vulnerable outside the cell. Transduction is a process in which a bacteriophage enters a host cell, incorporates part of the host cell DNA into its genome, and subsequently transfers this DNA into a recipient cell. This mechanism has also been comprehensively reviewed by Ozeki and Ikeda (92). Conjugation is a process in which DNA passes from the donor cell to a recipient cell during a mating process in which the donor cell synthesizes specialized structures called sex pili which directly contact the recipient cell. This mechanism has been discussed at length by Brinton (18), and all three mechanisms have been compared by Richmond (98) and Watanabe (126). Transduction apparently plays a role in gene transfer particularly among gram positive cocci (65) and conjugation is apparently the predominant mechanism for gene transfer among the enteric bacteria and pseudomonads (5,97).

GENERAL DESCRIPTION OF TILLAMOOK BAY AREA

Geography and Climate

Tillamook, Oregon is located on the northeastern shore of Oregon approximately 50 miles south of the Columbia River, 70 miles north of Yaquina Bay, and 75 miles southwest of Portland. A map showing Tillamook's general location and a more detailed map of the Tillamook area are presented in Figures 1 and 2.

Three population centers surround the Tillamook Bay estuary: Garibaldi to the north, Bay City to the east, and Tillamook to the southeast. The population of Tillamook County in 1970 was approximately 18,000 persons about 11,000 of whom resided in the immediate Tillamook Bay area. The approximate 1970 populations of Garibaldi, Bay City, and Tillamook were 1100, 900, and 4000, respectively.

Tillamook has a Pacific marine coastal climate characterized by wet winters, dry summers, and a relatively narrow range of seasonal temperature variations. Winter storms frequently result in large amounts of precipitation in short periods of time. The Tillamook drainage basin receives about 115 inches of precipitation annually with ranges from 90 inches in the city of Tillamook to 150 inches at higher elevations. Seventy percent of this annual precipitation occurs between November and March. The average January temperature is 42^{o}F; the average July temperature is 58^{o}F; the daily mean maximum temperature average is 67^{o}F for the summer months of July, August, and September; and the daily mean maximum temperature average is 52^{o}F for the winter months of November, December, January, and February. This demonstrates the relatively stable year

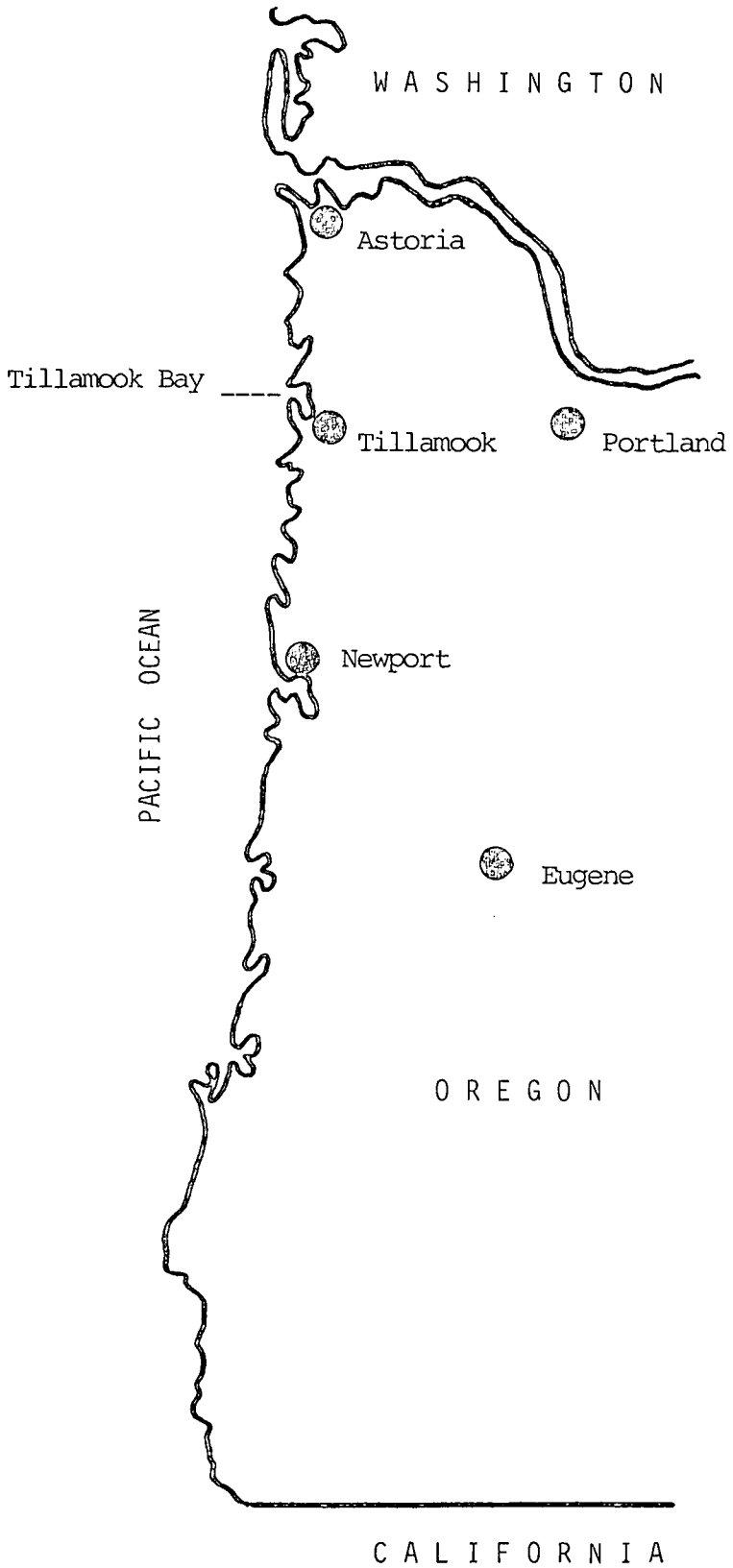

Figure 1. Map showing general location of Tillamook, Oregon.

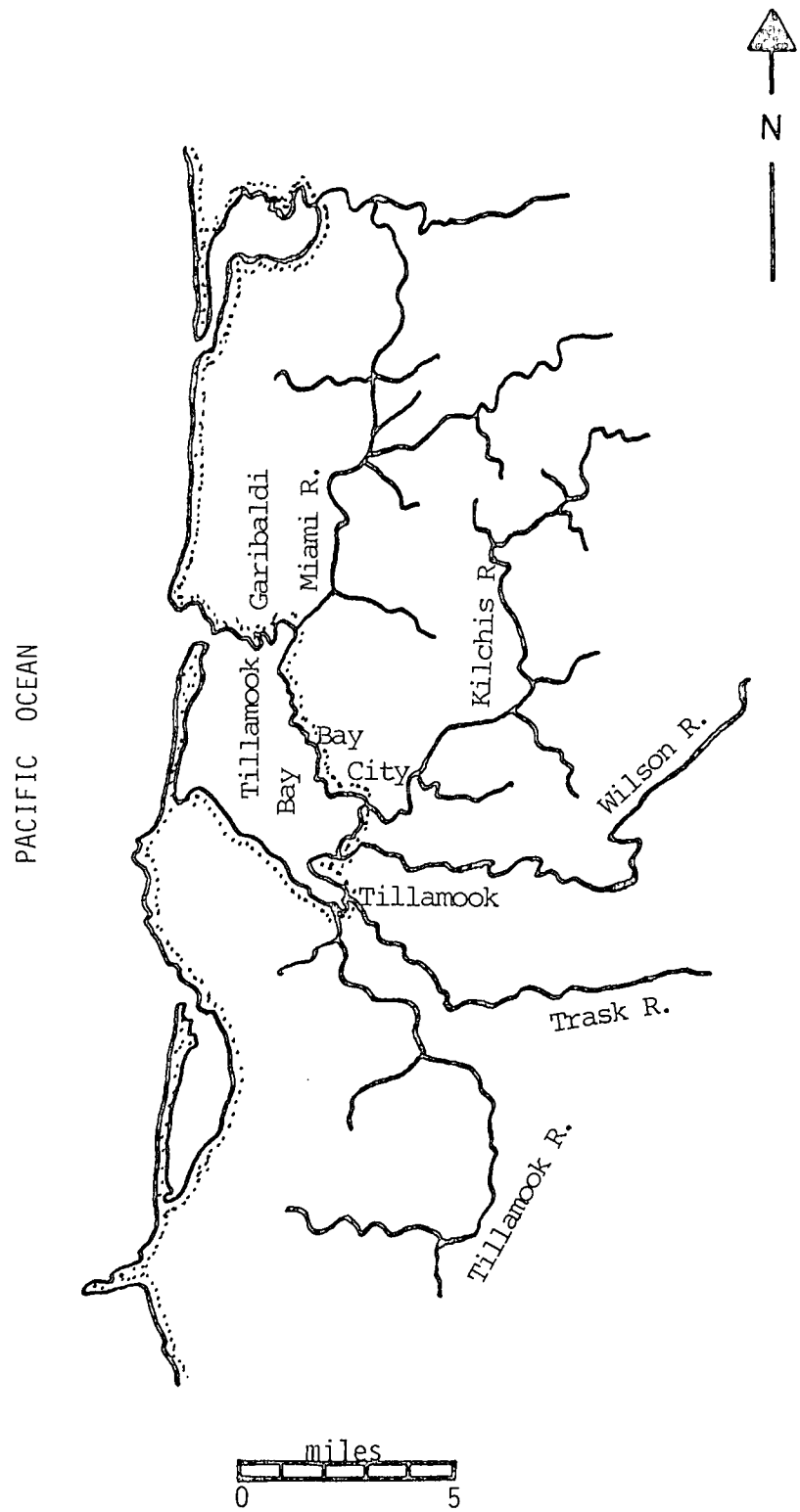

Figure 2. Map showing Tillamook Bay area..

round temperatures. Fog is common, particularly during the night and early morning, but freezing seldom occurs near the estuary resulting in a 190 day growing season with no killing frosts.

The terrain in the Tillamook area, excluding the flood plains, is very steep with a small percentage of land having slopes less than 20%. If the flood plains are included, over 38% of the land has less than a 3% slope, and, of this land, about 4% is either in the flood plains themselves or on the Bayocean peninsula. These flood plains include large pasture areas immediately adjacent to the bay which are frequently flooded during the rainy season. To the east of Tillamook is a coastal range of mountains with elevations to about 3000 feet.

Prevailing winds are from the southwest in the fall and winter months and from the northwest in the spring and summer months. During winter storms, winds frequently reach 50 miles per hour and occasionally exceed 100 miles per hour.

Estuary and Rivers

The Tillamook Bay estuary is the second largest estuary on the Oregon coast. It is about three miles wide and six miles long with approximate surface water area of 12-14 square miles at high tide. Of this approximately 8,800 acres, about 5,100 acres is tideland acreage. It is a very shallow bay with tide ranges from 0.0 feet at mean lowest low water to 7.5 feet at mean highest high water. The lowest tide is about minus three feet and the highest is 11.0 feet. The average lagoon depth at mean sea level is five feet.

There are five major watersheds as well as numerous streams and

sloughs which enter the Tillamook Bay. At the northern end of the bay is the Miami River which enters the bay through a 12 mile long narrow valley beginning in the coastal range to the northeast. At the southeastern end of the bay, four rivers, each of which arises in the coastal range, enter the bay within about 2 miles of each other. These are: the Kilchis River which originates about 17 miles to the northeast; the Wilson River which originates about 40 miles to the east; the Trask River which originates about 35 miles to the east; and the Tillamook River which originates about 15 miles to the southsoutheast. A broad flood plain exists in the area where these rivers discharge into the bay. The discharges of these rivers vary dramatically on a seasonal basis. Average summer discharges of all five rivers is 455 cubic feet per second (c.f.s.) while average winter flows are 28,300 c.f.s. Table 1 includes the seasonal flow rates for the rivers included in this study.

The Kilchis River drains at its mouth approximately 67 square miles (43,000 acres) and its annual discharge is approximately 350,000 acre feet. Approximately 320 persons not serviced by municipal sewage treatment facilities reside in its drainage basin. The Wilson River drains at its mouth approximately 193 square miles (124,000 acres) and its annual discharge is approximately 1,100,000 acre feet. Approximately 1000 persons not serviced by municipal sewage treatment facilities reside in its drainage basin. The Trask River drains at its mouth approximately 176 square miles (113,000 acres) and its annual discharge is approximately 850,000 acre feet. Approximately 2000 persons not serviced by municipal sewage treatment facilities reside in its

TABLE 1. Average monthly (80%) river flow[a] (cfs)[b].

	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
Kilchis	87	487	771	700	701	556	346	175	86	52	36	23
Wilson	227	1276	2020	1832	1835	1457	906	458	226	135	95	59
Trask	168	901	1548	1501	1496	1249	776	419	238	145	101	76
Tillamook	65	363	574	520	521	414	257	130	64	38	27	17

[a] Appendices, Proposed Water Quality Management Plan, North Coast-Lower Columbia River Basin, State of Oregon, Department of Environmental Quality, 1976.

[b] cfs = cubic feet per sec.

drainage basin. The Tillamook River drains at its mouth approximately 61 square miles (39,000 acres) and its annual discharge is approximately 315,000 acre feet. Approximately 800 persons not serviced by municipal sewage treatment facilities reside in its drainage basin.

These rivers and the bay itself provide year round recreational facilities which include boating, ocean, bay, and river fishing, crabbing, clamming, beachcombing, hiking and camping. Numerous parks and camping areas are provided along the river basins. The magnitude of recreational use is illustrated by angler-day[1] totals for 1970 of 7,900, 55,300, 31,300, and 4,400 for the Kilchis, Wilson, Trask, and Tillamook Rivers, respectively. A large marina at Garibaldi services both commercial and privately-owned boats.

Fish and Wildlife

Phytoplankton, zooplankton, and various crustacea are the primary food sources for commercial and sport estuarine fisheries and for offshore fisheries. A large population of benthic organisms includes burrowing worms, clams, and others. Other bay residents include salmon, steelhead, cutthroat trout, flounder, ling cod, sculpin, greenling, Pacific herring, northern anchovy, shad, dungeness crab, red rock crab, oysters, clams, and shrimp. Marine mammals including hair seals, fur seals, and sea lions are frequently seen in the north bay areas.

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[1]An angler-day is one angler fishing for four hours.

Three distinct marshland areas border Tillamook Bay. These include an area at Biggs Cove at the southern extreme of the Bayocean peninsula, a second at the Miami River delta, and a third on the delta between the Wilson and Kilchis rivers. The latter is by far the largest. These marshlands provide a habitat for over 30 species of waterfowl and 20 species of shorebirds. Ducks are abundant.

The flood plains surrounding the estuary are populated by deer, elk, and smaller mammals such as the meadow mouse and shrew. Similar wildlife are found in upland areas with the addition of bear to the list of large mammals. The flood plains also provide shelter for waterfowl, particularly ducks. Oregon Department of Fish and Wildlife estimates include 2300 elk, 11,000 deer, 300 bear, and 1300 coyote in the drainage areas of the Kilchis, Trask, Tillamook, and Wilson Rivers. This total population is apportioned about equally among these river drainage areas on an animal per square mile basis with a slightly larger deer and bear population in the Trask River basin and a slightly larger elk population in the Wilson River basin. Deer and elk are somewhat less abundant in the Tillamook River drainage area.

Industry

Lumbering, fishing, shellfishing, cheese manufacture, and tourism are the major industries in Tillamook. There are four major wood processing plants in the Tillamook Bay area and commercial fishing activities are conducted from Garibaldi. Some limited barge shipping is also conducted from Garibaldi. Cheese manufacture is an important industry supporting both processing workers and the related dairy industry.

Approximately 2650 acres of the Tillamook Bay estuary are leased from the Oregon Fish Commission for use in oyster cultivation. Oysters are not natural inhabitants of Tillamook Bay but will grow when seeded. About 80% of Oregon's annual oyster harvest of 120 tons comes from Tillamook Bay. The shellfish industry is directly affected by bacteriological standards for harvesting waters established by various governmental agencies including the Health Division, Oregon Department of Human Resources, the Oregon Department of Environmental Quality, and the Public Health Service, Food and Drug Administration.

Domestic Animals

The domestic animal population in the Tillamook Bay drainage area includes approximately 17,000 cattle, hogs, sheep, horses, and mules. Approximately 14,700 of these animals are dairy cows. Many of these animals graze on the low lying pastures immediately adjacent to the bay and to the rivers which enter the bay and deposit large quantities of manure on these pastures. In addition, manure deposited in animal holding areas is generally disposed of by spreading on pastures, often with liquid manure handling systems. Much of this manure finds its way into the bay via pasture runoff following rainfall and also by flooding of low-lying pasture areas directly adjacent to the bay.

Sewage Treatment Facilities

Five sewage treatment plants discharge effluent into Tillamook Bay (Figure 3). All of them provide at least secondary treatment

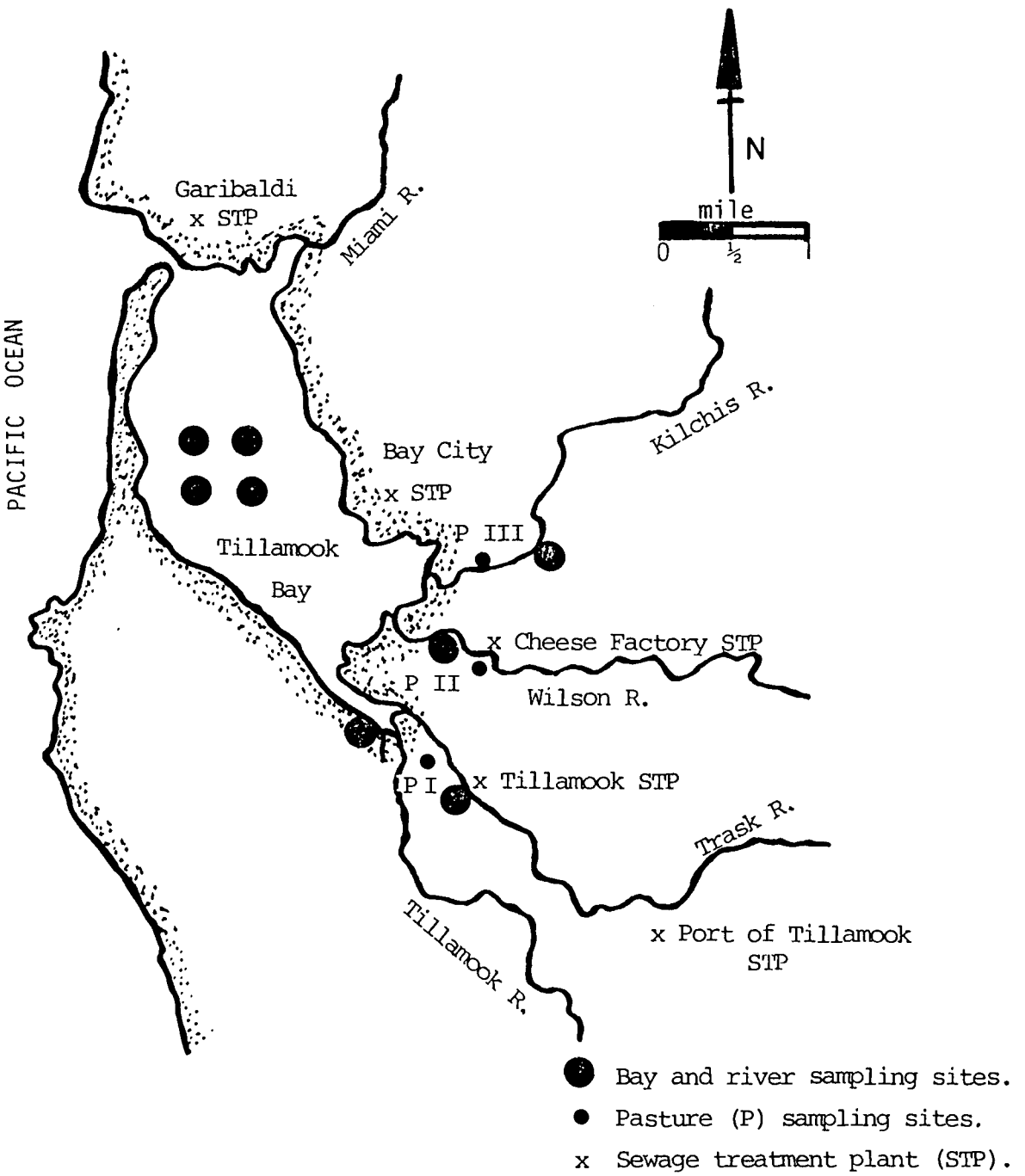

Figure 3. Map showing sampling sites and sewage treatment plants.

of domestic and industrial wastes.

The Garibaldi municipal sewage treatment plant is a complete mix-aeration, activated sludge type which serves approximately 1100 persons with effluent discharged directly into the bay. The Garibaldi plant has recently installed tertiary treatment facilities. The Bay City municipal sewage treatment plant is a lagoon type facility which serves approximately 900 persons. It discharges directly into the bay during ebbing tides. The Tillamook sewage treatment plant is a trickling filter type which serves approximately 4200 persons with discharge into the bay via the Trask River. The cheese plant sewage treatment plant processes industrial wastes including cheese waste and sanitary wastes by a complete mix-aeration method with discharge into the bay via the Wilson River. The Port of Tillamook sewage treatment plant is a lagoon type which processes wastes from one school and from nearby lumber operations. It discharges into the bay via the Trask River.

The Garibaldi, Tillamook, and Port of Tillamook plants all process storm water runoff, and, therefore, are sometimes overwhelmed during severe storms.

Miscellaneous sources of pollution for Tillamook Bay include near-shore houses, houseboats moored along the rivers and at Garibaldi, and a woodchip/paper company in Tillamook.

MATERIALS AND METHODS

Sampling Procedure

Samples were drawn monthly from the Kilchis, Trask, Tillamook, and Wilson Rivers from October, 1975 to March, 1976 as a part of a water quality study of the Tillamook Bay area during the rainy season. Monthly samples were also collected at sites representing the four corners of a square with sides equal to approximately 100 meters from the Tillamook Bay estuary approximately 1.5 miles from Bay City. In February, three water samples were taken directly from pasture areas. In March, one pasture sample was taken. Figure 3 shows the specific sampling sites.

Samples were collected in one gallon sterile Nalgene bottles by directly dipping the bottles into the surface of the water. Specific sampling dates and times are included in Table 2. Rough weather prevented the collection of a bay sample in February.

Samples were placed in insulated containers and transported to the laboratory. In all cases initial isolation procedures commenced within 2 hours of sample collection.

Isolation Procedure

Fecal coliform organisms were isolated using a standard membrane filter procedure (2). At least two different volumes of water were filtered depending on the expected fecal coliform levels.

Counting and Colony Selection Procedure

Fecal coliform colonies develop as blue colonies on an mFC medium

TABLE 2. Sampling dates, sites, and times.

DATE	SITE	TIME
16 OCT 75	Kilchis River	1145
	Trask River	1100
	Tillamook River	1025
	Wilson River	1130
	Tillamook Bay	1335
19 NOV 75	Kilchis River	1110
	Trask River	1030
	Tillamook River	1015
	Wilson River	1050
	Tillamook Bay	1430
17 DEC 75	Kilchis River	1435
	Trask River	1405
	Tillamook River	1350
	Wilson River	1420
	Tillamook Bay	1540
20 JAN 76	Kilchis River	1645
	Trask River	1715
	Tillamook River	1740
	Wilson River	1657
	Tillamook Bay	1555
17 FEB 76	Kilchis River	1025
	Trask River	0940
	Tillamook River	0905
	Wilson River	0955
	Pasture III	1030
	Pasture II	0955
	Pasture I	0940
31 MAR 76	Kilchis River	1040
	Trask River	0940
	Tillamook River	1000
	Wilson River	1025
	Tillamook Bay	1525
	Pasture III	1035

(2). Therefore, blue colonies were counted after approximately 18 hours incubation at 44.5° C. Blue colonies were selected from filter disks and transferred with sterile wooden toothpicks to 100 mm Petri plates containing tryptone-peptone-extract agar (TPE)(67). Table 3 lists the complete ingredients. Fecal coliform colonies were transferred to this plate in a pattern which permitted the subsequent use of a 30 colony per plate nichrome wire stab replicator (68).

Aerobic-heterotrophic counts were determined by the direct spread plating of water samples onto TPE agar and counting colonies after 24 hours incubation at 25°C.

Antibiotic Resistance Pattern Determination

The TPE agar base was also used to determine the antibiotic resistance patterns of fecal coliform organisms. Antibiotics were added directly to molten agar or were first diluted in distilled water and then added to agar. Table 4 lists the antibotics and their sources. Antibiotic media was prepared in the following concentrations: chloramphenicol (Cm) 2.5 ug/ml; streptomycin (Sm) 10 ug/ml; ampicillin (Am) 10 ug/ml; tetracycline (Tc) 25 ug/ml; chlortetracycline (Ct) 25 ug/ml; oxytetracycline (Ot) 25 ug/ml; neomycin (Nm) 50 ug/ml; nitrofurazone (Ni) 25 ug/ml; nalidixic acid (Na) 25 ug/ml; sulfathiazole (Su) 500 ug/ml; kanamycin (Km) 25 ug/ml; and procaine pencillin G (Pe) 75 IU/ml.

Twenty-four hour cultures of fecal coliforms were transferred from TPE agar plates with a 30 colony nichrome wire stab replicator to the antibiotic containing media, eosin-methylene-blue (EMB) agar

TABLE 3. Tryptone-peptone-extract agar[a] (TPE).

INGREDIENT	QUANTITY
Bacto-tryptone[b]	5 g
Bacto-peptone[b]	5 g
Sodium chloride, analytical reagent[c]	5 g
Yeast extract[b]	2.5 g
D-glucose anhydrous (granular), analytical reagent[c]	1 g
Distilled water	1000 ml

[a]Agar was sterilized at 121°C for 20 minutes.
[b]Difco Laboratories.
[c]Mallinckrodt Chemical Works.

TABLE 4. Antibiotics used in sensitivity testing.

ANTIBIOTIC (symbol)	TRADE NAME	MANUFACTURER
Chloramphenicol (Cm)	Chloromycetin kapseals	Parke-Davis
Streptomycin (Sm)	Streptomycin Sulfate injectable	Eli Lilly
Ampicillin (Am)	Polycillin-N injectable	Bristol
Tetracycline (Tc)	Achromycin injectable	Lederle
Chlortetracycline (Ct)	Aureomycin capsules or injectable	Lederle
Oxytetracycline (Ot)	Terramycin capsules or injectable	Pfizer
Neomycin (Nm)	Mycifradin tablets or injectable	Upjohn
Nitrofurazone (Ni)	Furacin soluble powder	Eaton
Nalidixic acid (Na)	Nalidixic acid powder, grade B	Calbiochem
Sulfathiazole (Su)	Sulfathiazole powder	Merck
Kanamycin (Km)	Kantrex injection	Bristol
Procaine Penicillin G (Pe)	Crysticillin injectable	Squibb

media[2], citrate agar media[2], and a TPE agar plate. The TPE agar control was replicated last to confirm successful inoculation of all preceding plates. Plates were incubated at 37° C for approximately 24 hours and results were read.

An organism was considered resistant to an antibiotic agent only if it grew as well on the antibiotic plate as on the control plate. Any sign of growth inhibition was scored as sensitivity to that antibiotic. Organisms which grew on EMB agar with a metallic sheen and which failed to grow on citrate agar were considered to be Escherichia coli.

R-Factor Transfer

Twenty-four hour cultures of the donor fecal coliform organism and recipient Escherichia coli K-12 (strain W3110, nalidixic acid resistant) were inoculated into ten ml TPE broth and also into Mueller-Hinton broth[2]. These broth cultures were incubated at 37°C for approximately four hours to obtain exponentially multiplying cells. Conjugation experiments were conducted by mixing equal volumes of donor and recipient cells in 25 ml Erlenmeyer flasks and incubating unshaken at 37°C overnight (approximately 16 hours). Volume of broth compared to flask size was deliberately made small to provide maximum air surface contact.

Conjugated cultures were tested for the transfer of resistance

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[2]Difco .

to tetracycline (Tc) and streptomycin (Sm) in the concentrations listed above. Ampicillin (Am) was tested at 20 ug/ml. TPE broth was used for Sm and Tc testing; Mueller-Hinton broth was used for Am testing. Donor cultures were spotted on Mueller-Hinton agar plates containing Am, Tc, or Sm with one drop from a Pasteur pipette. This was to confirm the ability of the donor to grow on the antibiotic. Donors were similarly spotted on nalidixic acid (Na) plates to confirm the susceptibility of the donor to Na. Likewise, the recipient culture was spotted on Am, Tc, and Sm media to confirm its susceptibility to these antibiotics and on Na media to confirm resistance to this antibiotic.

Donor, recipient, and conjugated cultures were all spotted on plates containing Am-Na, Sm-Na, or Tc-Na. The ability of conjugated cultures to grow on these media with the simultaneous failure of both donor and recipient cultures to grow indicated R-factor transfer.

Conjugated cultures which demonstrated R-factor transfer were then streaked for isolation on appropriate dual antibiotic containing media (Am-Na, Sm-Na, or Tc-Na). Colonies so isolated were then tested for their antibiotic resistance to all 12 antibiotics. This was to determine the transfer of unselected resistance markers.

Statistical Procedure

The Control Data 3300 computer at the Oregon State University Computer Center was used with a multiple linear regression analysis program which uses widely accepted statistical procedures (87). The

program produces a standard multiple regression model with the form $Y = B_0 + B_1X_1 + B_2X_2 + \ldots B_nX_n + e$ in which Y is the dependent variable, B_0 is the Y-intercept, B's are regression coefficients, X's are the independent variables, and e is an error term.

Two separate analyses were conducted. First, the fecal coliform counts of Tillamook Bay were used as the dependent variable and regressed individually on the 135 independent variables listed in Table 23. Second, the logs of Tillamook Bay fecal coliform counts were similarly regressed on these 135 independent variables. The regression coefficient of each individual, independent variable (B_p) was then subjected to an F test to determine whether $B_p = 0$ or $B_p \neq 0$, where F equals the regression mean square (MSR) divided by the error mean square (MSE), i.e. $F = \frac{MSR}{MSE}$. Independent variables whose regression coefficients were significantly different from zero at a 90% confidence level were retained for further study (Table 24). Other independent variables were discarded.

Retained independent variables were further analyzed by a stepwise regression search procedure to obtain the most meaningful combinations of independent variables for predicting each dependent variable. This stepwise procedure sequentially adds the "best" independent variable where the "best" independent variable is defined as the independent variable which has the largest entering F value of those independent variables not already included in the model, i.e. the independent variable whose addition to the model explains the most variation between observed values of the dependent variable and the model.

Models thus obtained were then subjected to evaluation by three criteria to determine the "best" set of independent variables. These three criteria were: coefficient of multiple determination (R^2), error mean square (MSE), and total squared error (C) (87).

Ambient temperature and precipitation data used in regression analysis were obtained directly from the National Weather Service. Information about fish catch in each river was obtained from the Oregon Department of Fish and Wildlife and is intended to provide an estimate of the recreational use of these rivers. River flow data was gathered in 1972 by the Oregon State Water Resource Board, and use of this information assumes that the monthly water flow from October, 1975 through March, 1976 was the same as that in 1972. Tide information was obtained from the National Oceanic and Atmospheric Administration of the United States Department of Commerce.

Antibiotic use data for the human population in the Tillamook Bay area was gathered by screening approximately 600 patient records at one pharmacy located on Main Street in the City of Tillamook. This was one of three pharmacies located in the City of Tillamook, and, in the opinion of the pharmacist who owns and operates this drug store, the clientele of this establishment are a representative cross section of Tillamook area residents, i.e. the pharmacy records representated a random selection of antibiotic use by Tillamook area residents.

When statistical correlations between different parameters were desired, the following formula was used to determine this correlation:

$$\text{Correlation} = \sum_{k=1}^{n} (Yik-\overline{Y}i)\ (Yjk-\overline{Y}j)/(n-1)SiSj$$

where Yik is the $k^{\underline{th}}$ observation on variable i

Yjk is the $k^{\underline{th}}$ observation on variable j

$\overline{Y}i$ is the average of variable i

$\overline{Y}j$ is the average of variable j

n is the number of observations

Si is the standard deviation of variable i

Sj is the standard deviation of variable j

RESULTS AND DISCUSSION

Fecal Coliform Count Data

The fecal coliform counts are presented in Table 5. River and bay counts are graphically displayed in Figures 4 through 7. Figure 5 suggests a close relationship between Trask River counts and Tillamook Bay counts. Similarly, Figure 7 suggests a relation between Wilson River counts and Tillamook Bay counts. The absence of February count data for the Tillamook Bay is profoundly felt, particularly since the counts were highest in February from all sources except the Kilchis River. Figure 8 graphically displays a simple arithmetic mean of the counts of all four rivers and bay counts. This, as would be expected, suggests a close relation between the river counts and the bay count.

Table 6 is a matrix showing the correlations between river and bay counts. Correlations are high between Trask River and the bay and also between Wilson River and the bay. Correlations between the Kilchis and Tillamook Rivers and the bay are low. Notably, the Trask and Wilson River counts correlate highly as do the Kilchis and Tillamook River counts. This may suggest a similar fecal coliform load in the Trask-Wilson River pair and a similar fecal coliform load in the Kilchis-Tillamook River pair with the former pair being a greater determinant of fecal coliform loads in Tillamook Bay than the latter.

Kilchis River counts are lowest of all tributaries for each month sampled except during October, January, and March when they slightly exceeded the Trask River counts. This might be expected since the

TABLE 5. Fecal coliform counts (colony forming units per 100 ml).

MONTH	KILCHIS RIVER	TRASK RIVER	TILLAMOOK RIVER	WILSON RIVER	MEAN OF FOUR RIVERS	TILLAMOOK BAY[a]	PASTURE I	PASTURE II	PASTURE III
OCTOBER	13.9	8.8	66.0	20.0	27.2	3.6			
NOVEMBER	3.0	67.0	34.0	63.0	42.0	42.0			
DECEMBER	1.0	42.0	34.0	17.0	23.5	27.0			
JANUARY	2.7	0.0	13.5	8.5	6.2	4.7			
FEBRUARY	7.0	132.0	112.0	105.0	89.0		TNTC[b]	670.0	540.0
MARCH	0.5	0.0	32.0	11.0	10.9	7.3			740.0

[a]Average of four separate counts.

[b]Too numerous to count.

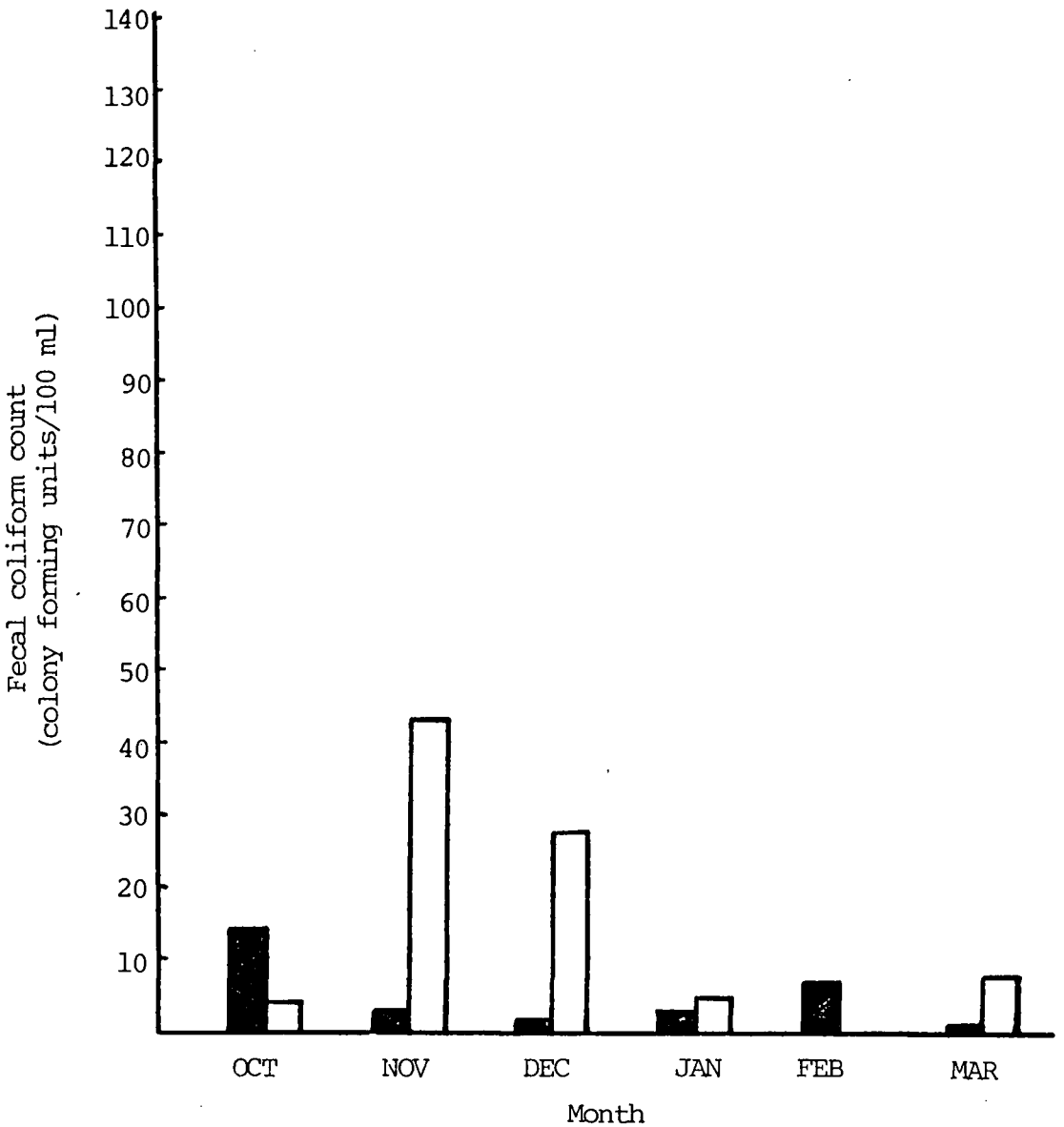

Figure 4. Fecal coliform counts in Kilchis River (darkened) and Tillamook Bay.

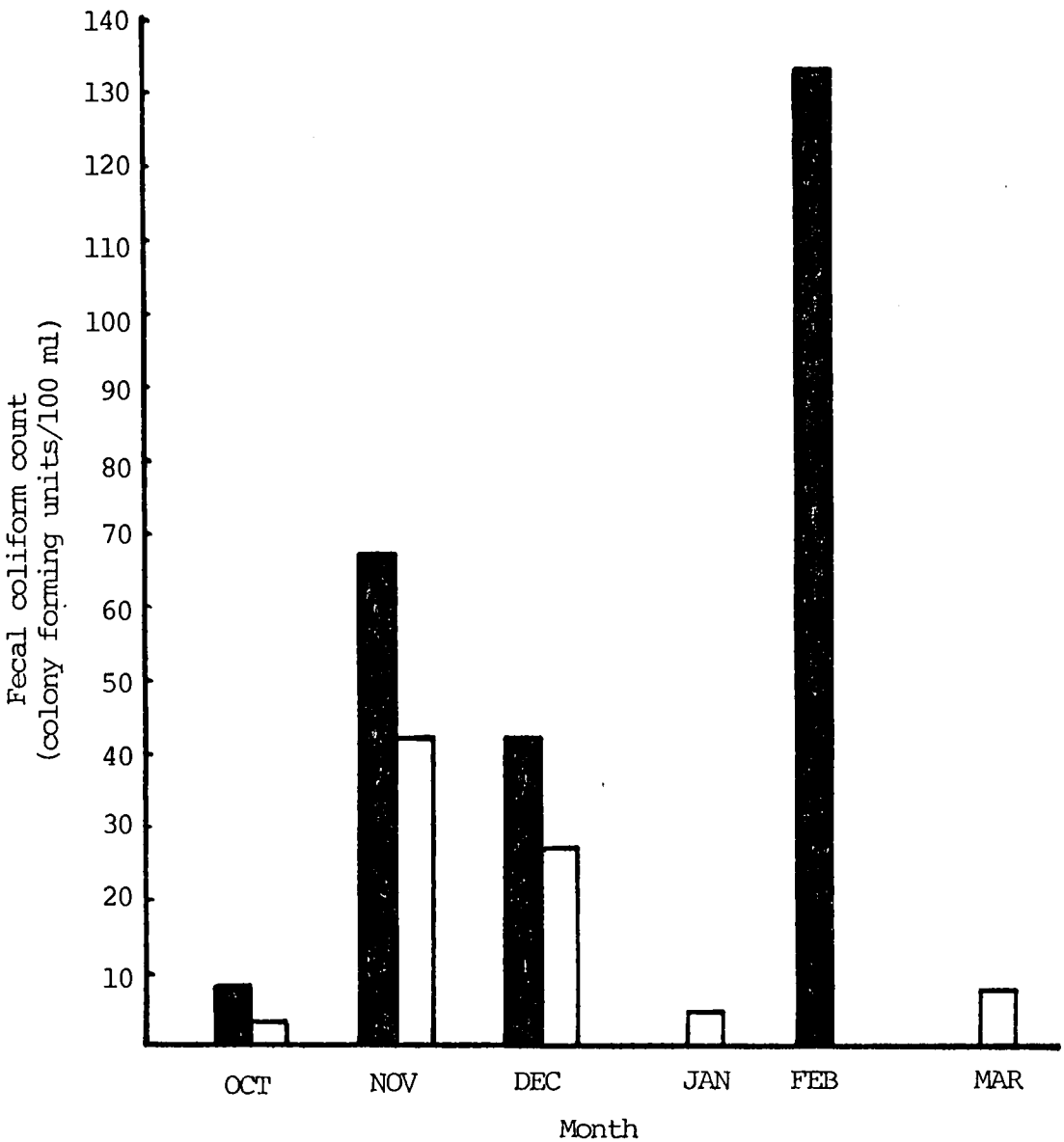

Figure 5. Fecal coliform counts in Trask River (darkened) and Tillamook Bay.

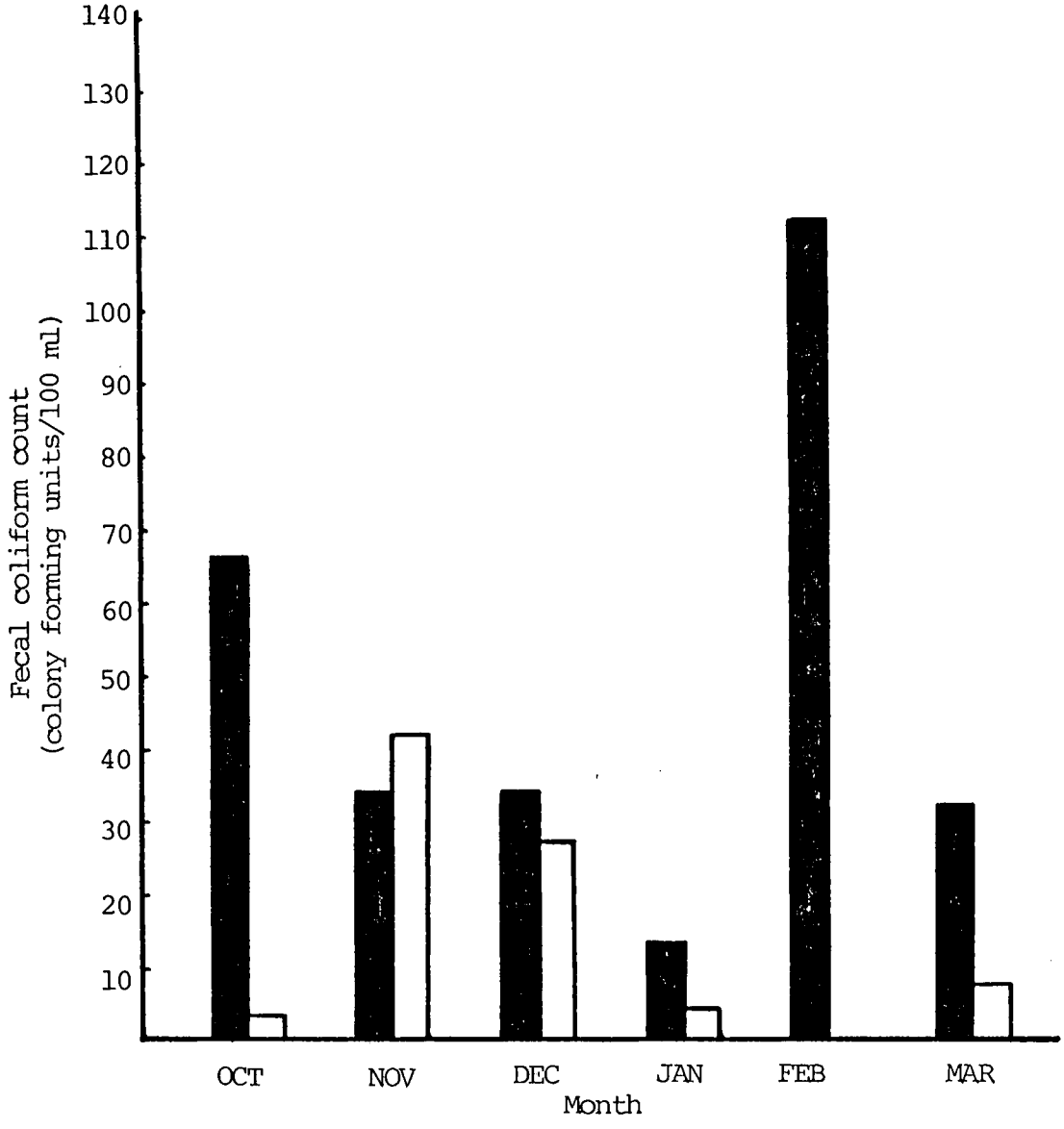

Figure 6. Fecal coliform counts in Tillamook River (darkened) and Tillamook Bay.

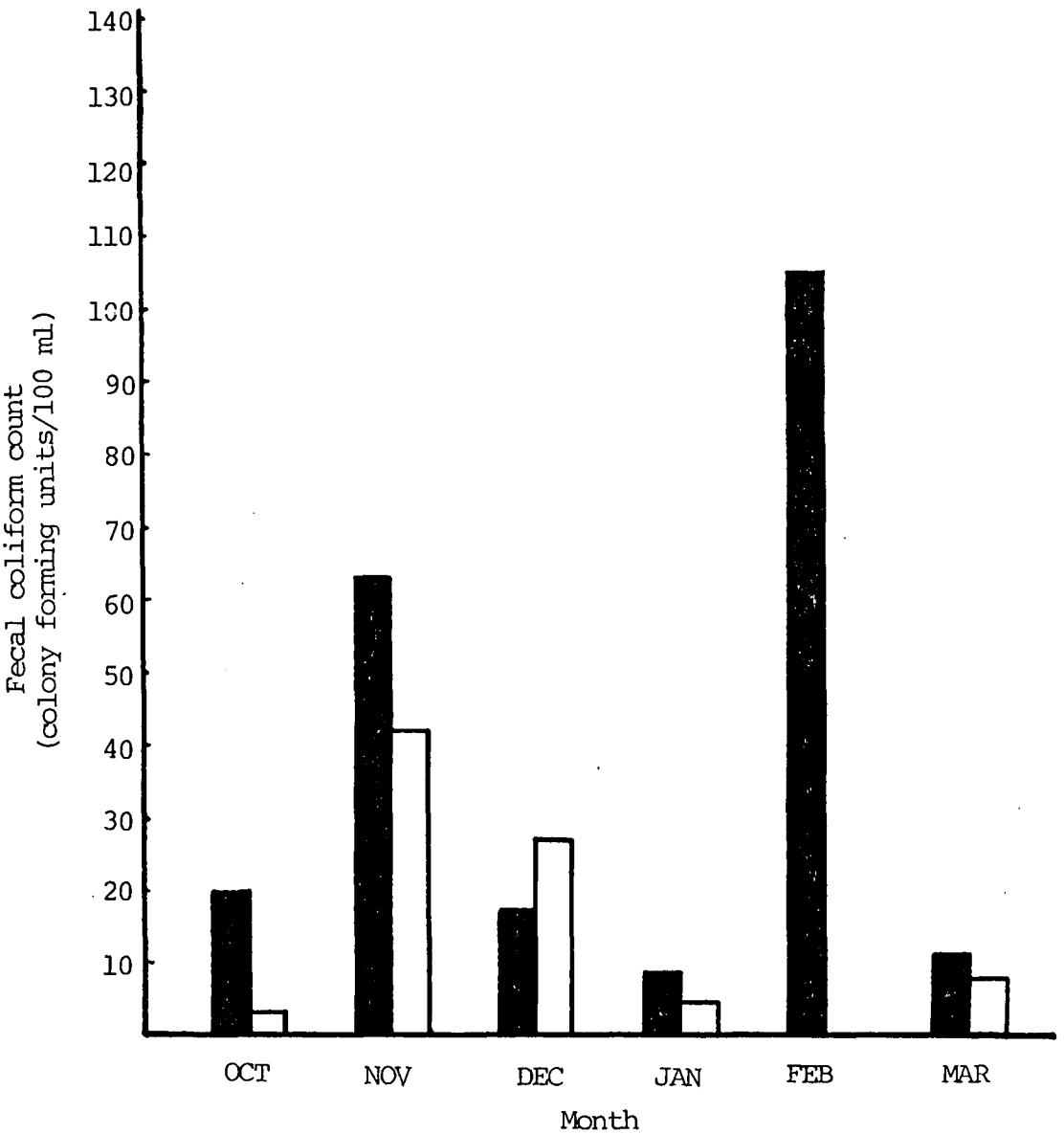

Figure 7. Fecal coliform counts in Wilson River (darkened) and Tillamook Bay.

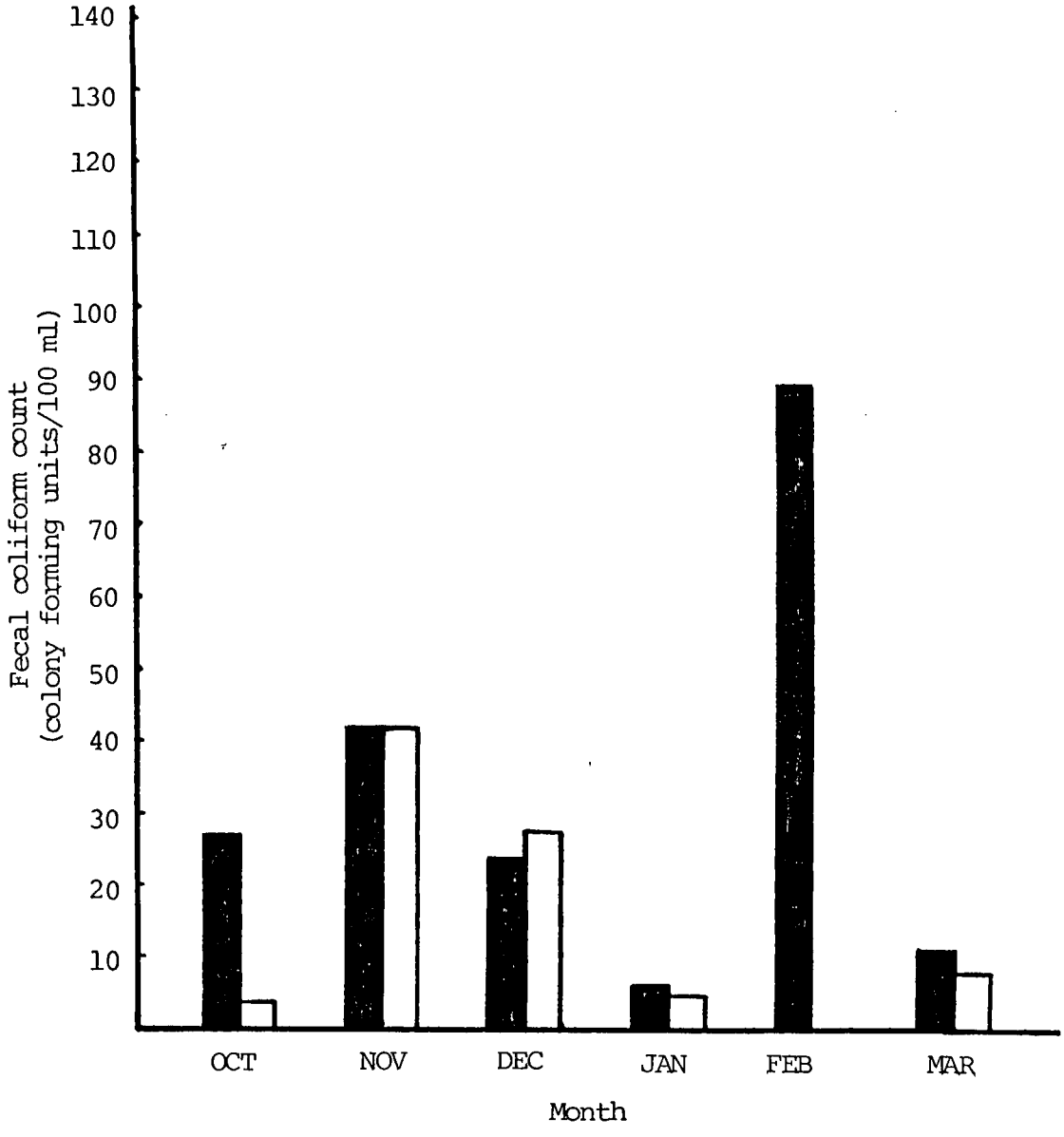

Figure 8. Fecal coliform counts, mean of four rivers (darkened) and Tillamook Bay.

TABLE 6. Correlation matrix of river and bay fecal coliform counts.

	KILCHIS RIVER	TRASK RIVER	TILLAMOOK RIVER	WILSON RIVER	TILLAMOOK BAY
KILCHIS RIVER		-0.2004	0.8350	0.0166	-0.3679
TRASK RIVER	-0.2004		0.0256	0.8619	0.9825
TILLAMOOK RIVER	0.8350	0.0256		0.1283	-0.1220
WILSON RIVER	0.0166	0.8619	0.1283		0.8420
TILLAMOOK BAY	-0.3679	0.9825	-0.1220	0.8420	

Kilchis River receives no effluent from sewage treatment plants, has the smallest human population not serviced by sewage treatment facilities of all the tributaries, is not used extensively for recreational purposes using angler-days as a measure of recreational use, and the sampling site was located above the drainage basin. Kilchis River counts did not fluctuate greatly during the study period.

The Trask River counts are notable because of their wide fluctuations during the study period. October, January, and March counts were very low (8.8, 0.0, and 0.0 FC per 100 ml, respectively), while November, December, and February counts were dramatically higher (67.0, 42.0, and 132.0 FC per 100 ml, respectively). Except possibly during February, these differences do not reflect increased rainfall preceding sampling. These differences may result from changes in the efficiency of sewage treatment at the Tillamook sewage treatment plant since the sampling site was immediately adjacent to this sewage treatment facility.

Counts from all tributaries, except the Kilchis River, were noticeably higher in February. This can be attributed to heavy rainfalls immediately preceding sampling. About 3.3 inches of rain fell in Tillamook in the 36 h period preceding sampling. Large quantities of debris from the river banks, particularly animal fecal wastes, enter the rivers under these conditions. In addition, the capacity of the sewage treatment plants would have been taxed under these heavy rainfall conditions. Notably, the FC count in the Kilchis River which does not receive sewage treatment plant effluent rose only slightly in February. The relationship between rainfall and FC counts will be discussed in more detail with the statistical analyses of the data.

The extremely high pasture counts seem to indicate that animal fecal wastes may be a significant source of fecal contamination in the bay although efforts to establish this causal relationship, to be discussed later, did not succeed.

Antibiotic Resistance Patterns

A total of 1917 fecal coliform organisms were screened to determine their antibiotic resistance patterns. Table 7 shows a representation by month and source of the number of organisms screened. Within this group of 1917 organisms, 176 different antibiotic resistance patterns were found. Table 8 lists a monthly representation of all resistance patterns which individually comprise 0.5% or more of the total number of organisms screened. Table 9 is a source representation of all resistance patterns which individually comprise 0.5% or more of the total organisms screened.

These resistance patterns were analyzed to determine if any resistance pattern or patterns were distinct enough to provide a useful point source indicator. In other words, efforts were made to discover whether any antibiotic resistance pattern(s) distinctly characterized a specific sampling location. If so, this resistance pattern(s) would be a characteristic indicator of contamination from that source, i.e. a point source indicator. Unfortunately, resistance patterns characteristic of specific sites were not found. Distribution of resistance patterns appeared to be essentially random. This must reflect either a homogeneity among fecal coliforms at the various sampling sites or a fundamental conceptual flaw in the use of this potential indicator system. Hope-

TABLE 7. Number of fecal coliform organisms screened for antibiotic resistance pattern.

MONTH	TOTAL	KILCHIS RIVER	TRASK RIVER	TILLAMOOK RIVER	WILSON RIVER	TILLAMOOK BAY[a]	PASTURE II	PASTURE III
OCTOBER	201	23	19	48	9	102		
NOVEMBER	435	17	55	46	59	258		
DECEMBER	243	8	28	32	65	110		
JANUARY	197	18	7	59	36	77		
FEBRUARY	440	24	107	75	107		71	56
MARCH	401	3	0	120	45	173		60
TOTAL	1917	93	216	380	321	720	71	116

[a]From all four bay sampling sites.

TABLE 8. Monthly distribution of fecal coliform organisms with antibiotic resistance patterns comprising >0.5% of total isolates examined.

ANTIBIOTIC RESISTANCE PATTERN[a]	NUMBER OF ORGANISMS (%) TOTAL	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	MARCH
ALL PATTERNS	1917 (100)	201 (100)	435 (100)	243 (100)	197 (100)	440 (100)	401 (100)
NO RESISTANCE	346 (18.05)	24 (11.94)	5 (1.15)	6 (2.47)	73 (37.06)	202 (45.91)	36 (8.98)
Ni	322 (16.80)	111 (55.22)	43 (9.89)	3 (1.23)	39 (19.80)	110 (25.00)	16 (3.99)
Sm,Ni	191 (9.96)	2 (1.00)	115 (26.44)	69 (28.40)	5 (2.54)		
Su	165 (8.61)	1 (.50)			1 (.51)	17 (3.86)	146 (36.41)
Sm	103 (5.37)		13 (2.99)	86 (35.39)	3 (1.52)	1 (.23)	
Ni, Su	83 (4.33)	3 (1.49)	1 (.23)			5 (1.14)	74 (18.45)
Sm, Nm, Ni	74 (3.86)		65 (14.94)	9 (3.70)			
Sm, Am, Tc, Ct, Ot, Su, Pe	53 (2.76)				26 (13.20)	26 (5.91)	1 (.25)
Ni, Pe	35 (1.83)	20 (9.95)	1 (.23)		1 (.51)	10 (2.27)	3 (.75)
Sm, Nm	31 (1.62)		9 (2.07)	22 (9.05)			
Pe	24 (1.25)	4 (1.99)	1 (.23)			17 (3.86)	2 (.50)
Sm, Am, Ni	23 (1.20)		20 (4.60)	3 (1.23)			
Su, Pe	16 (.83)					1 (.23)	15 (3.74)
Sm, Ni, Pe	16 (.83)	3 (1.49)	13 (2.99)				
Ni, Su, Pe	15 (.78)	7 (3.48)				1 (.23)	7 (1.75)
Tc, Ct, Ot, Ni, Sm	15 (.78)	3 (1.49)	11 (2.53)	1 (.41)			
Sm,Am,Tc,Ct,Ot, Nm,Ni,Su,Km,Pe	15 (.78)		3 (.69)	1 (.41)	1 (.51)	1 (.23)	9 (2.24)
Tc,Ct,Ot,Nm, Sm,Ni,Su,Km	14 (.73)		3 (.69)		1 (.51)	4 (.91)	6 (1.50)
Sm,Tc,Ct,Ot, Nm,Ni	12 (.63)		11 (2.53)	1 (.41)			
Sm,Am,Tc,Ct,Ot Nm,Su,Km,Pe	11 (.57)			2 (.82)		1 (.23)	8 (2.00)
Cm, Ni	10 (.52)				6 (3.05)	4 (.91)	

[a]Abbreviations used: ampicillin = Am; chloramphenicol = Cm; chlortetracycline = Ct; kanamycin = Km; nalidixic acid = Na; neomycin = Nm; nitrofurazone = Ni; oxytetracycline = Ot; procaine penicillin G = Pe; streptomycin = Sm; sulfathiazole = Su; tetracycline = Tc.

TABLE 9. Source distribution of fecal coliform organisms with antibiotic resistance patterns comprising >0.5% of total isolates examined.

ANTIBIOTIC RESISTANCE PATTERN[a]	NUMBER OF ORGANISMS (%)							
	TOTAL	KILCHIS RIVER	TRASK RIVER	TILLAMOOK RIVER	WILSON RIVER	TILLAMOOK BAY	PASTURE II	PASTURE III
ALL PATTERNS	1917 (100)	93 (100)	216 (100)	380 (100)	321 (100)	720 (100)	71 (100)	116 (100)
NO RESISTANCE	346 (18.05)	22 (23.66)	75 (34.72)	64 (16.84)	41 (12.77)	59 (8.19)	38 (53.52)	47 (40.52)
Ni	322 (16.80)	18 (19.35)	36 (16.67)	75 (19.74)	34 (10.59)	106 (14.72)	27 (38.03)	26 (22.41)
Sm, Ni	191 (9.96)	7 (7.53)	25 (11.57)	22 (5.79)	35 (10.90)	102 (14.17)		
Su	165 (8.61)	17 (18.28)	1 (.46)	73 (19.21)	12 (3.74	52 (7.22)		10 (8.62)
Sm	103 (5.37)	5 (5.38)	8 (3.70)	13 (3.42)	21 (6.54)	55 (7.64)	1 (1.41)	
Ni, Su	83 (4.33)	5 (5.38)	1 (.46)	19 (5.00)	13 (4.05)	31 (4.31)		14 (12.07)
Sm, Nm, Ni	74 (3.86)	1 (1.08)	2 (.93)	1 (.26)	18 (5.61)	52 (7.22)		
Sm,Am,Tc,Ct, Ot,Su,Pe	53 (2.76)			3 (.79)	49 (15.26)	1 (.14)		
Ni, Pe	35 (1.83)	2 (2.15)	5 (2.31)	5 (1.32)	7 (2.18)	14 (1.94)	1 (1.41)	1 (.86)
Sm, Nm	31 (1.62)			1 (.26)	17 (5.30)	13 (1.81)		
Pe	24 (1.25)		5 (2.31)	2 (.53)	14 (4.36)	2 (.28)		1 (.86)
Sm, Am, Ni	23 (1.20)	3 (3.23)	1 (.46)	2 (.53)		17 (2.36)		
Su, Pe	16 (.83)	1 (1.08)		5 (1.32)	5 (1.56)	5 (.69)		
Sm, Ni, Pe	16 (.83)	1 (1.08)	3 (1.39)		7 (2.18)	5 (.69)		
Ni, Su, Pe	15 (.78)	1 (1.08)	2 (.93)	7 (1.84)	2 (.62)	3 (.42)		
Sm, Tc, Ct, Ot, Ni	15 (.78)	1 (1.08)		3 (.79)		11 (1.53)		
Sm,Am,Tc,Ct,Ot, Nm,Ni,Su,Km,Pe	15 (.78)		1 (.46)	1 (.26)		13 (1.81)		
Tc,Ct,Ot,Nm, Sm,Ni,Su,Km	14 (.73)		2 (.93)	1 (.26)	4 (1.25)	5 (.69)		2 (1.72)
Tc,Ct,Ot, Sm,Nm,Ni	12 (.63)			2 (.53)	3 (.93)	7 (.97)		
Sm,Am,Tc,Ct, Ot,Nm,Su,Km,Pe	11 (.57)		2 (.93)	2 (.53)		7 (.97)		
Cm, Ni	10 (.52)		2 (.93)	8 (2.11)				

[a]Abbreviations same as Table 8.

fully the former applies, since the Tillamook area river sources appear to be relatively homogeneous in terms of fecal contamination sources. Nonetheless, the study might have been deficient in the number of organisms screened, might have used the wrong antibiotics or the wrong concentrations of these antibiotics, or might have been entirely ill-conceived.

Notwithstanding the apparent random occurrence of the antibiotic resistance patterns, there are some patterns which suggest, albeit very obtusely, that meaningful point source indicator patterns might be developed. For example, Table 9 includes the Sm, Nm pattern which occurred 31 times during the study. All 31 isolates bar one came either from the Wilson River or the Tillamook Bay. This suggests the possibility that the source of the organisms in the Tillamook Bay was the Wilson River. Similarly, the pattern Sm, Am, Tc, Ct, Ot, Su, Pe was heavily concentrated in the Wilson River (49 of 53 isolates) suggesting the possibility that this pattern might characterize the Wilson River origin.

Table 10 shows the percentages of organisms from each source resistant to a particular antibiotic. It is interesting to note that organisms from pasture samples were less resistant to each antibiotic than were the bay isolates. Kilchis River isolates were less resistant than bay isolates to all antibiotics except one (Su). Trask River isolates were less resistant than bay isolates to all antibiotics except two (Na and Pe). Tillamook River isolates were less resistant than bay isolates to all antibiotics except three (Cm, Na, and Su). The Wilson River isolates differed from this trend by being less resistant than bay

TABLE 10. Source listing of fecal coliform organism resistances to each antibiotic.

	PERCENTAGE RESISTANT TO EACH ANTIBIOTIC BY SOURCE							
	KILCHIS RIVER	TRASK RIVER	TILLAMOOK RIVER	WILSON RIVER	TILLAMOOK BAY	ALL PASTURES	WEIGHTED MEAN EXCLUDING BAY	WEIGHTED MEAN INCLUDING BAY
CHLORAMPHENICOL (Cm)	0.00	3.70	6.05	1.56	4.03	3.74	3.59	3.76
STREPTOMYCIN (Sm)	26.88	32.41	22.89	57.94	54.44	4.81	31.49	40.12
AMPICILLIN (Am)	9.68	12.04	9.21	20.56	13.89	2.14	11.70	12.52
TETRACYCLINE (Tc)	5.38	11.11	12.37	24.92	16.39	4.28	13.70	14.71
CHLORTETRACYCLINE(Ct)	4.30	9.72	11.84	22.74	17.50	5.35	12.78	14.55
OXYTETRACYCLINE (Ot)	5.38	12.04	12.11	25.55	19.03	6.42	14.29	16.07
NEOMYCIN (Nm)	6.45	11.11	6.05	17.45	24.03	3.21	9.61	15.02
NITROFURAZONE (Ni)	48.39	49.54	50.00	43.61	66.67	40.64	46.62	54.15
NALIDIXIC ACID (Na)	0.00	1.39	1.84	0.00	0.42	0.00	0.83	0.68
SULFATHIAZOLE (Su)	29.03	9.26	36.05	32.09	25.97	18.72	26.90	26.55
KANAMYCIN (Km)	1.08	6.02	3.95	2.18	8.19	2.67	3.43	5.22
PENICILLIN (Pe)	13.98	15.28	13.42	30.53	15.00	3.74	16.88	16.17

isolates to only five antibiotics (Cm, Nm, Ni, Na, and Km) and were more resistant than bay isolates to seven antibiotics (Sm, Am, Tc, Ct, Ot, Su, and Pe). A comparison of the resistance level of bay isolates with the weighted mean resistance level of all isolates exclusive of bay isolates reveals that for all antibiotics except three (Na, Su, and Pe) bay organisms were more resistant than the weighted mean. These results may indicate that antibiotic resistant organisms have a competitive advantage over non-resistant organisms. This would agree with the results of others who suggest that R-factor mediated antibiotic resistance may increase survival potential (36,47). Others have suggested that R-factor mediated antibiotic resistance may reduce survival potential (6) or may have no effect on survival potential (111).

Table 10 also presents some interesting information about the fecal coliforms isolated from the pastures. By comparing the antibiotic resistance of pasture isolates with that of river isolates and with the weighted mean excluding the bay, it is apparent that the antibiotic resistance of pasture isolates is lower in almost all cases. Exceptions include only Cm resistance in the Kilchis River, Trask River, Wilson River, and weighted mean, Ct and Ot resistance in the Kilchis River, Su resistance in the Trask River, and Km resistance in the Kilchis River and the Wilson River. These results seemingly lead to either or both of the following conclusions. First, antibiotic resistance may infer an increased survival potential on fecal coliform organisms, or, second, pasture areas are not a major source of fecal coliforms in these rivers. These conclusions must be viewed in light of the small number of pasture samples obtained. The first conclusion could be definitively tested by

studies of the die-off kinetics of resistant and non-resistant fecal coliforms. The second conclusion might be studied by the use of a more elaborate sampling scheme to determine fecal coliform sources for each individual river. Such an effort would, of course, depend on the use of some point source indicator system.

Table 11 is a correlation matrix showing the correlations between resistance percentages for each antibiotic when resistance data is separated by source, i.e. Table 11 is a correlation matrix between horizontal rows in Table 10. The correlations between any pair of the tetracycline group of antibiotics are extremely high which is to be expected since these antibiotics are so closely related chemically. The high correlation between Sm and Nm resistance may similarly be explained by the common chemical characteristics of the two aminoglycoside antibiotics. The Am-Pe correlation may be explained in the same way. The high correlations between Am-Sm and Am-Tc remain essentially unexplained although it might relate to the common presence of resistance determinants to these three antibiotics on R-factors. Na resistance correlated very poorly with resistance to any antibiotic except Cm. This may indicate different mechanisms for inactivation of Na and Cm. The total number of Na resistant organisms was quite small.

Table 12 shows the percentages of organisms from each month resistant to each antibiotic. A comparison of weighted mean percentages including bay isolates with weighted mean percentages excluding bay isolates reveals that in all cases, except Na, Su, and Pe, the former exceeded the latter. This would also tend to support the contention that antibiotic resistance may confer an increased survival potential.

TABLE 11. Matrix of correlations between percent antibiotic resistances from each source.

	CHLORAMPHENICOL (Cm)	STREPTOMYCIN (Sm)	AMPICILLIN (Am)	TETRACYCLINE (Tc)	CHLORTETRACYCLINE (Ct)	OXYTETRACYCLINE (Ot)	NEOMYCIN (Nm)	NITROFURAZONE (Ni)	NALIDIXIC ACID (Na)	SULFATHIAZOLE (Su)	KANAMYCIN (Km)	PENICILLIN (Pe)
CHLORAMPHENICOL (Cm)		-0.22	-0.29	0.00	0.08	0.03	-0.04	0.25	0.74	-0.03	0.57	-0.36
STREPTOMYCIN (Sm)	-0.22		0.94	0.88	0.87	0.88	0.92	0.52	-0.14	0.25	0.37	0.84
AMPICILLIN (Am)	-0.29	0.94		0.91	0.86	0.88	0.76	0.26	-0.06	0.28	0.16	0.96
TETRACYCLINE (Tc)	0.00	0.88	0.91		0.98	0.99	0.75	0.22	-0.01	0.36	0.22	0.89
CHLORTETRACYCLINE(Ct)	0.08	0.87	0.86	0.98		1.00	0.80	0.30	-0.03	0.36	0.32	0.81
OXYTETRACYCLINE (Ot)	0.03	0.88	0.88	0.99	1.00		0.81	0.27	-0.06	0.29	0.31	0.84
NEOMYCIN (Nm)	-0.04	0.92	0.76	0.75	0.80	0.81		0.71	-0.15	0.08	0.63	0.58
NITROFURAZONE (Ni)	0.25	0.52	0.26	0.22	0.30	0.27	0.71		0.19	0.07	0.81	0.02
NALIDIXIC ACID (Na)	0.74	-0.14	-0.06	-0.01	-0.03	-0.06	-0.15	0.19		-0.05	0.41	-0.11
SULFATHIAZOLE (Su)	-0.03	0.25	0.28	0.36	0.36	0.29	0.08	0.07	-0.05		-0.34	0.37
KANAMYCIN (Km)	0.57	0.37	0.16	0.22	0.32	0.31	0.63	0.81	0.41	-0.34		-0.08
PENICILLIN (Pe)	-0.36	0.84	0.96	0.89	0.81	0.84	0.58	0.02	-0.11	0.37	-0.08	

TABLE 12. Monthly listing of fecal coliform organism resistances to each antibiotic.

	PERCENTAGE RESISTANT TO EACH ANTIBIOTIC BY MONTH							
	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	MARCH	WEIGHTED MEAN EXCLUDING BAY	WEIGHTED MEAN INCLUDING BAY
CHLORAMPHENICOL (Cm)	1.49	2.76	2.88	8.12	3.18	4.99	3.59	3.76
STREPTOMYCIN (Sm)	7.96	81.61	94.24	31.98	12.73	12.47	31.49	40.12
AMPICILLIN (Am)	3.98	18.62	8.23	23.86	8.86	11.22	11.70	12.52
TETRACYCLINE (Tc)	8.46	16.32	8.64	24.87	13.18	16.46	13.70	14.71
CHLORTETRACYCLINE (Ct)	6.47	19.08	5.76	23.86	12.27	16.96	12.78	14.55
OXYTETRACYCLINE (Ot)	9.95	17.47	8.64	26.40	14.32	18.95	14.29	16.07
NEOMYCIN (Nm)	1.99	35.86	22.22	7.61	4.09	10.22	9.61	15.02
NITROFURAZONE (Ni)	84.08	91.03	41.98	37.56	33.64	37.16	46.62	54.15
NALIDIXIC ACID (Na)	0.00	0.46	0.41	1.02	0.68	1.25	0.83	0.68
SULFATHIAZOLE (Su)	6.47	6.21	4.94	21.32	17.27	84.54	26.90	26.55
KANAMYCIN (Km)	0.50	6.67	2.88	4.57	3.41	9.73	3.43	5.22
PENICILLIN (Pe)	19.90	15.86	4.94	21.83	16.59	18.20	16.88	16.17

A careful examination of these percentages also shows some instances where resistance to a particular antibiotic during certain months was noticeably greater or lesser than during most other months. Examples include the greater resistance to Sm during November and December, the greater resistance to Nm during November and December, the greater resistance to Ni during October and November, the greater resistance to Su in March, the lesser resistance to Km in October, and the lesser resistance to Pe in December. The possibility exists that the seasonal fluctuation in the use of antibiotics by either the human or domestic animal population in the Tillamook area might have selected resistant organisms and hence contributed to the change in antibiotic resistance patterns of the fecal coliform isolates. Prescription records of area pharmacies were examined and the monthly fluctuation of the antibiotic use data was compared with the fluctuation of the fecal coliform antibiotic resistance. Three antibiotics, Am, Tc, and Pe, accounted for virtually all the antibiotic use reflected in the pharmacy records and therefore this comparison was limited to these three antibiotics. Table 13 lists the quantities of each antibiotic used during our study period in the Tillamook area obtained from 600 pharmacy records. Also included are these same quantity figures standardized by assigning the October figure a value equal to the October resistance percentage and assigning other months standardized values using this as a baseline. Table 14 shows the correlation between each standardized antibiotic use value and the resistance percentages. Clearly, the correlations are very low and in no way would support a hypothesis that antibiotic resistance percentages reflect antibiotic use by the

TABLE 13. Antibiotic use by representative sample of Tillamook Bay area residents[a].

	AMPICILLIN ($mg \times 10^5$)	AMPICILLIN USE STANDARDIZED TO OCTOBER PERCENTAGE RESISTANCE	TETRACYCLINE ($mg \times 10^5$)	TETRACYCLINE USE STANDARDIZED TO OCTOBER PERCENTAGE RESISTANCE	PENICILLIN ($mg \times 10^5$)	PENICILLIN USE STANDARDIZED TO OCTOBER PERCENTAGE RESISTANCE
OCTOBER	2.715	3.98	3.402	8.46	2.140	19.90
NOVEMBER	2.110	3.09	3.260	8.11	1.928	17.93
DECEMBER	1.930	2.83	4.138	10.29	2.999	27.89
JANUARY	2.825	4.14	3.520	8.75	2.375	22.09
FEBRUARY	5.248	7.69	4.715	11.73	3.712	34.52
MARCH	2.712	3.98	3.938	9.79	1.618	15.05

[a]Obtained from 600 pharmacy records.

TABLE 14. Correlation between standardized antibiotic use and percentage resistant to the antibiotic.

	STANDARDIZED AMPICILLIN USE	STANDARDIZED TETRACYCLINE USE	STANDARDIZED PENICILLIN USE
PERCENTAGE RESISTANT TO AMPICILLIN	-0.1880		
PERCENTAGE RESISTANT TO TETRACYCLINE		-0.2656	
PERCENTAGE RESISTANT TO PENICILLIN			-0.3827

human population. It is indeed regrettable that similar antibiotic use data could not be obtained for domestic animals.

Table 15 is a correlation matrix showing the correlations between resistance percentages for each antibiotic when resistance data is separated by month, i.e. Table 15 is a correlation matrix between horizontal rows in Table 12. The correlations between pairs of tetracycline group antibiotics are again high (see also Table 11) as might be expected. Sm-Nm resistance correlation is again quite high although the Am-Pe correlation is not as pronounced as in Table 11. This probably points out the inherent danger of pooling data over a six month period as was done in Table 11. Of course, Table 15 suffers from a similar deficiency since it pooled sources rather than months. The most reliable correlation data would be that obtained by pooling neither source nor time period, but seldom would the number of isolates be large enough to permit this kind of analysis unless multiple samples had been obtained from each source at the same time. This was beyond the scope of this investigation.

Table 15 also shows a high correlation between Am and each of the three tetracycline antibiotics and between Cm and both Tc and Ot. The reason for these correlations is not apparent although, again, it may relate to their simultaneous presence in an R-factor.

Table 16 presents the percentage of fecal coliform organisms from each source resistant to a stated number of antibiotics. This table suggests that a higher level of antibiotic resistance exists among bay organisms and also suggests that organisms from pasture samples have

Table 15. Matrix of correlations between percent antibiotic resistances by month.

	CHLORAMPHENICOL (Cm)	STREPTOMYCIN (Sm)	AMPICILLIN (Am)	TETRACYCLINE (Tc)	CHLORTETRACYCLINE (Ct)	OXYTETRACYCLINE (Ot)	NEOMYCIN (Nm)	NITROFURAZONE (Ni)	NALIDIXIC ACID (Na)	SULFATHIAZOLE (Su)	KANAMYCIN (Km)	PENICILLIN (Pe)
CHLORAMPHENICOL (Cm)		-0.16	0.77	0.91	0.79	0.90	-0.18	-0.57	0.80	0.39	0.41	0.41
STREPTOMYCIN (Sm)	-0.16		0.24	-0.13	-0.08	-0.21	0.88	0.22	-0.24	-0.46	0.02	-0.75
AMPICILLIN (Am)	0.77	0.24		0.92	0.92	0.88	0.35	-0.07	0.52	0.03	0.44	0.34
TETRACYCLINE (Tc)	0.91	-0.13	0.92		0.97	0.99	0.01	-0.28	0.72	0.30	0.51	0.58
CHLORTETRACYCLINE(Ct)	0.79	-0.08	0.92	0.97		0.97	0.16	-0.12	0.70	0.33	0.62	0.58
OXYTETRACYCLINE (Ot)	0.90	-0.21	0.88	0.99	0.97		-0.04	-0.26	0.75	0.38	0.55	0.64
NEOMYCIN (Nm)	-0.18	0.88	0.35	0.01	0.16	-0.04		0.43	-0.11	-0.24	0.35	-0.50
NITROFURAZONE (Ni)	-0.57	0.22	-0.07	-0.28	-0.12	-0.26	0.43		-0.69	-0.45	-0.21	0.13
NALIDIXIC ACID (Na)	0.80	-0.24	0.52	0.72	0.70	0.75	-0.11	-0.69		0.79	0.79	0.27
SULFATHIAZOLE (Su)	0.39	-0.46	0.03	0.30	0.33	0.38	-0.24	-0.45	0.79		0.78	0.28
KANAMYCIN (Km)	0.41	0.02	0.44	0.51	0.62	0.55	0.35	-0.21	0.79	0.78		0.14
PENICILLIN (Pe)	0.41	-0.75	0.34	0.58	0.58	0.64	-0.50	0.13	0.27	0.28	0.14	

TABLE 16. Levels of antibiotic resistance in fecal coliform organisms.

		SOURCE							
		KILCHIS RIVER	TRASK RIVER	TILLAMOOK RIVER	WILSON RIVER	TILLAMOOK BAY	ALL PASTURES	WEIGHTED MEAN INCLUDING BAY	WEIGHTED MEAN EXCLUDING BAY
	8 or more	3.23	4.63	3.16	4.05	7.50	2.14	5.01	3.51
NUMBER	7	0.00	1.85	2.11	15.58	2.78	0.53	4.33	5.26
OF	6	1.08	3.70	2.37	2.80	2.50	0.53	2.40	2.34
ANTIBIOTICS	5	1.08	0.93	2.89	0.62	3.75	1.07	2.35	1.50
TO	4	2.15	2.31	3.42	2.49	4.86	0.00	3.29	2.34
WHICH	3	7.53	5.56	6.32	10.59	13.89	1.07	9.34	6.60
RESISTANT	2	18.28	20.83	19.21	24.30	25.97	11.23	21.96	19.55
	1	43.01	25.46	43.68	26.79	30.56	37.97	33.28	34.92
	0	23.66	34.72	16.84	12.77	8.19	45.45	18.05	23.97

relatively low levels of antibiotic resistance. For example, bay organisms have the highest level of resistance to eight or more antibiotics, five antibiotics, four antibiotics, three antibiotics, and two antibiotics, and have the lowest occurrence of resistance to zero antibiotics. Pasture isolates have the lowest level of resistance to eight or more antibiotics, six antibiotics, four antibiotics, three antibiotics, and two antibiotics, and have the highest occurrence in the zero antibiotic resistance category. The resistance of bay organisms exceeds the weighted mean resistance excluding bay organisms in all categories except resistance to seven antibiotics and one antibiotic. Bay organisms have the lowest occurrence in the zero antibiotic resistance category. The weighted mean resistance including bay organisms exceeds the weighted mean resistance excluding bay resistance in all categories except resistance to seven antibiotics and one antibiotic. The weighted mean resistance including bay organisms has a lesser occurrence of resistance to zero antibiotics than the weighted mean resistance excluding bay organisms. These results may also indicate that antibiotic resistance increases the survival potential of fecal coliforms or that the pasture areas are not a primary source of fecal coliforms in the Tillamook Bay.

Table 17 presents the same data as Table 16 except the resistance percentages are maintained cumulatively, e.g. in Table 16 resistance to seven antibiotics means resistance to exactly seven antibiotics while in Table 17 resistance to seven antibiotics means resistance to seven or more antibiotics. Table 17 illustrates the high level of antibiotic resistance in bay organisms compared to other sources. Bay organisms

TABLE 17. Levels of antibiotic resistance in fecal coliform organisms - cumulative.

		SOURCE							
		KILCHIS RIVER	TRASK RIVER	TILLAMOOK RIVER	WILSON RIVER	TILLAMOOK BAY	ALL PASTURES	WEIGHTED MEAN INCLUDING BAY	WEIGHTED MEAN EXCLUDING BAY
NUMBER OF ANTIBIOTICS TO WHICH RESISTANT	8 or more	3.23	4.63	3.16	4.05	7.50	2.14	5.01	3.51
	7 or more	3.23	6.48	5.27	19.63	10.28	2.67	9.34	8.77
	6 or more	4.31	10.18	7.64	22.43	12.78	3.20	11.74	11.11
	5 or more	5.39	11.11	10.53	23.05	16.53	4.27	14.09	12.61
	4 or more	7.54	13.42	13.95	25.54	21.39	4.27	17.38	14.95
	3 or more	15.07	18.98	20.27	36.13	35.28	5.34	26.72	21.55
	2 or more	33.35	39.81	39.48	60.43	61.25	16.57	48.68	41.10
	1 or more	76.36	65.27	83.16	87.22	91.81	54.54	81.96	76.02
	0 or more	100.02	99.99	100.00	99.99	100.00	99.99	100.00	99.99

have the highest level of resistance to eight or more, two or more, and one or more antibiotics; bay organisms have the second highest level of resistance in all other categories being second to only the Wilson River organisms. In no category were bay organisms less resistant than organisms from any source except the Wilson River. This might imply that the Wilson River is a primary source of the antibiotic resistant organisms found in the bay. Bay organisms are more resistant than the weighted mean resistance excluding bay organisms in all categories. Weighted mean resistance including bay organisms is higher in all categories than weighted mean resistance excluding bay organisms. Bay organisms are more resistant than pasture organisms at all levels of resistance. These results may similarly indicate that antibiotic resistance increases the survival potential of fecal coliforms or that the pasture areas are not a primary source of fecal coliforms in Tillamook Bay.

Table 18 presents the percentage of fecal coliform organisms excluding bay organisms from each month resistant to a stated number of antibiotics. This information indicates that the general level of antibiotic resistance was highest during the month of November. November resistance percentages are highest for six antibiotics, four antibiotics, three antibiotics, and two antibiotics, and in addition has the lowest occurrence of resistance to no antibiotics. November resistance percentages exceed the weighted mean percentages at all antibiotic resistance levels except resistance to seven antibiotics and one antibiotic and the November occurrence of resistance to no antibiotics is dramatically lower than the weighted mean percentage. January percentages are highest for eight or more antibiotics and for

TABLE 18. Levels of antibiotic resistance in fecal coliform organisms (excluding bay organisms).

		MONTH						
		OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	MARCH	WEIGHTED MEAN
	8 or more	1.01	4.52	3.76	5.00	3.41	3.07	3.51
NUMBER	7	2.02	1.69	0.75	21.67	6.59	0.88	5.26
OF	6	2.02	6.21	3.01	4.17	1.14	0.44	2.34
ANTIBIOTICS	5	3.03	2.82	1.50	0.83	0.68	1.75	1.50
TO	4	2.02	6.21	2.26	1.67	0.91	2.63	2.34
WHICH	3	13.13	18.08	12.03	4.17	0.45	4.82	6.60
RESISTANT	2	14.14	37.29	36.84	10.83	6.59	27.63	19.55
	1	54.55	21.47	36.09	16.67	34.32	46.93	34.92
	0	8.08	1.69	3.76	35.00	45.91	11.84	23.97

seven antibiotics while October percentages are highest for five antibiotics and one antibiotic.

Table 19 presents the same data on a cumulative basis. This method of displaying the data seems to indicate that the general resistance level was highest in January since it is highest for eight or more antibiotics, seven or more antibiotics, six or more antibiotics, five or more antibiotics, and four or more antibiotics. It also exceeds the weighted mean at all levels except one or more antibiotics. This high level of antibiotic resistance in January results largely from the number of organisms resistant to seven antibiotics during that month. November isolates had the highest level of resistance to three or more antibiotics, two or more antibiotics, and one or more antibiotics, and exceeded the weighted mean at all levels except seven or more antibiotics.

Table 20 presents the percentage of fecal coliform organisms including bay organisms from each month resistant to a stated number of antibiotics. This information suggests that the overall antibiotic resistance level was highest in November. November resistance level was highest for six antibiotics, five antibiotics, four antibiotics, and three antibiotics, and the November occurrence of resistance to no antibiotics was lowest of all months. November resistance exceeded the weighted mean resistance at all levels except resistance to seven antibiotics and one antibiotic. March isolates were most resistant to eight or more antibiotics, January isolates were most resistant to seven antibiotics, December isolates were most resistant to two antibiotics, and October isolates were most resistant to one antibiotic.

Table 21 presents the same information cumulatively. This data

TABLE 19. Levels of antibiotic resistance in fecal coliform organisms (excluding bay organisms) - cumulative.

		MONTH						
		OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	MARCH	WEIGHTED MEAN
NUMBER OF ANTIBIOTICS TO WHICH RESISTANT	8 or more	1.01	4.52	3.76	5.00	3.41	3.07	3.51
	7 or more	3.03	6.21	4.51	26.67	10.00	3.95	8.77
	6 or more	5.05	12.42	7.52	30.84	11.14	4.39	11.11
	5 or more	8.08	15.24	9.02	31.67	11.82	6.14	12.61
	4 or more	10.10	21.45	11.28	33.34	12.72	8.77	14.95
	3 or more	23.23	39.53	23.31	37.51	13.18	13.59	21.55
	2 or more	37.37	76.82	60.15	48.34	19.77	41.22	41.10
	1 or more	91.92	98.29	96.24	65.01	54.09	88.15	76.02
	0 or more	100.00	99.98	100.00	100.01	100.00	99.99	99.99

TABLE 20. Levels of antibiotic resistance in fecal coliform organisms (including bay organisms).

		MONTH						
		OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	MARCH	WEIGHTED MEAN
	8 or more	0.50	5.75	2.06	5.58	3.41	9.73	5.01
NUMBER	7	1.00	2.53	1.23	14.72	6.59	2.24	4.33
OF	6	1.00	5.52	2.47	2.54	1.14	1.00	2.40
ANTIBIOTICS	5	3.48	4.37	1.65	1.02	0.68	2.49	2.35
TO	4	1.49	8.28	2.88	2.54	0.91	2.00	3.29
WHICH	3	7.96	25.75	8.23	3.55	0.45	5.49	9.34
RESISTANT	2	14.43	32.87	40.33	8.63	6.59	26.18	21.96
	1	58.21	13.79	38.68	24.37	34.32	41.90	33.28
	0	11.94	1.15	2.47	37.06	45.91	8.98	18.05

TABLE 21. Levels of antibiotic resistance in fecal coliform organisms (including bay organisms) - cumulative.

		MONTH						
		OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	MARCH	WEIGHTED MEAN
NUMBER OF ANTIBIOTICS TO WHICH RESISTANT	8 or more	0.50	5.75	2.06	5.58	3.41	9.73	5.01
	7 or more	1.50	8.28	3.29	20.30	10.00	11.97	9.34
	6 or more	2.50	13.80	5.76	22.84	11.14	12.97	11.74
	5 or more	5.98	18.17	7.41	23.86	11.82	15.46	14.09
	4 or more	7.47	26.45	10.29	26.40	12.73	17.46	17.38
	3 or more	15.43	52.20	18.52	29.95	13.18	22.95	26.72
	2 or more	29.86	85.07	58.85	38.58	19.77	49.13	48.68
	1 or more	88.07	98.86	97.53	62.95	54.09	91.03	81.96
	0 or more	100.01	100.01	100.00	100.01	100.00	100.01	100.01

indicates that resistance levels were highest during January and November. January isolates were most resistant to seven or more antibiotics, six or more antibiotics, and five or more antibiotics. They exceeded the weighted mean resistance at all levels except resistance to two or more antibiotics and one or more antibiotics. November isolates were most resistant to four or more antibiotics, three or more antibiotics, two or more antibiotics, and one or more antibiotics. They exceeded the weighted mean resistances at all levels except resistance to seven or more antibiotics.

R-Factor Transfer

Fifty fecal coliform organisms which were initially resistant to at least Sm, Am, and Tc were tested to detect the presence of transferable R-factors. The sample was deliberately biased toward those organisms which were resistant to a large number of antibiotics. Of these 50 organisms, 28 (56%) had maintained resistance after 6 months to Sm, Am, and Tc, one (two percent) to Sm only, one (two percent) to Am only, one (two percent) to Tc only, and four (eight percent) to Sm and Am. Fifteen organisms (30%) lost their resistance to all these antibiotics. This may indicate a fairly high incidence of R-factor mediated resistance among the initial isolates since the spontaneous loss of R-factors has been frequently reported (26,86,128,129), while chromosomal resistance is presumably much more stable since mutation of a chromosomal gene occurs only about once in ten million to one billion cell divisions (126). It should be noted here that the Am concentration for the drug resistance transfer study was 20 ug/ml while the Am concentration in the screening

was 10 ug/ml. This was necessary because the recipient could grow in the presence of 10 but not 20 ug/ml of Am.

Of 29 organisms which were resistant to Am, six (20.7%) of them transmitted this resistance to the recipient strain. Of 33 organisms resistant to Sm, 24 (72.7%) transmitted this resistance. Of 33 organisms resistant to Tc, three (9.1%) transmitted this resistance.

Table 22 lists all organisms in which transferable R-factors were present. The most common resistance pattern transferred was Sm, Su. Of the 27 donors which were resistant to both Sm and Su, 23 (85.2%) transferred this resistance to the recipient. The transfer of only Sm, Su occurred 11 times. This result is similar to that obtained using Sm and sulphonamide where Sm and sulphonamide were transferred together 63.3% of the time (72). It is also consistent with two proposed R-factor genetic maps which place sulphonamide and Sm resistance determinants either very near to each other (128) or immediately adjacent (126).

The next most common resistance pattern transferred was Sm, Am, Tc, Ct, Ot, Su, Pe. Of the 27 donors which had resistance to these antibiotics, eight (29.6%) transferred resistance to at least these antibiotics to the recipient. The transfer of only Sm, Am, Tc, Ct, Ot, Su, and Pe occurred five times (18.5%). Although this resistance marker is much larger than those commonly reported, this probably resulted from the much larger number of antibiotics used in this study than in most other studies. Since only two or three percent of R-factor DNA is needed to code for the resistance markers commonly reported (35), it is conceivable that one R-factor could code for a very large number of resistances.

TABLE 22. Drug resistance patterns in fecal coliforms and resistance marker of R-factors.

ANTIBIOTIC RESISTANCE PATTERN[a]	NO. OF STRAINS	MEDIA ON WHICH SELECTED	RESISTANCE MARKER[b] OF R-FACTOR
Sm,Am,Tc,Ct,Ot, Nm,Ni,Su,Km,Pe	2	Sm-Na	Sm,Am,Tc,Ct,Ot,Nm,Su,Km,Pe (2)
Sm,Am,Tc,Ct,Ot, Nm,Ni,Km,Pe	2	Sm-Na	Sm,Am,Nm,Km,Pe (1); Sm,Am, Tc,Ct,Ot,Nm,Km,Pe (1)
Sm,Am,Tc,Ct,Ot, Su,Pe	16	Sm-Na	Sm,Am,Tc,Ct,Ot,Su,Pe (3); Sm, Su (11); Sm,Su,Pe (1); Sm, Am, Tc,Ct,Ot,Ni,Su,Pe (1)
Sm,Am,Tc,Ct,Ot, Ni,Su,Pe	4	Sm-Na	Sm,Su (2); Sm,Su,Pe (1); Sm Tc,Ct,Ot (1)
Sm,Am,Tc,Ct,Ot, Nm,Ni,Su,Km,Pe	1	Am-Na	Sm,Am,Tc,Ct,Ot,Pe (1)
Sm,Am,Tc,Ct,Ot, Nm,Ni,Km,Pe	2	Am-Na	Sm,Am,Nm,Km,Pe (1); Sm,Am,Tc, Ct,Ot,Nm,Km,Pe (1)
Sm,Am,Tc,Ct,Ot, Su,Pe	2	Am-Na	Sm,Am,Tc,Ct,Ot,Su,Pe (2)
Sm,Am,Tc,Ct,Ot, Ni,Su,Pe	1	Am-Na	Am,Su,Pe (1)
Sm,Am,Tc,Ct,Ot, Nm,Ni,Km,Pe	2	Tc-Na	Sm,Am,Tc,Ct,Ot,Pe (1); Sm,Am, Tc,Ct,Ot,Nm,Ni,Km,Pe (1)
Sm,Am,Tc,Ct,Ot, Ni,Su,Pe	1	Tc-Na	Sm,Tc,Ct,Ot (1)

[a]Abbreviations used: ampicillin = Am; chloramphenicol = Cm; chlortetracycline = Ct; kanamycin = Km; nalidixic acid = Na; neomycin = Nm; nitrofurazone = Ni; oxytetracycline = Ot; procaine penicillin G = Pe; streptomycin = Sm; sulfathiazole = Su; tetracycline = Tc.

[b]Number in parentheses is number of isolates with each resistance marker.

Although resistance to nitrofuran antibiotics was high in the screening study (54.15% of isolates) and in others (131), this resistance is considered chromosomal in nature and R-factors generally do not participate in nitrofuran resistance. R-factor transfer of resistance to very low levels (0.2-1.0 ug/ml) of nitrofurans has been demonstrated using a mean inhibitory concentration method (8). This study described the observed phenomenon as a "reduced sensitivity" rather than an actual resistance suggesting that a resistance per se is not actually conferred but rather that a sensitive organism become less sensitive. Table 22 includes two cases in which nitrofurazone resistance to 25 ug/ml was transferred. In one case, the resistance did not manifest itself in the donor strain but did in the recipient after conjugation. The recipient control did not grow on Ni. This suggests a donor-recipient interaction which caused the manifestation of a previously masked trait. This transfer of resistance to Ni may be interpreted in any of four ways. First, this may truly be the first occasion where resistance transfer to this level of a nitrofuran agent has been demonstrated, and, if so, it is likely because other nitrofuran agents used have been furazolidone (8,131), nifuriprinol (8), and nifurprazine (8), while the present study used nitrofurazone. Second, the difference between this and other studies may be attributable to differences in method. BTB-lactose-nutrient agar[3] with a furazolidone concentration of 12.5 ug/ml was used in one study (131) while a mean inhibitory concentration method was used

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[3]Difco

in the other (8). Third, the observed phenomenon may be another manifestation of the "reduced sensitivity" phenomenon mentioned above (8). Slightly "reduced sensitivity" of a sensitive organism may well be equivalent to slightly "increased resistance" of a resistant organism. Thus, an organism which has a low level of resistance to a nitrofuran which might not normally manifest itself when confronted with 25 ug/ml of nitrofurazone might well have the balance tipped in its favor by the introduction of an R-factor capable of slightly increasing an already existing resistance. Fourth, and last, two observations are not enough to justify a definitive conclusion. This indicates a need for further study. All of the above antibiotic resistance transfer information must be viewed with an understanding that the sampling was very biased, i.e. organisms with high levels of antibiotic resistance were deliberately chosen.

Statistical Results

Information used in regression analysis is included in Table 23. This includes bacterial count, temperature and precipitation, antibiotic resistance, recreational use, river flow, and tide data.

Initial screening revealed that 26 of the 135 independent variables listed in Table 23 correlated significantly with bay fecal coliform counts using the 90% confidence level F-test of regression coefficients. Similarly, 15 of the independent variables correlated significantly with the log bay fecal coliform counts using the same criterion. These results are summarized in Table 24.

Realizing that these statistical relationships do not infer any

sort of cause-effect relationship between variables, it is nonetheless interesting to note that fecal coliform counts in the bay (represented for discussion purposes by either dependent variable) seem to reflect count data from the Trask and Wilson Rivers (X_2, X_4, and X_{11} in Table 24). This might be expected since most fecal coliform organisms in the bay probably arise from the rivers.

Ambient temperature also relates highly with bay counts (X_{16}, X_{18}, X_{19}, X_{25}, X_{27}, and particularly X_{28} in Table 24). Approximately 15,000 dairy cattle reside in the drainage areas of these four rivers. A large part of their fecal material is deposited on exposed pastures and eventually finds its way into these rivers. Ambient temperature might profoundly affect the survival of fecal coliforms in this fecal material on these pastures. If this fecal material on these pastures is a primary source of fecal coliforms for the bay, one would also expect some positive correlation between bay counts and precipitation since the fecal material on the pastures presumably is flushed into the rivers by rainfall. Indeed, average precipitation in the six days preceding sampling, including the sampling day, does correlate positively with bay counts (X_{36} in Table 24). Ambient temperature correlates negatively with bay counts and more negatively at the higher temperature extremes. This suggests that increasing temperatures might adversely affect fecal coliform survival. Several investigators have reported a significant inverse relationship between water temperature and coliform survival (22,37,120,121) although others have not found this to be true (16,73). Ambient temperatures may affect coliform survival on wet pastureland in the same way as water temperatures would affect coliform survival in

water. The negative correlation found between temperature and coliform count is consistent with the findings of the former investigators (37,120,121).

The correlation of bay counts with recreational use of the Kilchis River (X_{96} in Table 24) is not unexpected. Recreational use of these rivers could well contribute directly to coliform levels or may simply reflect other meaningful parameters such as ambient temperature or precipitation.

The relationship between various antibiotic resistance patterns and bay counts was common (X_{39}, X_{40}, X_{44}, X_{46}, X_{47}, X_{48}, X_{67}, X_{71}, X_{85}, X_{90}, X_{94}, X_{117}, X_{122}, and X_{124} in Table 24), although difficult to explain. As discussed earlier, some of these relationships might be explained by different survival potentials among antibiotic resistant and nonantibiotic resistant bacteria. Evidence available to date neither supports nor disputes this hypothesis. Some have suggested that R-factor mediated antibiotic resistance might reduce survival potential (6), others that R-factor mediated antibiotic resistance might increase survival potential (45,47), and still others that R-factor mediated antibiotic resistance has no effect on survival potential (111). It is interesting to note that in this study increasing antibiotic resistance always correlated positively with bay counts. In no case was the regression coefficient negative. In this study at least, this suggests a positive effect of antibiotic resistance on survival.

The correlation between tide data and bay counts (X_{129}, X_{133}, and X_{135} in Table 24) may reflect some kind of flushing effect that

accompanies tidal extremes.

Results of the stepwise regression search procedure for bay fecal coliform counts (Y_1) are included in Table 25. The results of evaluation of these regression functions by the criteria coefficient of determination (R^2), error mean square (MSE), and total squared error (C) are also presented. The three criteria agree that Model III is the "best" model. Similarly, results for log bay fecal coliform counts (Y_2) are included in Table 26. Model VI is the "best" model based on the three evaluation criteria.

It is interesting that the "best" models for Y_1 and Y_2 both contain parameters which reflect count data in the rivers (both contain X_2) and also contain data which reflect antibiotic resistance information (X_{94} and X_{48}). The models differ in that the third parameter reflects recreational use in the Y_1 model (X_{96}) and reflects ambient temperature in the Y_2 model (X_{28}).

Although the statistical tools utilized in this analysis account for the small sample size, more samples would have been very desirable and would have permitted further refinement of these models. These, or similar models, might be used to predict bay fecal coliform levels provided that the inherent limitations of such predictions are understood. Antibiotic resistance parameters might be eliminated from the models since they are the only parameters not easily obtained. Probably more important than the models themselves is the method of systematic screening of variables designed to produce useful predictive models, and to provide clues to the dynamic interrelationships at work in a given microbial ecosystem.

TABLE 23a. Data for regression analysis.

VARIABLE	BAY FC/ 100 ml	LOG BAY FC/ 100 ml	KIL R FC/ 100 ml	TRA R FC/ 100 ml	TIL R FC/ 100 ml	WIL R FC/ 100 ml	KIL R AHB/ 0.01 ml	TRA R AHB/ 0.01 ml	TIL R AHB/ 0.01 ml	WIL R AHB/ 0.01 ml
SYMBOL FOR VARIABLE	Y_1	Y_2	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8
OCTOBER	3.6	0.5563	13.9	8.8	66.0	20.0	23.0	31.0	24.0	34.0
NOVEMBER	42.0	1.6232	3.0	67.0	34.0	63.0	3.6	15.0	24.0	5.7
DECEMBER	27.0	1.4314	1.0	42.0	34.0	17.0	4.3	15.8	24.8	5.8
JANUARY	4.7	0.6721	2.7	0.0	13.5	8.5	1.2	2.7	9.4	1.7
MARCH	7.3	0.8633	0.5	0.0	32.0	11.0	1.5	0.1	11.0	3.4

TABLE 23b. Data for regression analysis.

VARIABLE	LOG KIL R FC/ 100 ml	LOG TIL R FC/ 100 ml	LOG WIL R FC/ 100 ml	LOG KIL R AHB/ 0.01 ml	LOG TRA R AHB/ 0.01 ml	LOG TIL R AHB/ 0.01 ml	LOG WIL R AHB/ 0.01 ml	MEAN LO TEMP (^{o}F) -6 PRE da.	MEAN HI TEMP (^{o}F) -6 PRE da.	LO TEMP (^{o}F) -PRE da.
SYMBOL FOR VARIABLE	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}
OCTOBER	1.1430	1.8195	1.3010	1.3617	1.4914	1.3802	1.5315	45	63	49
NOVEMBER	0.4771	1.5315	1.7993	0.5563	1.1761	1.3802	0.7559	37	52	26
DECEMBER	0.0000	1.5315	1.2304	0.6335	1.1987	1.3945	0.7634	35	51	29
JANUARY	0.4314	1.1303	0.9294	0.0792	0.4314	0.9731	0.2304	40	56	38
MARCH	-0.3010	1.5051	1.0414	0.1761	-1.0000	1.0414	0.5315	38	51	42

TABLE 23c. Data for regression analysis.

VARIABLE	HI TEMP (°F) -PRE da.	LO TEMP (°F) -SAMP da.	HI TEMP (°F) -SAMP da.	AVR MIN TEMP (°F) -PRE mo.	AVR MAX TEMP (°F) -PRE mo.	AVR TEMP (°F) -PRE mo.	LOG MEAN LO TEMP (°F) -6 PRE da.	LOG MEAN HI TEMP (°F) -6 PRE da.	LOG LO TEMP -PRE da.	LOG HI TEMP -PRE da.
SYMBOL FOR VARIABLE	X_{19}	X_{20}	X_{21}	X_{22}	X_{23}	X_{24}	X_{25}	X_{26}	X_{27}	X_{28}
OCTOBER	67	50	61	43.5	71.5	57.5	1.6532	1.7993	1.6902	1.8261
NOVEMBER	48	36	48	44.0	59.3	51.7	1.5682	1.7160	1.4150	1.6812
DECEMBER	50	34	58	38.0	53.5	45.8	1.5441	1.7076	1.4624	1.6990
JANUARY	62	39	65	38.5	50.5	44.5	1.6021	1.7482	1.5798	1.7924
MARCH	59	36	51	34.9	52.4	43.7	1.5798	1.7076	1.6232	1.7709

TABLE 23d. Data for regression analysis.

VARIABLE	LOG LO TEMP (^{o}F) -SAMP da.	LOG HI TEMP (^{o}F) -SAMP da.	LOG AVR MIN TEMP (^{o}F) -PRE mo.	LOG AVR MAX TEMP (^{o}F) -PRE mo.	LOG AVR TEMP (^{o}F) -PRE mo.	PRECIP (in.) -PRE mo.	PRECIP (in.) -PRE da.	AVR PRECIP (in.) -6 PRE da.	LOG PRECIP (in.) -PRE mo.	LOG AVR PRECIP (in.) -6 PRE da.
SYMBOL FOR VARIABLE	X_{29}	X_{30}	X_{31}	X_{32}	X_{33}	X_{34}	X_{35}	X_{36}	X_{37}	X_{38}
OCTOBER	1.6990	1.7853	1.6385	1.8543	1.7597	0.07	0.52	0.11	-1.1549	-0.9586
NOVEMBER	1.5563	1.6812	1.6435	1.7731	1.7135	13.77	0.08	0.67	1.1389	-0.1739
DECEMBER	1.5315	1.7634	1.5798	1.7284	1.6609	14.15	0.00	0.30	1.1508	-0.5229
JANUARY	1.5911	1.8129	1.5855	1.7033	1.6484	19.80	0.00	0.30	1.2967	-0.5229
MARCH	1.5563	1.7076	1.5428	1.7193	1.6405	11.38	0.04	0.27	1.0561	-0.5686

TABLE 23e. Data for regression analysis.

VARIABLE	% FC RES Sm -KIL R	% FC RES Am -KIL R	% FC RES Tc -KIL R	% FC RES Ct -KIL R	% FC RES Ot -KIL R	% FC RES Nm -KIL R	% FC RES Ni -KIL R	% FC RES Su -KIL R	% FC RES Km -KIL R	% FC RES Pe -KIL R
SYMBOL FOR VARIABLE	X_{39}	X_{40}	X_{41}	X_{42}	X_{43}	X_{44}	X_{45}	X_{46}	X_{47}	X_{48}
OCTOBER	4.35	0.00	8.70	4.35	8.70	0.00	86.96	0.00	0.00	8.70
NOVEMBER	94.12	52.94	17.65	17.65	17.65	35.29	88.24	17.65	5.88	41.18
DECEMBER	100.00	0.00	0.00	0.00	0.00	0.00	25.00	0.00	0.00	25.00
JANUARY	0.00	0.00	0.00	0.00	0.00	0.00	11.11	0.00	0.00	0.00
MARCH	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE 23f. Data for regression analysis.

VARIABLE	% FC RES Cm -TRA R	% FC RES Sm -TRA R	% FC RES Am -TRA R	% FC RES Tc -TRA R	% FC RES Ct -TRA R	% FC RES Ot -TRA R	% FC RES Nm -TRA R	% FC RES Ni -TRA R	% FC RES Na -TRA R	% FC RES Su -TRA R
SYMBOL FOR VARIABLE	X_{49}	X_{50}	X_{51}	X_{52}	X_{53}	X_{54}	X_{55}	X_{56}	X_{57}	X_{58}
OCTOBER	10.53	36.84	5.26	10.53	10.53	10.53	0.00	100.00	0.00	15.79
NOVEMBER	1.82	47.27	25.45	7.27	7.27	7.27	21.82	83.64	1.82	3.64
DECEMBER	0.00	96.43	25.00	25.00	25.00	25.00	14.29	60.71	0.00	25.00
JANUARY	0.00	28.57	14.29	14.29	14.29	28.57	14.29	0.00	0.00	28.57
MARCH	NO ISOL	NO ISOL	NO ISOL	NO ISOL	NO ISOL	NO ISOL	NO ISOL	NO ISOL	NO ISOL	NO ISOL

TABLE 23g. Data for regression analysis.

VARIABLE	% FC RES Km -TRA R	% FC RES Pe -TRA R	% FC RES Cm -TIL R	% FC RES Sm -TIL R	% FC RES Am -TIL R	% FC RES Tc -TIL R	% FC RES Ct -TIL R	% FC RES Ot -TIL R	% FC RES Nm -TIL R	% FC RES Ni -TIL R
SYMBOL FOR VARIABLE	X_{59}	X_{60}	X_{61}	X_{62}	X_{63}	X_{64}	X_{65}	X_{66}	X_{67}	X_{68}
OCTOBER	0.00	52.63	2.08	6.25	12.50	12.50	12.50	12.50	2.08	85.42
NOVEMBER	9.09	16.36	2.17	54.35	15.22	34.78	30.43	28.26	17.39	97.83
DECEMBER	10.71	14.29	0.00	100.00	15.63	3.13	3.13	3.13	9.38	50.00
JANUARY	14.29	14.29	22.03	25.42	10.17	8.47	8.47	10.17	10.17	47.46
MARCH	NO ISOL	NO ISOL	3.33	4.17	5.00	8.33	8.33	9.17	0.83	25.83

TABLE 23h. Data for regression analysis.

VARIABLE	% FC RES Na -TIL R	% FC RES Su -TIL R	% FC RES Km -TIL R	% FC RES Pe -TIL R	% FC RES Cm -WIL R	% FC RES Sm -WIL R	% FC RES Am -WIL R	% FC RES Tc -WIL R	% FC RES Ct -WIL R	% FC RES Ot -WIL R
SYMBOL FOR VARIABLE	X_{69}	X_{70}	X_{71}	X_{72}	X_{73}	X_{74}	X_{75}	X_{76}	X_{77}	X_{78}
OCTOBER	0.00	10.42	2.08	27.08	0.00	22.22	0.00	11.11	0.00	22.22
NOVEMBER	0.00	2.17	13.04	17.39	1.69	100.00	0.00	5.08	5.08	6.78
DECEMBER	0.00	0.00	3.13	3.13	1.54	86.15	7.69	10.77	3.08	10.77
JANUARY	1.69	6.78	3.39	11.86	2.78	86.11	86.11	86.11	86.11	86.11
MARCH	4.17	100.00	0.83	11.67	0.00	13.33	2.22	13.33	13.33	13.33

TABLE 23i. Data for regression analysis.

VARIABLE	% FC RES Nm -WIL R	% FC RES Ni -WIL R	% FC RES Su -WIL R	% FC RES Km -WIL R	% FC RES Pe -WIL R	% FC RES Cm -A S	% FC RES Sm -A S	% FC RES Am -A S	% FC RES Tc -A S	% FC RES Ct -A S
SYMBOL FOR VARIABLE	X_{79}	X_{80}	X_{81}	X_{82}	X_{83}	X_{84}	X_{85}	X_{86}	X_{87}	X_{88}
OCTOBER	0.00	66.67	22.22	0.00	0.00	3.03	13.13	7.07	11.11	9.09
NOVEMBER	23.73	98.31	0.00	0.00	13.56	1.69	71.19	16.95	14.69	13.56
DECEMBER	55.38	16.92	1.54	1.54	3.08	0.75	92.48	12.78	11.28	7.52
JANUARY	0.00	19.44	86.11	0.00	77.78	11.67	40.00	31.67	30.83	30.83
MARCH	8.89	53.33	84.44	8.89	26.67	2.38	6.55	4.17	9.52	9.52

TABLE 23j. Data for regression analysis.

VARIABLE	% FC RES Ot -A S	% FC RES Nm -A S	% FC RES Ni -A S	% FC RES Na -A S	% FC RES Su -A S	% FC RES Km -A S	% FC RES Pe -A S	SAL FROM KIL R	SAL FROM TRA R	SAL FROM TIL R
SYMBOL FOR VARIABLE	X_{89}	X_{90}	X_{91}	X_{92}	X_{93}	X_{94}	X_{95}	X_{96}	X_{97}	X_{98}
OCTOBER	12.12	1.01	86.87	0.00	10.10	1.01	25.25	47	1564	259
NOVEMBER	13.56	22.60	92.66	0.56	3.39	6.78	18.08	326	751	122
DECEMBER	11.28	32.33	34.59	0.00	6.02	3.76	6.77	51	71	31
JANUARY	32.50	5.83	30.83	0.83	30.83	2.50	30.00	28	74	16
MARCH	10.12	2.98	32.74	2.98	94.05	2.98	15.48	0	0	0

TABLE 23k. Data for regression analysis.

VARIABLE	SAL FROM WIL R	SAL FROM All R	STL FROM KIL R	STL FROM TRA R	STL FROM TIL R	STL FROM WIL R	STL FROM KIL R	FLOW IN KIL R (cfs)	FLOW IN TRA R (cfs)	FLOW IN TIL R (cfs)
SYMBOL FOR VARIABLE	X_{99}	X_{100}	X_{101}	X_{102}	X_{103}	X_{104}	X_{105}	X_{106}	X_{107}	X_{108}
OCTOBER	696	2566	32	114	4	174	324	87	168	65
NOVEMBER	1030	2229	233	169	55	851	1308	487	901	363
DECEMBER	130	283	530	480	320	1779	3118	771	1548	574
JANUARY	0	118	371	846	321	2758	4296	700	1501	520
MARCH	67	67	60	618	60	860	1598	556	1249	414

TABLE 23 1. Data for regression analysis.

VARIABLE	FLOW IN WIL R (cfs)	FLOW IN All R (cfs)	% FC[a] RES >7 ANTI	% FC[a] RES >6 ANTI	% FC[a] RES >5 ANTI	% FC[a] RES >4 ANTI	% FC[a] RES >3 ANTI	% FC[a] RES >2 ANTI	% FC[a] RES >1 ANTI	% FC[a] RES >0 ANTI
SYMBOL FOR VARIABLE	X_{109}	X_{110}	X_{111}	X_{112}	X_{113}	X_{114}	X_{115}	X_{116}	X_{117}	X_{118}
OCTOBER	227	547	1.01	3.03	5.05	8.08	10.10	23.23	37.37	91.92
NOVEMBER	1276	3027	4.52	6.21	12.42	15.24	21.45	39.53	76.82	98.29
DECEMBER	2020	4913	3.76	4.51	7.52	9.02	11.28	23.31	60.15	96.24
JANUARY	1832	4553	5.00	26.67	30.84	31.67	33.34	37.51	48.34	65.01
MARCH	1457	3676	3.07	3.95	4.39	6.14	8.77	13.59	41.22	88.15

TABLE 23m. Data for regression analysis.

VARIABLE	% FC[a] RES 7 ANTI	% FC[a] RES 6 ANTI	% FC[a] RES 5 ANTI	% FC[a] RES 4 ANTI	% FC[a] RES 3 ANTI	% FC[a] RES 2 ANTI	% FC[a] RES 1 ANTI	% FC[a] RES 0 ANTI	DIFF BET SAMP TIME & TIME OF NXT PRE HI TIDE (min.)	HT OF NXT PRE HI TIDE (ft.)
SYMBOL FOR VARIABLE	X_{119}	X_{120}	X_{121}	X_{122}	X_{123}	X_{124}	X_{125}	X_{126}	X_{127}	X_{128}
OCTOBER	2.02	2.02	3.03	2.02	13.13	14.14	54.55	8.08	160	670
NOVEMBER	1.69	6.21	2.82	6.21	18.08	37.29	21.47	1.69	89	6.28
DECEMBER	0.75	3.01	1.50	2.26	12.03	36.84	36.09	3.76	225	8.55
JANUARY	21.67	4.17	.83	1.67	4.17	10.83	16.67	35.00	24	7.58
MARCH	0.88	0.44	1.75	2.63	4.82	27.63	46.93	11.84	96	6.43

TABLE 23n. Data for regression analysis[b,c].

VARIABLE	HT OF NEXT PRE LO TIDE (ft.)	HT OF NEXT PRE HI TIDE -mo. DTL (ft.)	HT OF NEXT PRE HI TIDE -mo. MTL (ft.)	HT OF NEXT PRE HI TIDE -mo. MSL (ft.)	Mo.DTL -HT OF NEXT PRE LO TIDE (ft.)	Mo.MTL -HT OF NEXT PRE LO TIDE (ft.)	Mo.MSL -HT OF NEXT PRE LO TIDE (ft.)
SYMBOL FOR VARIABLE	X_{129}	X_{130}	X_{131}	X_{132}	X_{133}	X_{134}	X_{135}
OCTOBER	0.77	2.87	2.60	2.58	3.06	3.33	3.35
NOVEMBER	2.39	2.31	2.00	1.98	1.58	1.89	1.91
DECEMBER	2.24	4.73	4.43	4.40	1.58	1.88	1.91
JANUARY	0.99	3.80	3.56	3.48	2.79	3.03	3.11
MARCH	-0.24	2.64	2.52	2.48	4.03	4.15	4.19

[a]Excluding bay organisms.

[b]Abbreviations used: AHB = aerobic heterophic bacteria; ANTI = antibiotic(s); A S = all sources; AVR = average; BET = between; cfs = cubic feet/second; DIFF = difference; DTL = diurnal tide level; FC = fecal coliform; HI = high; HT = height; ISOL = isolates; KIL = Kilchis; LO = low; LOG = base 10 logarithm; MAX = maximum; MIN = minimum; MSL = mean sea level; MTL = mean tide level; NXT = next; PRE = previous; PRECIP = precipitation; R = river; RES = resistant; SAL = salmon caught; SAMP = sampling; STL = steelhead caught; TEMP = temperature; TIL = Tillamook; TRA = Trask; WIL = Wilson.

[c]Abbreviations for antibiotics same as Table 22.

TABLE 24. Results of preliminary statistical analysis (significant F-values only)[a]

INDEPENDENT VARIABLE (X)	REGRESSION COEFFICIENT	STANDARD ERROR OF REGRESSION COEFFICIENT	F VALUE	P VALUE	SIGNIFICANCE LEVEL	COEFFICIENT OF MULTIPLE DETERMINATION	INTERCEPT
DEPENDENT VARIABLE = BAY FC/100 ml (Y_1)							
X_2, TRA R FC/100 ml	0.55078	0.06116	83.48	<.005	0.005	0.965	3.7551
X_4, WIL R FC/100 ml	0.63919	0.23648	7.31	<.100	0.10	0.709	1.6434
X_{11}, LOG WIL R FC/100 ml	41.786	16.39631	6.49	<.100	0.10	0.684	-35.743
X_{18}, LO TEMP (°F) -PRE da.	-1.6254	0.44691	13.23	<.050	0.05	0.815	76.733
X_{19}, HI TEMP (°F) -PRE da.	-1.9699	0.43304	20.69	<.025	0.025	0.073	129.60
X_{27}, LOG LO TEMP (°F) -PRE da.	-139.31	30.70039	20.5	<.025	0.025	0.073	233.43
X_{20}, LOG HI TEMP (°F) -PRE da.	-259.92	50.60271	26.38	<.025	0.025	0.898	472.79
X_{36}, AVR PRECIP (in.) -6 PRE da.	71.423	23.71304	9.07	<.100	0.10	0.751	-6.6495
X_{39}, % FC RES Sm - KIL R	0.30098	0.06820	19.47	<.025	0.025	0.866	4.9730
X_{40}, % FC RES Ap - KIL R	0.59218	0.23252	6.49	<.100	0.10	0.684	10.650
X_{44}, % FC RES Nm - KIL R	0.88835	0.34881	6.49	<.100	0.10	0.684	10.650
X_{46}, % FC RES Su - KIL R	1.7762	0.69742	6.49	<.100	0.10	0.684	10.650
X_{47}, % FC RES Km - KIL R	5.3316	2.09343	6.49	<.100	0.10	0.604	10.650
X_{48}, % FC RES Pe - KIL R	0.91674	0.14310	41.04	<.010	0.01	0.932	3.1910
X_{67}, % FC RES Nm - TIL R	2.0455	0.84865	5.81	<.100	0.10	0.659	0.61713
X_{71}, % FC RES Km - TIL R	2.9516	1.05612	7.01	<.100	0.10	0.722	3.6557
X_{90}, % FC RES Nm - A S	1.0236	0.39267	6.80	<.100	0.10	0.694	3.6644
X_{94}, % FC RES Km - A S	7.4112	1.64054	20.41	<.025	0.025	0.872	-8.3227
X_{96}, SAL FROM KIL R	0.10875	0.03816	8.12	<.100	0.10	0.730	7.0094
X_{117}, % FC RES 1 ANTI	1.0243	0.15786	42.10	<.010	0.10	0.933	-37.144
X_{122}, % FC RES 4 ANTI	7.7258	2.83970	7.40	<.100	0.10	0.712	-5.9328
X_{124}, % FC RES 2 ANTI	1.1685	0.40850	8.10	<.100	0.10	0.732	-12.696
X_{129}, HT OF NEXT PRE LO TIDE (ft.)	13.027	4.03290	7.26	<.100	0.10	0.708	0.89676
X_{133}, Mo. OTL - HT OF NEXT PRE LO TIDE (ft.)	-13.279	5.37363	6.11	<.100	0.10	0.670	51.551
X_{134}, Mo. MTL - HT OF NEXT PRE LO TIDE (ft.)	-14.404	5.59548	6.63	<.100	0.10	0.688	58.058
X_{135}, Mo. MSL - HT OF NEXT PRE LO TIDE (ft.)	-14.410	5.45957	6.97	<.100	0.10	0.699	58.624
DEPENDENT VARIABLE = LOG BAY FC/100 ml (Y_2)							
X_2, TRA R FC/100 ml	-0.014774	0.0033168	19.84	<.025	0.025	0.869	0.68119
X_{16}, MEAN LO TEMP (°F) -6 PRE da.	-0.10044	0.042086	5.70	<.100	0.10	0.655	4.9463
X_{18}, LO TEMP (°F) - PRE da.	-0.046823	0.010412	20.22	<.025	0.025	0.871	2.7524
X_{19}, HI TEMP (°F) - PRE da.	-0.057990	0.0054406	113.61	<.005	0.005	0.974	4.3463
X_{25}, LOG MEAN LO TEMP (°F) -6 PRE da.	-9.3659	3.79277	6.10	<.100	0.10	0.670	15.916
X_{27}, LOG LO TEMP (°F) - PRE da.	-3.9545	0.73822	20.70	<.025	0.025	0.905	7.1750
X_{28}, LOG HI TEMP (°F) - PRE da.	-7.5884	0.53656	200.0	<.001	0.001	0.985	14.339
X_{36}, AVR PRECIP (in.) -6 PRE da.	1.8571	0.77979	5.67	<.100	0.10	0.654	0.41640
X_{39}, % FC RES Sm - KIL R	0.0085512	0.0016420	27.12	<.025	0.025	0.900	0.68983
X_{48}, % FC RES Pe - KIL R	0.023839	0.0066395	12.89	<.050	0.05	0.811	0.67224
X_{85}, % FC RES Sm - A S	0.010605	0.0041304	6.59	<.100	0.10	0.687	0.55555
X_{90}, % FC RES Nm - A S	0.030694	0.0097770	12.23	<.050	0.05	0.803	0.63177
X_{94}, % FC RES Km - A S	0.20128	0.053001	14.42	<.050	0.05	0.828	0.34370
X_{117}, % FC RES >1 ANTI	0.027403	0.0063830	18.43	<.025	0.025	0.860	-0.41707
X_{124}, % FC RES 2 ANTI	0.035409	0.0090764	19.22	<.025	0.025	0.875	-0.13179

[a] Abbreviations same as Table 23.

TABLE 25. Results of stepwise regression search procedure (Y_1)[a].

	MODELS[b]	COEFFICIENT OF MULTIPLE DETERMINATION (R^2)	ERROR MEAN SQUARE (MSE)	TOTAL SQUARED ERROR (C)	STANDARD ERROR OF REGRESSION COEFFICIENTS	t VALUES
I	$Y_1 = 3.7551 + .55878\ X_2$	0.965	13.30	400.60	X_2, 0.061156	9.137
II	$Y_1 = -1.4462 + .39376\ X_2$	0.994	3.36	68.65	X_2, 0.060854	6.470
	$+2.6686\ X_{94}$				X_{94}, 0.84929	3.142
III	$Y_1 = -2.6336 + .44001\ X_2$	0.999	0.10	4.00	X_2, 0.011898	36.978
	$+3.2699\ X_{94}$				X_{94}, 0.16355	19.993
	$-.021574\ X_{96}$				X_{96}, 0.0026427	-8.164

[a] Abbreviations same as Table 23.

[b] Y_1 = BAY FC/100 ml

X_2 = TRA R FC/100 ml

X_{94} = % FC RES Km -A S

X_{96} = SAL FROM KIL R

TABLE 26. Results of stepwise regression search procedure (Y_2)[a].

	MODELS[b]	COEFFICIENT OF MULTIPLE DETERMINATION (R^2)	ERROR MEAN SQUARE (MSE)	TOTAL SQUARED ERROR (C)	STANDARD ERROR OF REGRESSION COEFFICIENTS	t VALUES
IV	$Y_2 = 14.339 - 7.5884\ X_{28}$	0.985	4.40×10^{-3}	30,400	X_{28}, 0.53656	-14.142
V	$Y_2 = 11.848 - 6.2161\ X_{28}$	0.998	9.38×10^{-4}	4,320	X_{28}, 0.46615	-13.335
	$+.0056084\ X_{48}$				X_{48}, 0.0016139	3.475
VI	$Y_2 = 16.591 - .035748\ X_2$	0.999	4.34×10^{-7}	4	X_2, 0.00054411	-65.700
	$-8.8815\ X_{28}$				X_{28}, 0.041792	-212.519
	$+.057268\ X_{48}$				X_{48}, 0.00078707	72.761

[a] Abbreviations same as Table 23.

[b] Y_2 = LOG BAY FC/100 ml

X_2 = TRA R FC/100 ml

X_{28} = LOG HI TEMP (^{o}F) -PRE da.

X_{48} = % FC RES Pe - KIL R

SUMMARY

Fecal coliform bacteria were isolated from Tillamook Bay, Oregon and its tributaries during the rainy season and attempts were made to establish the origin of the bay fecal coliforms by comparing the antibiotic resistance patterns of the isolated bacteria. The major findings of this study are:

1. Except the Kilchis River site, which was above the drainage basin, the fecal coliform levels of the tributaries exceeded those of the bay.
2. The count fluctuated by month, being the highest after heavy rainfall.
3. The 176 antibiotic resistance patterns exhibited by 1,917 isolates did not show site specific characteristics.
4. The antibiotic resistance was readily transferable to E. coli K-12 (strain W3110) with frequencies of 72.7% for streptomycin (Sm), 20.7% for ampicillin (Am), and 9.1% for tetracycline (Tc).
5. The bay fecal coliform counts were highly correlated with the counts of the tributaries, antibiotic resistance, recreational use of the rivers, and precipitation.
6. The ambient temperature showed a negative correlation with the bay count.
7. Two linear regression models that predicted the bay fecal coliform count were developed by the use of a computerized stepwise multiple linear regression program.

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