Seasonal Comparison of Fecal Coliform Concentrations in the Trask River and a Study of the Survival of *Escherichia coli* in Tillamook Bay Water

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INTRODUCTION

Fecal contamination has been identified as a priority environmental problem in Tillamook Bay because of the health risk it poses for the shellfish industry and for those who use the bay for recreational activities. The Oregon Department of Agriculture and county health departments monitor water quality in the bay monthly by measuring the most probable number (MPN) of fecal coliforms at 16 sampling sites using a five-tube lactose fermentation test. None of the shellfish growing areas in the bay is currently classified as "approved" for continuous shellfish harvest. The Main Bay and Cape Meares growing areas are classified as "conditionally approved", while the Flower Pot area is classified as "restricted" and the Upper Bay is classified as "prohibited" (42). Shellfish harvesting is allowed in conditionally approved or restricted areas only when the concentration of fecal coliforms does not exceed the U. S. Food and Drug Administration's standards for shellfish growing waters or when purification of the shellfish products can be assured. No shellfish products may be harvested from a prohibited area for use in interstate commerce. According to the most recent Oregon Department of Environmental Quality (DEQ) Water Quality Status Report (42), the classification of six of the 16 sampling sites in Tillamook Bay decreased between 1991 and 1993 due to increases in fecal coliform concentrations.

Between October 1991 and September 1993, the Main Bay growing area was closed for 55 days and the Cape Meares area was closed for 77 days under a management plan to control fecal contamination of shellfish products. Closures such as these, which are based upon stream gauge levels in the Wilson River, have a severe negative impact on shellfish growers and on the local economy of the counties surrounding Tillamook Bay. Oyster production has decreased by 80 percent since the largest commercial grower went out of business in 1990, and water quality has continued to worsen due to increases in fecal contamination.

Fecal coliforms are the most commonly used indicators of fecal contamination in natural bodies of water. Traditional methods for detecting coliform bacteria use standard bacteriological media to selectively culture gram negative species and to differentiate lactose-fermenting from lactose-nonfermenting organisms (21). The concentration of bacterial contaminants in water is determined by membrane filtration or by multiple-tube fermentations which estimate the MPN of coliform bacteria. Fecal (thermotolerant) coliforms are

differentiated from the more temperature-sensitive coliforms that may arise from non-fecal sources by incubating the cultures at an elevated temperature (44.5 °C).

Microbiologists have identified several problems associated with traditional methods that rely upon selective cultivation to identify coliform bacteria. These methods fail to detect bacterial contaminants that do not survive handling between the time of sample collection and the time at which the samples are inoculated into selective and differential culture media, and they do not detect bacteria that are viable but nonculturable due to environmental stresses (22, 52). Another disadvantage of viable culture methods is the time required (days) to confirm the presence of fecal contaminants in the water.

Alternative methods designed to circumvent some of these problems include colorimetric or fluorimetric tests for specific enzymes involved in lactose fermentation (3, 7) and detection of the genes that encode these enzymes by extracting DNA from water samples and amplifying specific sequences with the polymerase chain reaction (2). Enzymatic techniques provide more rapid results than traditional detection methods, but in many cases still require cultivation of viable bacteria and fail to detect organisms that are viable but metabolically inactive due to environmental factors (46). Genetic detection methods can also provide rapid and reliable results, but the specificity and sensitivity of detection varies depending upon the method used to extract DNA from the water, the annealing temperature and specificity of the primer used for the amplification reaction, and the type of probe that is used to identify the target sequences (2).

Several potential point and nonpoint sources of fecal contamination have been identified, including runoff from agricultural land, outfall from sewage treatment facilities, and failing septic systems, but no systematic studies have been conducted to assess the relative impacts of these sources on water quality in Tillamook Bay. We have therefore conducted a study to measure the concentrations of fecal coliforms in the Trask River, one of five rivers that drain into the bay, at six sites representing different land use areas and potential sources of fecal contamination. The normal habitat of these bacteria is the intestinal tract of warm-blooded animals, so their presence in surface waters indicates that the water has been contaminated with human or animal waste. The multiple-tube fermentation method was chosen to measure the MPN of fecal coliforms because it is the method used by the Oregon Department of Agriculture to monitor fecal contamination in the

bay and because the time required to obtain results was not a primary concern. Water temperature, salinity, and dissolved oxygen concentrations were measured at each sampling site to determine whether a correlation exists between these physical parameters and the concentration of viable fecal coliforms in the water.

The severity of the public health risk associated with fecal contamination depends on a number of factors, including how long the bacteria survive in the water. Several environmental factors have been shown to influence bacterial survival in aquatic habitats. Sunlight is thought to be the primary factor limiting bacterial survival in seawater (5). Other important factors include salinity (45), temperature (48), the growth stage of the bacteria (17), competition with other bacteria (27), predation by protozoa (14), low nutrient concentrations (18), and toxic substances in the water (28).

Estuaries are profoundly affected by tidal movements which produce wide variations in salinity and temperature on a daily and seasonal basis. Organisms that live in estuaries must have wide ranges of tolerance to survive the stressful physical conditions of the habitat. Indigenous species have acquired adaptations that enable them to tolerate the changing conditions of an estuary, and nonindigenous species must exhibit similar tolerance in order to survive. Enteric bacteria are not indigenous to aquatic habitats, but increasingly find their way into rivers, lakes, and estuaries through human activity. The longer these bacteria survive in contaminated water, the more likely they are to become dispersed over a large geographic area and the greater the risk to human health and to the wildlife that inhabit the area. For this reason we have also studied the survival of *Escherichia coli*, a common indicator of fecal contamination, in water samples collected from Tillamook Bay and from two major sources of water that enter the bay – the Trask River and the Pacific Ocean. Survival was monitored over a wide range of temperatures and natural extremes of salinity by inoculating the water samples with a high concentration of stationary phase bacterial cells and periodically measuring the number of surviving cells by standard plate counts on a selective and differential culture medium.

MATERIALS AND METHODS

Trask River sample collection and handling. Midstream water samples were collected at five sites on the Trask River and one site on Hoquarten slough during low tides on

31 July 1996 (tide level = -1.8 ft) and 19 October 1996 (tide level = 2.9 ft). No measurable rain had fallen for at least 7 days prior to the July (dry season) sample collection. Almost 12 cm of rain fell during the week preceding the October (wet season) sampling, including 4.8 cm on the day that the samples were collected.

Site 1 (Memaloose Point site) was located 0.8 mi downstream of river mile 0 on the Trask River levee, northwest of Tillamook, Oregon (Fig. 1). Site 2 (Hoquarten slough site) was located at the point where U. S. Highway 101 crosses Hoquarten slough, north of Tillamook. Site 3 (Third Street bridge site) was located at river mile 1.2 on the Trask River levee, 0.1 mi downstream of the Tillamook sewage treatment plant. Site 4 (Highway 101 bridge site) was located at river mile 4.2 on the Trask River, 1 mi downstream from the sewage treatment ponds near the Tillamook airport. Site 5 (Peninsula site) was located at river mile 14.5 on the Trask River, 0.5 mi east of a horseshoe bend in the river known as The Peninsula. Site 6 (North Fork site) was located at river mile 20.2 on the North Fork of the Trask River.

Three samples were collected at each site in critically clean, sterile 300-ml glass BOD bottles. All of the bottles were filled to the neck and immediately capped with a sterile glass stopper. At sites 2, 3, and 4, the samples were collected by lowering each bottle into the river at midstream from a highway bridge. At sites 1 and 6, the bottles were tied to the end of a 10-foot extension rod and placed into the water from the bank of the river. The 10-foot length was sufficient to reach midstream at site 6, but not at site 1, where the channel is 0.1 mi wide. At site 5, the river was shallow enough to wade to midstream and collect the samples by placing the bottles into the water by hand. The bottles were placed in the water neck downward and moved forward as they filled. Vinyl gloves were worn while collecting the samples to reduce the risk of contamination with skin microflora. The gloves were changed between sampling sites to avoid cross contamination.

Water temperature and salinity were measured in the field with a YSI Model 33 salinity meter (Yellow Springs Instrument Co., Yellow Springs, OH). All measurements were made at midstream except at site 1, where the probe was placed in the water at the end of a boat ramp. Dissolved oxygen was measured with a YSI Model 58 dissolved oxygen meter equipped with a YSI Model 5739 dissolved oxygen probe. Midstream measurements were recorded at sites 5 and 6. The remaining measurements were recorded at a distance of 10 feet from the bank of the river by placing the probe into the water with the extension rod. The probe's cable was

not long enough to reach the water from the highway bridges at sites 2, 3, and 4. The data obtained from these measurements are listed in Table 1.

MPN determination. The water samples were transported on ice to the Microbiology Laboratory at the University of Portland and immediately inoculated into Phenol Red Lactose broth (Difco) to determine the MPN of fecal coliform bacteria. A five-tube MPN determination (33) was performed for each of the three replicate samples collected at each site. Each water sample was aseptically inoculated into five replicate tubes of lactose broth at each of three successive 10-fold dilutions. The tubes were incubated at 44.5 °C for 48 h and examined for evidence of lactose fermentation after 24 and 48 h. Tubes exhibiting an acid reaction and a gas volume of at least 10% in the Durham tube after 24 h were scored as positive reactions. The number of positive reactions at each dilution was used to determine the MPN of fecal coliforms from a standard MPN table. When acid and gas production occurred only after 48 h incubation, the tubes were scored as doubtful positive reactions and not included in the MPN determinations.

Every tube in the five-tube dilution series yielded a positive reaction in the initial analysis of the wet season samples from the Memaloose Point, Hoquarten slough, and Third Street bridge sites. It was therefore necessary to dilute these water samples 100-fold and inoculate a three-tube dilution series on the following day to obtain MPN indices for the three downstream sites. The tubes were incubated at 44.5 °C for 48 h and evaluated for evidence of lactose fermentation as described above.

Confirmation of fecal coliform contamination. Eosin Methylene Blue (EMB) agar (Difco) plates were inoculated from the lactose broths at the highest dilutions exhibiting a positive or doubtful positive reaction. The plates were examined for the presence of typical coliform colonies after 24 h incubation at 44.5 °C . The presence of small colonies with a dark center or with a metallic green sheen confirmed that the lactose fermentation in the phenol red medium was produced by coliform bacteria. Selected colonies (three from each water sample) were aseptically transferred to Nutrient agar (Difco) slants and Phenol Red Lactose broths and incubated at 44.5 °C for 24 h. Isolation of thermotolerant, gram negative, nonsporeforming rods which fermented lactose to acid and gas constituted a completed test for the presence of fecal coliforms in the water samples.

Survival experiments. *Escherichia coli* strain K12 (Ward's, Rochester, NY) was obtained from the culture collection at the University of Portland. To prepare the inoculum for each experiment, the bacterium was grown to stationary phase in 5 ml of Nutrient broth (Difco) with moderate shaking in a 30 °C water bath. The cells were harvested by centrifuging the cultures at 3000 x *g* for 10 min. The harvested cells were washed once with 10 ml sterile distilled water and resuspended in 5 ml sterile distilled water.

Surface water samples were collected from Tillamook Bay during a low tide (–0.8 ft) on 14 July 1996. The sampling site was located approximately 0.9 km ESE of the Pacific Oyster Company in Bay City, Oregon, near the Oregon Department of Agriculture sampling site 1. The samples were obtained by placing clean, sterile 500-ml screw-cap bottles into the water neck downward and moving the bottles forward as they filled. Vinyl gloves were worn while collecting the samples to reduce the risk of contamination with skin microflora.

Water samples were collected in a similar manner from the Pacific Ocean shore and from the Trask River during a low tide (0.5 ft) on 21 July 1996. The ocean water sample was collected at Rockaway Beach, Oregon, by wading into the surf to a depth of approximately 1 m. The river water sample was collected at the Peninsula site (river mile 14.5) on the Trask River. All water samples were transported on ice to the Microbiology Laboratory at the University of Portland, where they were stored at 4 °C. Water temperature and salinity were measured in the field with a YSI Model 33 salinity meter. The pH of the samples was measured in the laboratory with a Corning pH meter equipped with an Orion combination pH probe. The data obtained from these measurements are summarized in Table 2.

Temperature experiment. Nine 50-ml aliquots of the Tillamook Bay water were aseptically transferred into separate sterile 125-ml flasks. A 100-µl sample of the water in each flask was spread onto an EMB agar (Difco) plate to test for background levels of bacteria that produce typical coliform colonies. Each flask was subsequently inoculated with 500 µl of washed cells from a stationary phase *E. coli* culture. Serial dilutions were prepared immediately after adding the inoculum (0 h) and a standard plate count method was used to determine the concentration of cells added to each flask. Three flasks were incubated at 4 °C in a laboratory refrigerator, three were incubated at 11 °C in a low temperature incubator (Precision/GCA, Chicago, IL), and three were incubated at 23 °C in a climate-controlled laboratory. The number of viable cells in each flask was determined at 24-h intervals for 96 h

using a standard plate count method. EMB agar was used for all plate counts to select and differentiate *E. coli* colonies.

Salinity experiment. Three 50-ml aliquots of the Trask River, Tillamook Bay, and Pacific Ocean shore water samples were aseptically transferred into sterile 125-ml flasks. A 100-µl sample of the water in each flask was spread onto an EMB agar plate to test for background levels of bacteria that produce typical coliform colonies. Each flask was inoculated with 500 µl of washed cells from a stationary phase *E. coli* culture and the concentration of the inoculum in each flask was determined by a standard plate count. The water samples were incubated at 11 °C in the low temperature incubator and plate counts were performed at 24-h intervals for 96 h to assess the survival of *E. coli* in water from the three locations. EMB agar was used for all plate counts to select and differentiate *E. coli* colonies.

Statistical analyses. The MPN index provided a statistical estimate of the concentration of fecal coliforms in each river water sample within a 95% confidence limit. A Kruskal-Wallis One-Way ANOVA on Ranks was performed to compare the median MPNs of fecal coliforms at the six sampling sites. Pairwise comparisons between sampling sites were made with the Student-Newman-Keuls test. The Mann-Whitney rank sum test was used to compare dry season and wet season median MPNs at each site. A Pearson Product Moment Correlation test was run to determine whether correlations existed between the MPN of fecal coliforms, water temperature, salinity, and the concentration of dissolved oxygen.

Each experimental group consisted of two factors in both survival experiments – temperature and incubation time in the temperature experiment and water source and incubation time in the salinity experiment. A two-way repeated measures ANOVA was performed to determine whether either factor produced significant differences in the mean concentration of viable *E. coli* cells (log CFU/ml) in each experiment and to test for significant interactions between the two factors. The data at each sampling time were then analyzed independently with a one-way ANOVA to pinpoint when significant differences appeared between temperatures or between the different sources of water. The Student-Newman-Keuls method was used for pairwise comparisons between experimental groups. Simple linear regression analyses were performed to determine whether changes in the concentration of viable cells with time fit a linear model.

RESULTS

Seasonal MPNs of fecal coliforms. The concentration of fecal coliforms was significantly higher ($P < 0.05$) at the Hoquarten slough site than at any of the other sampling sites in the dry season samples (Fig. 3). The median MPN of fecal coliforms in the slough at that time was 900/100 ml. No significant differences were detected among the remaining sites ($P > 0.05$), where the median MPN ranged from 30 to 130/100 ml. Subsequent tests confirmed that the lactose fermentation observed in the MPN dilution series was produced by fecal coliforms. All of the bacteria isolated from the tubes that yielded positive or doubtful positive reactions were gram negative, nonsporeforming rods which produced acid and gas in Phenol Red Lactose broth at 44.5 °C.

The concentration of fecal coliforms remained highest at the Hoquarten slough site following the October rains (Fig. 4). The median MPN in the slough at that time was 110,000/100 ml, a 122-fold increase over the dry season median. Despite the substantial increase in the concentration of fecal coliforms in the slough, the median MPN of the wet season samples did not differ significantly from that of the dry season samples. It also did not differ significantly from the median MPNs at the Memaloose Point and Third Street bridge sites. The median MPN of the wet season samples at Memaloose Point (2300/100 ml) was 32 times greater than that of the dry season samples, while the wet season median at the Third Street bridge site (1600/100 ml) was 12 times greater than the dry season median. Neither of these differences was statistically significant. Much lower concentrations of fecal coliforms were detected at the three upstream sites. The median MPN at the Highway 101 site (240/100 ml) did not differ significantly from those at the Memaloose Point and Third Street bridge sites, but it did differ significantly from the median MPNs at the Peninsula site (130/100 ml) and at the North Fork site (50/100 ml). Variation in the MPNs at the downstream sites, where the coliform concentrations were very high, had a profound effect on the outcome of the statistical comparisons.

Correlation of MPNs with temperature, salinity, and dissolved oxygen. The MPNs of fecal coliforms did not correlate significantly with water temperature ($P = 0.39$) or salinity (0.83) in the dry season samples, but did correlate strongly with the concentration of dissolved oxygen ($P < 0.01$). The correlation of MPNs with dissolved oxygen (DO) was negative ($r = -0.80$), with the lowest DO concentration (2.5 mg/l) recorded at the Hoquarten slough site,

where the concentration of fecal coliforms was highest. The only other significant correlation noted at that time was a weak negative correlation between temperature and DO ($r = -0.52$).

Median MPNs of fecal coliforms correlated significantly with water temperature ($P < 0.01$) and DO ($P < 0.01$) in the wet season samples. In both cases, the correlations were negative ($r = -0.93$ for the correlation between MPN and water temperature; $r = -0.93$ for the correlation between MPN and DO). The salinity of the water was below the limit of detection of the field meter (< 0.1 g/kg) at all six sites.

Survival of *E. coli* at different temperatures. The concentration of viable *E. coli* in Tillamook Bay water varied significantly with temperature and with time ($P < 0.01$), and there was a significant interaction between temperature and time ($P < 0.01$). The concentrations of the inocula added to the flasks did not differ significantly at the beginning of the experiment ($P = 0.14$) (Fig. 5). The concentration of viable *E. coli* remained statistically indistinguishable after 24 h at 4, 11, or 23 °C ($P = 0.11$), but at 48 h the flasks incubated at 4 °C had a significantly higher concentration of viable *E. coli* than the flasks incubated at 11 or 23 °C ($P < 0.05$). At 72 and 96 hours, the concentration of surviving *E. coli* differed significantly at all three temperatures ($P < 0.05$). The flasks incubated at 4 °C maintained the highest concentration of viable cells, while the flasks incubated at 23 °C had the lowest concentration. No data point appears for the 23 °C treatment at 96 h in Fig. 5 because the concentration of *E. coli* at this point was below the level of detection in this experiment (less than 10^3 CFU/ml). The concentration of viable *E. coli* did not change significantly throughout the experiment at 4 °C ($P = 0.14$), while it decreased linearly at 11 °C ($r = 0.98$) and at 23 °C ($r = 0.97$).

Survival of *E. coli* at different salinities. The concentration of viable *E. coli* in river water, ocean water, and bay water varied significantly with the source of the water and with incubation time ($P < 0.01$), and there was a significant interaction between the source of water and time ($P < 0.01$) in this experiment. No differences were detected among the three sources of water at 0 h ($P = 0.73$) and 24 h ($P = 0.96$), but at 48 h both the bay water and the ocean water contained significantly lower concentrations of viable *E. coli* than the river water ($P < 0.05$) (Fig. 6). The concentration of *E. coli* remained lower in the bay water and ocean water than in the river water at 72 h and 96 h, but at no time was a difference detected between the bay and ocean water ($P < 0.05$). The concentration of viable *E. coli* decreased

linearly in the bay water ($r = 0.97$) and ocean water ($r = 0.97$), while it remained unchanged in the river water ($P = 0.98$) throughout the experiment.

DISCUSSION

Tillamook Bay is a drowned river valley estuary in which fresh water from five major rivers mixes with salt water from the Pacific Ocean. The Miami, Kilchis, Wilson, Trask, and Tillamook rivers drain a watershed consisting of 147,200 ha. Almost 10,000 ha within the watershed is agricultural land which is used primarily for pasture, hay, and silage production for dairy herds that occupy the lowlands adjacent to the bay and its tributaries. The dairy herds produce approximately 292,500 metric tons of manure annually.

The climate of the Tillamook Bay watershed is characterized by short summers and mild, extended, wet winters. Annual rainfall in the area averages 230 to 380 cm, and surface water runoff from dairy farms constitutes a major nonpoint source of fecal contamination in the bay. In 1982 funds were received from the Rural Clean Water Program (RCWP) to initiate a project to improve water quality in the Tillamook Bay watershed. Information regarding the effectiveness of the project is inconsistent. According to an RCWP report (47), implementation of best management practices on 48% of the contracted critical areas in the watershed reduced the number of restricted oyster beds sites from twelve to six between 1982 and 1991. The most recent Oregon DEQ Water Quality Status Report (42) states that six of the 16 sites that are sampled monthly to monitor levels of fecal contamination were downgraded in their classification for shellfish harvesting between 1991 and 1993. Fecal contamination from agricultural land and other sources constitutes a potential health risk for the commercial shellfish industry in Tillamook Bay and for those who use the area for fishing, boating, clam digging, or other recreational activities. Continued efforts to monitor and improve water quality in the bay and its tributaries are essential.

Our data indicate that the Trask River carries a substantial load of fecal coliforms and that the concentration increases sharply following a heavy rainfall. Statistical analyses failed to detect significant differences between the dry season and wet season concentrations of fecal coliforms, despite the much higher MPNs at the downstream sites following the October rains. The failure to substantiate the differences between sampling times with statistical tests reveals the inherent inaccuracy of MPN estimations of bacterial populations. There is a seven- to ten-

fold difference between the upper and lower limits of the 95 percent confidence interval for a five-tube MPN index, and this range is amplified when a dilution factor is included in the estimation of population density. The large differences in the MPNs of water samples from the same site, which differed by as little as one positive tube in the fermentation series, made it impossible to substantiate differences between sampling times at the most heavily contaminated sites.

We were able to detect significant differences among the six sites at each sampling time. The three downstream sites contained the highest concentrations of fecal coliforms. Hoquarten slough yielded substantially higher concentrations than any other site at both sampling times, and the 122-fold increase in the MPN following the October rains was the largest difference between the wet season and dry season samples at any site . The concentration of dissolved oxygen in the slough was very low (2.5 mg/l) at both sampling times, which is consistent with a high level of biological activity in a stagnant or slow moving body of water. DO concentrations at the other sites were 1.4 to 3.0 mg/l higher after the fall rains, when the current of the river was noticeably stronger. We did not measure stream velocity in this study, but were aware of the difference due to the difficulty in obtaining samples at the later sampling time.

The Hoquarten slough site is located on U. S. Highway 101, within the city limits of Tillamook, Oregon. Several commercial developments are located alongside the slough at this location, and the surrounding land is predominantly agricultural. In May 1995 the U. S. Environmental Protection Agency (USEPA) cited a local dairy for discharging pollutants into the slough in violation of the federal Clean Water Act. The complaint against the dairy was settled in 1996 without an admission or denial of responsibility (58).

The next highest concentrations of fecal coliforms occurred at the Memaloose Point and Third Street bridge sites. In the dry season samples, neither site differed significantly from the other sites on the Trask River, though the Third Street bridge site was the only location other than Hoquarten slough at which the median MPN was higher than 100/100 ml. In the wet season samples, the MPNs at both sites exceeded 2000/100 ml. The median MPN at the next upstream site, the Highway 101 bridge, was 10-fold lower, despite its close proximity to pasture land. Memaloose Point is located in the channel formed by the convergence of the Tillamook River and Trask River as they enter Tillamook Bay. The site was chosen to represent a nonpoint source of fecal contamination from agricultural runoff and to determine

the concentration of fecal coliforms entering the bay at a major inlet. The Third Street and Highway 101 sites were chosen to represent point sources of fecal contamination resulting from outfall from sewage treatment facilities. The Third Street bridge site is located 0.1 river mile downstream from the Tillamook sewage treatment plant while the Highway 101 bridge site is located 1 river mile downstream from the sewage treatment ponds near the Tillamook airport.

Our data suggest that outfall from the Tillamook sewage treatment facility might contribute to fecal contamination in the Trask River following a heavy rainfall, though we have been unable to determine whether the facility discharges excess waste during storms. It is also possible that the high concentrations of fecal coliforms at the Memaloose Point and Third Street bridge sites resulted from an accumulation of bacterial contaminants in the lowland area where the flow rate of the river slows considerably. We did not measure stream velocity in this study, but recommend that it be included in future bacteriological studies. Fecal coliform concentrations were progressively lower at each site upstream of the Highway 101 bridge site in the wet season samples, which is consistent with a downstream accumulation of pollutants. The MPNs at the two uppermost locations, the Peninsula and North Fork sites, were similar to those measured during the dry season. The MPNs at these sites, which ranged from 23 to 170/100 ml, may represent a base concentration of fecal coliforms that enter the Trask River from sources that are not affected by seasonal changes in surface water runoff.

Much more information must be gathered to accurately identify the primary sources of fecal contamination in the Tillamook Bay watershed and to design effective resource management practices that will minimize the risk to human health and to the estuarine environment. Similar conditions forced the closure of several commercial shellfish growing areas in Puget Sound by the Washington State Department of Social and Health Services in 1978 [57]. Preliminary investigations identified failing septic systems and small, noncommercial farms as the primary sources of fecal contamination [12, 15]. Subsequent repair of the septic systems and implementation of improved livestock management practices significantly reduced the load of fecal coliforms, but not sufficiently to comply with state water quality standards. Further investigations revealed that fecal bacteria had adapted to the freshwater stream environment and that the bacteria were capable of proliferating in

stream sediment when organic nutrients were deposited by runoff from fields and livestock waste (57).

Several studies have shown that fecal bacteria can survive and, under certain conditions, even grow to a limited extent in marine and freshwater sediments (1, 11, 19, 24, 36). Bacteria adsorbed to sediment particles may feed on nutrients associated with the particles and receive protection from ultraviolet radiation (4), high salinity (20), heavy metal toxicity (28), and bacteriophage infection (51). These bacteria may act as a reservoir of fecal contaminants in aquatic environments. Physical forces such as storms, natural upwellings, sediment disturbances by benthic invertebrates, currents, or wave action can resuspend the bacteria in the overlying water.

The survival of fecal bacteria in estuarine and marine habitats has been studied extensively for many years. The conclusion reached in most studies is that nonindigenous bacteria do not survive for more than a few days or hours in saltwater environments. Several factors contribute to the steady decline in the number of culturable bacteria, the most important being short wavelength ultraviolet radiation in sunlight (5, 10, 32, 55). Other important factors include salinity (45), temperature (48), the growth stage of the bacteria (17), competition with other organisms (27), predation by protozoa (14, 49), low nutrient concentrations (18), and toxic substances in the water (28).

We found that temperature significantly affected the survival of *E. coli* in water samples from Tillamook Bay. The water temperatures that we measured in the bay varied from a low of 10 °C near the center of the bay during a preliminary sampling trip in March 1996 to a high of 19 °C in a shallow tide pool on a mild, sunny day in April 1996. The 11 °C incubation temperature most closely represented the mean daily temperature of the water in Tillamook Bay. The concentration of viable *E. coli* decreased by 98% over a 96-h period at this temperature, though a substantial number of bacteria remained in the water due to the high concentration of the inoculum in this experiment. The bacteria died more rapidly in bay water incubated at 23 °C.

None of the temperatures included in the experiment is lethal for *E. coli*, a mesophilic bacterium which grows optimally at 37 °C and tolerates temperatures as high as 45 °C. Therefore, additional abiotic or biotic factors must have caused the bacterium to die more rapidly at the higher temperatures, such as a more rapid depletion of available nutrients,

increased predation by protozoa, or increased sensitivity to toxic substances that may have been present in the water. The linear decrease in the log number of cells with time at 11 and 23 °C indicates that the bacteria died exponentially at these temperatures. Exponential death occurs when a bacterial population is unable to maintain itself in stationary phase and enters the death phase of its growth cycle.

Salinity also affected the survival of *E. coli* in our experiments, though the effect was not as pronounced as that of incubation temperature. We selected river water, bay water, and ocean water to represent the natural extremes of salinity that fecal contaminants would be expected to encounter in the Tillamook Bay watershed, and high salinity was probably a major factor contributing to the more rapid death rate in the bay water and ocean water as compared to the river water. The salinity of the bay water (10.2 g/kg) apparently exceeded a threshold level that *E. coli* tolerates, causing the bacterium to die exponentially. The rate of death in this experiment was similar to that observed in bay water at the same incubation temperature (11 °C) in the temperature experiment. The higher salinity of the ocean water (18.8 g/kg) did not produce a more rapid death rate. The bay water used in this experiment was collected during a low tide (–0.8 ft), so the salinity of the water was likely to be near the low end of the normal range encountered in the bay.

Salinity was not the only factor that differed among the water samples used in this experiment. The pH varied from 7.5 in the river water to 8.1 in the ocean water, and several other abiotic and biotic factors that we did not measure may have also affected the survival of the bacteria. The lack of any evident decline in the concentration of viable *E. coli* in the river water over a 96-h period has important implications for the quality of the water in Tillamook Bay. The results suggest that fecal contaminants entering the Trask River, and possibly the other rivers that drain into the bay, are capable of surviving in river water for several days and may not begin to decline in numbers until they reach the more saline environment of the bay.

Our measurements of bacterial survival in bay water, like those in many previous investigations, depended upon the ability to culture *E. coli* from water samples inoculated onto a standard bacteriological culture medium. Several studies have shown that measurements of this type may underestimate the actual number of viable, and potentially infective, bacteria in marine or estuarine water (22). Studies comparing the number of viable

bacteria detected by standard plate counts with the number detected by a direct viable count method [34] have demonstrated that enteric bacteria may enter a viable but not culturable state upon exposure to a saltwater environment [44, 61]. These dormant [56] or starved [40] bacteria remain physiologically active, and pathogens may remain capable of producing an infection, but they lose the ability to grow in standard culture media. Cells that have entered this physiological state are not detected as "viable" cells by standard plate counts or by MPN determinations. It is possible that the apparent decline in the number of viable *E. coli* in our experiments may have resulted from such a change in the physiology of the bacteria. This phenomenon also has important implications for the data collected by routine monitoring of Tillamook Bay, which relies on MPN determinations that may underestimate the level of fecal contamination.

Most enteric bacteria are not indigenous to estuarine or marine habitats, though some species – notably *Vibrio cholerae*, *V. parahemolyticus*, and *Aeromonas hydrophila* – naturally inhabit these environments [9, 29, 31]. Both indigenous and nonindigenous populations may enter the viable but not culturable state [8, 43, 53]. Indigenous species not only survive in this condition, but also can grow when essential nutrients become available. Suitable nutrients may be supplied by surface water runoff or wastewater outfall in coastal environments [50, 60]. These nutrients may also enable animal pathogens to grow, thereby affecting the health of aquatic wildlife [26, 30] or waterfowl [16].

It was once thought that large bodies of water rapidly and effectively dilute pollutants to the point at which the concentration of bacterial pathogens was well below that required to affect human or animal health. Several studies have shown, however, that particulate wastes remain associated rather than dispersing. Bacterial cells may settle into marine sediments, move collectively in ocean currents, attach to surfaces or to one another to form stable biofilms, or aggregate into droplets that are carried to shore by waves or in windblown aerosols [13, 23, 37, 39]. Stable aggregates of bacterial cells may be counted as single colonies on standard plate counts or as single units in MPN determinations, contributing to the underestimation of bacterial contamination by these enumeration methods. Careful, repeated monitoring is essential to understand the fate of fecal contaminants in coastal waters and to assess the potential impact on human health and on the health of the wildlife that inhabits these areas.

We have collected a limited amount of data in this study, but hope that our results will provide a useful starting point for the design of more extensive investigations of the sources and fate of fecal contaminants in the Tillamook Bay watershed. Future studies should examine the rate at which fecal bacteria enter each of the rivers that drain into the bay and correlate these loading rates with potential point and nonpoint sources of fecal contamination and with hydrologic parameters such as rainfall and stream flow. It would also be useful to study the survival of fecal bacteria in freshwater and estuarine sediments and to correlate these observations with bacterial concentrations in the overlying water. The effects of key environmental factors such as organic and inorganic nutrients, sunlight, temperature, and salinity should be included in these investigations. Other enumeration methods should be used to supplement MPN estimates, and other indicator organisms should be examined in addition to fecal coliforms. Numerous studies have shown that concentrations of fecal coliforms often correlate poorly with concentrations of other enteric organisms, such as vibrios, salmonellae, and enteric viruses [25, 35, 41, 48, 54]. As a result, water quality regulations based on concentrations of enterococci and *E. coli* have been proposed by the U. S. Environmental Protection Agency and adopted by several states [6, 59].

REFERENCES

1. **Ashbolt, N. J., G. S. Grohmann, and C. Kueh.** 1993. Significance of specific bacterial pathogens in the assessment of polluted receiving waters of Sydney. Water Sci. Technol. **27**: 449-452.

2. **Bej, A. K., R. J. Steffan, J. DiCesare, L. Haff, and R. M. Atlas** 1990. Detection of coliform bacteria in water by polymerase chain reaction and gene probes. Appl. Environ. Microbiol. **56**:307-314.

3. **Berg, J. D., and L. Fiksdal.** 1988. Rapid detection of total and fecal coliforms in water by enzymatic hydrolysis of 4-methylumbelliferone-β-D-galactoside. Appl. Environ. Microbiol. **54**:2118-2122.

4. **Bitton, G., Y. Henis, and N. Lahav.** 1972. Effect of several clay minerals and humic acid on the survival of *Klebsiella aerogenes* exposed to ultraviolet irradiation. Appl. Microbiol. **23**:870-874.

5. **Chamberlin, C. E., and R. Mitchell.** 1978. A decay model for enteric bacteria in natural waters, pp. 325-348. *In* R. Mitchell (ed.), Water Pollution Microbiology, vol. 2. Wiley, New York.

6. **Chasis, S., K. Mooney, A. McLain, and B. Hanson.** 1992. Testing the waters: A national perspective on beach closings. Report of the Natural Resources Defense Council. Natural Resources Defense Council, New York.

7. **Clark, D. L., B. B. Milner, M. H. Stewart, R. L. Wolfe, and B. H. Olson.** 1991. Comparative study of commercial 4-methylumbelliferyl-β-D-glucuronide preparations with the *Standard Methods* membrane filtration fecal coliform test for the detection of *Escherichia coli* in water samples. Appl. Environ. Microbiol. **57**:1528-1534.

8. **Colwell, R. R., P. R. Brayton, D. J. Grimes, D. B. Roszak, S. A. Huq, and L. M. Palmer.** 1985. Viable but non-culturable *Vibrio cholerae* and related pathogens in the environment: implications for release of genetically engineered microorganisms. Bio/Technol. **3**:817-820.

9. **Colwell, R. R., R. J. Seidler, J. Kaper, S. W. Joseph, S. Garges, H. Lockman, D. Maneval, H. Bradford, N. Roberts, E. Remmers, I. Huq, and A. Huq.** 1981. Occurrence of *Vibrio cholerae* serotype 101 in Maryland and Louisiana estuaries. Appl. Environ. Microbiol. **41**:555-558.

10. **Davies, C. M., and L. M. Evison.** 1991. Sunlight and the survival of enteric bacteria in natural waters. J. Appl. Bacteriol. **70**:265-274.

11. **Davies, C. M., J. A. H. Long, M. Donald, and N. J. Ashbolt.** 1995. Survival of fecal microorganisms in marine and freshwater sediments. Appl. Environ. Microbiol. **61**:1888-1896.

12. **Determan, T., B. Carey, W. Chamberlain, and D. Norton.** 1985. Sources affecting the sanitary conditions of water and shellfish in Minter Bay and Burley Lagoon. Washington State Department of Ecology Report No. 84-10.

13. **Duedall, I. W., B. H. Ketchum, P. K. Park, and D. R. Kester** (eds.). 1983. Wastes in the Ocean, vol. 1. Industrial and Sewage Wastes in the Ocean. Wiley, New York.

14. **Enzinger, R. M., and R. C. Cooper.** 1976. Role of bacteria and protozoa in the removal of *Escherichia coli* from estuarine waters. Appl. Environ. Microbiol. **31**:758-763.

15. **Erkenbreche, C.** 1981. Sediment bacterial indicators in an urban shellfishing subestuary of the Lower Chesapeake Bay. Appl. Environ. Microbiol. **42**:484-492.

16. **Friend, M.** 1985. Wildlife health implications of sewage disposal in wetlands, pp. 262-267. *In* P. J. Godfrey, E. R. Kaynor, S. Pelczarski, and J. Benforado (eds.), Ecological Considerations in Wetlands Treatment of Municipal Wastewaters. Van Nostrand Reinhold, New York.

17. **Gauthier, M. J., G. N. Flatau, R. L. Clément, and P. M. Munro.** 1992. Sensitivity of *Escherichia coli* cells to seawater closely depends on their growth stage. J. Appl. Bacteriol. **73**:257-262.

18. **Gauthier, M. J., P. M. Munro, and V. A. Breittmayer.** 1989. Influence of prior growth conditions on low nutrient response of *Escherichia coli* in seawater. Can. J. Microbiol. **35**:379-383.

19. **Gerba, C. P., and J. S. McLeod.** 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. Appl. Environ. Microbiol. **32**:114-120.

20. **Ghoul, M., T. Bernard, and M. Cormier.** 1986. Evidence that *Escherichia coli* accumulates glycine betaine from marine sediment. Appl. Environ. Microbiol. **56**:551-554.

21. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (ed.).** 1992. Standard methods for the examination of water and wastewater, 18th ed. American Public Health Association, Washington, D.C.

22. **Grimes, D. J., R. W. Atwell, P. R. Brayton, L. M. Palmer, D. B. Roszak, F. L. Singleton, M. L. Tamplin, and R. R. Colwell.** 1986. The fate of enteric pathogenic bacteria in estuarine and marine environments. Microbiol. Sci. **3**: 324-329.

23. **Hargrave, B. T.** 1984. Sinking of particulate matter from the surface water of the ocean, pp. 155-178. *In* J. E. Hobbie and P. B. Williams (eds.), Heterotrophic Activity in the Sea. Plenum Press, New York.

24. **Hood, M. A., and G. E. Ness.** 1982. Survival of Vibrio cholerae and Escherichia coli in estuarine waters and sediments. Appl. Environ. Microbiol. **43**:578-584.

25. **Hood, M. A., G. E. Ness, G. E. Rodrick, and N. J. Blake.** 1983. Distribution of *Vibrio cholerae* in two Florida estuaries. Microb. Ecol. **9**:65-75.

26. **Huq, A., E. B. Small, P. A. West, M. I. Huq, R. Rahman, and R. R. Colwell.** 1983. Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. Appl. Environ. Microbiol. **45**:275-283.

27. **Jannasch, H. W.** 1988. Competitive elimination of Enterobacteriaceae from seawater. Appl. Environ. Microbiol. **16**:1616-1618.

28. **Jones, G. E.** 1964. Effect of chelating agents on the growth of *Escherichia coli* in seawater. J. Bacteriol. **87**:484-499.

29. **Joseph, S. W., R. R. Colwell, and J. Kaper.** 1982. *Vibrio parahaemolyticus* and related halophilic vibrios. CRC Crit. Rev. Microbiol. **10**:77-124.

30. **Kaneko, T., and R. R. Colwell.** 1978. The annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. Microb. Ecol. **4**:135-155.

31. **Kaper, J., H. Lockman, R. R. Colwell, and S. W. Joseph.** 1981. *Aeromonas hydrophila*: ecology and toxigenicity of isolates from an estuary. J. Appl. Bacteriol. **50**:359-377.

32. **Kapuscinski, R. B., and R. Mitchell.** 1981. Solar radiation induces sublethal injury in *Escherichia coli* in seawater. Appl. Environ. Microbiol. **41**:670-674.

33. **Koch, A. L.** 1994. Growth measurement. *In* P. Gerhardt, R. G. E. Murray, W. A. Wood, and N. R. Krieg (ed.), Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, D.C.

34. **Kogure, K., U. Simidu, and N. Taga.** 1979. A tentative direct microscopic method for counting living marine bacteria. Can. J. Microbiol. **25**:415-420.

35. **Koh, E. G. L., J-H. Huyn, and P. A. LaRock.** 1994. Pertinence of indicator organisms and sampling variables to vibrio concentrations. Appl. Environ. Microbiol. **60**:3897-3900.

36. **LaLiberte, P., and D. J. Grimes.** 1982. Survival of *Escherichia coli* in lake bottom sediment. Appl. Environ. Microbiol. **43**:623-628.

37. **Liu, D., Y. L. Lau, Y. K. Chau, and G. J. Pacepavicius.** 1993. Characterization of biofilm development on artificial substratum in natural water. Water Res. **27**:361-367.

38. **Marshall, K. C.** 1985. Bacterial adhesion in oligotrophic habitats. Microbiol. Sci. **2**:321-326.

39. **McFeters, G. A., and D. G. Stuart.** 1972. Survival of coliforms in natural water: Field and laboratory studies with membrane filter chambers. Appl. Microbiol. **24**:805-811.

40. **Morita, R. Y.** 1985. Starvation and miniaturisation of heterotrophs, with special emphasis on maintenance of the starved viable state, pp. 111-130. *In* M. Fletcher and G. D. Floodgate (eds.), Bacteria in Their Natural Environments. Academic Press, Orlando.

41. **Oliver, J. D., R. A. Warner, and D. L. Cleland.** 1983. Distribution of *Vibrio vulnificus* in the marine environment. Appl. Environ. Microbiol. **45**:985-998.

42. **Oregon Department of Environmental Quality**. 1994. Estuary and coastal water quality. Oregon's 1994 Water Quality Status Assessment Report, Section 3.5.

43. **Palmer, L. M., A. M. Baya, D. J. Grimes, and R. R. Colwell**. 1984. Molecular genetic and phenotypic alteration of *Escherichia coli* in natural water microcosms containing toxic chemicals. FEMS Microbiol. Lett. **21**:169-173.

44. **Peele, E. R. and R. R. Colwell**. 1981. Application of a direct microscopic method for enumeration of substrate-responsive marine bacteria. Can. J. Microbiol. **27**:1071-1075.

45. **Pike, E. B., A. H. L. Gameson, and D. J. Gould**. 1970. Mortality of coliform bacteria in seawater samples in the dark. Rev. Int. Oceanogr. Med. **18/19**:97-107.

46. **Pommepuy, M., J. J. F. Guillard, E. Duprey, A. Derrien, F. LeGuyader, and M. Cormier**. 1992. Enteric bacteria survival factors. Water Sci. Technol. **25**:93-103.

47. **Rural Clean Water Program Report**. 1996. Oregon Tillamook Bay (RCWP 18). http://h2osparc.wq.ncsu.edu/info/rcwp/orprof.html.

48. **Rhodes, M. W., and H. I. Kator**. 1988. Survival of *Escherichia coli* and *Salmonella* spp. in estuarine environments. Appl. Environ. Microbiol. **54**:2902-2907.

49. **Rhodes, M. W., and H. I. Kator**. 1990. Effects of sunlight and autochthonous microbiota on *Escherichia coli* survival in an estuarine environment. Curr. Microbiol. **21**:65-73.

50. **Rippey, S. R., and V. J. Cabelli**. 1979. Membrane filter procedure for enumeration of *Aeromonas hydrophila* in fresh waters. Appl. Environ. Microbiol. **38**:108-113.

51. **Roper, M. M., and K. C. Marshall**. 1979. Effects of salinity on sedimentation and of particulates on survival of bacteria in estuarine habitats. Geomicrobiol. J. **1**:103-116.

52. **Roszak, D. B., and R. R. Colwell**. 1987. Survival strategies of bacteria in the natural environment. Microbiol. Rev. **51**:365-379.

53. **Roszak, D. B., D. J. Grimes, and R. R. Colwell**. 1984. Viable but nonrecoverable stage of *Salmonella enteritidis* in aquatic systems. Can. J. Microbiol. **30**:334-338.

54. **Shiaris, M. P., A. C. Rex, G. W. Pettibone, K. Keay, P. McMacus, M. A. Rex, J. Ebersole, and E. Gallagher.** 1987. Distribution of indicator bacteria and *Vibrio parahaemolyticus* in sewage-polluted sediments. Appl. Environ. Microbiol. **53**:1756-1761.

55. **Sinton, L. W., R. J. Davies-Colley, and R. G. Bell.** 1994. Inactivation of enterococci and fecal coliforms from sewage and meatworks effluents in seawater chambers. Appl. Environ. Microbiol. **60**:2040-2048.

56. **Stevenson, L. H.** 1978. A case for bacterial dormancy in aquatic systems. Microb. Ecol. **4**:127-133.

57. **Struck, P. H.** 1988. The relationship between sediment and fecal coliform levels in a Puget Sound estuary. J. Environ. Health **50**:403-407.

58. **Tillamook Headlight-Herald.** 1996. Clean water act allegedly violated. 4 September 1996, p. A2.

59. **U. S. Environmental Protection Agency.** 1985. Test methods for *Escherichia coli* and enterococci in water by the membrane filter procedure. EPA-600/4-85/076, pp. 1-25. U. S. Environmental Protection Agency, Washington, D.C.

60. **Watkins, W. D., and V. J. Cabelli.** 1985. Effect of fecal pollution on *Vibrio parahaemolyticus* densities in an estuarine environment. Appl. Environ. Microbiol. 49:1307-1313.

61. **Xu, H-S., N. Roberts, F. L. Singleton, R. W. Atwell, D. J. Grimes, and R. R. Colwell.** 1982. Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. Microb. Ecol. **8**:313-323.

TABLE 1. Water temperature, salinity, and dissolved oxygen concentrations at the six sampling sites on the Trask River and Hoquarten slough.

Sampling site	Temperature (°C)	Salinity (g/kg)	Dissolved Oxygen (mg/l)
31 July 1996			
Memaloose Point	16.5	7.5	6.8
Hoquarten slough	16.5	1.2	2.5
Third Street bridge	18.0	0.8	7.4
Hwy 101 bridge	16.0	0.1	8.6
Peninsula	15.0	0.1	9.5
North Fork	12.5	0.0	9.8
19 October 1996			
Memaloose Point	9.0	0.0	8.5
Hoquarten slough	10.5	0.0	2.4
Third Street bridge	8.5	0.0	10.4
Hwy 101 bridge	9.0	0.0	11.0
Peninsula	8.5	0.0	11.1
North Fork	8.0	0.0	11.2

TABLE 2. Water temperature, salinity, and pH of water samples collected from Tillamook Bay, the Pacific Ocean shore (Rockaway Beach), and the Trask River for the bacterial survival experiments.

Sampling site	Temperature (°C)	Salinity (g/kg)	pH
Trask River	15	0.1	7.5
Tillamook Bay	14	10.2	7.7
Rockaway Beach	15	18.8	8.1

FIGURE LEGENDS

FIG 1. Location of the sampling sites on the Trask River and Hoquarten slough. Site 1 = Memaloose Point. Site 2 = Hoquarten slough. Site 3 = Third Street bridge. Site 4 = Highway 101 bridge. Sites 5 and 6 (Peninsula and North Fork sites) are not shown.

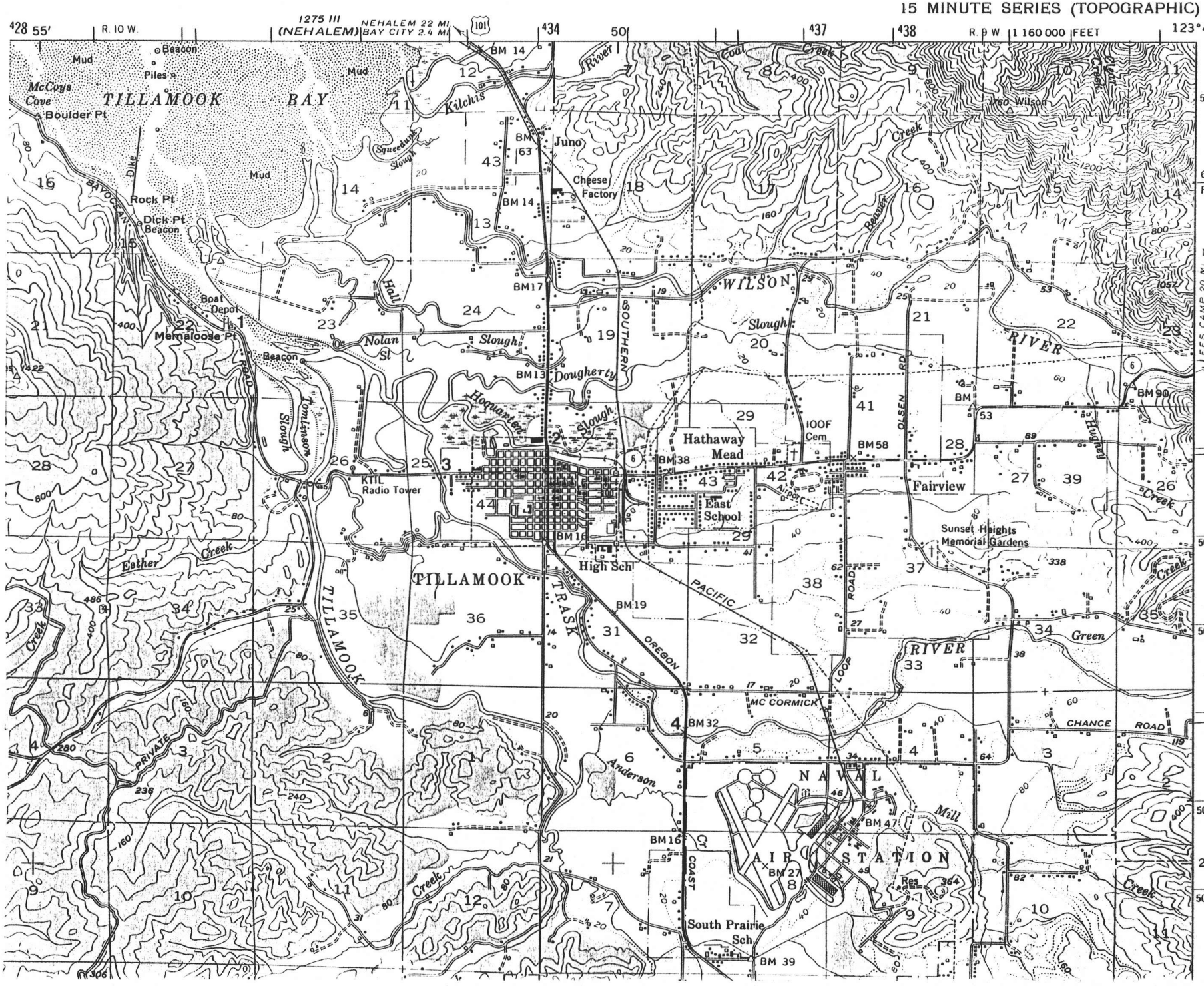
FIG 2. Views of the sampling sites on the Trask River and Hoquarten slough. (A) Memaloose Point. (B) Hoquarten slough. (C) Third Street bridge. (D) Highway 101 bridge. (E) Peninsula. (F) North Fork.

FIG 3. Mean MPN of fecal coliform bacteria at the six sampling sites on the Trask River and Hoquarten slough on 31 July 1996 (dry season samples). Data are the means of three replications. Error bars are the standard error of the means.

FIG 4. Mean MPN of fecal coliform bacteria at the six sampling sites on the Trask River and Hoquarten slough on 19 October 1996 (wet season samples). Data are the means of three replications. Error bars are the standard error of the means.

FIG. 5. Survival of *Escherichia coli* strain K12 in Tillamook Bay water incubated at 4, 11, or 23 °C. Data are the means of three replications. Error bars indicate the standard error of the means. No data point appears for the 23 °C treatment at 96 h because the concentration of *E. coli* at this point was below the level of detection in this experiment ($< 10^3$ CFU/ml).

FIG 6. Survival of *Escherichia coli* strain K12 at 11 °C in water samples collected from the Trask River, Tillamook Bay, or the Pacific Ocean shore. Data are the means of three replications. Error bars indicate the standard error of the means.


TILLAMOOK QUADRANGLE
OREGON—TILLAMOOK CO.
15 MINUTE SERIES (TOPOGRAPHIC)
1275 II (ENRIGHT)
1275 III (NEHALEM)
NEHALEM 22 MI.
BAY CITY 2.4 MI.
R. 10 W.
R. 9 W.
1 160 000 FEET
123°45'
45°30'
680 000 FEET
TILLAMOOK BAY
McCoys Cove
Boulder Pt
Rock Pt
Dick Pt Beacon
Mud
Piles
Beacon
Dike
BAY OCEAN
Boat Depot
Memaloose Pt
Beacon
Nolan St
Hall
Squeedunk Slough
Juno
Cheese Factory
Kilchis River
Coal Creek
Beaver Creek
Wilson Creek
Wilson Slough
Wilson River
Lees Camp 20 Mi.
Glenwood 34 Mi.
Hoquarten Slough
Tomlinson Slough
Dougherty Slough
Southern Pacific
Olsen Rd
Hathaway Mead
100F Cem
Fairview
East School
Sunset Heights Memorial Gardens
KTIL Radio Tower
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Pacific Oregon
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Mc Cormick
Chance Road
Green Creek
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Private
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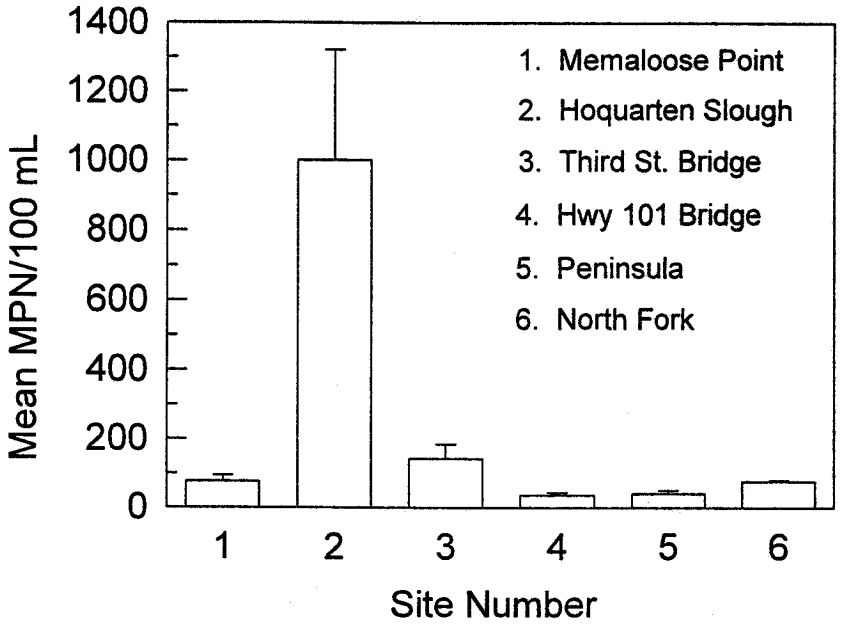
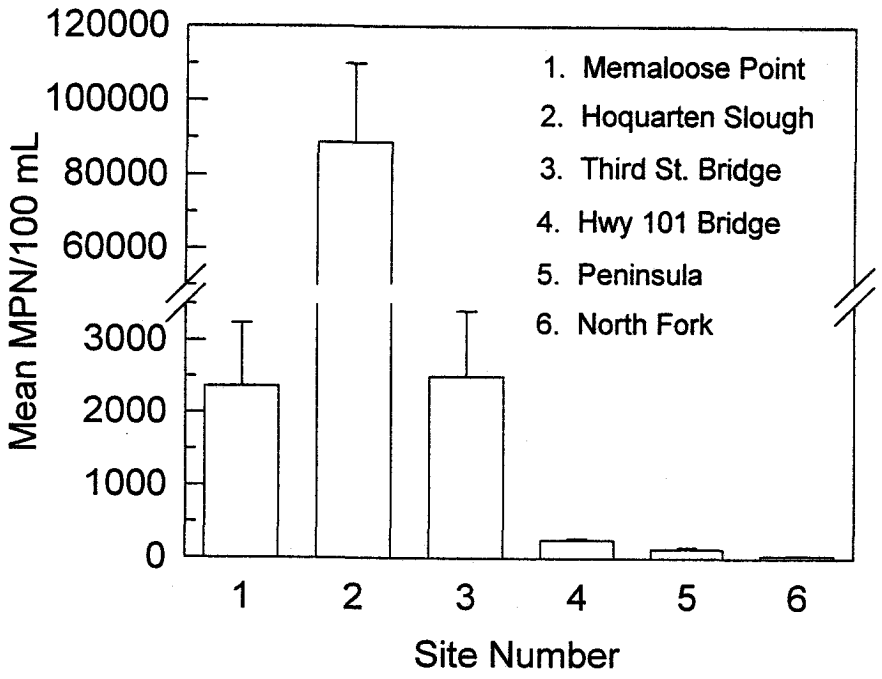
Fecal Coliform Concentrations in the Trask River

31 July 1996

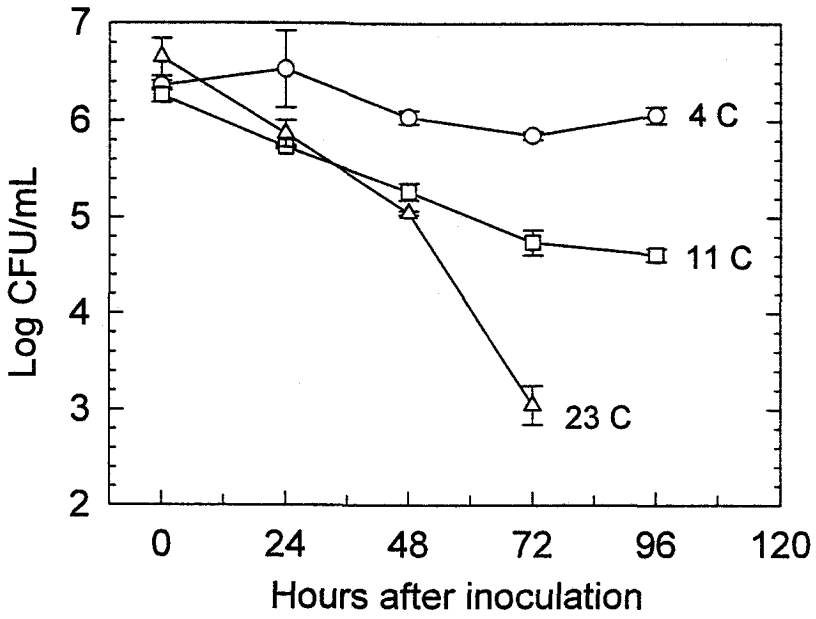


Fecal Coliform Concentrations in the Trask River

19 October 1996



Effect of temperature on survival of *E. coli*



Effect of salinity on survival of *E. coli*