

# Spatial and Temporal Changes in Bacterial Assemblages of the Cuyahoga River<sup>1</sup>

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**ABSTRACT.** Bacterial assemblage- and population-level variables were assessed at five sites along the Cuyahoga River in Northeastern Ohio during three seasons. Assemblage-level parameters (total, culturable, and lactose-positive bacteria) increased in a downstream direction during spring when discharge was elevated. Population-level responses were examined using colony hybridization with species-specific probes for *Burkholderia cepacia* and *Pseudomonas putida*. Also a group-specific probe for the  $\gamma$ -proteobacteria was used to monitor the abundance of this widespread bacterial taxon. Abundances of culturable individuals of these taxa exhibited spatio-temporal differences; patterns typically were different from those observed for assemblage-level parameters. Seasonal changes in the abundances of these taxa were frequently large; for example, in winter and spring the  $\gamma$ -proteobacteria represented 20 to 80% of the colony forming units (CFU) while in summer this group accounted for 5% or less of the CFU. Likewise, *B. cepacia* populations peaked in spring and became nearly undetectable in summer. Assemblage-level variations appeared to often mask population dynamics. These changes in bacterial populations imply that studies designed to monitor abundances for bioremediation or ecological purposes: 1) must account for seasonal blooms of bacteria of different species and 2) consider documentation of bacterial populations to detect more sensitive environmental responses.

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## INTRODUCTION

Stream bacterial ecology studies have typically focused on assemblage-level parameters, such as total numbers or production (McDowell 1984). Although a few studies have examined species composition of stream bacterial assemblages (Baker and Farr 1977; Bell and others 1980; Brown and Leff 1996; Leff and others 1998; Lemke and others 1997; Nuttall 1982; Suberkropp and Klug 1976), methodological limitations associated with identifying freshwater bacteria using physiological tests have restricted comparisons of bacterial populations (Leff 1994). Recently, rRNA sequence data have been used to develop species-specific gene probes making identification of selected species of environmental bacteria quicker and more efficient than when using traditional methods (Olsen and others 1986).

A limited number of studies have used rDNA probes to identify bacteria cultured from stream environments (Leff and others 1995; Leff and others 1998; Lemke and others 1997; Wise and others 1995). In these studies, colony hybridization (in which DNA from cultured bacteria is hybridized with taxon-specific probes) allowed examination of thousands of bacterial cultures which facilitated comparison of temporal and spatial dynamics of populations. Both Lemke and others (1997) and Leff and others (1998) discovered that spatio-temporal changes in populations were dissimilar to changes in assemblage-level parameters (total number of bacteria and number of culturable bacteria). Changes at the assemblage-level masked dynamic spatial or temporal changes in bacterial populations and population-level

changes varied among the species examined.

In this study, spatio-temporal changes in assemblage-level parameters (numbers of total bacteria, culturable bacteria,  $\gamma$ -proteobacteria, and lactose-positive bacteria) were compared to population-level parameters (numbers of culturable *Burkholderia cepacia* and *Pseudomonas putida*) in the Cuyahoga River, a large "use-reuse" river in Northeastern Ohio. Each of the three bacterial taxa examined are common in streams (Brown and Leff 1996; Leff and others 1995; Leff and others 1998; Lemke and others 1997) and were selected because of their abundance and ecological and economic importance. The two species examined are both gram negative bacteria common in soil and water; *B. cepacia* is a wide spread species with a great degree of catabolic flexibility and a unique genetic structure (Ballard and others 1970; Lessie and others 1996) and *P. putida* is widely studied because of its ability to degrade certain xenobiotic compounds, such as naphthalene and toluene (Guerin and Boyd 1995; Schwartz and Bar 1995). Representing a higher taxonomic level, the  $\gamma$ -proteobacteria are a diverse group of gram negative bacteria which includes *Acinetobacter*, *Pseudomonas*, and *Aeromonas*.

## MATERIALS AND METHODS

### Study Site

The Cuyahoga River originates in an agricultural region of Northeastern Ohio and traverses the cities of Akron and Cleveland before entering Lake Erie. The five study sites, listed from upstream to downstream, were Hiram Rapids (HR), Kent (KT), Old Portage (OP), Boston Mills (BM), and Rockside Road (RR) and were selected from among locations monitored by the Ohio EPA (Ohio EPA 1994). HR was located in the agricultural portion of the river and sites KT and OP were in a region modified for residential and commercial use that receives combined-

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sewer overflows. Discharge from the Akron Wastewater Treatment Plant enters the Cuyahoga downstream from OP. The last two sites (BM and RR) were located in the last 20 km before the Cuyahoga flows into Lake Erie which is extensively modified for residential and commercial purposes.

### Sample Collection and Analysis

Three mid-channel water samples were collected from bridges at each site on 9 December 1994, 8 March 1995, and 7 June 1995 using a small bucket and rope. A subsample from each was preserved with Formalin (5% final concentration) and the total number of bacteria was determined using the DAPI (4',6-diamidino-2-phenylindole) direct count procedure (Porter and Feig 1980).

Modified nutrient agar (2 g nutrient broth, 15 g agar, Difco; 2.5 g NaCl, 0.1g MgSO<sub>4</sub>, 0.01g CaCl<sub>2</sub>, 7.0 g Na<sub>2</sub>HPO<sub>4</sub>, 3.0 g KH<sub>2</sub>PO<sub>4</sub>, 1.0 g NH<sub>4</sub>Cl, 0.1 g cycloheximide l<sup>-1</sup>; Leff and Meyer 1991, used to enumerate culturable heterotrophic bacteria) and MacConkey agar (BBL, used to enumerate lactose fermenters) were inoculated with 0.1, 0.01, and 0.001 ml of water using sterile, bent glass rods. Modified nutrient agar plates were incubated for 3 days at 24° C and MacConkey plates were incubated for 24 h at 37° C.

After colonies were enumerated, up to 50 colonies per sample from the modified nutrient agar plates were re-cultured by transferring them individually to new nutrient agar plates using sterile toothpicks. Colonies were blotted onto nylon filters (Magna Lift, MSI) after 24 h, then colonies were lysed with 0.5 M NaOH, neutralized in 0.5 M Tris HCl (pH 8), immersed in 2 x SSC (1 x SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.2), and washed in 95% ethanol (Shimkets and Asher 1988). Filters were baked at 80° C for 2 h. DNA on filters was hybridized with either a 23S rDNA *B. cepacia*-specific probe (5' CCC ATC GCA TCT AAC AAT 3'; Schleifer and others 1992), a 16S rDNA *P. putida*-specific probe (5' GCT GGC CTA ACC TTC 3'; Schleifer and others 1992), or a 16S rDNA probe for  $\gamma$ -proteobacteria (5' GCC TTC CCA CAT CGT TT 3'; Manz and others 1992) to identify colonies as described in Leff and others (1995). Oligonucleotide probes were labeled with digoxigenin (Boehringer-Mannheim, Indianapolis, IN) and excess label was removed using a Nuctrap column (Stratagene, La Jolla, CA). Hybridizations were performed in 5 x SSC, 0.5% blocking reagent, 0.1% sodium N-lauroyl sarcosine and 0.02% SDS at the empirically determined hybridization temperatures (47° C for *B. cepacia*, 50° C for *P. putida*, and 53° C for  $\gamma$ -proteobacteria) for 12 to 18 h. Filters were washed three times in 0.1 x SSC and 0.1% SDS for 20 minutes at the hybridization temperature, and results were visualized using Lumi-Phos (Boehringer-Mannheim). For the species-specific probes, controls were *B. cepacia* (ATCC 24561), *P. putida* (ATCC 12633), and *B. solanacearum* (ATCC 11696). For the  $\gamma$ -proteobacteria probe, positive controls were *P. fluorescens* (ATCC 13525), *P. putida*, *P. aeruginosa* (ATCC 10145), *P. chlororaphis* (ATCC 9446) and negative controls were *Comamonas acidovorans* (ATCC 15668),

*C. testosteroni* (ATCC 11996), *B. cepacia*, and *B. solanacearum*.

Statistical comparisons were made using two-way ANOVA ( $\alpha = 0.05$ ; StatView Ver. 4.02, Abacus Concepts, Berkeley, CA) with site and date as treatments.

### RESULTS

The total abundance of bacteria, based on epifluorescence microscopy of DAPI stained samples, varied among sites and dates (Fig. 1, site, date and site\*date effects,  $P < 0.001$ ). As suggested by the significant site by date interaction, spatial patterns were not consistent across dates. In December, bacterial numbers were lowest in the mid-reaches; in March, at most downstream sites, abundances were twice as high as at upstream sites. In June, numbers were highest in the mid-reaches.

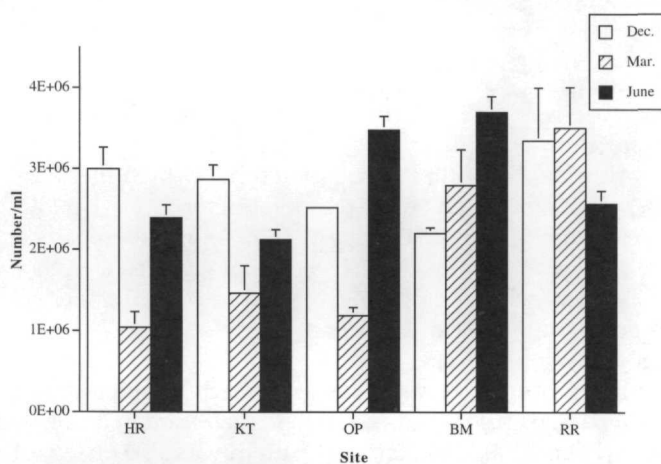


FIGURE 1. Total bacterial abundances based on epifluorescence microscopy at the five study sites on three dates. Values are means and standard errors.

The abundance of CFU on modified Nutrient agar also varied among sites and dates but the pattern and magnitude of change differed from that observed for total bacterial abundances (Fig. 2, site, date and site\*date effects,  $P < 0.001$ ). In December, no regular spatial pattern in CFU abundances was observed, while in March there was an increase in a downstream direction. CFU abundances were generally much lower in June than on other dates and were similar among sites.

The abundance of culturable *B. cepacia* also varied among sites and dates and there was a significant site-date interaction (Fig. 3A, site, date and site\*date effects,  $P < 0.001$ ). In December, abundances were moderate and similar among sites and in June, *B. cepacia* was barely detectable. In March, there was an increase in *B. cepacia* abundance in a downstream direction; the same pattern was observed for total bacteria and CFU abundances.

*P. putida* exhibited spatial and temporal changes (Fig. 3B, site, date and site\*date effects,  $P < 0.001$ ) but patterns of change differed from those observed for *B. cepacia*. Similar to *B. cepacia*, *P. putida* abundances were very low in June. However, *P. putida* abundances were also low in March when *B. cepacia* abundances

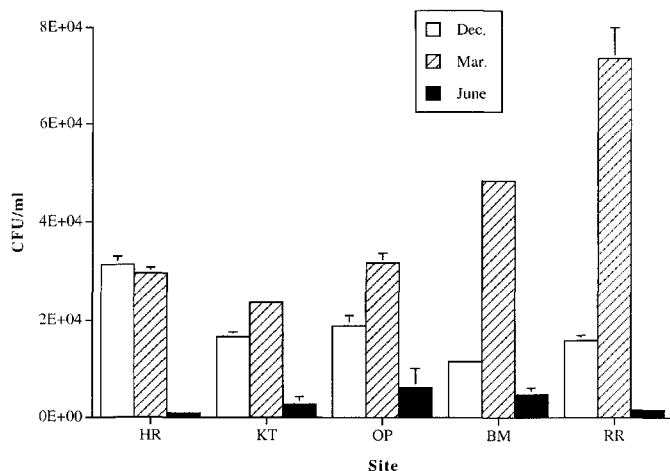


FIGURE 2. Colony forming unit (CFU) abundances based on growth on modified nutrient agar at the five study sites on three dates. Values are means and standard errors.

peaked and for *P. putida* no pattern of downstream increase was observed. In December, *P. putida* numbers were elevated at the most upstream site.

Abundance of the  $\gamma$ -proteobacteria (which includes *P. putida*) exhibited spatio-temporal differences (Fig. 3C, site, date and site\*date effects,  $P < 0.001$ ) and there was a distinct decline in June. Differences among sites were not consistent when comparing March and December; no regular pattern of downstream increase was observed on any sampling date.

Three of the five variables examined (abundances of total bacteria, CFU, and *B. cepacia*) increased in a downstream direction in March. This pattern was also observed for lactose-fermenting bacteria abundances (Fig. 4). These lactose-positive cells, which were dominated by coliforms (Brown and Leff 1996), were not detectable or were at low abundances on the other two sampling dates.

Some of the spatial and temporal changes observed seemed to be related to discharge (Fig. 5). The number of CFU and lactose-positive colonies were strongly correlated with discharge ( $r = 0.97$  and  $0.87$ , respectively). In addition, *B. cepacia* abundance was correlated with discharge ( $r = 0.60$ ); other variables did not exhibit statistically significant correlations with discharge.

## DISCUSSION

Similar to the observations of Lemke and others (1997), the two species of bacteria examined in the present study exhibited different patterns of spatio-temporal change. Both *B. cepacia* and *P. putida* had lowest abundances in June; low abundances of *B. cepacia* in summer were also observed in two other Ohio streams (Leff and others 1998). Seasonal changes in abundances of these species in these lotic ecosystems may be attributable to changes in population sizes or culturability of the target species. Thus *B. cepacia* may be nearly absent from the Cuyahoga River during summer or it may be present and non-culturable. In either case, the number of active individuals of the species would be reduced and the role of that population in dissolved organic carbon (DOC) and nutrient utilization would be reduced.

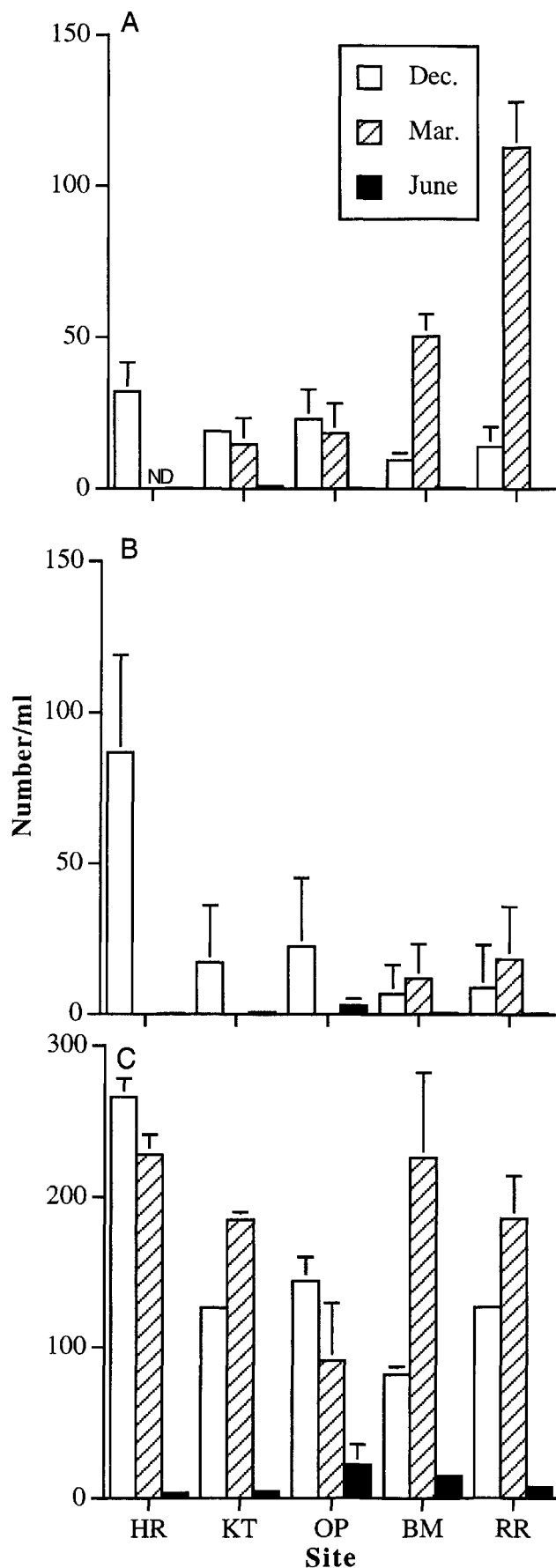


FIGURE 3. Abundances of culturable *B. cepacia* (A), *P. putida* (B), and  $\gamma$ -proteobacteria (C) at the five study sites on three dates. Values are means and standard errors.

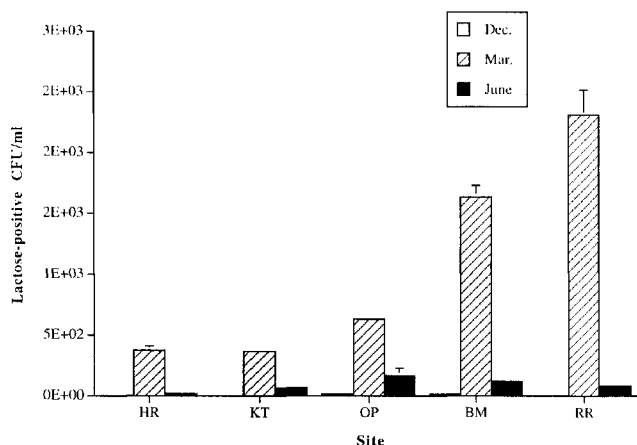


FIGURE 4. Lactose-positive colony forming unit abundances based on growth on MacConkey agar at the five study sites on three dates. Values are means and standard errors.

Downstream increases in *B. cepacia* abundance in March coincided with increased abundances of total bacteria, CFU, and lactose-positive bacteria. This pattern was quite similar to spatial changes in discharge (Fig. 5) and the effect of discharge on these variables is also implied by the lack of downstream increases on the other two sampling dates when discharges were much lower. Increases in lactose-positive bacteria are likely attributable to inputs from combined sewer overflows that release untreated sewage into the river during storm events (Ohio EPA 1994). Increases in total numbers, *B. cepacia*, and CFU abundances are likely due to inputs of cells from the soil surrounding the river, release of cells from sediments in the channel, or stimulation of bacteria activity by increased nutrient concentrations during elevated discharge.

For *P. putida* or the  $\gamma$ -proteobacteria, abundances were not correlated with discharge and were independent of site location, suggesting that either these organisms are not responsive to nutrient inputs that may be associated with high discharge or that they are typically not derived from outside the stream channel. The latter

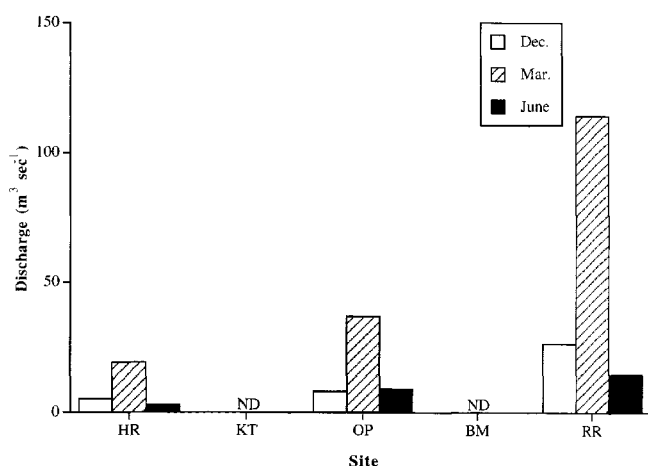


FIGURE 5. Discharge in the Cuyahoga on the three sampling dates from USGS Water Resources Data, OH.

possibility may be the most likely since *B. cepacia* ( $\beta$ -proteobacteria), which was responsive to elevated discharge, are common in soil (especially in the rhizosphere) and are classified as soil-borne plant pathogens (Schroth and others 1991). In contrast, *P. putida* is grouped with saprophytic pseudomonads and occurs in a variety of habitats (Palleroni 1991).

Other researchers have documented temporal differences in assemblage-level parameters using CFU or total bacteria and have found a relationship with discharge and flooding (McDowell 1984). Although increased bacterial abundance at elevated discharge may be attributable to either inputs of allochthonous cells or stimulation of bacteria by nutrient and carbon inputs, it is likely that soil, leaf litter, and other substrates in the catchment are sources of bacteria found in stream water. Evidence for this phenomenon was found by Leff and others (1993) in which an increase in soil-type bacteria in the water column coincided with flooding in a South Carolina stream. In addition, Wainwright and others (1992) demonstrated that floodplain soils and stream sediments were a significant source of bacteria suspended in a Georgia river. Thus, downstream increases in bacterial numbers and the number of culturable *B. cepacia* at times of elevated discharge may reflect increased inputs of allochthonous bacteria from the catchment.

In a similar study conducted in South Carolina streams, *B. cepacia* densities ranged from 100 to 150 CFU/ml in summer (Lemke and others 1997). Culturable *B. cepacia* were not detected in summer in two other Ohio streams and reached abundances of up to 9000 CFU/ml in fall (Leff and others 1998). Seasonal changes documented in the present study and the other Ohio study (Leff and others 1998) were similar but smaller peaks in *B. cepacia* abundance were observed in the Cuyahoga River. *P. putida* abundances ranged from 75 to 400 CFU/ml in the South Carolina streams in summer (Lemke and others 1997) compared to less than 10 CFU/ml in the Cuyahoga.

Hidden beneath spatio-temporal changes in assemblage-level parameters, such as total bacterial numbers or CFU abundances, dynamic shifts in bacteria populations occurred. The two bacterial species examined differed in spatio-temporal patterns of abundance; *B. cepacia* abundances increased at elevated discharge in a manner similar to assemblage-level changes but *P. putida* did not. In general, the magnitude of temporal changes exceeded spatial changes. Perhaps one of the most noteworthy observations demonstrating this phenomenon is that the culturable bacteria were dominated by the  $\gamma$ -proteobacteria in December and March (representing 20 to 80% of CFU) while in June this group accounted for 5% or less of the CFU. Such dynamic seasonal changes are consistent with observations of Tuomi and others (1997) and Leff and others (1998) who reported blooms of freshwater bacteria at different times of the year.

The phenomena of seasonal blooms of different bacterial species has implications for studies aimed at monitoring abundances of bacteria for bioremediation or ecological concerns. Specifically, the season of introduction of bacteria may effect their abundances and

prolonged temporal sampling may be required to detect population changes.

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