Benthic Responses to Wet-Weather Discharges in Urban Streams in Southern Ontario

Lee Grapentine,* Quintin Rochfort and Jiri Marsalek

National Water Research Institute, Environment Canada, 867 Lakeshore Road, Burlington, Ontario L7R 4A6

Urban stormwater and combined sewer overflow (CSO) discharges are important sources of sediment and contaminants (trace metals, PAHs, nutrients and road salts), and cause changes in flow, sediment, chemical and thermal regimes of receiving waters. Over the past several years, benthic conditions of streams representing a range of exposure environments were assessed in Hamilton, Toronto, Oshawa and Kingston, Ontario. Studies progressed from initial surveys of sediment contaminant levels, sediment toxicity and benthic invertebrate community structure to more spatially intensive sampling and experimental approaches that included the use of artificial substrates, in situ water toxicity tests and measurements of contaminant bioaccumulation.

Results showed that while sediments and some biota at sites exposed to wet-weather discharges were often contaminated with metals and PAHs and enriched with nutrients, significant biological degradation measured by sediment toxicity or depauperated benthic communities was not evident. Exposure to stormwater discharges at sites below outfalls could alter the composition of benthic communities, but these effects were not strongly related to contaminant concentrations in sediment or invertebrate tissue. No outfall-associated toxicity was observed for caged amphipods held in the water column.

Effects of wet-weather discharges on benthic communities at the urban stream sites studied appear to be small, and their detection was limited by several inherent conditions, including natural heterogeneity in the distribution of benthic invertebrates, episodic (intermittent) exposure to discharges and contaminant fluxes allowing some recovery, “background” levels of disturbance, poorly delineated changes in communities caused by physical effects such as flow and sediment transport, and community response dynamics. Detection of stormwater discharge effects should be improved by sampling on smaller temporal and multiple spatial scales to better quantify stressor exposure and invertebrate responses.

Key words: benthic invertebrates, bioaccumulation, sediment conditions, toxicity, urban streams, wet-weather discharges

Introduction

Urbanization results in increased volume and intensity of surface runoff, along with suspended sediment and associated chemical contamination (polycyclic aromatic hydrocarbons [PAHs], metals, nutrients and road salts). Runoff arising from rainfall and snowmelt in urban areas and draining into receiving waters as stormwater is recognized as a major potential source of disturbance to aquatic ecosystems (Marsalek et al. 2001; U.S. EPA 2002). Passage through the urban environment increases runoff flows and degrades runoff quality. Wet-weather flows can have adverse impacts in receiving streams through changes in stream flow, erosion, and transport and deposition of sediment (Roesner and Bledsoe 2003). In addition to physical impacts, there can be an influx of nutrients, metals and organic compounds, both adsorbed to suspended solids and in soluble form. Urban wet-weather discharges resulting from increased development thus present a group of stressors, resulting in both an increased severity and frequency of disturbance, as well as long-term changes to stream conditions (Pitt 2003).

In densely urbanized regions, contaminant and nutrient loadings are of particular concern. In terms of discharge volume and solids load, urban runoff from all sources (treated and untreated) significantly exceeds those associated with municipal wastewater (Chambers et al. 1997). In the Canadian Great Lakes region, urban runoff discharges annually in the order of 10^5 tonnes of suspended solids, 10^4 tonnes of chloride, 10^3 tonnes of oil and grease, and 10^2 to 10^3 tonnes of trace metals (Marsalek and Schroeter 1989). Combined sewer overflows (CSOs) represent another form of wet-weather pollution which incorporates both wastewater and stormwater runoff. The most significant pollutants of concern associated with CSOs include pathogens, solids, oxygen-demanding substances, nutrients and chemicals from small industrial, municipal and commercial sources. Evidence of serious impacts of these discharges was found in half of the Areas of Concern in the Great Lakes region, in which stormwater and CSO discharges caused medium-high pollution problems impeding the delisting of these areas (Weatherbe and Sherbin 1994).

Although stormwater discharges are typically episodic and result in short-term pulses of exposure, sediments in receiving waters frequently accumulate contaminants. Hydraulic action may disperse pollutants asso-
associated with sediments and make them temporarily bioavailable to the organisms in nondepositional areas (Hatch and Burton 1999). Among biological communities in streams, benthic invertebrates may have a greater degree of contact with pollutants derived from these discharges than other organisms. Their sedentary nature, ubiquity, responsiveness to disturbances, ease of sampling and importance to other ecosystem components make benthic invertebrate communities highly relevant in environmental studies (Johnson et al. 1993). In addition, their responses integrate the effects of both water and sediment exposures over time. Assessing sediment and benthic invertebrate conditions is thus a practical alternative to the continued monitoring of water quality affected by wet-weather discharges.

Numerous studies have assessed benthic invertebrate communities in urban streams exposed to wet-weather runoff (reviewed by Pitt 2003). Benthic communities in urbanized areas and watersheds were frequently depauperate (reduced total invertebrate abundance and taxon richness), dominated by pollution-tolerant taxa, such as oligochaetes and chironomids, and poorly populated by pollution-sensitive taxa, such as ephemeropterans, plecoptera and trichoptera, compared to communities in non-urbanized streams. A large body of evidence also has demonstrated in situ toxicity of sediment, porewater and overlying water in stormwater-exposed aquatic systems (Burton et al. 2000).

Linking benthic conditions to wet-weather discharges in general (rather than underlying stream conditions), and specific component stressors in particular, has been problematic. Variability in the magnitude, duration and frequency of the discharges and the relative importance of their component stressors, encumbers the quantification of exposure. As well, the length of recovery time (dry period) between events varies. Stream ecosystems are highly heterogeneous and dynamic at multiple spatial and temporal scales, resulting in often complex responses to disturbances (Resh et al. 1988). Urban streams are typically influenced by multiple anthropogenic factors. Establishing causal relationships between highly variable discharges and benthic conditions may, therefore, be a challenge unless either the discharge effect is large or the sampling effort substantial. Pitt (2003) suggests that there is a large amount of evidence that biological degradation occurs as a result of habitat changes, and that there is limited evidence for contaminant-induced impairment. On the other hand, Burton et al. (2000) and Walsh (2000) emphasize that pollutant effects are often not negligible.

In this paper, we review the results from several studies in which benthic conditions were assessed in a series of urban stream systems in the Lake Ontario watershed. The purpose of these assessments was to characterize benthic conditions of sites exposed to wet-weather runoff (primarily untreated stormwater), and to determine if observed alterations could be associated with runoff-related stressors.

Assessment Methods

Our studies focussed on assessing potential responses of benthic invertebrates and their immediate habitats to wet-weather discharges (mainly untreated stormwater) in streams (and a few standing water sites). Although the spatial and temporal scales of the observations differed among studies, our methods were most suitable for detecting alterations that extend over the whole site (≤100 m in length) and persist for a significant fraction of the period between runoff events. Shorter-term or microhabitat-level effects could be “averaged” across the sampling units and possibly not detected.

The assessments of wet-weather discharge effects progressed from a preliminary survey of broad conditions in sites exposed to a wide variety of wet-weather runoff to planned field experiments and more focussed observations for specific benthic conditions. In each assessment, several sets of benthic conditions were examined. Altogether, observations were made on sediment physicochemistry (grain size distribution, nutrients, metals, PAHs), sediment toxicity (involving 4 species and 10 endpoints), in situ water column toxicity, benthic invertebrate communities (resident and on artificial substrates), and contaminant (metals, PAHs) bioaccumulation in invertebrates. An overview of the study designs and methods are given in the next sections.

Survey of Benthic Environments Exposed to Wet-Weather Discharges

Study design. A survey of benthic conditions was conducted in 1998 at 10 locations in the vicinity of wet-weather outfalls representing a range of combined sewer overflow (CSO) and treated and untreated stormwater exposures. The objectives of the study were to assess whether 3 categories of benthic environment conditions—sediment chemistry and particle size, sediment toxicity and benthic invertebrate community structure (BCS)—varied according to the expected degree of runoff exposure at the sites, and to determine if among-site patterns were correlated between the categories. Five study areas were surveyed:

- Three sites were associated with a CSO treatment facility designed to protect the near-shore water quality in Lake Ontario (Dunker’s Wet-Weather Flow Balancing System, Scarborough). Site DU03 was located in the main settling pond, site DU02 was in a wetland polishing cell, and site DU01 was in a shallow embayment of Lake Ontario near the final outfall.
- Four sites were at a residential stormwater pond in Richmond Hill and the stream to which it discharges.
Site HAP01 was located in the sediment forebay and site HAP02 was in the main settling pond. Site HAP03 was in German Mills Creek, upstream of the pond outfall (but below untreated drainage outfalls from streets and housing lots) and site HAP04 was located approximately 10 m downstream of the stormwater pond outfall in German Mills Creek.

- Site RPO01 was in the Rouge River at the outfall from a stormwater pond, which receives runoff from a major multilane highway (Highway 401, east of Port Union Road) and an urban catchment in Scarborough.
- Site SC01 was in Spencer Creek in Dundas, approximately 50 m upstream of the confluence with Sulphur Springs Creek. Upstream of the site were multiple untreated stormwater and CSO inputs.
- Site RH01 (sampled for sediment) was in Red Hill Creek in Hamilton at a CSO outfall and approximately 40 m downstream of an untreated stormwater outfall. Sampling for benthic invertebrates occurred 50 m downstream of the CSO outfall at subsite RH02.

**Sample collections.** Sediment samples for physicochemical analyses and toxicity tests were obtained from accumulated deposits using two different methods. Where a sufficient amount of sediment was present, a Petite-Ponar grab was used (to a depth of approximately 10 cm). Collected sediment was homogenized in a pre-cleaned glass dish with a Teflon spatula before subsampling for analyses of PAHs, metals, nutrients and particle size. In the other locations, sediment was collected by scooping directly from the stream bed using a pre-cleaned plastic jar. Multiple collections were made to obtain five 2-L samples of fine-grained sediment for the toxicity tests.

Samples for benthic community structure were also obtained by two different methods, dependent on substrate type. In depositional sites (lake, pond or large stream habitat), sediments were collected by Petite-Ponar (up to 10 cm in depth). Three 2-L replicate samples were collected for community structure. Samples were sieved through a 500-µm mesh screen in the field prior to preservation with 5% formalin. Within 3 days, samples were transferred into 70% ethanol. Where the water was fast moving (erosional sites: HAP03, HAP04, RH02), benthic invertebrates were collected by a timed, traveling kick net method. A 400-µm mesh conical net on a triangular frame (38.5 cm to the side) was held firmly against the stream bottom as substrate upstream of the net was dislodged while traversing upstream in a zigzag pattern from bank to bank for 3 minutes. Dislodged material captured by the net was washed into a container and preserved as above.

**Sample and data analyses.** Sediment physicochemistry. For each site, sediment samples were analyzed for 28 PAH compounds, particle size distribution, 18 metals, total organic carbon (TOC), total Kjeldahl nitrogen (TKN) and total phosphorus (TP).

Wet samples for analyses of PAH compounds were extracted (using dichloromethane) and analyzed by gas chromatograph as described in Rochfort et al. (2000). Particle size was determined using a sediment apparatus, and results reported as percent gravel, sand, silt and clay. Mean particle size and particle sizes of the 25th and 75th percentiles were also indicated.

All other analyses were performed by a private (Canadian Association for Environmental Analytical Laboratories accredited) laboratory following Standard Methods (APHA 1989), and using appropriate QA/QC methods. Samples for analysis of elements were prepared by nitric acid extraction and analyzed by inductively coupled plasma (ICP). Total nitrogen and total phosphorus were extracted from the sediments using a standard Kjeldahl digestion (H2SO4/K2SO4/HgSO4) and analyzed colourimetrically on a spectrophotometer.

**Sediment toxicity tests.** Samples for toxicity tests were stored in the dark at 4°C until used. Each sediment sample was homogenized and sieved, where possible, through a 250-µm mesh sieve to remove indigenous macrofauna, using a 4:1 ratio of culture water:sediment (2 L culture water:500 mL sediment). The sieved sediment was allowed to settle for a minimum of 24 hours, after which the water was decanted and used as the overlying water in the experiment. Culture water was dechlorinated municipal tap water.

Four sediment toxicity tests were performed: *Chironomus riparius* 10-d survival and growth, *Hyalella azteca* 28-d survival and growth, *Hexagenia* spp. 21-d survival and growth, and *Tubifex tubifex* 28-d survival and reproduction. Details of the procedures are described in Rochfort et al. (2000) and elsewhere (Borgmann and Munawar 1989; Borgmann et al. 1989; Krantzberg 1990; Reynoldson et al. 1991, 1998). All tests were subject to acceptability criteria based on percent control survival in a reference sediment (from Long Point Marsh, Lake Erie): i.e., ≥80% for *H. azteca* and ≥70% for *C. riparius* (U.S. EPA 1994; ASTM 1995); ≥80% for *Hexagenia* spp. and ≥75% for *T. tubifex* (Reynoldson et al. 1998).

Water chemistry variables (pH, dissolved oxygen [mg/L], conductivity [µS/cm], temperature [ºC], and total ammonia + ammonium [mg/L]) were measured for each test in each replicate test beaker on day 0 (start of test—prior to introduction of organisms) and at completion of the test (day 10, 21 or 28). Tests were run under static conditions in environmental chambers at 23 ± 1ºC, under a photoperiod of 16L:8D and an illumination of 500 to 1000 lux, with the exception of the *T. tubifex* test which was run in the dark.

**Benthic invertebrate communities.** Macroinvertebrates were removed from benthic samples and sorted to family level using a low power stereo microscope. Kick
net samples were subsampled using a 100-cell Marchant box (Marchant 1989). Cells were randomly selected and their contents counted until at least 200 organisms were obtained. Total numbers of invertebrates in the sample were estimated by extrapolation based on the number of cells counted. The number of individuals for each taxon was enumerated and recorded.

Data analyses. Data analyses were aimed at (a) characterizing conditions at sampling sites in terms of sediment physicochemistry, sediment toxicity and benthic community composition, and (b) assessing the relationships between the variable categories. Characterization of sediment conditions at sites was achieved by univariate and multivariate descriptive statistics and graphs. For the sediment chemistry data, concentrations of metals and PAHs at each site were compared to existing provincial sediment quality guidelines (Persaud et al. 1993) as a means of identifying the degree of contamination. To provide a general characterization of sediment chemistry, principal components analysis (PCA) was performed on each of two subgroups of variables: (a) metal, nutrient and grain size variables, and (b) PAH variables. However, total PAH concentration was an equally meaningful descriptor as principal components.

Sediment toxicity results were evaluated by comparing endpoint means for each of the 10 sampling sites with criteria derived from tests with uncontaminated reference sediment from the Great Lakes (Reynoldson and Day 1998). The criteria are based on measurements of the 10 toxicity endpoints for 179 to 220 reference sediments collected from the nearshores of the Great Lakes over a three-year period.

PCA was also applied to the benthic invertebrate data set to produce several (3) descriptor component variables to characterize BCS. Because benthic invertebrates were collected by two sampling methods—Petite-Ponar grab at seven sites and kick net at three sites—the data were not commensurate and therefore not pooled. PCA was performed only on ln(x + 1)-transformed data from seven Ponar-sampled sites.

Relationships between sediment chemistry descriptors, toxicity test endpoints and BCS descriptors were assessed by correlation analysis and bivariate plots. Sediment chemistry descriptors included 3 PCs from the ordination of the metal, nutrient and grain size variables, and a separate grain size variable—ln(25th percentile of particle size)—as a means of assessing the influence of grain size alone. The toxicity endpoints were the 10 measured responses. The BCS descriptors were the first three PCs from the ordinations of the Ponar sample data subset, plus total abundance and taxa richness for each of the Ponar and kick net sample data subsets. Separate correlations were calculated for each of the Ponar and kick samples. Associations between the assumed degree of exposure to the wet-weather discharges and benthic conditions were examined.

Downstream-Upstream Comparisons of Paired Sites

Study design. The challenge of detecting moderate effects of wet-weather discharges on benthic communities in urban streams was to distinguish them from natural habitat-driven spatial variability and from alterations due to other anthropogenic stressors. Benthic invertebrate communities are influenced by a variety of abiotic and biotic factors (Power et al. 1988). It is possible that natural and/or human-induced differences in these factors among streams would make the detection of discharge-related effects difficult. Lotic benthic community structure typically varies sufficiently among locations within a watershed to require impractically large numbers of replicate samples to detect human impacts (Resh and McElravy 1993). Therefore, to reduce variability among sites due to these “nuisance” factors, benthic invertebrate communities were examined in two stream systems, at points immediately downstream and upstream of 3 stormwater outfalls. It was assumed that differences between paired downstream and upstream sites in conditions affecting benthic invertebrates would be minimal in all respects (at least compared to the range of conditions along the course of a stream or among streams), except for the level of stormwater-related physicochemical disturbance and associated biotic responses. By assessing differences in benthic communities between each of the paired sites, effects of the nuisance factors would be controlled. Thus, as in a split plot experiment, variability among sites at different outfalls is not incorporated into an “error term” against which variability due to the treatment (outfall exposure) is compared.

It was recognized that stormwater discharges from different outfalls were not necessarily equivalent in quantity or quality, that some degree of difference in habitat conditions would exist between any sites in the field, and that it was not possible to achieve true replication of both the stressor treatment and the observational units. Nevertheless, this sampling design (in which the 2 streams are blocks, exposure to outfall discharges is the treatment, and the paired sites at each outfall are the observational units) should offer an improved in situ assessment of stormwater impacts through repeated observations of similar natural benthic communities exposed to the same class of physicochemical disturbances. As a check on the assumption of habitat homogeneity for paired sites, several habitat attributes known to affect benthic invertebrates were measured, assessed and tested for association with patterns in the benthic community observations. At the same time, concentrations of PAHs in sediment were determined as an indication of exposure to stormwater effluent.

The study areas involved multiple sites along two different stream systems in Oshawa and Kingston, Ontario.
In each stream, sampling was conducted upstream and downstream of 3 stormwater outfalls in October 1999:

- **Farewell Creek (Oshawa)**—Site FAR01, located downstream from an untreated stormwater discharge from a major 6-lane freeway (Highway 401), paired with Site FAR03 upstream of the freeway; Site FAR04 on the main stem of Farewell Creek, located below several small discharges of untreated runoff from a major road (Nash Road), paired with Site FAR05 upstream of these discharges; Site FAR06 on Black Creek, a tributary of Farewell Creek, downstream of untreated discharges from a major traffic intersection (Highway 2 and Courtice Road) and effluent from a residential stormwater pond, paired with Site FAR07 above these discharges.

- **West Branch of Little Cataracaui Creek (Kingston)**—Site LCC02, located downstream of the untreated stormwater outfall from a 4-lane major traffic artery (Princess St., Highway 2), paired with Site LCC01 above Princess St.; Site LCC04, located below several untreated discharges from residential and commercial developments, as well as road runoff from Gardeners Rd., a 4-lane major traffic artery, paired with Site LCC03 above the series of discharges; Site LCC07, immediately downstream of a discharge of treated stormwater pond effluent, paired with Site LCC05, upstream of the stormwater pond outflow, but just below the outfall from a separate stormwater pre-treatment pond, which was designed to remove heavy sediments from the runoff generated by the Cataracaui Town Centre parking lot; a far-field site (LCC08) that had no nearby stormwater inputs located at the end of a long reach downstream of the stormwater pond was also sampled.

**Sample collections. Habitat descriptors.** Each site was characterized by a series of descriptors, using methods outlined in Rosenberg et al. (2001) and detailed in Rosenberg et al. (1999). These descriptors included geographical location (latitude, longitude), stream characteristics (flow state, water velocity, macrophyte coverage) and channel measurements (stream width, floodplain width, channel depth, slope). Stream bed interstitial material was also collected for particle size analysis.

**Sediment.** Sediment samples were collected by the same methods (scooping surficial sediment using a pre-cleaned jar) as for the survey of sites (above).

**Benthic invertebrates.** Assessment of the benthic invertebrate communities focussed on the dominant habitat of the study sites—erosional (riffle-run) zones. Invertebrates were collected by a timed, travelling kick net method as described above.

**Sample and data analyses. Sediment.** PAHs and particle size analyses were conducted using the same methods as for the survey of sites (above).

**Benthic invertebrates.** Kick net samples were subsampled using a Marchant box. Macroinvertebrates were sorted and identified to the lowest taxonomic level possible (genus or species, otherwise family for early life-stages) using a low-power stereo microscope (16X with 10X eye piece) and, for chironomids and oligochaetes, slide mounts and a high-power microscope. Porifera, nematodes, copepods and cladocerans were excluded. The number of individuals for each taxon was enumerated and recorded.

**Data analyses.** Analyses of the observations on stream conditions above and below stormwater outfalls were performed to (a) determine if benthic invertebrate communities below outfalls differed from those above, (b) test if natural habitat attributes of the sites accounted for such differences, and (c) assess whether sediment PAH concentrations were elevated below outfalls and correlated with benthic invertebrate community structure. A split-block-type data analysis was conducted, in which the 2 streams were blocks, exposure to outfall discharges was the “treatment,” and the paired sites at each outfall were the observational units. It was assumed that variability between the paired downstream/upstream sites, which are on the order of <100 m apart, would be minimal, whereas variability among sites at different outfalls could be high. To adjust for this (i.e., factor out the among-outfall variance), differences in responses between downstream and upstream sites within outfalls were analyzed as replicate observations, rather than comparing responses for the 3 downstream sites against responses for the 3 upstream sites in each stream.

Data reductions were achieved by ordinating the habitat data with PCA separately for each stream. For the benthic invertebrate data, non-metric multidimensional scaling (NMDS; McClune and Mefford 1999) was conducted. Separate ordinations were performed for each of the Farewell and Little Cataracaui Creek sites because the faunal assemblages were sufficiently distinct to obscure relatively smaller differences likely to exist between upstream and downstream paired sites. Benthic communities were also described by total abundance, taxonomic richness and Simpson’s evenness. PAH conditions were quantified based on total PAH concentration.

Effects of outfalls on BCS were tested using a permutation test described in Legendre and Legendre (1998). A repeated change in the descriptors in the same direction at locations downstream of outfalls relative to those upstream would be predicted if stormwater discharges affect BCS, whereas no change on average would be predicted in the absence of discharge effects. Responses of individual invertebrate taxa (as downstream/upstream differences in abundances) were also assessed graphically.

Differences in habitat attributes between sites above and below outfalls were analyzed in a similar manner to
that used for the benthic community data. Differences in the scores for each of the first 3 components (PCs) from the habitat variable PCA between up- and down-stream site pairs within each stream were calculated and examined to determine if natural habitat attributes were confounded with exposure to outfall discharges. Relationships between habitat PC scores and benthic community descriptors were examined graphically and by regression analysis to assess if the invertebrate assemblages varied with the habitat attributes. The habitat PCs were also plotted with the most widely distributed individual taxa. The correspondence of site similarities in habitat attributes with site similarities in benthic community structure was evaluated by Mantel’s test. Site similarities were quantified by Euclidean distance for the habitat variables and Bray-Curtis distance for the taxon variables.

Benthic invertebrate axis scores were plotted and regressed against PAH concentrations in sediments separately for each stream to test for relationships between BCS and PAH exposure.

Field Experimental Exposures

**Study design.** The sensitivity of assessments of benthic community responses to wet-weather discharges is in part related to the amount of habitat-induced spatial variability influencing the observational units. Substrate type is an important factor in benthic community composition, but substrate heterogeneity is difficult to reduce when sampling natural stream beds from distinct locations. In addition, commonly used devices such as kick nets, which collect invertebrates from several microhabitats (pool-riffle sequence) over a scale of several metres or more, effectively homogenize rather than partition the effects of small-scale spatial variability. Therefore, to minimize effects due to substrate variability, pre-colonized artificial substrates were deployed upstream and downstream of outfalls in 2 streams for 6 weeks to measure effects of exposure to stream conditions. Epilithic benthic communities of the artificial substrates serve as standardized observational units. As well as assessing benthic communities of the substrates after exposure to stream conditions, in situ toxicity tests were conducted using caged amphipods positioned at the same sites. Contaminant accumulation was also measured in semipermeable membrane devices (SPMDs), field-collected resident invertebrates and amphipods from the toxicity test cages.

The experiments were conducted September to October 2000 in 2 streams in the Toronto area:

- Etobicoke Creek at a large outfall (by Dundas St.) that carries untreated stormwater drainage from a major expressway (Highway 427) and the surrounding residential neighborhood. The upstream site (EU) was several hundred metres above the outfall; the downstream site (ED) was ~100 m below the outfall in the same type of stony-bottom “run” habitat.

- East Humber River near King City at an outfall that receives untreated runoff from a major road (King Road). The upstream site (HU) was approximately 50 m above two outfalls transporting untreated stormwater from King Road. The downstream site (HD) was about 100 m below the outfalls.

**Experimental procedures.** Artificial substrates. Ten concrete substrates (approximately 15 cm square × 4 cm thick with 20-mm stone partially embedded on the upper surface) were placed on stream beds to be colonized by native benthic organisms at each of the 4 experimental locations in July 2000. After 8 to 9 weeks in the streams, 5 substrates from each site were reciprocally transplanted to the corresponding upstream or downstream locations. Substrates for transfer were randomly selected. This transplantation allows assessment of two types of effects: (1) transfer from unexposed to exposed conditions and (2) transfer from exposed to unexposed conditions. It also allows adjustment for any effects due to the transplantation process itself (i.e., a non-specific change of location). Disturbance of the epilithic communities was minimized during removal, transport and reinstallation. Imported substrates were placed in the locations previously occupied by the exported substrates. Six weeks after reciprocal transplantations, all substrates were covered with a close-fitting inverted plastic tub and removed to a bucket of stream water, uncovered and gently scrubbed with brushes to remove attached biota and accumulated sediment and other debris. Bucket contents were poured through a 400-µm mesh net, and material retained was preserved with formalin. The scrubbed substrate was also placed in formalin, in a plastic bag, to drive out invertebrates from crevices which were not removed by brush. After 1 day, these invertebrates were added to the jar of scrubbed substrate material. After 1 week, the invertebrates were transferred from formalin to 70% ethanol. Invertebrates were counted based on taxonomic identifications to the family level.

**Amphipod toxicity tests.** Concurrent with the benthic community transplantation experiment, in situ toxicity tests were conducted using caged amphipods (*Hyalella azteca*). In each of the 4 exposure areas assessed for the transplantation experiment (upstream and downstream of 2 stormwater outfalls), 5 cages each containing 20 to 22 randomly distributed adult amphipods were positioned on the stream bed. The cages were made of two sections of clear acrylic tube that fit securely together (7.6 cm diameter, 7.6 cm long) covered at each end by 250-µm mesh plastic screen, and contained approximately 5-cm² of cotton gauze and 2.5 mg TetraMin dried food. For approximately 7 weeks, amphipods were exposed to the water column and any suspended material that passed through the screen. Survival for the group of amphipods in each cage was measured weekly, at which times cage screens were cleaned and amphipods fed dried fish food. At the
end of the experiment, surviving amphipods were retained for analyses of contaminant concentrations in tissues.

Contaminant accumulation. At the end of the toxicity test (47 and 48 days for East Humber River and Etobicoke Creek, respectively), the cages were removed from the streams and returned to the laboratory. Amphipods were taken out of each cage, counted, and placed into a solution of 40 mL 50-µM EDTA + 40 mL stream water from the site to allow clearance from the gut of material from the site. Also in the container was a square of cotton gauze and 2.5 mg TetraMin dried food. After 1 day, amphipods were removed from the gut clearance solution, weighed wet as a cage group, and dried at 60°C for 3 days. Dry weights were then measured for each cage group.

Six amphipods were selected from 2 cages from each of the 4 study sites for metal analyses. Amphipods were selected to roughly represent the interquartile range of body lengths for the cage group. For each of the 8 cage groups, amphipods were digested in 150 µL of concentrated nitric acid. Six days later hydrogen peroxide was added, followed by Milli-Q water. Total metals (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Tl and Zn) in amphipod digests were determined by inductively coupled plasma-mass spectroscopy (ICP-MS).

In each of the 4 study sites, kick net samples were taken on November 22 to obtain invertebrates for analyses of PAHs. Samples were collected by travelling longitudinally upstream through the area where artificial substrates had been placed previously. This was done 4 times, with each kick sample covering roughly the same length of stream. Material retained on the triangular net was washed into a pail (kick samples composited within sites) and brought back to the laboratory for sorting. After a preliminary examination of the invertebrate collections, isopods and hydropsychids were selected from the Etobicoke Creek sites and heptageniids and hydropsychids were picked from the East Humber River sites. Each of the 8 groups of invertebrates (2 taxa x 4 sites) were held for 20 to 24 hours in site stream water in plastic weigh boats to clear gut contents, then rinsed in Milli-Q water, transferred to pre-cleaned glass jars and frozen. Tissues from invertebrates were pooled within groups, digested and analyzed for PAH concentrations as described for sediments above.

Semipermeable membrane devices (Bennett et al. 1996) were exposed to stream water (2 per site) adjacent to the amphipods cages for the duration of the toxicity tests. Concentrations of PAHs in extractions from the SPMDs were determined as described for sediments above.

Data analyses. As with previous analyses, multivariate ordination was initially applied to the benthic community data to provide a reduced number of descriptors of community structure. In this case, hybrid multidimen-
centrations of all metals and nutrients, and directly related to grain size). Highest contaminant and nutrient levels were measured from mostly near-field sites (SC01, RH01, HAP01, HAP02, DU03), whereas samples from far-field or far-far-field sites (DU01, DU02, RPO01) were comparatively uncontaminated. An exception to the pattern was far-far-field site HAP04, which had high PAH concentrations, possibly from an unaccounted for local source. At both the Dunker’s and Harding Park wet-weather discharge treatment facilities, sediment samples collected furthest “upstream” in the treatment chain (primary settling areas), were higher in contaminant concentrations compared to samples collected further “downstream” (e.g., DU03 versus DU02 versus DU01; HAP01 and HAP02 versus HAP03 versus HAP04 [metals only]).

Concentrations of PAHs in sediment were also assessed in Farewell and Little Cataraqui Creeks. With the exception of the furthest upstream paired site (FAR 07/06), sites below the stormwater outfalls were substantially elevated compared to conditions above the outfalls (Fig. 2), but the range in Farewell Creek was greater than that in Little Cataraqui Creek. Some compounds commonly associated with vehicular pollution (e.g., phenanthrene, fluoranthene, pyrene, benzo[a]anthracene) were 2 to 5 times the Probable Effect Levels of the Canadian Sediment Quality Guidelines (CCME 2001) at several sites.

**Sediment Toxicity**

Acute and chronic sediment toxicity based on 4 benthic invertebrate taxa and 10 endpoints was assessed for the
sites sampled in the 1998 survey. Overall, toxicity was low and did not correspond to either sediment contaminant concentrations or the assumed level or exposure to wet-weather discharges (Table 2). Greatest overall toxicity was observed in samples from sites DU01 and HAP04, which were from sites furthest “downstream” in their discharge treatment systems. As well, samples from Spencer and Red Hill Creeks, which were exposed to untreated discharges, showed the lowest toxicity. Among the 50 correlations between toxicity endpoints and sediment physicochemistry descriptors calculated, only 5 were significant at the $\alpha = 0.05$ level. Of those, none indicate a relationship of increasing sediment toxicity with increasing contaminant concentration.

Although a lack of observed toxicity of the most contaminated sediments could be surprising given the review of Burton et al. (2000), concentrations of metals and PAHs may not have been high enough to cause adverse effects—SEls were exceeded at only 1 site (PAHs at RH01). Contaminant-laden particles from wet-weather discharges could have been carried downstream from some of the locations sampled. A similar lack of correlation was seen for sediments from sites surrounding a major CSO outfall in the Don River (Toronto); however, more highly contaminated stormwater ponds did exhibit elevated toxicity of sediments (Grapentine, unpublished data).

**Water Toxicity**

Survivorship of amphipods after 47 to 48 days in cages open to water flow close to the stream bed was high (mostly >80%) and not significantly different between locations upstream and downstream of stormwater outfalls in both streams (Fig. 3). Inferred growth of amphipods was more variable among sites: mean weight after 47 to 48 days was much lower below than above the outfall in Etobicoke Creek, but not in East Humber River. On some occasions, accumulation of fine particles was observed in some cages, indicating that the test amphipods

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**TABLE 2.** Toxicity of sediments from surveyed sites in southern Ontario, 1998

<table>
<thead>
<tr>
<th>Site code</th>
<th>DU01</th>
<th>DU02</th>
<th>DU03</th>
<th>HAP01</th>
<th>HAP02</th>
<th>HAP03</th>
<th>HAP04</th>
<th>RPO01</th>
<th>SC01</th>
<th>RH01</th>
</tr>
</thead>
</table>
| *H. azteca* —survival (%) | 50.7  
| —growth (mg) | 0.18  
| *C. riparius* —survival (%) | 93.3  
| —growth (mg) | 0.30  
| *Hexagenia spp.* —survival (%) | 90.0  
| —growth (mg) | 1.90  
| *T. tubifex* —survival (%) | 100  
| —coconns (no.) | 7.7  
| —hatch (%) | 74.2  
| —young/adult (no.) | 11.2  

---

$a$Toxic (bold).

$b$Non-toxic (regular).

$c$Potentially toxic (bold, italics).

$d$(Enriched) (bold, brackets).
could be exposed to suspended material as well as dissolved and colloidal substances in the water column.

**Benthic Invertebrate Communities**

Responses of intact, natural communities of benthic invertebrates were examined at all sites. The focus and the methods of the assessments differed among studies, but in all cases tests for effects of exposure to wet-weather discharges were conducted. At most sites, oligochaete worms (naidids and tubificids) and chironomids were the dominant taxa. Ephemeropterans, plecopterans and trichopterns (“EPT” taxa) were rare, and taxonomic richness, even at the minimally exposed sites was not high.

In terms of broad descriptors of community structure—total abundance and taxonomic richness—effects of runoff exposure were not evident in the 1998 survey or the 1999 upstream/downstream comparisons. As well, total abundance and richness were largely uncorrelated to sediment contaminant concentrations. While differences in the sampled habitat (pond, lake, stream sediment, stream riffle) and sampling method (Ponar grab, kick net) could have hindered detection of alteration in the 1998 study, these limits did not exist in 1999. Neither the total number of individuals, taxon richness, nor evenness of the invertebrate samples were recurrently altered in sites below stormwater outfalls, relative to those above, in Farewell and Little Cataraqui Creeks (Fig. 4). The sole significant difference (by randomization test) was for richness, which was elevated in the communities downstream of outfalls in Farewell Creek. Artificial substrate communities in Etobicoke and East Humber Creeks, however, showed minor effects of location (above versus below outfall) on total abundance, which was higher (or became higher after transplantation) below outfalls, and richness, which was (or became) lower below 1 of the 2 outfalls (Fig. 5).

Benthic community structure (taxonomic composition) was characterized by multivariate methods; i.e., using the ordination axis scores from PCA, NMS or HMDS. These provide a means of describing the dominant patterns of variability in the data, as the information carried by numerous taxon counts is compressed...
into a reduced set of component variables. The axis scores, which are readily relatable to the original measured variables, “preserve” the majority of information on site-to-site similarity, and can be further analyzed or plotted to compare sites with each other, or to correlate with descriptors from other sets of variables. An effect of wet-weather runoff on BCS would be suggested if sites plotted by ordination scores were arranged in groups or a sequence corresponding to different levels of exposure of runoff to the sites.

Taxonomic composition of samples from the 1998 survey sites was not related to discharge exposure level (at least at the family level of resolution). In terms of the dominant axis of variability for the depositional sites, which corresponds to oligochaete and chironomids density, near-field communities were not distinct from those of far-field sites. There were also no significant correlations between any of the BCS descriptors (first 3 PCs, accounting for 90% of total variation) and sediment contaminant concentrations. However, as noted above, differences in benthic habitat type could account for some of the variation in BCS.

For the below and above outfalls comparisons, it was hypothesized that outfall-related alterations of benthic communities could appear in the ordination plots as repeated differences of downstream sites in the same direction relative to their paired upstream sites. In Farewell Creek (Fig. 6A), this is evident for 2 of the 3 outfalls—sites FAR04 and FAR06 score higher on axis 2 than their paired upstream sites (FAR05 and FAR07). Sites at the third outfall (FAR01 and FAR03), located further downstream, do not differ in the same direction and, in fact, appear comparatively similar. In Little Cataraqui Creek (Fig. 6B), common displacements of downstream sites relative to their paired upstream sites are observed for all 3 outfalls, in terms of both axis 1 (downstream sites displaced negatively) and axis 2 (downstream sites displaced positively). Furthermore, far-field site LCC08, which is further downstream from site LCC07, shifts back in the direction of the 3 upstream sites. These differences are significant based on the permutation tests for axes 1 and 2 of Little Cataraqui Creek ordination, but not for either axis in Farewell Creek. Similar displacements are thus apparent for 2 of 3 paired sites in one stream and 3 of 3 paired sites in the other stream. A similar pattern of differences between paired sites downstream and upstream of outfalls was not seen for the habitat attributes assessed, nor were the habitat attributes strongly correlated with BCS descriptors across sites within streams. Therefore, recurrent differences in BCS between paired sites are not explainable by differences in the observed habitat factors.

To identify the taxa that account for these alterations of taxonomic composition in the benthic communities, differences in the abundances of individual taxa between downstream and upstream sites were analyzed. A minority of taxa (16–21%) exhibited recurrent downstream-upstream differences within streams. In Farewell Creek, these included unidentified Enchytraeidae, Nais bretcheri, Nais elinguis, Pisidium compressum, Stenelmis...
crenata, Hydropsyche spp., Cricotopus spp., Parakieferiella spp. and Paratanytarus spp. higher in downstream sites; and Chaetogaster diaphanus, Dubiraphia spp. and Polypedilum spp. higher in upstream sites. In Little Cataraqui Creek, Nais bretcheri, Cricotopus spp., Hemerodromia spp. and Hydropsyche spp. were higher in downstream sites; and Dero digitata, unidentified Tubificidae, unidentified Candoniidae and Limnocythere spp. were higher in upstream sites. These are the invertebrates most likely to be exhibiting responses to discharges from the stormwater outfalls. Only 3 of these taxa demonstrated similar patterns in both streams: Nais bretcheri, Cricotopus spp. and Hydropsyche spp. All were higher downstream of all outfalls.

Across all sites within each stream, relationships between the benthic community descriptors (total abundance, taxon richness, evenness, NMS axis 1 and 2 scores) and sediment total PAH concentration were largely not significant. An exception was for NMS axis 1 scores for the Little Cataraqui Creek sites (linear regression P = 0.023), which declined with total PAH concentration. Among the widespread taxa, only unidentified Tubificidae (which is strongly correlated with the NMS axis 1 scores) declined with sediment PAH level (polynomial regression P < 0.001). However, this taxon did not show the same relationship for the Farewell Creek sites, nor did any of the other 3 oligochaetes (Nais bretcheri, N. elinguis, N. simplex) examined. Furthermore, growth and reproduction of T. tubifex in the toxicity tests of the 1998 survey of sites study were not correlated to sediment total PAH concentration.

Benthic communities of artificial substrates in the transplant experiments were strongly affected by location relative to stormwater outfalls. In both Etobicoke and East Humber streams, the untransplanted communities downstream of the outfall were distinct from those upstream, as indicated by non-overlapping areas occupied by the 2 solid symbols in each of the ordination plots (Fig. 7). Furthermore, the structure of transplanted communities (open symbols) was more similar to untransplanted communities in their destination than to untransplanted communities in their initial source locations (ANOVA P < 0.001). Although the data from the 2 streams were ordinated by HMDS separately, differences between downstream and upstream substrates involved similar taxonomic alterations. Univariate comparisons of family abundances indicated that oligochaetes, chironomids and physid snails were more abundant downstream of outfalls, whereas mites (hydrphantids and hygrobatids) and limnocytherid ostracods were less abundant downstream. Hydropsychid caddisflies were strongly reduced in the downstream site of Etobicoke Creek, but not in the East Humber River.

Overall, based on observations from 27 sites in 3 studies, alterations of benthic invertebrate communities in response to wet-weather discharges were not strong (Table 1). It seems that as the potential influence of nuisance factors (non-discharge conditions) was increasingly controlled from survey to experiment, detection of effects increased. Effects on total abundance and taxon richness were weak or not detected. Changes in community composition involved a minority of the taxa. In some instances, common taxa, such as the amphipod Gammarus pseudolimnaeus in Little Cataraqui Creek and asellid isopods in Etobicoke Creek, showed no significant differences in abundance between exposed and unexposed sites. Hydropsychid caddisflies, considered sensitive to disturbance, showed conflicting responses: increasing in abundance below outfalls in Farewell and Little Cataraqui Creeks, but decreasing below...
the outfall in Etobicoke Creek. The strongest agreement between studies in BCS alteration was the increased dominance of oligochaetes and chironomids, which are typically opportunistic and tolerant of disturbance.

Contaminant Accumulation

Concentrations of 10 metals in whole tissues of gut-cleared amphipods that were held for almost 7 weeks in cages exposed to the water column were not elevated in the sites below stormwater outfalls relative to sites above outfalls in Etobicoke Creek and East Humber River. Tissue metal levels in general were low, and well below concentrations determined to be lethal to 25% of laboratory test organisms (estimated for Cd, Cr, Cu, Ni, Pb, Ti and Zn by U. Borgmann, pers. comm.). Given the low mortality observed in the cages, these levels are not unexpected. Perhaps what is surprising is the lack of elevation of Cu and Zn (metals commonly associated with runoff from roads [Burton and Pitt 2002]) in amphipods held downstream of the outfalls.

Semipermeable membrane devices exposed to water flow in the same locations as caged amphipods accumulated more than 2× the total PAHs below outfalls relative to above in Etobicoke Creek (90–92 versus 42–48 µg/mL extract). However, in East Humber River, total PAH concentration in the downstream SPMDs averaged lower than those in the upstream SPMDs (25–26 versus 24–34 µg/mL extract).

Similar differences were shown in tissues of resident benthic invertebrates from above and below the outfalls. Concentrations of most PAH compounds were elevated in hydropsychid caddisflies and isopods downstream (relative to upstream) in Etobicoke Creek, but not in hydropsychids and heptageniid mayflies in East Humber River (Fig. 8). Insects from the East Humber sites had pronounced accumulations of naphthalene and alkyl naphthalene compounds, especially the hydropsychids from above the outfall, suggesting a possible non-runoff source of PAHs (Irwin et al. 1998). On a basis of total PAHs excluding naphthalenes, PAH bioaccumulation by downstream invertebrates exceeded upstream amounts for the Etobicoke caddisflies and isopods, and the mayflies of East Humber, but not for the caddisflies of East Humber due to significant levels of phenanthrene, fluoranthene and pyrene.

Impacts of Wet-Weather Runoff on Benthic Conditions

It has been well established that warm-weather urban runoff exposes streams to (a) physical disturbance through changes in stream flow and sediment redistribution (Roesner and Bledsoe 2003), and (b) an influx of dissolved and particle-bound nutrients, metals and organic compounds (Burton and Pitt 2002). Wet-weather discharges thus subject the benthic environments of receiving waters to a group of stressors, involving an increased frequency of disturbance and episodic exposure to hydraulically dispersed pollutants, as well as long-term, cumulative changes to stream conditions.

![Fig. 7.](https://iwaponline.com/wqrj/article-pdf/39/4/374/228842/wqrj0390374.pdf) Effects of transplantation treatment on benthic community structure (BCS) of artificial substrates in Etobicoke (A) and East Humber (B) streams. BCS is described by axes from hybrid multidimensional scaling. Substrates are represented as points in 2 of the 3 ordination dimensions. Point symbols indicate experimental treatments; i.e., initial and final location downstream (D) or upstream (U) of the outfall.

![Fig. 8.](https://iwaponline.com/wqrj/article-pdf/39/4/374/228842/wqrj0390374.pdf) Comparison of concentrations of PAH compounds in resident benthic invertebrates from below and above outfalls in Etobicoke Creek and East Humber River, October 2000, during field experimental exposures. Bars represent the total PAH concentrations (including alkyl naphthalenes) in a pooled sample of gut-cleared invertebrates for 2 taxa.
Conditions in Urban Streams of Lake Ontario

Overall, it was found that in sites exposed to discharges, sediments were slightly to moderately enriched in nutrients, metals and PAHs. Highest nutrient and metal concentrations reached levels associated with potential adverse effects; highest PAH concentrations exceeded by severalfold lower limits for levels associated with probable adverse effects (e.g., lowest and severe effect levels from Ontario sediment quality guidelines [Persaud et al. 1993]). There was also some evidence of elevated uptake of PAHs by SPMDs and resident benthic invertebrates below an outfall relative to above.

Adverse biological responses, however, were not strongly associated with runoff exposure. Acute and chronic toxicity of sediments, based on 4 invertebrate species, did not vary with site exposure to runoff. Overlying water column conditions were not chronically toxic to amphipods. At sites below outfalls, benthic invertebrate communities were not generally depauperate relative to sites above outfalls, but did show some alteration in composition involving a minority of taxa. Whether these differences represent ecologically adverse effects of stormwater discharges was not clear. Based on the weight of evidence, therefore, wet-weather discharges appeared to impact physicochemical conditions of the benthic environment in the stream systems assessed, but not to a level deleterious to the majority of the benthic fauna.

Identification of the stressor(s) through which wet-weather discharges affect biota (i.e., water flow, sediment redistribution, contaminants) is limited in correlational, field observation studies. However, acute toxicological responses to contaminants were not evident as shown in the sediment and water toxicity tests, and by the low levels of metal and PAH bioaccumulation by invertebrates. Effects of sediment movement were not directly examined, but stream sites above and below stormwater outfalls did not differ significantly in particle size distributions. Stream hydrology and geomorphology are dynamic and difficult to characterize by one-time observations, but could potentially have large influences on benthic conditions through adjustments of channel width, depth, slope, velocity and substrate characteristics among other variables (Roesner and Bledsoe 2003).

Other studies of wet-weather discharges effects on urban streams report persistent physical disturbance, contamination and degraded benthic communities. However, as noted by Pitt (2003) in his review of receiving water impacts associated with wet-weather discharges, field observations for these studies were typically obtained from either urbanized and nonurbanized areas within stream drainage basins or from separate basins. Few studies involved sampling invertebrate communities above and below individual outfalls. Consequently, distinguishing discharge-related effects from responses to natural and other anthropogenic conditions has been difficult.

Among studies designed to examine impacts of individual outfalls, degradation of benthic communities at stormwater-exposed sites is not universally observed. Two recent comprehensive studies of benthic invertebrate assemblages below individual stormwater outfalls found variable community patterns. Maltby et al. (1995) sampled upstream and downstream of 7 runoff discharges from a major highway (in separate streams) in the UK. Although sediments in the downstream sites were elevated in PAHs (dominated by phenanthrene, pyrene and fluoranthene) and heavy metals (Zn, Cd, Cr and Pb), total invertebrate abundance and taxon richness were not significantly depressed. Lowered diversity and altered taxonomic composition (fewer pollution-sensitive taxa) were observed at 4 of the 7 downstream sites. Carr et al. (2000) assessed sediment chemistry, toxicity (solid phase and porewater), and benthic community structure for 36 sites in Corpus Christi Bay, Texas, U.S., of which 15 were near stormwater outfalls, 8 were in reference locations, and 13 were potentially exposed to other human stressors. The stormwater sites were heavily contaminated with PAHs and metals. Of the 5 most degraded sites (based on integrating contaminant, toxicity and benthic community data), 4 were near stormwater outfalls. However, not all sites near stormwater outfalls had disturbed benthic communities, and the benthic community descriptors (integrated by ordination) were not correlated to sediment contaminant levels.

Methodological Power, Natural Variability and Impact Detection

To account for apparent minor biological responses to wet-weather discharges, 2 possible situations are suggested: (1) impacts to the benthic community existed but were not detected by our assessment approach, or (2) impacts were nonexistent, or ecologically insignificant compared to effects of other factors influencing the communities.

Regarding the first situation, the conclusion of “minor impact” is limited by the spatial and temporal boundaries of the studies. Our methods were most suitable for detecting alterations that extend over the whole site (≤100 m in length) and persist for a significant fraction of the period between runoff events. Wet-weather discharge-related effects on benthic invertebrates that were limited to smaller spatial scales (or different seasons of the year) or specific microhabitats could escape detection, as could impacts from which recovery was rapid. However, it is arguable whether or not impacts at small spatial and temporal scales are ecologically significant.

Benthic invertebrate communities are influenced by a variety of natural abiotic and biotic factors (Power et al. 1988). Urban streams are typically affected by multiple
anthropogenic stressors. It is possible that natural and/or human-induced differences in these factors among streams and sites obscured detection of discharge-related effects. To minimize the “nuisance” variability, we narrowed the scope of the assessments to involve sites as similar as possible, except in exposure to runoff. As a possible consequence, discharge-related impacts were strongest in the field experiment, in which nuisance factors were the most controlled. However, it was recognized that some degree of difference in habitat conditions likely exists among sites in the field that will contribute to the residual variability of the observational units.

The statistical approach for establishing significance of outfall-related differences in benthic communities between sites depends on an assumption that each stormwater-exposed community would “respond” in a similar manner relative to its paired unexposed community, even if the habitats and faunal assemblages differed among outfall locations. If this assumption did not hold, real impacts varying in direction and magnitude would not be identified as recurrent effects.

Another assumption of the statistical approach was that the stormwater discharges represented the same type of stressor or “treatment” applied to the stream. This is unlikely to some degree. The relative importance of the various stressors presented through stormwater discharges (altered stream flow, sediment transport and deposition, and an influx of nutrients, metals and organic compounds) reflects the nature of the drainage basin, which certainly varies with location. Thus, different outfalls will not deliver the same type or amount of runoff, and thus could affect the receiving streams differently. Consequently, the benthic responses could differ and add to the “error” variance of the analyses for stormwater effects. Carr et al. (2000) also noted that the stormwater outfalls in their study were not equivalent treatments and, therefore, limited detection of recurrent responses by benthic communities.

The second explanation offered to account for the observed responses of benthic invertebrate communities to wet-weather runoff is that the physicochemical disturbances from the outfall discharges were weak or unimportant compared to the influence of other natural (habitat) or human factors. Spatial “control” for effects of reach- and larger-scale habitat factors was attempted by the sampling designs in 2 of the 3 studies but, as noted above, it is possible that unmeasured natural factors (including those arising from within the spatial scale separating the paired sites) could have influenced the benthic communities. A more important natural factor could be temporal variability in stream conditions, due to seasonal cycles or weather-related disturbance.

In temporally variable habitats, such as those subjected to frequent disturbance, taxa that are resistant and/or resilient should be favoured (Townsend and Hildrew 1994). Exposure of habitats to wet-weather flows should increase the frequency and magnitude of disturbance (Roesner and Bledsoe 2003). However, if the magnitude of natural disturbances (e.g., peak flows of stream water) is sufficient to select for tolerant and resilient benthic taxa throughout the stream, communities below outfalls may not significantly differ from those above.

A similar hypothesis could be offered involving human disturbances; i.e., that the background level of degradation in urbanized watersheds is high enough to result in resistant benthic communities in upstream as well as downstream locations. Walsh (2000) commented that benthic invertebrate communities in streams of southeastern Australian watersheds with impervious surfaces covering >25% of their areas were widely degraded, making detection of localized impacts impossible. Pitt (2003) cited other studies finding similar conditions in streams with the percent watershed area of impervious surfaces as low as 10%. Cumulative effects of other anthropogenic stressors could contribute to whole stream impacts. It is noteworthy that in most of our sites, few ephemeropterans, plecopterans and trichopterans were collected. These are common benthic invertebrates, but generally pollution-intolerant, especially to metals (Barbour et al. 1999). Their absence suggests poor environmental quality.

Responses of benthic invertebrates to conditions at other times of the year (instead of late summer and autumn) could differ from what we observed, due to seasonal changes in the receiving environment and the quantity and quality of urban runoff. Life-history patterns of aquatic invertebrates alter benthic communities in terms of organism abundances and physiological condition. In north temperate zones, annual cycles involve the recruitment of small, early life-history stages for many taxa in the spring, increase in size and abundance through to the autumn, and reductions in populations with late autumn senescence (Allan 1995). Small, young individuals can be more sensitive to contaminants than larger ones (McKimm 1985). The fewer individuals exposed to wet-weather discharges, the fewer adverse responses. Food supply and interactions with predators and competitors vary through the year, and could mediate runoff effects. Abiotic habitat conditions, such as water temperature and stream flow, also change seasonally. Tolerance to contaminants often increases at lower temperatures (Sprague 1985). Periods of high stream flow (e.g., spring melt water runoff, high rainfall seasons) might “dampen” the severity of effects from individual outfalls. Consequently, benthic communities could be more vulnerable during warmer times of summer and early autumn, when invertebrate densities are highest, compared to early and late in the ice-free part of the year.

Concentrations of pollutant constituents of urban runoff differ between warm and cold weather periods. In a study of baseflow, stormwater and snowmelt runoff from residential + commercial and industrial catchments
in the Humber River drainage basin (Pitt and McLean 1986 [cited in Burton and Pitt 2002]), chloride and other dissolved solids in the discharges (due to road salt applications) were substantially higher, and bacteria populations lower, during cold weather (December 15 to March 15) than during warm weather. Warm-weather stormwater runoff was a significant contributor to the total annual loading of particulate residue, phosphorus, phosphates, copper, lead and zinc from the industrial catchment. During winter, pollutants from urban areas can be stored in snowpacks on the catchment surface, and later released in highly concentrated pulses of meltwater (Marsalek et al. 2003). Various processes (e.g., freezing and thawing, infiltration to soil) affect pollutant movements, but soluble constituents are typically flushed out in the early stages of snowmelt; solids and associated hydrophobic substances, such as PAHs, are generally released towards the end or after final melt. Toxicity of urban meltwater has been observed (mainly due to chlorides and metals), and linked to adverse effects on benthic communities in some systems, particularly urban impoundments (Mayer et al. 1999; Marsalek et al. 2003). However, it is not clear whether benthic communities in streams would be more vulnerable to discharges during cold weather than during warm weather.

**Enhancing Assessment Power to Detect Impacts**

Key components of comprehensive assessments of effects of stormwater runoff on receiving waters recommended by Burton and Pitt (2002) include (i) sediment and water physicochemistry (including contaminant analyses), (ii) laboratory and in situ toxicity of water and sediment, (iii) benthic invertebrate and fish community structure, (iv) contaminant bioaccumulation in benthic species and fishes, and (v) water flow and physical habitat characterizations. With the exception of observations of fish conditions, our assessments have included all components. Among these response variables, greatest improvement in the sensitivity of bioassessments is likely to occur through more quantitative habitat observations at multiple spatial scales with repeated measurements to describe temporal variability (Roesner and Bledsoe 2003). These data should advance understanding of the interactions of hydrologic and geomorphic processes with stream biota.

More detailed characterization of stream bed exposures to wet-weather discharges would improve determination of relationships between stressors and the benthic response variables. This could involve delineations of runoff plume dilution, benthic drift or more detailed measurements of indicators of runoff exposure, such as changes in water flow, sediment and contaminant conditions. In particular, time series observations of in situ conditions before and after runoff events would enhance detection of short-term alterations.

Consideration should also be given to the cumulative effects of other wet-weather discharges, and other human stressors, in the watershed. It is possible that impacts of urban runoff are more apparent at the catchment level than at the pool-riffle sequence scale of our assessments. Walsh et al. (2001) concluded that catchment-scale variables that describe urban density and the form of urban drainage best explained degradation of benthic invertebrate communities in the Melbourne region of Australia. Expanding the spatial scale of the assessment to include whole drainage basins would provide a comprehensive examination of impacts due to wet-weather runoff, but at a cost of increased sampling effort and additional complications in accounting for effects of other human stressors and natural factors that are confounded with exposure to urban discharges.

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