Effects of an azinphos-methyl runoff event on macroinvertebrates in the Wilmot River, Prince Edward Island, Canada

Lisa A. Purcell,[1,2] Donna J. Giberson

Department of Biology, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island, Canada C1A 4P3

Abstract—High levels of azinphos-methyl (0.4–0.8 μg/L) were detected in the Wilmot River, Prince Edward Island, Canada, following runoff from an agricultural field after a heavy rainfall on 19 July 2002. Benthic macroinvertebrate abundance and diversity were sharply lower in samples collected 1 d after the event compared with samples collected in the same manner in July or October 2001. The greatest effects were noticed on the aquatic insects, whose abundance declined from >10 000 individuals per 3-min kick sample in July 2001 to <900 individuals per 3-min kick sample in July 2002. One family of Diptera, one family of Plecoptera, and three families of Trichoptera disappeared entirely from the study reach after the runoff event, and several other families were severely depleted in number. This led to low taxonomic similarity values between the communities before and after the runoff event and a change relative to reference streams on PEI. Examination of biological metrics (including indices such as % EPT (Ephemeroptera, Plecoptera, or Trichoptera), % chironomids, % burrowers, *etc.*) confirmed that aquatic insects were more heavily targeted by the insecticide than non-insect invertebrates. This resulted in a shift in the community towards non-insect taxa that were better able to avoid or tolerate this type of pollution.

Résumé—De fortes teneurs d'azinphos-méthyle (0,4–0,8 μg/L) ont été détectées dans la Wilmot, Île-du-Prince-Édouard, Canada, résultant du ruissellement d'un champ agricole après une forte pluie le 19 juillet 2002. L'abondance et la diversité des macroinvertébrés benthiques ont fortement diminué dans les échantillons récoltés 1 j après l'événement, par rapport à des échantillons recueillis de la même manière en juillet et en octobre 2001. Les effets les plus prononcés se sont produits chez les insectes aquatiques dont l'abondance a décliné de >10 000 individus par échantillonnage de 3 min au filet troubleau par coups de pied (« kick sample ») en juillet 2001 à <900 individus par échantillon de 3 min au filet troubleau en juillet 2002. Une famille de diptères, une de plécoptères et trois de trichoptères ont entièrement disparu de la zone d'étude après l'événement et les densités de plusieurs autres ont été considérablement réduites. Cela a eu comme conséquence de diminuer les valeurs de similarité taxonomique entre les communautés avant et après l'événement et de produire un changement de communauté par rapport aux cours d'eau témoins à l'IPE. L'analyse des métriques biologiques (en particulier des indices tels que le % d'EPT (éphéméroptères–plécoptères–trichoptères), le % de chironomidés, % de fouisseurs, *etc*.) confirme que les insectes aquatiques ont été plus fortement ciblés par l'insecticide que les autres invertébrés non insectes. Il en est résulté un glissement de la composition de la communauté en faveur des taxons autres que les insectes qui ont mieux réussi à éviter et à tolérer ce type de pollution.

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[1]Corresponding author (e-mail: lisa.purcell@mail.mcgill.ca).
[2]Present address: Institute of Parasitology, McGill University, 21 111 Lakeshore Road, Sainte-Anne-de-Bellevue, Quebec, Canada H9X 3V9.

Introduction

Streams on Prince Edward Island (PEI), an island province on the eastern coast of Canada, are small (mostly 1st to 3rd order) and typically have at least some agricultural activity in the watershed. Nearly half of the province's land mass of 5500 km^2 is used for agriculture (PEI Environment 2000), and ~40 fish kills documented since 1962 have been related to pesticide runoff from agricultural fields (Mutch *et al.* 2002). Over half of these kills occurred in the period 1994–2002, and 16 of these could be attributed to specific pesticides from adjacent agricultural (mostly potato) fields (Mutch *et al.* 2002). Ten of the 16 involved the pesticide azinphos-methyl (*O*,*O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorodithioate). Azinphos-methyl is an organophosphate insecticide used on a number of food crops, including potatoes, and its high water solubility (25.10 mg/L at 25 °C) means that it can enter watercourses in runoff after heavy rains (US EPA 1998).

Heavy rainfalls in July 2002 resulted in two runoff events (10 and 19 July) that carried soil and agricultural chemicals into two sites on the Wilmot River, apparently from adjacent potato fields. Both events resulted in fish kills: >4500 dead salmonids were recovered from the stream after the first runoff, and >6500 dead fish were recovered after the second runoff event (Gormley *et al.* 2005). Samples analyzed immediately after each event (within 24 h, for pesticides known to have been used in the watershed) revealed high levels of azinphos-methyl in stream water, in standing water beside the river, and in fish tissue. Streamwater concentrations were 0.8 μg/L for the 10 July event and 0.39–0.8 μg/L for the 19 July event (Gormley *et al.* 2005). An earlier study of runoff events in the Wilmot River (10, 24, and 27 August 2000) yielded azinphos-methyl levels ranging from non-detectable to 0.45 μg/L, with a mean of 0.27 μg/L (SE 0.1) (Bill Ernst, Environment Canada, Dartmouth, Nova Scotia, personal communication). Azinphos-methyl has been reported in ~25% of stream samples tested on PEI in previous pesticide monitoring, with median levels of 0.073 μg/L (Mutch *et al.* 2002). Both fish-kill events in July 2002 were subsequently attributed to azinphos-methyl runoff from potato fields adjacent to the stream.

The 19 July 2002 event affected a site that had been monitored for invertebrates during the summer and autumn of 2001 as part of a larger study of the relationship between stream macroinvertebrates and land use (Purcell 2003). This provided an opportunity to evaluate responses of aquatic insects and other macroinvertebrates to a confirmed pesticide runoff event. Although fish mortalities have been documented frequently in PEI streams, no previous attempts have been made to evaluate responses of benthic macroinvertebrates to runoff events, mainly because of the lack of baseline data for comparison. Azinphos-methyl is highly toxic to fish (Pesticide Action Network 2006) and has been implicated in >20% of all pesticide-related fish-kill events in the United States (US EPA 2001). Toxicity to invertebrates, however, is highly variable, both within and among major taxonomic groups (Wogram and Liess 2001). For example, effects (usually LC_{50}) have been reported at concentrations ranging from 0.26 to 10 000 μg/L for Mollusca (though few freshwater species have been evaluated) and from 1000 to 5000 μg/L for freshwater Oligochaeta (US EPA 2006). Even in groups such as the Ephemeroptera (mayflies), which are considered to be highly susceptible to azinphos-methyl (Pesticide Action Network 2006), effect concentrations in the ECOTOX database (US EPA 2006) range from 1.2 μg/L to 6990 μg/L, though data exist on very few species within the order. These disparate toxicity values make prediction of field effects on invertebrate populations difficult, but generally, non-insects such as Mollusca and Annelida are expected to be more tolerant to organic insecticides than arthropods (Wogram and Liess 2001). Within the Insecta, groups such as Coleoptera, Trichoptera, Diptera, and Hemiptera are usually reported to be more tolerant than Ephemeroptera and Plecoptera (Wogram and Liess 2001). These differences are believed to be due to a combination of feeding and respiratory differences among the taxonomic groups, which can affect the amount of toxicant coming into contact with the body surface of the invertebrate (Wogram and Liess 2001).

Macroinvertebrates are useful indicators of stress in streams because they are relatively sedentary (and so should remain in the location of potential stressors) and are a diverse group that shows a range of responses to different stressors (Rosenberg and Resh 1993). However, benthic macroinvertebrate samples can be time-consuming and difficult to process and identify, which has led to a paucity of benthic baseline studies relative to fish studies; therefore, data are rarely available for comparisons following a runoff event. The goal of this study was to evaluate the effects of the 19 July pesticide runoff event on the benthic macroinvertebrate community in one site on the Wilmot River by comparing post-runoff data with baseline information from the same site and with data from a suite of similar reference (low agriculture) streams throughout Prince Edward Island.

Methods

Study site

Wilmot River is located in central Prince Edward Island and is a 3rd-order stream (1 : 50 000 map scale) with an overall catchment area of >150 km^2. The study reach (46°23′35.8″N, 63°39′36.4″W) was located about halfway along the length of the river and drained a catchment area of 45.7 km^2. More than 80% of the land upstream of the study site is used for agriculture, and most of this land is in potato rotation, with approximately 35% of the area planted to potatoes in any given year. At the time of the study, only 9.4% of the watershed contained forest, most of which was found along the stream edges. This forest consisted mainly of white spruce (*Picea glauca* (Moench) Voss) and deciduous trees including maple (*Acer* L. spp.), birch (*Betula* L. spp.), willow (*Salix* L. spp.), and alder (*Alnus* L. spp.). Riparian zones along the river are poorly managed; at the time of the study, >60% of the stream possessed a forested buffer zone <10 m wide (calculated from digital land use data: PEI Environment 2000).

The study reach was approximately 1 km downstream of the documented point of entry of the azinphos-methyl runoff. It consisted of a low-gradient riffle, approximately 30 m long, with gravel/cobble substrate embedded with sand and silt. Approximately 53% of the substrate of the study reach was sand or silt, and the median particle size found in the reach was 0.1 mm. The stream banks were mainly sand and silt and had a 27% slope on one side and a 6% slope on the other.

Pre- and post-runoff Wilmot River samples were compared with each other and with samples from a suite of 26 similar-sized reference (low agriculture) streams that were monitored for a larger land use study (Purcell 2003). These were assessed from digital land use data (PEI Environment 2000) using MapInfo® v. 7 (MapInfo Canada, 26 Wellington Street East, Suite 500, Toronto, ON M5E 1S2). All reference streams were chosen so that (*i*) they were shaded by similar amounts and types of riparian forest regardless of land use, (*ii*) they had ≥60% of their watershed in forest, and (*iii*) ≥75% of their length had a forested buffer zone ≥10 m wide on each side of the stream. The reference stream sites were similar in physical characteristics (stream width approximately 5 m, average depth of flow 0.12 m, average watershed area >22 km^2) but differed slightly in watershed land use and nutrient composition. The Wilmot River, with <10% forest and <40% of the river with a 10 m wide buffer zone, had been designated as a "test" stream in this larger study to determine whether the stream was showing effects from the high level of agricultural land use in the watershed.

Benthic macroinvertebrate sampling and processing

Benthic macroinvertebrate samples were collected from the study riffle on 26 July and 29 October 2001, as part of the larger PEI land use study, and again on 20 July 2002, 1 day after the reported fish-kill event on 19 July. The invertebrates were sampled on all dates by taking a standard 3-min "travelling kick sample". This was done by walking the entire reach in a tight "zigzag" from one side of the stream to the other and disturbing substrate materials in front of a D-frame net (400 μm Nitex® mesh) at approximately 0.5 m intervals to dislodge invertebrates, which were then carried into the net by the current. Samples were emptied into a clear 1 L collecting jar, fixed in 4% buffered formalin, and transported to the laboratory for processing. Samples from 2001 (prior to the runoff event) were observed in the collecting jar prior to fixing, and invertebrates were seen to be very active in the jar, with no dead or immobile specimens noted. In contrast, the sample from 2002 (after the runoff event) had a number of specimens that were immobile and apparently dead. Therefore, the collecting jar was emptied into a white sorting pan to determine visually whether the invertebrates that were captured were alive or dead (based on body swelling and discolouration as well as immobility). Because intensive examination of dead *versus* live invertebrates was not made in 2001, all invertebrates (living or dead) were retained and the sample was fixed in formalin as above. All samples were washed and transferred to 70%

ethanol, then subsampled for counting and identification. Subsampling was carried out by emptying the sample into a sorting tray divided into 20 squares. The entire sample was checked for large (>2 cm) invertebrates to ensure that large and potentially rare taxa were included in the sample. Then 4 of the 20 squares were selected, based on randomly generated grid locations in the tray, and the material in each of these squares was removed and examined using a stereoscopic dissecting microscope. The number of squares to sort was determined by completely sorting and processing all 20 grid squares for samples from each of several streams (Purcell 2003) and then using a power analysis (Minitab® v. 12, Minitab Inc., Pine Hall Road, State College, Pennsylvania, USA) to assess the number of grid squares that needed to be sorted to provide a representative sample. Macroinvertebrates were identified using the keys in Merritt and Cummins (1996) and Thorpe and Covich (2001).

Water chemistry analysis

Water samples were collected prior to sampling for benthic macroinvertebrates in July and October 2001 and transported to the Prince Edward Island Government laboratory (Charlottetown) for analysis of standard water chemistry variables, including alkalinity, hardness, nitrates, and total phosphorus. Temperature, pH, conductivity, and dissolved oxygen were determined onsite using a Hanna 9812 pH/conductivity meter (Hanna Instruments, Tampa, Florida; supplied by VWR International, Mississauga, Ontario) and a YSI model 55 oxygen meter (Yellow Springs Instrument Co., Inc., Ohio; supplied by Hoskins Scientific, Burlington, Ontario). Pesticides were not analysed in 2002 pre-runoff samples, but detectable levels of atrazine (0.00136 μg/L), azinphos-methyl (0.27 μg/L), chlorothalonil (0.204 μg/L), dimethoate (0.0116 μg/L), mancozeb (3.107 μg/L), metalaxyl (0.0733 μg/L), and metribuzin (0.0045 μg/L) were detected in a preliminary study in the river over three sampling dates during August 2000 (Purcell 2003). Stream water was collected on 19 July 2002, the night that the fish kill was discovered by the PEI Division of Fish and Wildlife, and transported to the Environment Canada laboratory in Moncton, New Brunswick, for pesticide analysis. Levels of azinphos-methyl in field depressions reached 1225 μg/L, while in-stream concentrations ranged from 0.39 to 0.8 μg/L (Gormley *et al.* 2005).

Macroinvertebrate analysis

The Wilmot River post-runoff sample (20 July 2002) was compared with samples collected the previous summer (26 July 2001) and autumn (29 October 2001) to assess relative abundance and diversity of benthic macroinvertebrates. Wilmot River samples were also compared with samples from the suite of 26 similarly sized reference streams examined in the concurrent land use study (Purcell 2003), using the reference condition approach (Reynoldson *et al.* 1997). The basis of the reference condition approach is that even in fairly homogeneous regions such as PEI, no two streams have identical aquatic insect communities, but an average "reference condition" can be determined for an area by examining a large number of streams (Reynoldson *et al.* 1997). Communities from other streams can then be compared with those from the reference streams to see whether they deviate from the average conditions.

Taxonomic composition in the Wilmot River was compared between the different sampling periods (before and after the runoff event) by calculating the Bray–Curtis similarity index using a similarity percentages (SIMPER) analysis (PRIMER [Plymouth Routines in Multivariate Ecological Research], v. 5, Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth, UK). Wilmot River summer samples (2001 and 2002) were then compared with samples from the reference streams using (*i*) ordination (multidimensional scaling [MDS]; Reynoldson *et al.* 1997) and (*ii*) evaluation of a variety of biological metrics that have been shown in previous studies to be related to agricultural effects (Wallace 1996).

To evaluate potential differences among streams through MDS ordination, the original group of streams in the study (37 streams; Purcell 2003) was evaluated using a stepwise discriminant function analysis (DFA) (Tabachnik and Fidell 1996) to establish groups based on physical stream characteristics. Two groups of streams were identified (Purcell 2003), based mainly on stream size: "Group 1" consisted of small, 1st-order, spring-dominated streams, and "Group 2" consisted of 2nd- to 3rd-order streams with shallow gradients and higher temperatures. Wilmot River was compared with Group 2 reference streams, since it fell within that grouping in the DFA. Taxonomic composition patterns were then compared by generating ordination scores using non-metric MDS for the reference streams (summer and fall combined) and for the test stream (Wilmot River). Probability ellipses were generated using the distance measures generated in the MDS by plotting axis scatterplots using the BEnthic Assessment of SedimenT (BEAST) predictive model (Reynoldson and Wright 2000). These ellipses show the probability that points on the ordination plot (each representing a sample site) are similar to each other with respect to the benthic community. Test sites were considered "unstressed" or equivalent to the reference group if they were located within the innermost or 90% ellipse. A site was considered "potentially stressed" if it was located between the 90% and 99% ellipses, "stressed" if it was between the 99% and 99.9% ellipses, and "severely stressed" if it was outside the 99.9% ellipse (Reynoldson and Wright 2000). All analyses were carried out using SYSTAT® v. 8 (Systat Software Inc., Technology Drive, San Jose, California, USA).

Metrics were evaluated visually by calculating each metric, plotting the distribution and range of values for each metric onto box and whisker graphs, and then plotting additional points representing the values obtained for the Wilmot River in July 2001 (before the runoff event) and July 2002 (after the runoff event). The Wilmot River values were also compared with the probability distribution of the metric over the range of reference streams (following transformation to fit the assumption of normality, where applicable) to determine the probability that the value belonged to that distribution (STATISTICA® v. 6, StatSoft Inc., East 14th Street, Tulsa, Oklahoma, USA). The metrics evaluated included density (number of individuals captured during a 3-min kick sample), taxonomic richness (number of families), % Chironomidae (proportion of benthic fauna made up by Diptera: Chironomidae), % EPT (proportion of fauna composed of Ephemeroptera, Plecoptera, or Trichoptera), % Baetidae in Ephemeroptera (proportion of Ephemeroptera made up by the family Baetidae), % Coleoptera, % burrowers, % Oligochaeta, and % scrapers (proportion of fauna that feed by scraping biofilm from rocks).

Results

For the sampling periods in 2001, the Wilmot River water was slightly alkaline (pH values ranged between 7.3 and 8 during 2001), with alkalinity readings close to 100 mg/L $CaCO_3$ and hardness values of 120 mg/L $CaCO_3$. Specific conductance readings ranged from 100 to 170 μS/cm. Nutrient concentrations were relatively high: nitrate (NO_3-N) concentrations were 6.7–7.5 mg/L and total phosphorus concentrations were 0.042 mg/L. Water temperatures ranged from 12 to 15 °C over several visits to the stream, with a maximum of 18.6 °C. The dissolved oxygen level of stream water ranged between 10 and 12 mg/L. No water samples were analysed in 2002.

The benthic community in the sample taken after the pesticide runoff event differed dramatically from that in the same period in the previous year in virtually every measured variable. The most obvious changes were differences in density and taxonomic diversity. Over 11 000 invertebrates were collected in the 3-min kick sample in July 2001, and numbers climbed to >24 000 in October 2001, but only about 1500 invertebrates were collected in the sample after the runoff event (Table 1, Fig. 1*a*). Visual examination of invertebrates in the collecting jars showed vigorous movement by the invertebrates in 2001 but little or no invertebrate activity in 2002. Subsequent intensive examination of the 2002 sample in the field confirmed that approximately one-half of the macroinvertebrates in that sample were dead (many swollen and blackened, others immobile), and the swollen and discoloured specimens were also obvious in preserved samples. No specimens that showed similar swellings or discolouration were noted in preserved samples from 2001. In 2001, the Wilmot River summer sample (Fig. 2*a*, open circle) had higher invertebrate abundance (probability of belonging to specified distribution, $P = 0.045$) than the reference streams sampled during the same period, but after the 2002 runoff event (Fig. 2*a*, closed circle), the abundance was lower (probability of belonging to specified distribution, $P = 0.001$) than that found in the reference streams.

Taxonomic patterns also changed in Wilmot River between 2001 and 2002. The taxonomic richness declined between 2001 and 2002, though not to the same extent as abundance, falling from 19 taxa in July 2001 to 15 in July 2002 (Fig. 1*b*). Although summer and fall samples collected in 2001 had a number of taxa in common (SIMPER analysis: 58% similarity; Table 1), the summer 2002 (post-runoff) sample showed low similarity to both

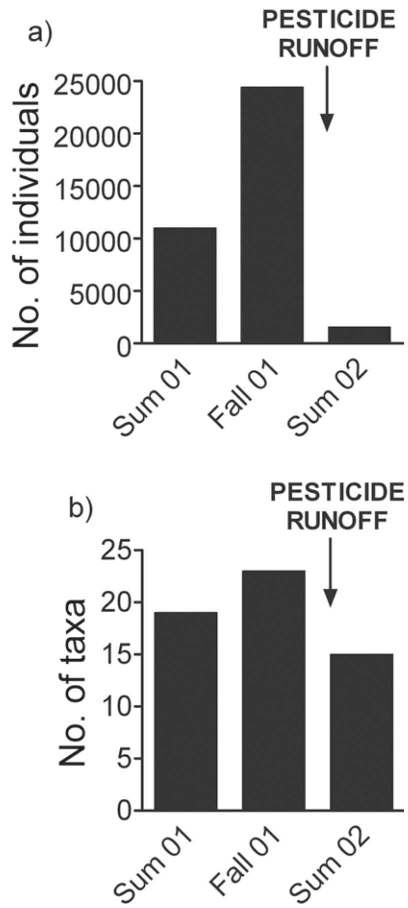
Table 1. Number of individuals within each taxon collected in the Wilmot River, at the same study site, in the summer and fall of 2001 and after a pesticide runoff event in the summer of 2002.

Taxon	Summer 2001	Fall 2001	Summer 2002
Nematoda	0	0	10
Annelida: Oligochaeta	290	656	290
Annelida: Hirudinea	20	5	0
Mollusca: Sphaeriidae	395	745	405
Arthropoda: Crustacea: Ostracoda	5	55	30
Arthropoda: Arachnida: Hydracarina	65	120	5
Arthropoda: Insecta			
Diptera			
Tipulidae	300	0	20
Athericidae	5	280	0
Simuliidae	250	210	10
Chironomidae	6 575	10 660	700
Ceratopogonidae	150	160	5
Coleoptera			
Elmidae	715	890	40
Dytiscidae	30	15	5
Ephemeroptera			
Baetidae	1 980	1 645	20
Ephemerellidae	85	7 490	10
Heptageniidae	0	30	0
Leptophlebiidae	0	120	0
Plecoptera			
Nemouridae	20	0	0
Perlodidae	0	220	0
Trichoptera			
Glossosomatidae	0	5	0
Hydroptilidae	0	5	0
Hydropsychidae	55	555	0
Limnephilidae	45	60	10
Polycentropodidae	0	5	0
Rhyacophilidae	20	40	0
Lepidostomatidae	5	0	0
Brachycentridae	0	460	0
Total	11 010	24 431	1 555
	Similarity*		
Summer 2001 to summer 2002	24.11%		
Summer 2001 to fall 2001	58.01%		
Fall 2001 to summer 2002	11.77%		

*Similarity percentages (SIMPER; PRIMER v. 5) were calculated for the differences in the abundance of fauna between each of the samples.

the summer 2001 (<25%) and fall 2001 (<12%) samples in terms of taxonomic composition (Table 1). The differences between the pre- and post-runoff summer samples were caused mainly by large declines in aquatic insects (from >10 000 individuals to <900), especially Chironomidae (Diptera), Baetidae (Ephemeroptera), and Elmidae (Coleoptera) (Table 1). In contrast, numbers of non-insects remained nearly constant between the two sampling periods (740–770 individuals; Table 1). When taxonomic richness was compared, Wilmot River was similar to the reference streams prior to the runoff event

Fig. 1. Comparison of (*a*) abundance and (*b*) taxa richness (based on Table 1) for benthic macroinvertebrate samples taken from Wilmot River on 26 July 2001 (Sum 01), 29 October 2001 (Fall 01), and 20 July 2002 (Sum 02). The timing of the pesticide runoff event (1 d prior to the 2002 sample) is shown by the arrow.



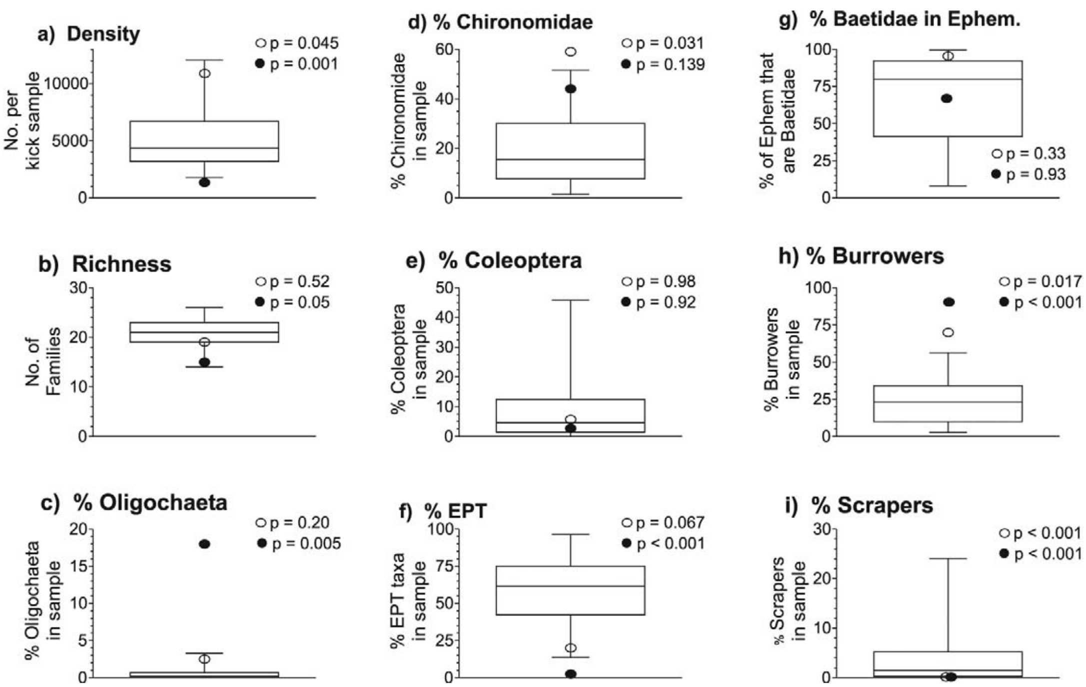
(Fig. 2*b*, open circle, $P = 0.52$) and was at the low end of the range after the runoff event, though not significantly different (Fig. 2*b*, closed circle, $P = 0.05$). Total community patterns (abundance and composition) were compared among streams by evaluating the MDS plots for the three major axes from the ordination (Fig. 3); these indicate that Wilmot River, both before and after the runoff event, showed evidence of being "potentially stressed", or only slightly different from the average condition for streams of similar size and character. However, the points for the Wilmot River July 2001 and 2002 samples are located in opposite quadrants of the graph, indicating a radical change in overall composition of the community between the two sampling periods.

Differences between pre- and post-runoff samples were also seen by comparing values for the Wilmot River with ranges for the reference streams for the various biomonitoring metrics (Fig. 2). The most prominent differences were reductions in the EPT group (Ephemeroptera, Plecoptera, Trichoptera; Fig. 2*f*) and increases in the proportion of the fauna made up by oligochaete worms (Fig. 2*c*) and overall burrowing taxa (including oligochaetes, sphaerid clams, and some Diptera; Fig. 2*h*). Values for both the % EPT and % Oligochaeta metrics did not differ significantly between Wilmot River and the reference streams prior to the runoff event, but significant differences ($P < 0.001$ and $P = 0.005$, respectively) were seen after the event. The proportion of burrowing taxa (indicative of fine substrates) was higher in Wilmot River than in the reference streams during both study periods. Another important pattern was a decline in the proportion of the Ephemeroptera composed of the relatively nutrient-tolerant Baetidae (Fig. 2*g*).

Discussion

Although streams in Prince Edward Island (PEI) have experienced a number of pesticide-related fish kills in recent decades (Mutch *et al.* 2002), information has not been available on pesticide-related effects on the benthic invertebrate community in PEI. Insecticides can cause catastrophic mortality of stream insects (Whiles and Wallace 1995), and azinphos-methyl is toxic to both fish and stream invertebrates (Kidd and James 1991; Pesticide Action Network 2006; US EPA 2006; US National Library of Medicine 2006). Dominant salmonids in the Wilmot River are brook trout (*Salvelinus fontinalis* (Mitchill)) and rainbow trout (*Oncorhynchus mykiss* (Walbaum)) (Gormley *et al.* 2005). Kidd and James (1991) reported the 96 h LC_{50} of azinphos-methyl for rainbow trout to be 3 µg/L, though levels as high as 28 µg/L have been reported for different series of 96 h LC_{50} studies (US EPA 2006). Brook trout are more susceptible to azinphos-methyl than rainbow trout, with recorded 96 h LC_{50} values of 1.2 µg/L (US EPA 2006). In contrast, aquatic invertebrates show highly variable responses to azinphos-methyl exposure, even within taxonomic groups. For example, the ECOTOX and

Fig. 2. Summary of biological metric results for each Wilmot River summer sample (○, summer 2001; ●, summer 2002) and the group of PEI reference streams. Reference stream results are shown as boxplots with the boxes representing the quartile values for the 26 streams, the horizontal lines within the boxes representing the median values, and the total range shown by the upper and lower horizontal lines. Reported *P* values refer to the probability that the Wilmot River value falls within the frequency distribution of the reference streams.



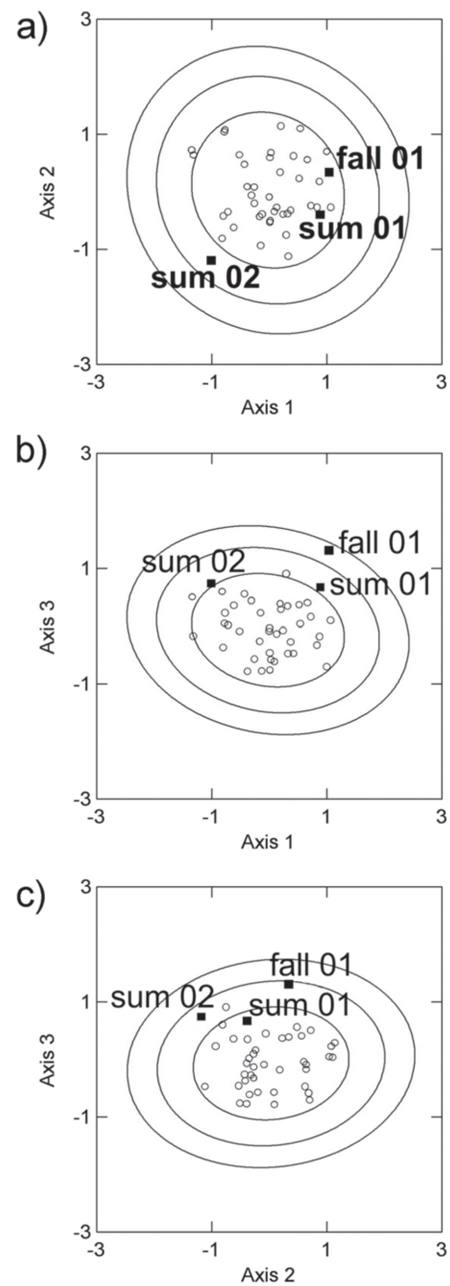
Hazardous Substances databases (US EPA 2006; US National Library of Medicine 2006) list LC_{50} values of 6.4, 14, and 6990 µg/L for ephemerellid mayflies, and 7.4 and 6990 µg/L for hydropsychid caddisflies, all higher than the concentrations of 0.4–0.8 µg/L recorded in this study the day after the runoff event. However, azinphos-methyl concentrations in standing water beside the river were much higher (up to 1255 µg/L), suggesting that levels during the actual runoff event could have been responsible for the observed effects.

The observation of dead invertebrates following the runoff event was critical in interpreting the results of this study. Aquatic invertebrate responses to insecticides are difficult to document in nature owing to the presence of other stressors that can cause site-specific declines in abundance. For example, flooding associated with high rainfall and runoff events can disrupt the streambed, also leading to catastrophic declines in macroinvertebrate numbers (Lancaster and Hildrew 1993; Giberson and Cobb 1995). In this study, direct observations of invertebrate and fish mortality, and recorded levels of azinphos-methyl known to cause mortality in salmonids and aquatic invertebrates, indicate that the observed declines were due to toxicity from the runoff.

Evaluation of the Wilmot River event in the context of reference streams allowed us to evaluate the pesticide effect in a more comprehensive manner than might have been possible without the reference stream data. Prior to the runoff event, Wilmot River was assessed as "nutrient enriched" compared with 26 similarly sized reference streams in PEI (Purcell 2003). Nutrients (nitrates, phosphorus) were higher in the Wilmot River than in the reference streams, and invertebrate abundance was significantly higher in both seasons, reflecting a response to the increased nutrients (Purcell 2003). The main differences in taxonomic composition patterns between the Wilmot River (before the pesticide event) and the reference streams were increased proportions of a few taxa known to be tolerant of siltation and organic pollution (Chironomidae, Baetidae, burrowing taxa;

Fig. 2). The runoff-related decline in overall abundance is dramatic when comparing Wilmot River pre- and post-runoff samples but is also evident when placed in the context of the reference streams. The most dramatic declines were noted in the Ephemeroptera, Plecoptera, and Trichoptera (EPT group), and some numerically important families in these orders were nearly or completely eliminated from the stream site. These orders are known to be sensitive to chemical-induced disturbances (Wogram and Liess 2001), and Wallace (1996) suggested that the EPT richness was the single most reliable metric for detecting pesticide effects. However, the Chironomidae, which are usually (as a group) considered to be tolerant to pollutants (van den Brink *et al.* 1996), also declined, indicating a general effect on all the insects, as might be expected with a broad-spectrum insecticide such as azinphos-methyl. In contrast, several of the non-insect taxa, such as oligochaete worms and sphaerid clams, showed virtually no change from one year to the next. The few oligochaete worms that have been tested have shown relatively high tolerance to azinphos-methyl exposure (1000–5000 μg/L; US EPA 2006), so this lack of any change might be due to simple tolerance to the insecticide. Molluscs are also reported to be tolerant to azinphos-methyl, but care should be taken in extrapolating to the group as a whole, since studies have focussed on large marine forms such as oysters and clams (LC_{50} up to 10 000 μg/L; US EPA 2006), and the few studies of small freshwater forms have shown much lower tolerance (*e.g.*, LC_{50} of 0.02 μg/L for a freshwater snail; US EPA 2006). Although azinphos-methyl toxicity to sphaerids has not been assessed, these organisms show variable toxicity to other organic pesticides. In one study of long-term organic pesticide effects on stream invertebrates, one genus of sphaerids persisted in orchard ditches that received multiple pesticide runoffs, whereas another disappeared entirely (Heckman 1981). The response of the non-insect groups may also be related to behaviour, specifically their habit of burrowing into the substrate. Since stream water during floods flows above the substrate with little mixing with the water in the substrate interstices (Giberson and Cobb 1995), burrowing into the substrate could provide shelter from full pesticide contact (Wallace and Hynes 1975; Sibley and Kaushik 1991; van den Brink *et al.* 1996).

Fig. 3. Three-dimensional assessment (MDS ordination) of the Wilmot River samples relative to the summer and fall samples of 26 similarly sized reference streams in PEI. Probability ellipses (from the inner to the outer circle) indicate the 90%, 99%, and 99.9% probabilities of a point falling within the average reference condition, corresponding to predictions of unstressed, potentially stressed, and stressed sites. ■, Wilmot River samples: sum 01, 26 July 2001; fall 01, 29 October 2001; and sum 02, 20 July 2002. ○, reference samples.



Pesticides have well-documented negative effects on stream invertebrates (Muirhead-Thomson 1987; Whiles and Wallace 1995), but pesticide effects can be difficult to document in field studies because of a lack of baseline data, confounding factors such as flood disturbance and invertebrate behaviour, difficulty in measuring pesticide concentrations, and the rapid breakdown or carry of the pesticide from the stream. In this study we were able to compare invertebrate abundance and taxonomic composition before and after a documented pesticide runoff event and assess patterns by comparing the affected stream with reference streams influenced only weakly or moderately by agriculture. MDS ordination graphs (reference condition approach; Reynoldson *et al.* 1997) were not particularly useful in detecting the stress caused by the runoff event, since the Wilmot River samples fell only in the "potentially stressed" category relative to reference streams. However, several metrics were useful, especially the density, EPT, and Oligochaeta metrics, in showing differences between the pesticide-stressed stream and the reference streams.

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