Impact of Methoxychlor on Selected Nontarget Organisms in a Riffle of the Souris River, Manitoba

Robert J. Sebastien[1] and Reinhart A. Brust

Department of Entomology, University of Manitoba, Winnipeg, Man. R3T 2N2

David M. Rosenberg

Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, Man. R3T 2N6

Sebastien, R. J., R. A. Brust, and D. M. Rosenberg. 1989. Impact of methoxychlor on selected nontarget organisms in a riffle of the Souris River, Manitoba. Can. J. Fish. Aquat. Sci. 46: 1047–1061.

The insecticide methoxychlor was applied at 300 μg·L^{-1} for 15 min to a riffle on the Souris River, located about 18 km downstream from Souris, Manitoba. Physical, chemical, and biological variables were measured and aquatic insect community structure was monitored using drift, emergence trap, and artificial substrate samplers. All taxa monitored, irrespective of functional feeding group, drifted catastrophically for 4–24 h immediately following methoxychlor addition. Different species demonstrated varying abilities to recolonize artificial substrates following treatment. Species having a high propensity to drift naturally, recolonized most rapidly. Taxa that required the longest period to recolonize following methoxychlor treatment were generally univoltine, had a low propensity to drift, and a limited ability to disperse as adults. Impact of methoxychlor was influenced by the prevalent life-cycle stage of some species at the time of treatment. *Catostomus commersoni* fry and juvenile *Orconectes virilis* were more sensitive to methoxychlor than previous research on mature individuals has indicated. Invertebrate drift appeared to be more sensitive to pesticide treatment than benthic invertebrates on artificial substrates. Species richness and total numbers of drift were significantly reduced for at least 33 d following treatment, whereas richness and numbers on artificial substrates were significantly lower for only 4 and 8 d, respectively.

On a traité un seuil de la rivière Souris, situé à environ 18 km en aval de la ville de Souris au Manitoba, avec un insecticide, le méthoxychlore, à une dose de 300 μg·L^{-1} pendant 15 min. On a mesuré des variables physiques, chimiques et biologiques et on surveillé la structure des populations d'insectes au moyen des techniques suivantes : dérive, piège à émergence et dispositifs d'échantillonnage sur substrat artificiel. Tous les taxons surveillés, sans tenir compte du groupe d'alimentation fonctionnelle, ont dérivé de façon catastrophique pendant 4–24 h, immédiatement après l'addition de métoxychlore. Différentes espèces ont présenté des aptitudes diverses à recoloniser les substrats artificiels à la suite du traitement. La recolonisation a été très rapide chez les espèces ayant une nette tendance naturelle à dériver. Les taxons chez lesquels la recolonisation demande la plus longue période à la suite d'un traitement au métoxychlore étaient généralement des taxons univoltins ayant une faible tendance à dériver naturellement et peu d'aptitude à se disperser à l'état adulte. L'impact du traitement au méthoxychlore a été influencé par le stade dominant du cycle biologique de certaines espèces au moment du traitement. Le frai de *Catastomus commersoni* (Lacépède) et les jeunes *Orconectes virilis* (Hagen) se sont montrés plus sensibles au méthoxychlore que les recherches précédentes sur les individus adultes ne l'avaient indiqué. La dérive des invertébrés a semblé plus sensible aux répercussions du traitement au pesticide, comparativement aux invertébrés benthiques vivant sur des substrats artificiels. On a constaté une diminution significative de la richesse des espèces et du nombre d'organismes ayant dérivé pendant au moins 33 d après le traitement; toutefois cette diminution n'a duré que 4 et 8 jours en ce qui concerne la richesse des espèces et le nombre total d'organismes vivants sur des substrats artificiels, respectivement.

Received December 29, 1987
Accepted January 17, 1989
(J9530)

Reçu le 29 décembre 1987
Accepté le 17 janvier 1989

Blood-sucking black flies are the most serious insect pests affecting both humans and livestock in some regions of western Canada. The most spectacular outbreaks affecting livestock are those of *Simulium arcticum* Malloch from the Saskatchewan and Athabasca rivers. For example, between 1944 and 1948, more than 1300 cattle were killed by *S. arcticum* in Saskatchewan (Fredeen 1977). Black flies landing and crawling about the head, face, and other parts of the body may be unbearable for people engaged in outdoor activities.

[1]Present address: Environment Canada, Pesticides Division, Commercial Chemicals Branch, Ottawa, Ont. K1A 0H3.

In the past, chemicals have been the only significant means by which black flies have been controlled. The earliest toxic chemical used extensively for control was DDT because of its low cost, ease of application, supposed greater selectivity to black fly larvae than to other members of the stream fauna, and its outstanding transport downstream. However, with the discovery of significant effects on nontarget organisms, long-term stability in the environment and biomagnification in the food chain, DDT was banned in 1969 for general use in Canada.

Methoxychlor (2,2-bis-(p-methoxyphenyl)-1,1,1-trichloroethane) was among the first chemicals to be tested in the field as a possible replacement for DDT. Over the years, methoxychlor has been the most extensively used chemical in Canada for the control of black files, and it is presently registered for the control of *S. arcticum* and *S. luggeri* Nicholson and Mickel in Saskatchewan and Alberta at a rate of 300 $\mu g \cdot L^{-1}$ for 7.5–15 min. Methoxychlor has also been used to control black flies in northeastern North America (Jamnback 1969; Burdick et al. 1974), and in the Humboldt River in northern Nevada (French et al. 1986).

The numerous conflicting Canadian studies regarding the effects of methoxychlor treatments on nontarget macroinvertebrates (Fredeen 1974, 1975, 1983; Wallace and Hynes 1975; Flannagan et al. 1979, 1980; Depner et al. 1980; Haufe et al. 1980) are usually difficult to interpret because of experimental design (e.g. lack of suitable control stations, inconsistency among habitats sampled, little or no collection of pretreatment data, insufficient replication, improper statistical analyses, identification of invertebrates to generic level or higher; National Research Council of Canada 1983). The objectives of the present study were: (1) to determine what taxa were most adversely affected by methoxychlor addition (300 $\mu g \cdot L^{-1}$ per 15 min) to a riffle of the Souris River; (2) to determine the recovery time for populations and the community of nontarget invertebrates in the riffle; and (3) to determine the relative importance of invertebrate drift from untreated areas upstream in recolonizing the treated riffle.

Methods

Study Sites

The Souris River enters Manitoba from the United States 6.58 km southwest of Lyleton, Manitoba (101°15′W, 49°00′N). It is characterized by a fairly slow current, a high concentration of suspended solids, and little emergent vegetation. Temperatures rise dramatically during the summer, often exceeding 26°C during mid-July. Depth is quite variable (30–400 cm; $\bar{x}$ = 80–100 cm), depending on location and time of year. Dams at Melita, Hartney, Souris, and Wawanesa, Manitoba regulate water flow, except in spring when severe flooding can occur. Discharge varies greatly at different localities during the course of the year (e.g. at Souris, Manitoba in 1976: 275 $m^3 \cdot s^{-1}$ in May; 0.50 $m^3 \cdot s^{-1}$ in October) and from year to year (Westwood and Brust 1981).

The "control" and "treatment" riffle sites selected were located about 18 km downstream from the town of Souris (Fig. 1). Both sites were shallow (mean depth 50 cm) and their substrates consisted of cobble-sized stones and large boulders. The control site was 34–43 m wide × 65 m long, whereas the treatment site, located ≈900 m downstream, was 32–45 m wide × 140 m long. Preliminary sampling indicated a close similarity in the species of aquatic invertebrates inhabiting both sites.

Methoxychlor Addition

An emulsifiable concentrate formulation of methoxychlor (240 $g \cdot L^{-1}$ active ingredient) was applied to the upstream end of the treatment riffle (Fig. 2) on 15 July 1982 at 1200 h. Four litres of methoxychlor (amount calculated to give a theoretical dosage of 300 $\mu g \cdot L^{-1}$ for 15 min, based on river discharge) were thoroughly mixed with 92 L of river water that had been filtered through a fine mesh screen into a 200-L drum. The chemical mixture was pumped through a 20-m length of garden hose that was divided into two additional 10-m long sections. Each 10-m portion was submerged and moved back and forth across half the width of the river just upstream of the treatment riffle to obtain even application of the chemical. The method simulated point-source application in a control program.

Measurements of Water Chemistry and Discharge

Water samples for chemical analysis were collected on 8, 15, 22, 29 July, 17 August, and 16 September 1982. Analysis

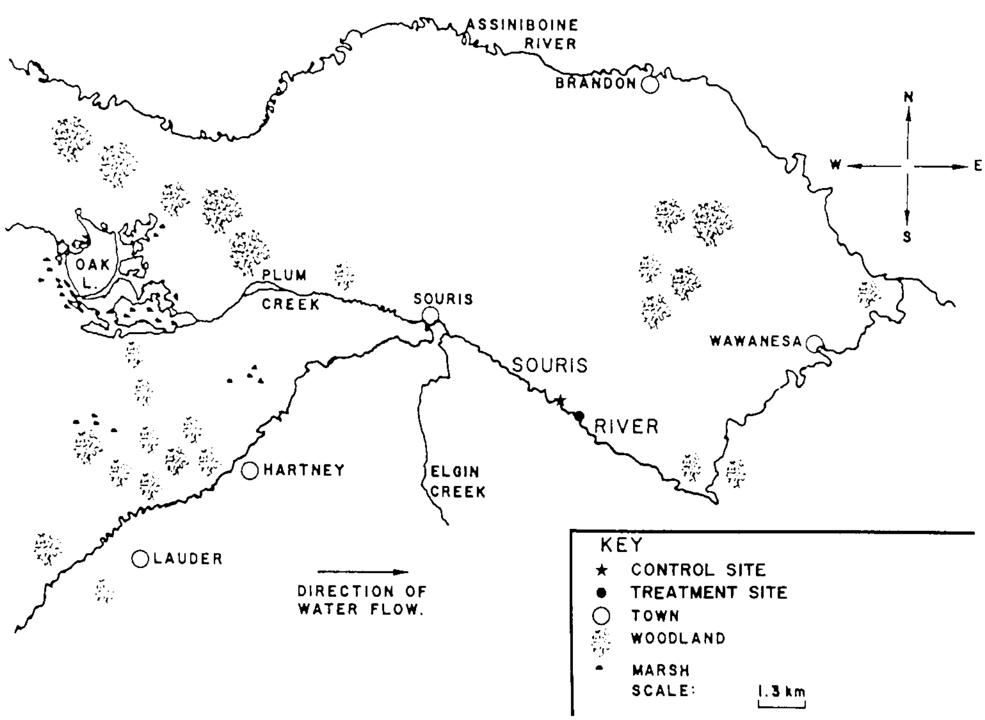

FIG. 1. Study area showing locations of the control and treatment sites on the Souris River, Manitoba.

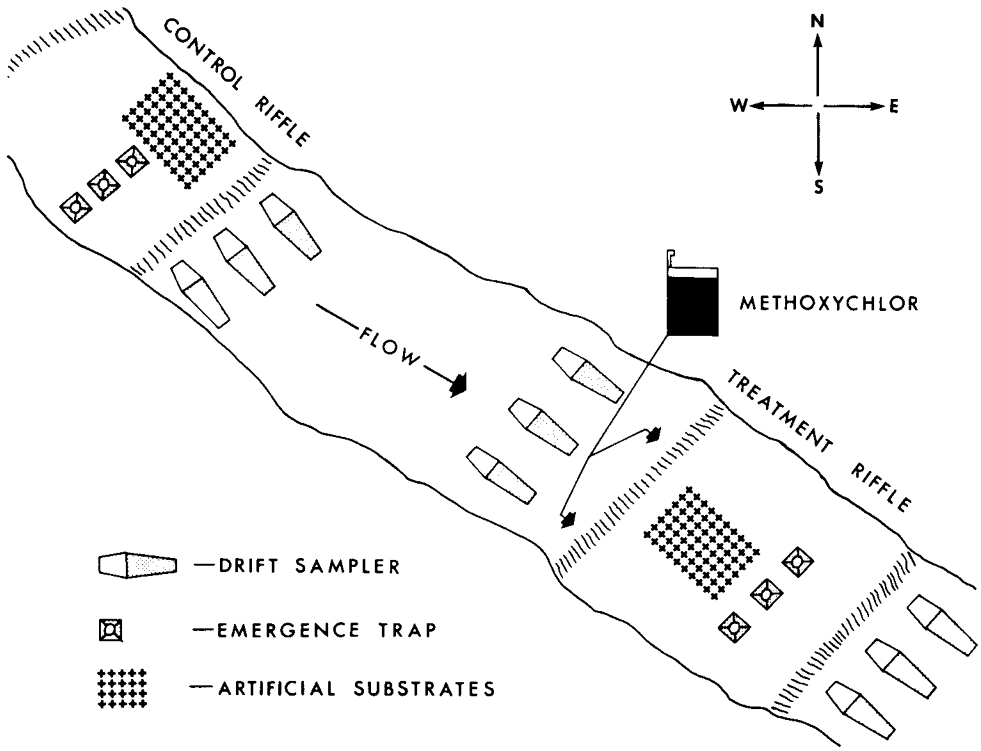

FIG. 2. Arrangement of sampling devices used at the control and treatment sites. Distance from the upstream end of the control riffle to the downstream end of the treatment riffle is ≃900 m.

included pH, total suspended solids (TSS), particulate carbon (PC) and particulate nitrogen (PN). Approximately 900 mL of water were collected in glass jars from the centre of control and treatment sites on each sampling date. Immediately after collection, 1 mL of saturated $HgCl_2$ solution was added to each jar to prevent microbial degradation. The jars were then placed in an ice cooler for transportation to the laboratory, and were stored at 5°C until analyzed. Analyses were performed by the Analytical Chemistry Unit, Water Chemistry Laboratory at the Freshwater Institute, Winnipeg (Stainton et al. 1977). In addition, water temperatures (degrees celsius) were recorded at midday at both sites throughout the study.

Daily discharge measurements (cubic metres per second) for 1982 and 1983 were obtained for the Souris River at Souris, Manitoba, from Manitoba Department of Mines, Resources, and Environmental Management (1982, 1983). Discharge was measured at the site of methoxychlor addition on 14 July 1982 in order to calculate the amount of chemical required to achieve the desired dosage.

Analysis of Methoxychlor Residues in River Water

Water samples for analysis of methoxychlor residues were collected in 1000-mL amber glass jars. Three 900-mL water samples were taken at both the control and treatment sites at each sample period from a transect across each riffle, one from the centre, and one from each side. CH_2Cl_2 (50 mL) was added immediately to each sample jar to ensure complete extraction of methoxychlor from the water sample. The jar was shaken repeatedly and then placed on a magnetic stirrer for 5 min to allow complete mixing of the solvent with the water. Samples were placed in an ice cooler for transportation to the laboratory, and were stored at 5°C until analyzed.

The control and treatment sites were sampled at 24 h before treatment and 10 min, 1, 3, 6, 12, 24, and 48 h after treatment. Three additional samples were taken at each of the 10-min and 1-h posttreatment sampling periods to which sodium azide was added to suppress microbial degradation. Since samples were not analyzed immediately, this provided a check to determine if microbial degradation exerted a significant effect on the concentration of methoxychlor in the samples.

In the laboratory, extraction, clean up, and injection of samples into the gas chromatograph (Tracor Model 565) for residue analysis essentially followed procedures described by Muir and Yarechewski (1984). The minimum detection limit was 0.01 $\mu g \cdot L^{-1}$.

Sampling of Fauna

Three biological variables were monitored: drift, benthos, and emergence. Drift and benthos provided information on recolonization and recovery after methoxychlor treatment. Emergence allowed confirmation of species-level identifications of immatures, and provided the life cycle information necessary to interpret methoxychlor effects on the fauna.

The taxa of interest to this study included all species of Ephemeroptera, Plecoptera, and Trichoptera, the amphipod *Hyalella azteca* (Saussure), the crayfish *Orconectes virilis* (Hagen), and white sucker fry (*Catostomus commersoni* Lacepede). Chironomidae (Diptera) were omitted because of limitations in taxonomy, time, and manpower necessary for species-level identification. All *Baetis* immatures were designated "*Baetis* spp." because most specimens were early instars or had been damaged, and thus could not be identified to species.

Drift

Modified Burton–Flannagan bomb samplers (Burton and Flannagan 1976; Flannagan et al. 1979) with 500-μm Nitex nets were used to collect drift. Three sets of three drift samplers were positioned in the river (Fig. 2). The "control" set was placed immediately downstream of the control site and measured drift leaving this riffle. The "treatment-inlet" set was placed just upstream of the methoxychlor addition. It sampled drift entering the treatment riffle and provided a measure of recolonization potential. The "treatment-outlet" set was placed immediately downstream of the treatment site, and measured drift leaving the treatment riffle. The three drift samplers at each site were positioned at mid-depth in the water column, evenly spaced across the river.

Drift was sampled prior to (7 and 14 July 1982), during (15 July 1982), and after (21, 29 July and 17 August 1982; 3 August 1983) treatment. On each sampling date, drift samples were collected at 4-h intervals beginning at 0800 h and continuing for 24 h. Additional samples were taken at 1300 and 1400 h on the treatment date at the treatment-outlet site in order to prevent clogging of nets by large numbers of invertebrates induced to drift by the methoxychlor treatment (1200 h). Samples were immediately preserved in 10% formalin. Rose Bengal stain was added to each sample to aid sorting efficiency.

Water velocities were measured directly upstream of the opening of each drift sampler, using a Gurley Model 622 current meter, at the beginning and end of each 24-h sampling period, and mean water velocities were calculated for the sampling period. Drift rates (No./4-h period) were standardized to drift densities (No./100 m^3), using mean water velocity, to allow comparisons between samplers and sites (Elliott 1970). Total drift density for individual taxa for each 24-h sample period was obtained by summing drift densities for the six intervals on each sampling date. Times of sunrise and sunset were also recorded for each drift sampling date.

Drift samples were quantitatively subsampled in the laboratory using the technique of Sebastien et al. (1988). However, subsampling of two invertebrate species (*Acroneuria lycorias* (Newman) and *Orconectes virilis*), and one vertebrate (*Catostomus commersoni*), was not necessary due to their low numbers and large sizes.

Benthos

Coarse substrate in the Souris River experimental sites prohibited effective quantitative sampling of the benthos, so artificial substrates (concrete paving bricks (Trieste design), surface area ≈840 cm^2, made by Genstar Materials Ltd., Manitoba Region) were used. A 15 × 10 grid of substrates was placed in the areas of greatest flow in both the control and treatment sites on 8 and 9 June 1982 (Fig. 2), which allowed 4 wk for invertebrate colonization prior to the first sampling date. Ten bricks were removed, downstream-most row first, from each of the three sites on each sampling date. A 500-μm Nitex dip net was held immediately downstream of each brick, the brick was rapidly lifted and placed in a 12-L plastic pail. Any invertebrates found in the net were added to the pail. The samples were preserved in 75% ethyl alcohol and returned to the laboratory for sorting and identification.

Artificial substrates were sampled prior to (7, 12, and 14 July 1982), and up to a year following treatment (16, 19, 23, 29 July, 16 August, and 17 September 1982; 18 July and 3 August 1983) to assess recolonization and recovery of the benthos (National Research Council of Canada 1983). Artificial substrates sampled in 1983 were installed on 21 June 1983.

Insect emergence

Three pyramidal emergence traps (Westwood and Brust 1981; Sebastien 1986) were placed in each of the control and treatment riffles (Fig. 2), and were emptied after a 48-h collecting period at various dates before (8 and 14 July 1982) and up to 1 mo following methoxychlor addition (17, 19, 22, 28 July, and 18 August 1982). Adult insects were aspirated from the collection jar and the inside of the trap after slowly raising the trap above the water. Specimens were preserved in 75% ethyl alcohol for later identification.

Statistical Analysis

Numbers

The effects of methoxychlor on macroinvertebrate drift densities, numbers on artificial substrates, and numbers emerging, for individual taxa and totals of taxa, were compared between control and treatment sites using two-way analysis of variance (ANOVA) (Snedecor and Cochran 1981). The mean square for the time and site (control, treatment) interaction was used as an error term to test for treatment effects (Green 1979). A $\ln(x+1)$ transformation, as indicated by Taylor's power law (Taylor 1961), was used to remove the dependence of the variance on the mean observed for individual taxa in drift, artificial substrate, and emergence samples.

The two-way ANOVA's used a repeated measures design with two treatments (control, treatment) and no replication of treatments. The time and site interaction was further partitioned among dates using a priori multiple comparisons to compare the difference between the overall control and treatment site means prior to treatment, with the difference between the control and treatment site means on each posttreatment sampling date (Snedecor and Cochran 1981). The H_0 for these comparisons was: methoxychlor treatment does not affect observed pretreatment differences between control and treatment sites for drift density, numbers on artificial substrates or numbers emerging. The above analysis was used to determine the impact of methoxychlor on invertebrates at the treatment site, in comparison to the control site, by taking into account the difference between the control and treatment sites prior to treatment. An example ANOVA table using drift density data for *Caenis tardata* McDunnough is presented in Appendix A.

Like most ecosystem-level manipulations, this study involved "pseudoreplication" because there was no true replication of treatments (Hurlbert 1984). Therefore, in a strict sense, statistical differences between control and treatment sites cannot be attributed to treatment effects. Significant differences actually only demonstrate that species did not show the same (parallel) trends at control and treatment sites for the variable being measured, although departure from these parallel trends coincident with methoxychlor addition strongly implies an effect due to the pesticide.

Species richness

Numbers of species were counted for each replicate sample on each date for both control and treatment sites, so that means and standard errors could be calculated. Species richness in the drift was expressed as mean number of species/24-h period; on artificial substrates it was expressed as mean number of species/840 cm^2 of surface area. Taylor's power law indicated that the variance of species counts was independent of the mean and no transformation was necessary for statistical analysis. The same analyses as described above were used to test for differences in numbers of species in the drift and on artificial substrates between the control and treatment sites.

TABLE 1. Methoxychlor residues (micrograms per litre) in water samples taken from the north (N), middle (M), and south (S) sides of the treatment site before and after methoxychlor addition to the Souris River, Manitoba. Concentrations at the control site were always below minimum detection limits (0.01 $\mu g \cdot L^{-1}$; N/D). * Indicates samples to which sodium azide was added immediately following collection. N/A = not available.

	Treatment site		
Sampling period	N	M	S
Pretreatment (24 h):	N/D	N/D	N/D
Posttreatment:			
10 min	284.44	358.97	223.85
10 min*	282.20	306.88	229.74
1 h	2.08	N/A	2.21
1 h*	2.86	3.08	1.11
3 h	0.26	0.41	0.24
6 h	0.05	0.12	0.03
12 h	0.02	0.06	0.02
24 h	N/D	N/D	N/D
48 h	N/D	N/D	N/D

Results

Water Chemistry and Discharge

The control and treatment sites were similar based on temperature, pH, PC, PN, and TSS. Mid-day water temperatures at control and treatment sites reached a maximum of 25.0°C on 15 July 1982, and declined steadily to 12.5°C on 16 September 1982. pH ranged from 7.6–8.4 over the sampling period but no pattern was obvious between sites or dates. Similarly, there were variations in PC (890–1880 $\mu g \cdot L^{-1}$ at control; 1150–2220 $\mu g \cdot L^{-1}$ at treatment), PN (134–289 $\mu g \cdot L^{-1}$ at control; 121–290 $\mu g \cdot L^{-1}$ at treatment), TSS (9–15 $mg \cdot L^{-1}$ at control; 10–14 $mg \cdot L^{-1}$ at treatment) over the sampling period. Only one sample was collected from each site on each sampling date for chemical analyses so no estimates of variation were possible.

Peak discharges of 78–90 $m^3 \cdot s^{-1}$ occurred in spring and declined to levels below 10 $m^3 \cdot s^{-1}$ by 1 July in 1982 and 1983 (Manitoba Department of Mines, Resources, and Environmental Management 1982, 1983). Discharge at the study site on the day of methoxychlor treatment was 3.3 $m^3 \cdot s^{-1}$.

Methoxychlor Residues in River Water

No detectable methoxychlor residues were observed at the control site on any sampling period before or after methoxychlor addition. At the treatment site, water samples taken 10 min after beginning the addition contained methoxychlor concentrations very close to the desired concentration of 300 $\mu g \cdot L^{-1}$ (Table 1). Trace levels were detected up to 12 h after treatment, but no residues were detected by 24 h. A dense growth of filamentous algae may have retarded passage of methoxychlor through the riffle, which could account for the relatively long period that methoxychlor was detected in the water column. Methoxychlor residues in water samples to which sodium azide was added were similar to residues observed in untreated samples collected simultaneously, indicating that microbial degradation did not significantly alter the methoxychlor concentrations prior to analysis.

Drift

Total drift of taxa

Catastrophic drift of aquatic invertebrates occurred immediately following methoxychlor treatment (Table 2), similar to responses shown in other lotic ecosystems (Burdick et al. 1968; Wallace et al. 1973; Wallace and Hynes 1975; Flannagan et al. 1979). Drift densities at the treatment-outlet site were significantly greater ($p<0.0001$) than at the control site for all invertebrate taxa during the 24-h period after treatment. Moreover, all functional feeding groups were simultaneously affected (predators: *A. lycorias* and *Polycentropus cinereus* (Hagen); scrapers: *Baetis* spp., *C. tardata*, *Leucrocuta maculipennis* (Walsh), *Hydroptila ajax* Ross, *Psychomyia flavida* Hagen, and *Stenacron interpunctatum* (Say); filter feeders: *Cheumatopsyche campyla* (Ross), *Hydropsyche alternans* (Walker), and *Isonychia sicca* Walsh; and collector-gatherers: *Ephoron album* (Say)). Total drift density, and drift densities of most species, were significantly depressed ($p<0.05$) at the treatment-outlet site relative to the control site for all subsequent sampling dates in 1982. Absence in the drift, extremely low drift densities, or high variability prevented the detection of any statistically significant differences ($p<0.05$) between the sites on other than the treatment date for *P. flavida, P. cinereus, A. lycorias*, and *O. virilis*. Drift densities of only *I. sicca* and *E. album* remained significantly ($p<0.05$) reduced at the treatment-outlet site relative to the control on 3 August 1983.

Species richness

A significantly higher ($p<0.05$) number of species was observed in the drift at the treatment-outlet site relative to the control site on the treatment date (Table 2). The number of species drifting was significantly ($p<0.05$) reduced at the treatment-outlet site compared with the control site for all sampling dates after treatment in 1982 but not by 3 August 1983.

Drift patterns of individual taxa

The response of individual taxa following methoxychlor treatment was categorized according to drift densities at the treatment-inlet site and the length of time required to recover on artificial substrates (see below). Five categories were established: (1) species naturally drifting in high numbers and showing no reduction on artificial substrates (e.g. *C. tardata*); (2) species naturally drifting in moderate to high numbers and recovering rapidly (<8 d) on artificial substrates (e.g. *Baetis* spp. and *L. maculipennis*); (3) species naturally drifting in low to moderate numbers, emerging during the treatment period and showing either no effect, or recovering moderately quickly (<64 d) on artificial substrates (e.g. *H. ajax* and *C. campyla*); (4) species naturally drifting in very low numbers, and recovering slowly on artificial substrates (>64 d, and possibly up to 1 yr following treatment) (e.g. *P. flavida* and *H. alternans*); and (5) species usually occurring in low numbers in the drift, and either absent or occurring in very low numbers on the artificial substrates (e.g. *E. album, I. sicca, S. interpunctatum, P. cinereus, A. lycorias, H. azteca*, and *O. virilis*). Treatment effects on group (5) species were difficult or impossible to assess, so most drift data presented below concern representative species from each of the first four categories. Complete drift data can be found in Sebastien (1986).

Group 1 species — The mayfly *C. tardata* drifted in high numbers under natural conditions at all sites, and demonstrated a diel periodicity with peak densities occurring during periods of darkness (Fig. 3). The catastrophic drift observed exceeded

TABLE 2. Geometric means and SE factors[a] for 24-h drift densities (No. · 100 m^{-3}) of invertebrates and sucker fry at the control (C) and treatment-outlet (T) sites in the Souris River before and after treatment with methoxychlor. Significant differences are based on a priori multiple comparisons (see methods for explanation).

		Pretreatment 1982	Treatment 1982	Posttreatment 1982			1983
		7 and 14 July[b]	15 July	21 July	29 July	17 August	3 August
Baetis spp.[c]	C	107.4×÷1.2	175.0×÷1.4**	199.7×÷1.1*	395.2×÷1.4*	650.6×÷1.4*	225.7×÷1.2
	T	259.5×÷1.1	12755.4×÷1.4	16.5×÷1.3	123.8×÷1.5	189.5×÷1.4	689.9×÷1.2
Caenis tardata	C	144.4×÷1.1	303.8×÷1.2**	135.6×÷1.1*	222.5×÷1.6*	972.8×÷1.2*	225.5×÷1.3
	T	648.7×÷1.1	18882.4×÷1.3	22.6×÷1.4	75.7×÷1.7	577.6×÷1.2	373.3×÷1.2
Leucrocuta maculipennis	C	52.7×÷1.2	78.6×÷1.4**	59.6×÷1.3*	114.9×÷1.5*	77.7×÷1.2*	43.9×÷1.1
	T	94.1×÷1.1	12688.0×÷1.2	3.9×÷2.0	27.6×÷1.8	31.5×÷1.3	131.4×÷1.4
Stenacron interpunctatum	C	4.8×÷1.7	16.2×÷1.5**	2.8×÷1.3*	3.6×÷1.4*	7.6×÷1.4*	42.8×÷1.3
	T	25.3×÷1.1	3886.1×÷1.4	0.0±0.0	1.8×÷1.8	4.0×÷1.1	142.8×÷1.4
Isonychia sicca	C	22.4×÷1.4	52.6×÷1.5**	26.9×÷1.1*	19.8×÷1.4*	9.3×÷1.4*	10.7×÷1.3*
	T	43.8×÷1.2	11067.4×÷1.3	1.6×÷1.6	0.0±0.0	0.0±0.0	2.2×÷1.5
Ephoron album	C	6.3×÷1.4	2.4×÷2.4**	0.0±0.0*	1.9×÷1.5*	2.4×÷1.3*	1.5×÷1.5*
	T	39.7×÷1.3	2344.8×÷1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Hydroptila ajax	C	145.8×÷1.3	127.2×÷1.5**	19.1×÷1.7*	141.8×÷2.2	37.4×÷1.2*	55.5×÷1.4
	T	326.9×÷1.3	2563.6×÷1.6	1.6×÷1.6	107.7×÷1.7	7.9×÷1.0	102.9×÷1.3
Cheumatopsyche campyla	C	5.9×÷1.0	25.8×÷1.1**	13.3×÷1.3*	5.5×÷2.4	87.3×÷1.3	18.5×÷1.7
	T	9.8×÷1.4	20549.0×÷1.4	0.0±0.0	4.4×÷2.1	29.7×÷1.2	54.4×÷1.3
Hydropsyche alternans	C	2.9×÷1.4	3.1×÷1.8**	2.5×÷1.2*	2.1×÷1.6*	19.5×÷1.1*	21.9×÷1.3
	T	13.1×÷1.1	3178.6×÷1.4	1.5×÷1.5	0.0±0.0	8.0×÷1.4	58.3×÷1.3
Pyschomyia flavida	C	1.2×÷1.1	1.6×÷1.6**	0.0±0.0	5.5×÷2.1	3.3×÷1.8	1.6×÷1.6
	T	1.9×÷1.5	12103.8×÷1.3	1.8×÷1.8	1.6×÷1.6	0.0±0.0	6.6×÷1.5
Polycentropus cinereus	C	2.6×÷1.7	6.1×÷1.5**	2.6×÷1.6	6.7×÷1.5	0.0±0.0	8.3×÷1.6
	T	0.0±0.0	304.8×÷1.7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Acroneuria lycorias	C	1.1×÷1.1	0.0±0.0**	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	T	0.0±0.0	10.4×÷1.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Hyalella azteca	C	92.7×÷1.3	92.7×÷1.1**	57.0×÷1.1*	50.0×÷1.4*	129.2×÷1.3*	19.9×÷1.2
	T	474.0×÷1.2	7208.4×÷1.3	5.7×÷1.6	16.7×÷1.2	65.5×÷1.2	41.4×÷1.2
Orconectes virilis	C	0.0±0.0	1.5×÷1.5**	1.4×÷1.4	0.0±0.0	0.0±0.0	0.0±0.0
	T	0.0±0.0	37.2×÷1.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Catostomus commersoni	C	10.8×÷1.6	9.9×÷1.7	0.0±0.0	5.2×÷1.9	7.1×÷1.8*	3.4×÷1.6
	T	9.0×÷1.5	4.7×÷1.2	0.0±0.0	1.1×÷1.1	0.0±0.0	4.7×÷2.4
Total of taxa	C	651.3×÷1.2	951.2×÷1.1**	521.0×÷1.1*	1015.0×÷1.6*	2032.0×÷1.3*	713.3×÷1.0
	T	2092.3×÷1.1	108190.0×÷1.3	51.5×÷1.4	365.7×÷1.6	929.7×÷1.2	1609.1×÷1.2
Species richness[d]	C	11.7±0.4	11.7±1.2*	10.0±0.0*	12.4±0.7*	11.7±0.4*	11.7±0.4
	T	11.2±0.3	15.0±0.0	5.0±0.0	6.7±0.4	8.0±0.0	10.7±0.4

[a]Since the transformation used was $Y = \ln(x+1)$, a value of 1 must be subtracted from the table values to return completely to the original scale. For example, the values in the original scale corresponding to 5.0×÷2.0 are $5-1$, $(5\div 2)-1$ and $(5\times 2)-1$, or a geometric mean of 4 and SE of 1.5 to 9.0. (If all observations are zero for any cell in the table, the table values should be 1×÷1 according to the above rule. But to avoid confusion, they were set to 0±0).

[b]Seven and 14 July combined to give overall pretreatment geometric mean and SE calculated from means of the three replicates over the two dates.

[c]Includes *Baetis pygmaeus* (Hagen), *B. flavistriga* McDunnough, *B. brunneicolor* McDunnough, *B. propinquus* (Walsh), *B. pallidulus* McDunnough, and *B. intercalaris* McDunnough.

[d]Since no transformation was used, table values are arithmetic mean (± 1SE) number of species in the drift.

*Means are significantly different at $p<0.05$.

**Means are significantly different at $p<0.0001$.

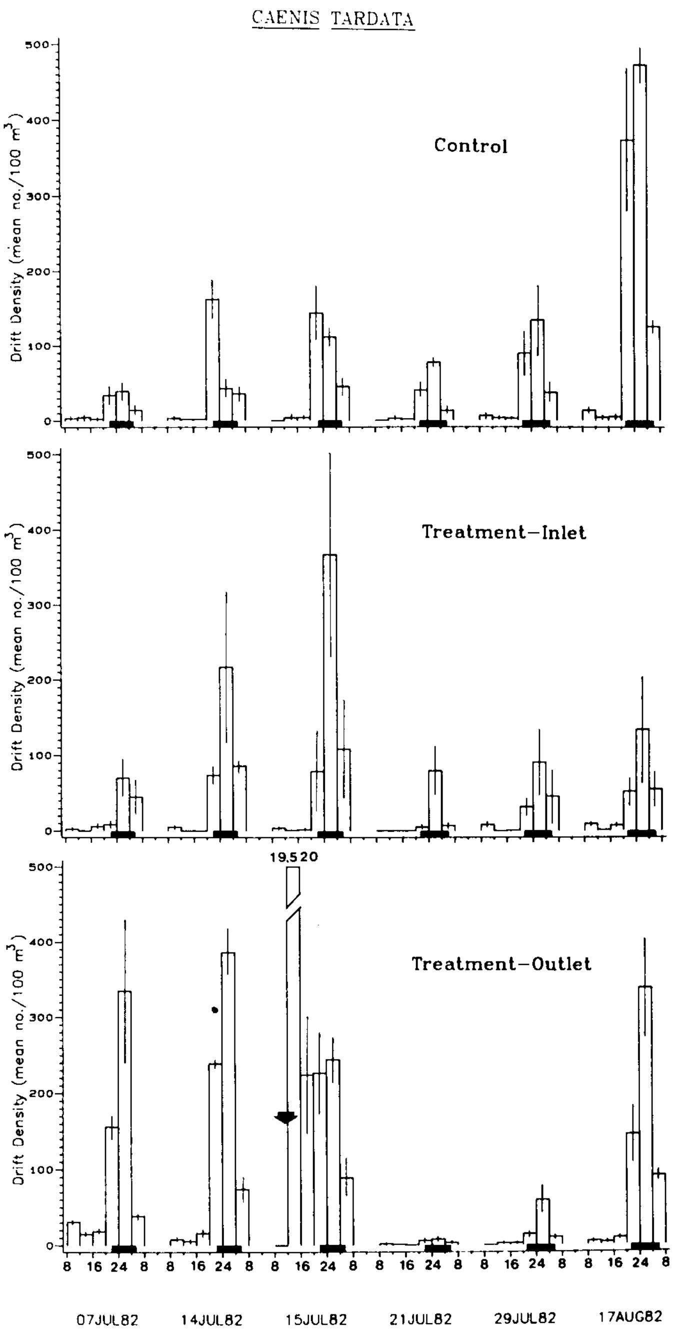

FIG. 3. Mean (± 1SE) drift density of *Caenis tardata* larvae at the three sampling sites in 1982. Dark bars = night. Numbers on *x* axis represent 24-h time. Arrow indicates methoxychlor addition.

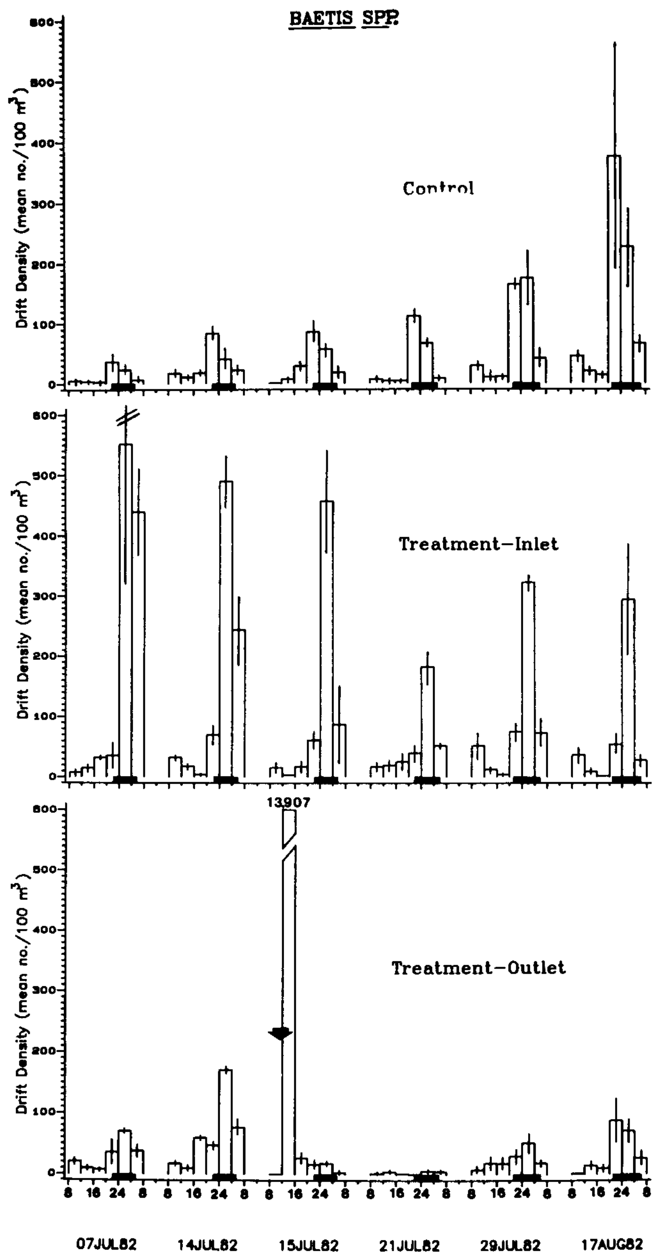

FIG. 4. Mean (± 1SE) drift density of *Baetis* spp. larvae at the three sampling sites in 1982. Dark bars = night. Numbers on *x* axis represent 24-h time. Arrow indicates methoxychlor addition.

control drift densities for ‹8 h and was significantly greater ($p<0.0001$) than at the control site for the 24-h period of 15 July 1982 (Table 2). Drift density increased ‹3900 times relative to the control site in the 4 h following treatment. Total 24-h drift densities were significantly lower ($p<0.05$) at the treatment-outlet site compared with the control for all the post-treatment sampling dates in 1982 (Table 2). The sites were not significantly different ($p>0.05$) for 24-h drift densities on 3 August 1983.

Group 2 species — Baetis spp. mayflies drifted naturally in high numbers (especially at the treatment-inlet site), and showed a diel periodicity with peaks corresponding to periods of darkness (Fig. 4). The catastrophic drift peak observed immediately following methoxychlor addition lasted for ‹4 h. Drift was significantly greater ($p<0.0001$) than at the control site for the 24-h period of 15 July 1982 (Table 2). The number of *Baetis* spp. drifting during the 4 h following treatment increased 1900-fold relative to the control site. Total 24-h drift densities were significantly lower ($p<0.05$) at the treatment-outlet site compared with the control site for all the posttreatment sampling dates in 1982. No significant differences ($p>0.05$) were observed for 24-h drift densities between the two sites on 3 August 1983.

Group 3 species — The caddisfly *H. ajax* drifted in relatively high densities at the control and treatment-outlet sites but in very low densities at the treatment-inlet site, and showed no

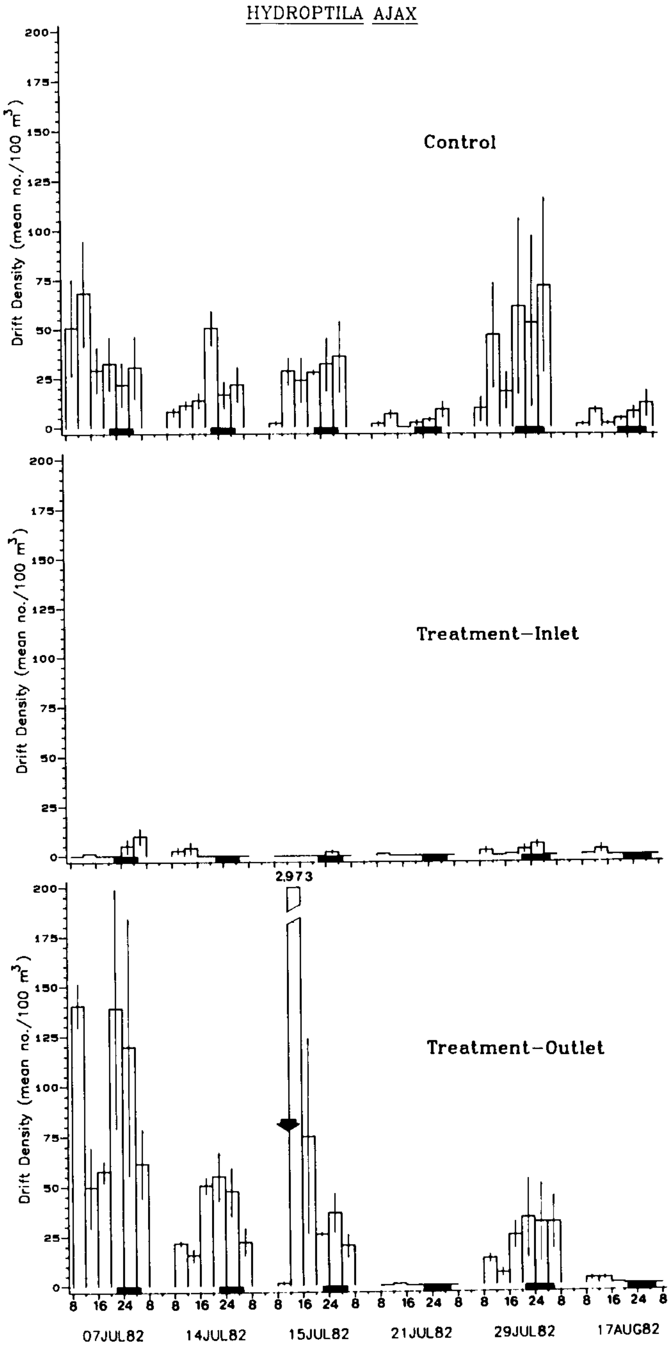

FIG. 5. Mean (± 1SE) drift density of *Hydroptila ajax* larvae at the three sampling sites in 1982. Dark bars = night. Numbers on *x* axis represent 24-h time. Arrow indicates methoxychlor addition.

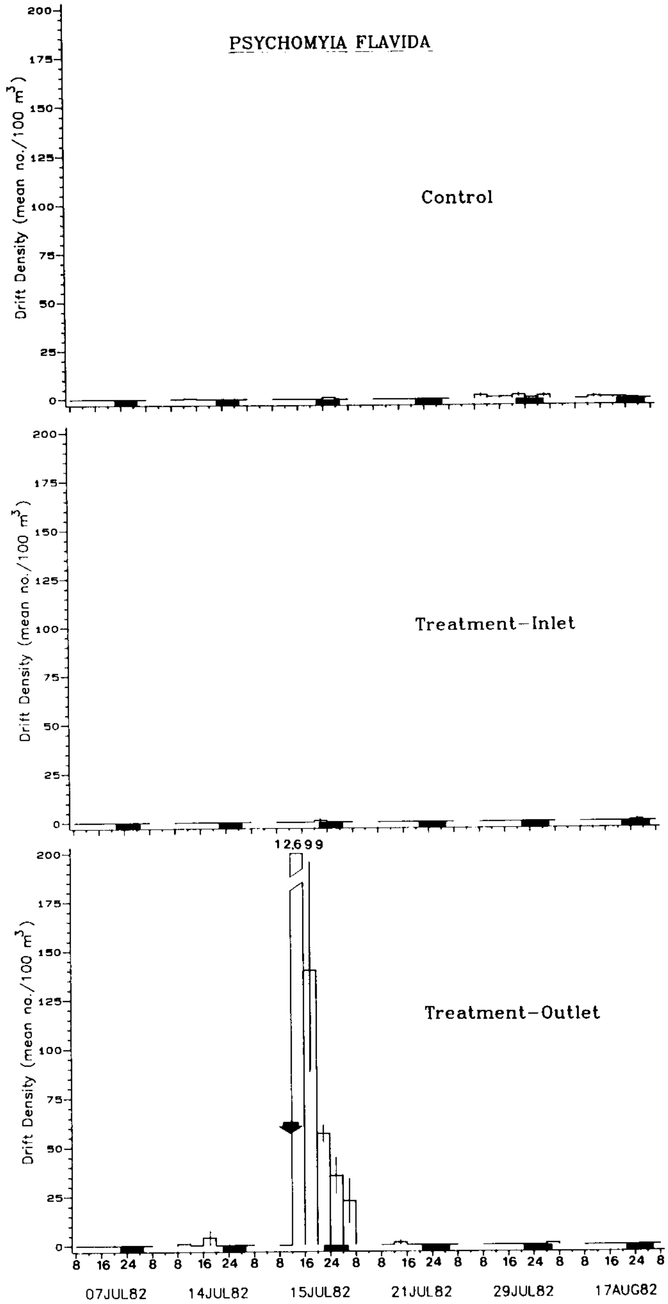

FIG. 6. Mean (± 1SE) drift density of *Psychomyia flavida* larvae at the three sampling sites in 1982. Dark bars = night. Numbers on *x* axis represent 24-h time. Arrow indicates methoxychlor addition.

diel drift periodicity (Fig. 5). The catastrophic drift observed exceeded drift densities at the control site for ‹8 h and was significantly greater ($p<0.0001$) than at the control site for the 24-h period of 15 July 1982 (Table 2). Drift densities increased 104 times relative to the control site in the 4 h following treatment. Drift density (24-h) was significantly lower ($p<0.05$) at the treatment-outlet site compared with the control on 21 July 1982. An increase in drift density occurred at both sites on 29 July 1982 that resulted in no significant difference ($p>0.05$) for 24-h drift density between the two sites. Drift density (24-h) was again significantly lower ($p<0.05$) at the treatment-outlet site relative to the control on 17 August 1982. The sites were not significantly different ($p>0.05$) on 3 August 1983.

Group 4 species — The caddisfly *P. flavida* rarely drifted under natural conditions, but large numbers drifted following methoxychlor treatment (Fig. 6). Drift density at the treatment-outlet site increased 12 700 times that of the control site in the 4 h following methoxychlor addition. Elevated drift densities lasted for ‹24 h following treatment, and they were significantly greater ($p<0.0001$) than at the control site for this period (Table 2). Drift densities (24-h) were not significantly different ($p>0.05$) between the control and treatment-outlet sites on any of the posttreatment sampling dates in 1982, or on 3 August 1983.

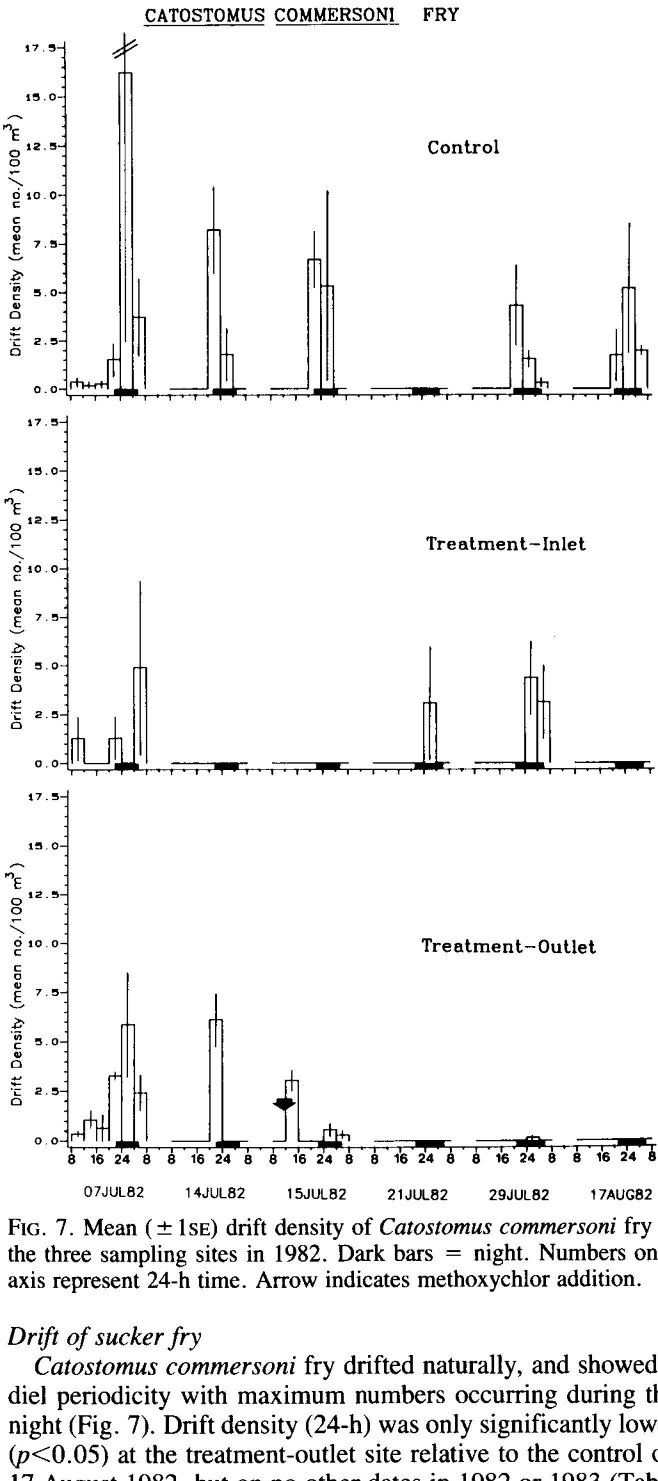

FIG. 7. Mean (± 1SE) drift density of *Catostomus commersoni* fry at the three sampling sites in 1982. Dark bars = night. Numbers on *x* axis represent 24-h time. Arrow indicates methoxychlor addition.

Drift of sucker fry

Catostomus commersoni fry drifted naturally, and showed a diel periodicity with maximum numbers occurring during the night (Fig. 7). Drift density (24-h) was only significantly lower ($p<0.05$) at the treatment-outlet site relative to the control on 17 August 1982, but on no other dates in 1982 or 1983 (Table 2). However, a slight increase in drift density of the fish fry occurred at the treatment-outlet site relative to the control that lasted for ‹4 h following methoxychlor treatment. Fry were virtually absent from the drift on posttreatment sampling dates in 1982 at the treatment-outlet site (cf. pretreatment levels at that site and posttreatment levels at both the control and treatment-inlet sites; Fig. 7).

Artificial Substrates

Total numbers

Total numbers (all taxa) on artificial substrates were significantly reduced ($p<0.05$) at the treatment site relative to the control site on all sampling dates following treatment in 1982, except at 14 d (Table 3). However, the significant differences ($p<0.05$) observed between sites on 16 August and 17 September 1982 were due to very large numbers of *H. alternans* (geometric mean numbers = 277.3 and 478.4, respectively) observed on control artificial substrates. The difference between control and treatment sites on these dates was not significant ($p>0.05$) when *H. alternans* was removed from the analysis. Significant differences were not found ($p>0.05$) between sites in 1983 (Table 3).

Species richness

Species richness was significantly reduced ($p<0.05$) at the treatment site relative to the control site for at least 4 d after treatment, but no significant differences ($p>0.05$) occurred between sites for the remaining sampling dates in 1982 and 1983 (Table 3).

Individual taxa

Individual taxa recolonized the artificial substrates at different rates following methoxychlor treatment (Table 3). Recolonization of some species was rapid. For example, numbers of *L. maculipennis* and *Baetis* spp., both Group 2 species, were significantly lower ($p<0.05$) at the treatment site only for day 1 and days 1 and 4, respectively (Table 3). Other taxa remained significantly reduced ($p<0.05$) at the treatment site relative to the control site for the majority of sampling dates, including the last one, in 1982 (e.g. *H. alternans* and *P. flavida*, both Group 4 species). *Cheumatopsyche campyla*, a Group 3 species, apparently had recovered by 17 September 1982 (64 d after treatment). Numbers of some taxa on the artificial substrates appeared to be enhanced following the treatment, e.g. *H. ajax* (Group 3) and *C. tardata* (Group 1). *Ephoron album*, juvenile *O. virilis* and *C. commersoni* fry were not included in Table 3 because they were never collected from artificial substrates, and numbers of *I. sicca, S. interpunctatum, A. lycorias, P. cinereus,* and *H. azteca*, all Group 5 species, were too low to allow meaningful statistical analysis.

Drift–Benthos Relationships

Although numbers of many taxa and total numbers on artificial substrates (exluding *H. alternans*) had recovered at the treatment site by 14 d after treatment (Table 3), drift densities of the majority of taxa and total drift density remained significantly reduced ($p<0.05$) for all sampling dates in 1982 (Table 2). In fact, numbers in the drift may have required until the following summer to reach pretreatment levels (Fig. 8).

Insect Emergence

Adults of *P. flavida, P. cinereus, I. sicca, H. alternans, S. interpunctatum, Baetis propinquus, B. pallidulus, B. intercalaris, B. flavistriga, H. ajax, C. campyla, Hydropsyche sparna* Ross, *H. bifida* Banks, *H. betteni* Ross, and *Nixe inconspicua* (McDunnough) were collected from emergence traps at both sites in 1982, but only *H. ajax* and *C. campyla* occurred in sufficient numbers for analysis.

Peak emergence of *H. ajax* occurred soon after treatment, and numbers emerging were significantly lower ($p<0.05$) at the treatment site relative to the control 4, 7, and 13 d after treatment (Table 4). However, the sites were not significantly different ($p>0.05$) by 18 August 1982, 34 d after treatment (Table 4). Emergence of *C. campyla* was significantly reduced ($p<0.05$) at the treatment site relative to the control on all sampling dates, except 2 and 13 d, following treatment (Table 4).

TABLE 3. Geometric means and SE factors[a] for numbers of invertebrates on artificial substrates at the control (C) and treatment (T) sites in the Souris River before and after treatment with methoxychlor. Methoxychlor was added on 15 July 1982. Significant differences are based on a priori multiple comparisons (see methods for explanation).

		Pretreatment 1982	Posttreatment 1982						1983	
		7, 12, and 14 July[b]	16 July	19 July	23 July	29 July	16 August	17 September	18 July	3 August
Baetis spp.[c]	C	39.0×÷1.1	21.5×÷1.2*	25.6×÷1.3*	18.9×÷1.3	35.5×÷1.2	42.1×÷1.2	5.6×÷1.3	12.0×÷1.2	35.4×÷1.2
	T	29.3×÷1.2	1.1×÷1.1	1.7×÷1.3	9.9×÷1.6	35.7×÷1.3	60.6×÷1.2	5.0×÷1.3	16.9×÷1.2	44.9×÷1.2
Caenis tardata	C	1.9×÷1.2	1.5×÷1.2	0.0±0.0	1.1×÷1.1*	1.2×÷1.2	0.0±0.0	1.4×÷1.2	1.1×÷1.1	0.0±0.0
	T	3.1×÷1.2	4.4×÷1.3	3.1×÷1.4	6.9×÷1.4	1.9×÷1.3	1.5×÷1.2	1.8×÷1.2	2.0×÷1.2	1.3×÷1.1
Leucrocuta maculipennis	C	5.6×÷1.2	9.4×÷1.5*	4.5×÷1.4	3.0×÷1.4	5.4×÷1.5	2.5×÷1.4	1.4×÷1.2	1.8×÷1.2	2.0×÷1.3
	T	6.6×÷1.2	1.4×÷1.2	1.8×÷1.3	1.6×÷1.2	2.5×÷1.4	1.4×÷1.2	1.3×÷1.2	4.8×÷1.4	2.6×÷1.4
Isonychia sicca	C	2.4×÷1.1	1.6×÷1.2*	3.7×÷1.4*	1.2×÷1.1	1.9×÷1.2*	1.3×÷1.1*	0.0±0.0	1.5×÷1.2	1.6×÷1.2*
	T	4.3×÷1.2	1.4×÷1.1	1.1×÷1.1	1.2×÷1.1	1.5×÷1.2	1.1×÷1.1	1.1×÷1.1	1.9×÷1.2	1.2×÷1.2
Stenacron interpunctatum	C	1.1×÷1.1	1.2×÷1.1	1.1×÷1.1	0.0±0.0	1.1×÷1.1	0.0±0.0	0.0±0.0	0.0±0.0	1.5×÷1.2
	T	1.1×÷1.1	0.0±0.0	1.1×÷1.1	1.3×÷1.2	1.3×÷1.2	0.0±0.0	1.1×÷1.1	1.3×÷1.2	2.0×÷1.3
Acroneuria lycorias	C	1.0×÷1.0	1.1×÷1.1	0.0±0.0	1.1×÷1.1	1.2×÷1.1	0.0±0.0	0.0±0.0	0.0±0.0	1.1×÷1.1
	T	1.0×÷1.0	0.0±0.0	1.1×÷1.1	0.0±0.0	1.1×÷1.1	0.0±0.0	1.1×÷1.1	1.1×÷1.1	1.1×÷1.1
Hydroptila ajax	C	32.2×÷1.3	3.2×÷1.5*	1.8×÷1.4	2.2×÷1.4	31.9×÷1.5	17.9×÷1.4	4.4×÷1.4*	3.7×÷1.4	159.4×÷1.1*
	T	65.8×÷1.2	37.8×÷1.1	3.7×÷1.4	13.5×÷1.7	117.6×÷1.3	87.0×÷1.2	2.3×÷1.4	13.3×÷1.5	55.6×÷1.1
Hydropsyche alternans	C	12.9×÷1.3	3.9×÷1.5	12.8×÷1.4*	6.4×÷1.6*	7.8×÷1.6*	277.3×÷1.3	478.4×÷1.4*	129.1×÷1.3	126.8×÷1.3
	T	5.3×÷1.2	1.2×÷1.1	1.4×÷1.2	0.0±0.0	2.0×÷1.4	63.9×÷1.4	58.9×÷1.5	75.6×÷1.3	81.4×÷1.4
Cheumatopsyche campyla	C	6.3×÷1.2	6.4×÷1.3	8.6×÷1.2*	10.2×÷1.6*	17.5×÷1.3*	50.2×÷1.2*	188.4×÷1.2	15.3×÷1.2	30.7×÷1.2
	T	8.3×÷1.1	3.0×÷1.3	1.1×÷1.1	1.2×÷1.1	2.8×÷1.3	17.6×÷1.3	110.9×÷1.2	21.6×÷1.2	38.6×÷1.2
Psychomyia flavida	C	3.8×÷1.2	74.8×÷1.3*	105.5×÷1.3*	18.0×÷1.6*	5.4×÷1.2	1.7×÷1.3	31.8×÷1.1*	25.5×÷1.2	4.3×÷1.3
	T	3.8×÷1.3	2.5×÷1.3	1.7×÷1.2	2.7×÷1.3	2.4×÷1.4	2.5×÷1.4	5.0×÷1.4	16.7×÷1.1	8.5×÷1.2
Polycentropus cinereus	C	1.2×÷1.1	1.9×÷1.2*	1.5×÷1.2	1.2×÷1.2	1.4×÷1.2	0.0±0.0	0.0±0.0	1.1×÷1.1	1.1×÷1.1
	T	1.3×÷1.1	0.0±0.0	1.2×÷1.1	1.1×÷1.1	1.7×÷1.2	0.0±0.0	0.0±0.0	1.1×÷1.1	1.1×÷1.1
Hyalella azteca	C	2.9×÷1.2	0.0±0.0	0.0±0.0	1.1×÷1.1	1.9×÷1.3	1.2×÷1.2	21.1×÷1.3*	1.1×÷1.1	0.0±0.0
	T	5.0×÷1.2	1.7×÷1.2	1.8×÷1.3	3.0×÷1.3	2.2×÷1.3	1.1×÷1.1	7.8×÷1.5	1.3×÷1.1	1.1×÷1.1
Total of taxa	C	146.8×÷1.1	144.5×÷1.1*	200.1×÷1.1*	94.7×÷1.2*	140.0×÷1.2	445.8×÷1.2*	830.6×÷1.2*	201.3×÷1.2	390.4×÷1.1
	T	177.5×÷1.1	50.9×÷1.1	13.7×÷1.2	44.3×÷1.5	185.3×÷1.3	268.9×÷1.2	221.7×÷1.3	178.5×÷1.1	259.5×÷1.2
Species richness[d]	C	7.1±0.2	6.7±0.5*	6.6±0.3*	5.7±0.3	7.7±0.3	5.3±0.4	6.4±0.3	6.1±0.3	6.5±0.5
	T	7.5±0.2	5.1±0.3	4.3±0.6	5.3±0.6	6.7±0.6	5.4±0.3	6.5±0.4	7.8±0.5	7.1±0.5

[a]See footnote "a", Table 2.
[b]7, 12, and 14 July combined to give overall pretreatment geometric mean and SE calculated from means of the 10 replicates over the three dates.
[c]See footnote "c", Table 2.
[d]Since no transformation was used, table values are arithmetic mean (±1SE) number of species on artificial substrates.
*Means are significantly different at $p<0.05$.

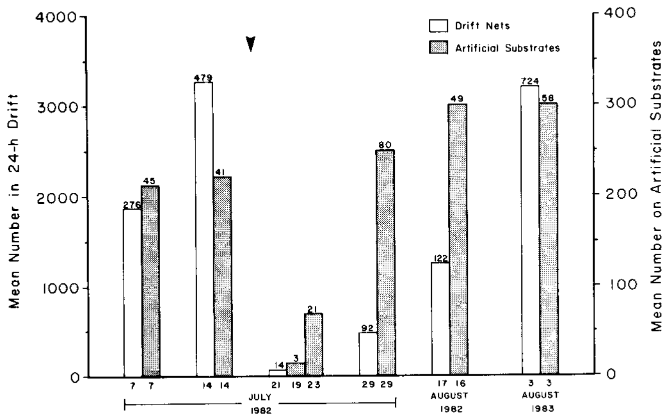

FIG. 8. Mean number of invertebrates drifting over a 24-h period ($n = 3$ for each sample date) from the treatment-outlet site, and on artificial substrates ($n = 10$ for each sample date) at the treatment site. Number above each bar is one standard error of the mean. Drift data for treatment data (15 July 1982) are not included. Arrow indicates methoxychlor addition.

Discussion

Recolonization

Different taxa demonstrated varying abilities to recolonize the artificial substrates following pesticide addition to the Souris River. Some taxa recolonized within days, whereas others required months to recover relative to the control site. This great variation in ability to recolonize probably can be best explained by the major pathways used for recolonization. Williams and Hynes (1976) demonstrated that animals recolonizing an area of denuded stream substrate came from four main sources: downstream drift, upstream migration, upward migration from the hyporheic zone, and egg laying by aerial adults. Drift from upstream areas was the most important source, accounting for ‹40% of the total number that settled in a denuded area. Townsend and Hildrew (1976) determined that drift from areas upstream was responsible for 82% of the recolonization observed in their study. Wallace et al. (1973) observed rapid repopulation of the stream surface substrate, after application of insecticides, and ascribed this to immigration from hyporheic sources. Recolonization by selected taxa in the Souris River is discussed below.

Group 1 species

Although *C. tardata* drifted catastrophically and did not recover until well after methoxychlor treatment (Fig. 3), numbers on artificial substrates were always higher at the treatment site than at the control, significantly so ($p<0.05$) 8 d after treatment (Table 3). The disparity in numbers between treatment and control sites in both types of samples before methoxychlor was added (Table 2 and 3), and the lack of response of *C. tardata* on artificial substrates afterward remain unexplained.

Colonization of artificial substrates can be selective (Rosenberg and Resh 1982), which may explain the overall low numbers of *C. tardata* at both treatment and control sites. The catastrophic drift of *C. tardata* observed must have originated in the natural substrate, but in the absence of samples directly from this habitat we are unable to comment on recolonization by this species.

Group 2 species

Baetis spp. have a high propensity to drift under natural conditions, which probably represents the main method used by nymphs of this genus to recolonize denuded areas of stream. Numbers of *Baetis* spp. on artificial substrates at the treatment site were significantly lower ($p<0.05$) than at the control on days 1 and 4 following methoxychlor addition (Table 3). The rapid recovery on the artificial substrates probably can be explained by the very high densities drifting into the treatment riffle at the treatment-inlet site (Fig. 4). Moreover, five of the six species of *Baetis* collected in this study have bivoltine life cycles; only *B. intercalaris* is univoltine (Clifford 1982). Production of more than one generation per year should enhance recolonization. Therefore, most of the species of *Baetis* probably did not suffer long lasting reductions following the single methoxychlor treatment.

Leucrocuta maculipennis also recovered very rapidly on artificial substrates following methoxychlor treatment (Table 3). This observation also can be explained by high densities drifting into the treatment riffle at the treatment-inlet site (Sebastien 1986, figure 25, appendix A).

Group 3 species

Hydroptila ajax also drifted in high numbers immediately following treatment (Fig. 5). However, the magnitude of its catastrophic drift was less than for most other species of Trichoptera examined (Table 2). Fredeen (1969) suggested that *Hydroptila* and *Brachycentrus* larvae tolerated DDD better than

TABLE 4. Geometric means and standard error factors[a] for numbers of Trichoptera emerging at the control (C) and treatment (T) sites in the Souris River before and after treatment with methoxychlor. Methoxychlor was added on July 15 1982. Significant differences are based on a priori multiple comparisons (see methods for explanation).

		Pretreatment 1982	Posttreatment 1982				
		8 and 14 July[b]	17 July	19 July	22 July	28 July	18 August
Hydroptila ajax	C	8.6⋇1.1	44.0⋇1.0	48.0⋇1.2	29.1⋇1.2	26.6⋇1.4	4.2⋇1.2
				*	*	*	
	T	12.1⋇1.2	38.9⋇1.3	24.9⋇1.3	5.3⋇1.1	0.0±0.0	4.6⋇1.6
Cheumatopsyche campyla	C	0.0±0.0	2.9⋇1.4	3.2⋇1.3	8.6⋇1.2	6.5⋇1.8	9.5⋇1.4
				*	*		*
	T	1.9⋇1.4	1.6⋇1.3	1.3⋇1.3	4.5⋇2.2	8.8⋇1.6	4.7⋇1.3

[a]See footnote "a", Table 2.
[b]Eight and 14 July combined to give overall pretreatment geometric mean and standard error; calculated from means of the three replicates over the two dates.
*Means are significantly different at $p < 0.05$.

net-spinning larvae because their cases provided protection from the chemical. *Hydroptila ajax* was the only case-bearing caddisfly of the five species examined here.

Hydroptila ajax on artificial substrates did not appear to be affected following methoxychlor addition (Table 3). In fact, numbers were almost always higher at the treatment site than the control site, both before and after treatment, an observation that remains unexplained. An emergence peak roughly coincided with methoxychlor addition (Table 4) indicating that emerging adults were not initially affected by the pesticide. Pupae about to emerge also may be resistant because they are enclosed in silken cases. Later, emergence was significantly depressed ($p<0.05$) at the treatment site relative to the control, perhaps because of an original adverse effect of the treatment on larvae that were about to pupate. *Hydroptila ajax* has a low propensity to drift, so the recolonization that occurred was probably due to egg-laying adults. Flannagan (1977) reported that *H. ajax* was trivoltine in the Roseau River, southeastern Manitoba.

Although emergence of *C. campyla* at the treatment site relative to the control decreased significantly following methoxychlor addition (Table 4), recolonization of the treatment riffle probably occurred mainly by egg-laying adults of this species (cf. Byrtus 1982). Because of its low behavioural drift rate (Sebastien 1986, figure 10) and univoltine life cycle (Flannagan 1977), recolonization at the treatment site probably would have required much longer had emergence not occurred prior to and during methoxychlor treatment.

Group 4 species

Numbers of *P. flavida* were significantly reduced ($p<0.05$) on artificial substrates at the treatment site relative to the control site on all posttreatment sampling dates in 1982 except for 29 July and 16 August (Table 3). However, numbers also were reduced on these dates at the control site, possibly due to an emergence of *P. flavida* that was observed from mid-July to mid-August in the Souris River (R.J. Sebastien, unpubl. data). Egg-laying adults probably were responsible for the subsequent recolonization of *P. flavida*, and increased numbers at the control site on 17 September 1982 likely resulted from a new generation of larvae. Low numbers of *P. flavida* drifting naturally (Fig. 6: see treatment-inlet site) may explain why this species did not recover until the following summer. Also, *P. flavida* is univoltine in the Roseau River (Flannagan 1977), and a univoltine species with a low propensity to drift would be susceptible to long-term reductions by methoxychlor larviciding operations. Because recolonization is most likely by egg-laying adults, a much longer period would be required for this species to recover.

Hydropsyche alternans also required a long time to recolonize the artificial substrates following methoxychlor treatment (Table 3). It too had a low propensity to drift naturally (Sebastien 1986, figure 29, appendix A).

Other invertebrate taxa

It is difficult to comment on recolonization by Group 5 species because of their relative scarcity in samples taken from the Souris River. However, limited data and observations are available for three of the taxa in this group.

Although *E. album* adults were not collected by emergence traps at any time during the study, 538 adults were recovered from the three drift samplers at the control site 14 d after treatment (R.J. Sebastien, unpubl. data). Only 14 adults were collected from the three drift samplers at the treatment-outlet site at the same time. *Ephoron album* nymphs drifted catastrophically at the treatment-outlet site following methoxychlor treatment (Table 2), so the adult population at the treatment site may have been severely reduced by effects of the methoxychlor on immatures. *Ephoron album* drifts in very low numbers under natural conditions (Sebastien 1986, figure 21, appendix A), has a univoltine life cycle (Clifford 1982), and a short-lived adult stage (Giberson and Galloway 1985). Therefore, it should be very susceptible to long-term numerical reduction by repeated methoxychlor treatments.

The predatory stonefly *A. lycorias* also increased in the drift following methoxychlor addition (Table 2), but drift densities were lower than other invertebrate taxa because of its relative rarity at the study sites. *Acroneuria lycorias* occurred on artificial substrates at the treatment site on various posttreatment sampling periods, but numbers were too low to permit any meaningful statistical analysis (Table 3). Although the species is susceptible to methoxychlor (Sebastien and Lockhart 1981; Scherer and McNicol 1986), some individuals were able to survive the methoxychlor injection, as suggested by their presence on artificial substrates at the treatment site only 4 d after treatment.

Orconectes virilis juveniles rarely drifted, but a substantial increase in numbers drifting was observed following methoxychlor treatment (Table 2). *Orconectes virilis* was the least susceptible of several organisms to methoxychlor in the laboratory tests of Merna and Eisele (1973), and Flannagan et al. (1979) found no significant effect on blood calcium and no mortality in caged *O. virilis* after an application of 300 $\mu g \cdot L^{-1} \cdot 15\ min^{-1}$ of methoxychlor to the Athabasca River, Alberta. However, mature specimens were used in these studies, and *O. virilis* juveniles may be far more sensitive to methoxychlor because of higher surface:volume ratios (Sanders and Cope 1968; Wallace and Hynes 1981). Many of the juveniles collected in the catastrophic drift from the Souris River were moribund or dead (R.J. Sebastien, unpubl. data), indicating their susceptibility to methoxychlor treatment.

Downstream effects

Although the experimental design of this study precluded examination of downstream responses to methoxychlor addition in the Souris River, some comment can be made about recovery in areas distant from pesticide application. In general, the theory of island biogeography (MacArthur and Wilson 1963, 1967) can be applied to invertebrate recolonization following black fly larviciding operations in large rivers (see also Gore 1982; Minshall et al. 1983).

Characteristically, methoxychlor is added directly to the water as a point-source application rather than being sprayed over the catchment. Sites closest to pesticide application, although exposed to the highest pesticide concentrations, should have the greatest potential to recolonize rapidly because of their proximity to sources of upstream colonization such as drift and aerial, egg-laying adults. The time required to recolonize should progressively increase with increasing distance downstream from the treatment site. One year after applying methoxychlor to the Athabasca River, invertebrate standing crops at the two furthest downstream sampling sites were still significantly reduced, whereas the upstream sampling sites had completely recolonized (Flannagan et al. 1979). The greatest long-term effect on nontarget invertebrates was observed at the furthest downstream sampling site, 400 km from the treatment site. In rivers, the effect of distance diminishes with time, because sources of colonization progressively shift downstream. Recolonization also can be influenced by tributaries that act as a source of colonists for treated sections of rivers, although no major tributaries exist in the Souris River downstream from the site of pesticide addition. Currently, no guidelines exist to estimate speed of recovery in lotic systems.

Responses of White Sucker Fry

Mortality of *C. commersoni* fry was not observed at any time during the experiment. However, slightly elevated drift immediately following methoxychlor treatment, during a period when the species does not normally drift (Fig. 7), indicated an avoidance response, and some fry were moribund in the pool downstream of the treatment riffle immediately following treatment. Numbers of alevins and fry of *C. commersoni* increased substantially in the drift 400 km downstream of methoxychlor addition to the Athabasca River (Flannagan et al. 1979), but it was not established that the increase was due to methoxychlor.

Larval fish should be more susceptible to acute poisoning that larger mature fish. Most of the research conducted on the acute toxicity of methoxychlor to native fish species during black fly larviciding operations (e.g. Lockhart et al. 1977; Lockhart 1980) has focused on fish specimens >100 g. However, exposure of 3- to 240-g rainbow trout to methoxychlor resulted in a statistically significant inverse relationship between fish size and methoxychlor uptake (Lockhart 1980). Also, methoxychlor was harmful to juvenile *C. commersoni* in pulse-dosed experiments done in the laboratory (Holdway et al. 1987). Spawning activities of *C. commersoni* could coincide with a methoxychlor larviciding operation, so more research is required to determine the impact of treatment on early life stages of this species.

Community Responses

The recovery of total abundances, species richness, and many individual taxa at the treatment site occurred more slowly in the drift than on the artificial substrates following methoxychlor treatment (Table 2 and 3). Either the artificial substrates provided a less-sensitive measure than drift of impact due to methoxychlor treatment, or invertebrates living in the stream benthos recovered more quickly than those in the drift (Fig. 8).

Dimond (1967) also observed that, in streams with different histories of DDT treatment, drift required one or two seasons after bottom populations had recovered to regain its normal abundance and composition. If stream drift represents a way for invertebrates to seek more preferable habitats and food resources, avoid predation, and regulate population densities (Minshall and Winger 1968; Hildebrand 1974; Walton et al. 1977; Bohle 1978; Corkum 1978; Walton 1980; Ciborowski 1983; Kohler 1985), then delayed recovery of drift in relation to bottom density may indicate long-term disruption by pesticides of fundamental biotic interactions in stream ecosystems. The similar delay in recovery of drift observed here and in Dimond's (1967) study indicates a need for research into the long-term effects of pesticides on drift phenomena in lotic systems, research that also may shed light on the basic causes of stream drift.

Drift is much easier to collect than benthic samples in rivers such as the Souris. In fact, a different artificial substrate benthic sampling method used in our preliminary studies had to be abandoned because the samplers became coated by filamentous algae, creating an atypical habitat. Thus, perhaps drift rather than benthic samples should be used to measure stream recovery or to indicate water quality (Larimore 1974). In large, deep, turbid rivers where benthos sampling in the centre of the river is very difficult, the use of drift sampling to indicate recovery after an insecticide treatment merits further investigation. Benthic samples taken close to the river banks, a common practise in many studies involving the impact of a toxicant on the benthos of large rivers, is likely to be a poor indicator of effects near the centre of the channel where concentration of the chemical and ecological conditions may be completely different. However, the disadvantages in using drift to indicate water quality also should be borne in mind: the habitats from which individuals originate are not always known, and not all benthic species drift.

Conclusion

Although the invertebrate community of the treated riffle had recovered by the following year, the results of this study should be put into proper perspective when attempting to determine the impact of methoxychlor treatments on invertebrates downstream of a treatment site. First, far-downstream species would be exposed under different conditions than those immediately downstream from methoxychlor addition. Pesticide concentration would be attenuated as it moved downstream, so invertebrates would be exposed to the chemical at lower concentrations, but for a longer period. Since the toxicity of methoxychlor increases with increasing exposure time for most organisms (Sanders and Cope 1968; Merna and Eisele 1973; Anderson and DeFoe 1980), taxa in downstream sites would be exposed to the pesticide under chronic conditions and could be at higher risk. Also, because of its physicochemical properties, much of the pesticide would be adsorbed onto suspended solids in the water column, so its availability to filter feeders downstream of application would be enhanced (Sebastien and Lockhart 1981). Second, downstream recovery could be prolonged because of the greater distance from upstream sources of colonization, although tributaries could hasten recolonization. Third, multiple methoxychlor treatments, as carried out in some black fly larviciding operations in western Canada (e.g. Byrtus 1981, 1982; Fredeen 1983) may be more harmful to benthic invertebrates than a single application such as investigated here. Last, potential effects of the treatment on biotic interactions and functional processes such as energy transfer and nutrient cycling (e.g. Wallace et al. 1982, 1986) also have to be considered in any decision to use methoxychlor — or any other nonselective pesticide — for black fly control in a river system.

Acknowledgements

Research support was provided to R. Brust from Agriculture Canada Grants 1013 and 1037, and to R. Sebastien by a University of Manitoba Graduate Fellowship. We are grateful to J. Serger, K. Nixon, and V. DeJong for their persistent dedication during the field and laboratory portions of the study. We are also grateful to D. Muir for advice and the use of equipment for analysis of methoxychlor residues in water, and to N. Holliday and S. Hurlbert for advice concerning statistical analysis. D. Fenwick kindly provided facilities on his farm at Bunclody, Manitoba for a camper-trailer during the study. We wish to thank B. deMarch, L. Dosdall, J. Flannagan, S. Hurlbert, T. Galloway, L. Lockhart, and an anonymous reviewer for their critical comments on the manuscript. D. Laroque guided the manuscript through its many drafts. A special thanks is extended to R. Lypka for his invaluable advice concerning computer analysis and graphics.

References

ANDERSON, R. L., AND D. L. DEFOE. 1980. Toxicity and bioaccumulation of endrin and methoxychlor in aquatic invertebrates and fish. Environ. Pollut. 22: 111–121.

BOHLE, H. W. 1978. Relation between food supply, drift and microdistribution of larvae of *Baetis rhodani*: Investigations in a stream model. Arch. Hydrobiol. 84: 500–525.

BURDICK, G. E., H. J. DEAN, E. J. HARRIS, J. SKEA, C. FRISA, AND C. SWEENEY. 1968. Methoxychlor as a black fly larvicide, persistence of its residues in fish and its effect on stream arthropods. NY Fish Game J. 15: 121–142.

BURDICK, G. E., H. J. DEAN, J. C. SKEA, AND C. N. FRISA. 1974. Effects of black fly larviciding in some Adirondack streams. NY Fish Game J. 21: 1–17.

BURTON, W., AND J. F. FLANNAGAN. 1976. An improved river drift sampler. Can. Fish. Mar. Serv. Tech. Rep. 641. 8 p.

BYRTUS, G. 1981. Athabasca River monitoring program — 1980. Report to Pollution Control Division, Alberta Environment, Edmonton, Alta.

1982. Athabasca River monitoring program — 1981. Report to Pollution Control Division, Alberta Environment, Edmonton, Alta.

CIBOROWSKI, J. J. H. 1983. Influence of current velocity, density and detritus on drift of two mayfly species (Ephemeroptera). Can. J. Zool. 61: 119–125.

CLIFFORD, H. F. 1982. Life cycles of mayflies (Ephemeroptera), with special reference to voltinism. Quaest. Entomol. 18: 15–90.

CORKUM, L. D. 1978. The influence of density and behavioural type on the active entry of two mayfly species (Ephemeroptera) into the water column. Can. J. Zool. 56: 1201–1206.

DEPNER, K. R., W. A. CHARNETSKI, AND W. O. HAUFE. 1980. Effect of methoxychlor on resident populations of the invertebrates of the Athabasca River, p. 141–150. *In* W. O. Haufe and G. C. R. Croome [ed.] Control of black flies in the Athabasca River. Alberta Environment, Edmonton, Alta.

DIMOND, J. B. 1967. Evidence that drift of stream benthos is density related. Ecology 48: 855–857.

ELLIOTT, J. M. 1970. Methods of sampling invertebrate drift in running waters. Ann. Limnol. 6: 133–159.

FLANNAGAN, J. F. 1977. Emergence of caddisflies from the Roseau River, Manitoba, p. 183–197. *In* M. I. Crichton [ed.] Proceedings of the 2nd International Symposium on Trichoptera. Dr. W. Junk Publishers, The Hague.

FLANNAGAN, J. F., B. E. TOWNSEND, AND B. G. E. DEMARCH. 1980. Acute and long term effects of methoxychlor larviciding on the aquatic invertebrates of the Athabasca River, Alberta, p. 151–157. *In* W. O. Haufe and G. C. R. Croome [ed.] Control of black flies in the Athabasca River. Alberta Environment, Edmonton, Alta.

FLANNAGAN, J. F., B. E. TOWNSEND, B. G. E. DEMARCH, M. K. FRIESEN, AND S. L. LEONHARD. 1979. The effect of an experimental injection of methoxychlor on aquatic invertebrates: accumulation, standing crop, and drift. Can. Entomol. 111: 73–89.

FREDEEN, F. J. H. 1969. A new procedure allowing replicated miniature larvicide tests in a large river. Can. Entomol. 101: 713–725.

1974. Tests with single injections of methoxychlor black fly (Diptera:Simuliidae) larvicides in large rivers. Can. Entomol. 106: 285–305.

1975. Effects of a single injection of methoxychlor black fly larvicide on insect larvae in a 161 km (100 mile) section of the North Saskatchewan River. Can. Entomol. 107: 807–817.

1977. A review of the economic importance of black flies (Simuliidae) in Canada. Quaest. Entomol. 13: 219–229.

1983. Trends in numbers of aquatic invertebrates in a large Canadian river during four years of black fly larviciding with methoxychlor (Diptera:Simuliidae). Quaest. Entomol. 19: 53–92.

FRENCH, J. L., M. S. MULLA, AND L. DE MATTEI. 1986. Conditions leading to the toxicity of methoxychlor to game fish in Humboldt River, Nevada. Bull. Soc. Vector Ecol. 11: 264–267.

GIBERSON, D. J., AND T. D. GALLOWAY. 1985. Life history and production of *Ephoron album* (Say) (Ephemeroptera: Polymitarcidae) in the Valley River, Manitoba. Can. J. Zool. 63: 1668–1674.

GORE, J. A. 1982. Benthic invertebrate colonization: source distance effects on community composition. Hydrobiologia 94: 183–193.

GREEN, R. H. 1979. Sampling design and statistical methods for environmental biologists. John Wiley and Sons, New York, NY. 257 p.

HAUFE, W. O., K. R. DEPNER, AND W. A. CHARNETSKI. 1980. Impact of methoxychlor on drifting aquatic invertebrates, p. 159–168. *In* W. O. Haufe and G. C. R. Croome [ed.] Control of black flies in the Athabasca River. Alberta Environment, Edmonton, Alta.

HILDEBRAND, S. G. 1974. The relation of drift to benthos density and food level in an artificial stream. Limnol. Oceanogr. 19: 951–957.

HOLDWAY, D. A., E. J. KEMPE, AND D. G. DIXON. 1987. Acute toxicity of methoxychlor to larval white sucker (*Catostomus commersoni*) as modified by age, food availability, and egg preexposure. Can. J. Fish. Aquat. Sci. 44: 227–232.

HURLBERT, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54: 187–211.

JAMNBACK, H. 1969. Field tests with larvicides other than DDT for control of black flies (Diptera:Simuliidae) in New York. Bull. WHO 40: 633–635.

KOHLER, S. L. 1985. Identification of stream drift mechanisms: an experimental and observational approach. Ecology 66: 1749–1761.

LARIMORE, R. W. 1974. Stream drift as an indication of water quality. Trans. Am. Fish. Soc. 103: 507–517.

LOCKHART, W. L. 1980. Methoxychlor studies with fish: Athabasca River exposures and experimental exposures, p. 183–196. *In* W. O. Haufe and G. C. R. Croome [ed.] Control of black flies in the Athabasca River. Alberta Environment, Edmonton, Alta.

LOCKHART, W. L., D. A. METNER, AND J. SOLOMON. 1977. Methoxychlor residue studies in caged and wild fish from the Athabasca River, Alberta, following a single application of black fly larvicide. J. Fish. Res. Board Can. 34: 626–632.

MACARTHUR, R. H., AND E. O. WILSON. 1963. An equilibrium theory of insular zoogeography. Evolution 17: 373–387.

1967. The theory of island biogeography. Monographs in population biology, Vol. 1. Princeton University Press, Princeton, NJ. 203 p.

MANITOBA DEPARTMENT OF MINES, RESOURCES, AND ENVIRONMENTAL MANAGEMENT. 1982. Water flow reports, January to December 1982. Province of Man., Winnipeg, Manitoba.

1983. Water flow reports, January to December 1983. Province of Manitoba, Winnipeg, Man.

MERNA, J. W., AND P. J. EISELE. 1973. The effects of methoxychlor on aquatic biota. Rep. No. EPA R3-73-046. U.S. Environmental Protection Agency, Washington, DC. 59 p.

MINSHALL, G. W., D. A. ANDREWS, AND C. Y. MANUEL-FALER. 1983. Application of island biogeographic theory to streams: macroinvertebrate recolonization of the Teton River, Idaho, p. 279–297. *In* J. R. Barnes and G. W. Minshall [ed.] Stream ecology. Application and testing of general ecological theory. Plenum Press, New York, NY.

MINSHALL, G. W., AND P. V. WINGER. 1968. The effect of reduction in stream flow on invertebrate drift. Ecology 49: 580–582.

MUIR, D. C. G., AND A. L. YARECHEWSKI. 1984. Degradation of methoxychlor in sediments under various redox conditions. J. Environ. Sci. Health Part B Pestic. Food Contam. Agric. Wastes 19: 271–295.

NATIONAL RESEARCH COUNCIL OF CANADA. 1983. Impact assessments in lotic environments-methoxychlor. NRCC No. 20645. Associate Committee on Scientific Criteria for Environmental Quality. National Research Council of Canada, Ottawa, Ont. 130 p.

ROSENBERG, D. M., AND V. H. RESH. 1982. The use of artificial substrates in the study of freshwater benthic macroinvertebrates, p. 175–235. *In* J. Cairns, Jr. [ed.] Artificial substrates. Ann Arbor Science Publishers, Ann Arbor, MI.

SANDERS, H. O., AND O. B. COPE. 1968. The relative toxicities of several pesticides to naiads of three species of stoneflies. Limnol. Oceanogr. 13: 112–117.

SCHERER, E., AND R. E. MCNICOL. 1986. Behavioural responses of stream-dwelling *Acroneuria lycorias* (Ins., Plecopt.) larvae to methoxychlor and fenitrothion. Aquat. Toxicol. (Amst.) 8: 251–263.

SEBASTIEN, R. J. 1986. The impact of methoxychlor on selected non-target organisms in a riffle of the Souris River, Manitoba. Ph.D. thesis, University of Manitoba, Winnipeg, Man. 184 p.

SEBASTIEN, R. J., AND W. L. LOCKHART. 1981. The influence of formulation on toxicity and availability of a pesticide (methoxychlor) to black fly larvae (Diptera:Simuliidae), some non-target aquatic insects and fish. Can. Entomol. 113: 281–293.

SEBASTIEN, R. J., D. M. ROSENBERG, AND A. P. WIENS. 1988. A method for subsampling unsorted benthic macroinvertebrates by weight. Hydrobiologia 157: 69–75.

SNEDECOR, G. W., AND W. G. COCHRAN. 1981. Statistical methods. 7th ed. Iowa State College Press, Ames, IA. 507 p.

STAINTON, M. P., M. J. CAPEL, AND F. A. J. ARMSTRONG. 1977. The chemical analysis of fresh water. 2nd ed. Fish. Mar. Serv. Misc. Spec. Publ. 25: 166 p.

TAYLOR, L. R. 1961. Aggregation, variance and the mean. Nature (Lond.) 189: 732–735.

TOWNSEND, C. R., AND A. G. HILDREW. 1976. Field experiments on the drifting, colonization, and continuous redistribution of stream benthos. J. Anim. Ecol. 45: 759–772.

WALLACE, J. B., D. S. VOGEL, AND T. F. CUFFNEY. 1986. Recovery of a head-

water stream from an insecticide-induced community disturbance. J. North Am. Benthol. Soc. 5: 115–126.
WALLACE, J. B., J. R. WEBSTER, AND T. F. CUFFNEY. 1982. Stream detritus dynamics: regulation by invertebrate consumers. Oecologia (Berl.) 53: 197–200.
WALLACE, R. R., AND H. B. N. HYNES. 1975. The catastrophic drift of stream insects after treatments with methoxychlor. Environ. Pollut. 8: 255–268.
1981. The effect of chemical treatments against black fly larvae on the fauna of running waters, p. 237–258. *In* M. Laird [ed.] The future for biological methods in integrated control. Academic Press, New York, NY.
WALLACE, R. R., A. S. WEST, A. E. R. DOWNE, AND H. B. N. HYNES. 1973. The effects of experimental black fly (Diptera:Simuliidae) larviciding with abate, dursban and methoxychlor on stream invertebrates. Can. Entomol. 105: 817–831.
WALTON, O. E. 1980. Active entry of stream benthic macroinvertebrates into the water column. Hydrobiologia 74: 129–139.
WALTON, O. E., S. R. REICE, AND R. W. ANDREWS. 1977. The effects of density, sediment particle size and velocity on drift of *Acroneuria abnormis* (Plecoptera). Oikos 28: 291–298.
WESTWOOD, A. R., AND R. A. BRUST. 1981. Ecology of black flies (Diptera:Simuliidae) of the Souris River, Manitoba as a basis for control strategy. Can. Entomol. 113: 223–234.
WILLIAMS, D. D., AND H. B. N. HYNES. 1976. The recolonization mechanisms of stream benthos. Oikos 27: 265–272.

APPENDIX A. Sample ANOVA table — comparisons of drift density data for the mayfly, *Caenis tardata*.

Source	*df*	SS	MS	*F*-value	Pr > F
Site	1	3.86	3.86	15.88	0.0163
Replicate (site) (error A)	4	0.97	0.24		
Date	6	53.32	8.89	37.20	0.0001
Site × Date	6	36.33	6.06	25.34	0.0001
(PreC[a] + 15 Jul 82T[b]) − (PreT[c] + 15 Jul 82C[d])	1	6.90	6.90	28.90	0.0001
(PreC + 21 Jul 82T) − (PreT + 21 Jul 82C)	1	10.86	10.86	45.45	0.0001
(PreC + 29 Jul 82T) − (PreT + 29 Jul 82C)	1	6.66	6.66	27.89	0.0001
(PreC + 17 Aug 82T) − (PreT + 17 Aug 82C)	1	4.09	4.09	17.14	0.0004
(PreC + 03 Aug 83T) − (PreT + 03 Aug 83C)	1	1.00	1.00	4.17	0.0523
Replicates (site × date) (error B)	24	5.73	0.24		
Total	41	100.22			

[a]PreC = pretreatment control site.
[b]15 Jul 82T = 15 July 1982 treatment site.
[c]PreT = pretreatment treatment site.
[d]15 July 82C = 15 July 1982 control site.