AQUATIC FATE AND IMPACT STUDIES WITH DIMILIN®

File Report No. 78 January 1987

P. KINGSBURY, K.M.S. SUNDARAM, S. HOLMES
R. NOTT and D. KREUTZWEISER

Forest Pest Management Institute
Canadian Forestry Service
Sault Ste. Marie, Ontario
P6A 5M7

INTRODUCTION

Diflubenzuron is an insecticide whose activity is based on disruption of chitin deposition. Commercial products of this material have been developed, registered and marketed under the trade name DIMILIN®[1]. The mode of action of these products tends to make them more selective towards arthropods and safer to vertebrates than other broader spectrum insecticides whose activity is based on physiological systems common to invertebrates and vertebrates. This can be an advantage in use patterns such as forest insect control where a very wide range of non-target organisms may be exposed to the control agent.

Dimilin has been found to be a promising tool in protecting Canadian forests from defoliating lepidoptera such as gypsy moth *Lymantria dispar* L., hemlock looper, *Lambdina fiscellaria fiscellaria* (Guen.), and oak leaf shredder, *Croesia semipurpurana* (Kft.). Before Dimilin can be registered for forestry uses, regulatory agencies require the submission of extensive data packages detailing the effectiveness and safety of the compound. Extensive literature exists on the effects of diflubenzuron on aquatic ecosystems due to its testing and use for mosquito control programs and other purposes. It was, however, felt that further data were required on the fate and impacts of Dimilin entering forest ponds under Canadian conditions before registrations for forest insect control could be granted in Canada. The Forest Pest Management Institute conducted such studies in Ontario in 1986 in a co-operative program with Pfizer and Duphar B.V., the companies interested in registering and marketing Dimilin for forestry use in Canada. This report presents the details of the aquatic fate and impact work conducted.

---

[1] DIMILIN® is a registered trademark and product of DUPHAR B.V., Weesp, Holland. Subsequently referred to as Dimilin in this report.

STUDY SITE DESCRIPTION

The study was carried out on privately owned land in Kaladar Township, Lennox and Addington County, Ontario. A 25 ha forest block approximately 3 km South-East of Flinton, Ontario was treated with Dimilin to evaluate fate and impact on two pond ecosystems. The treatment block was situated in an area close to the southern edge of the Canadian shield whose topography has been heavily influenced by glacial activity. The area is characterized by long rock ridges running in a north-east south-west direction covered with a mixed forest of hardwoods and softwoods including oaks *Quercus* spp., pines *Pinus* spp., maples *Acer* spp., poplars *Populus* spp. and birches *Betula* spp. Between the rock ridges are low areas whose drainage patterns are heavily influenced by beaver activity. The treatment and control ponds utilized in this study are all part of the Flinton Creek drainage system (Fig. 1) and were all created by beaver dam building activity. The treatment ponds are at least seven years old according to available maps which show them present in 1979. This is supported by the extensive shrub growth on their beaver dams. Outflow from these ponds appeared to be minimal during the study period. The pond used as a control site for benthos studies is a damned up portion of Flinton Creek, with considerable outflow throughout the study period. A second adjacent pond above another beaver dam with negligible outflow was used as a control for zooplankton studies.

All the study ponds are rather shallow, but differ from each other in a number of other respects. Treatment pond 1 is substantially smaller and slightly deeper than the other ponds (Table 1) and exhibited

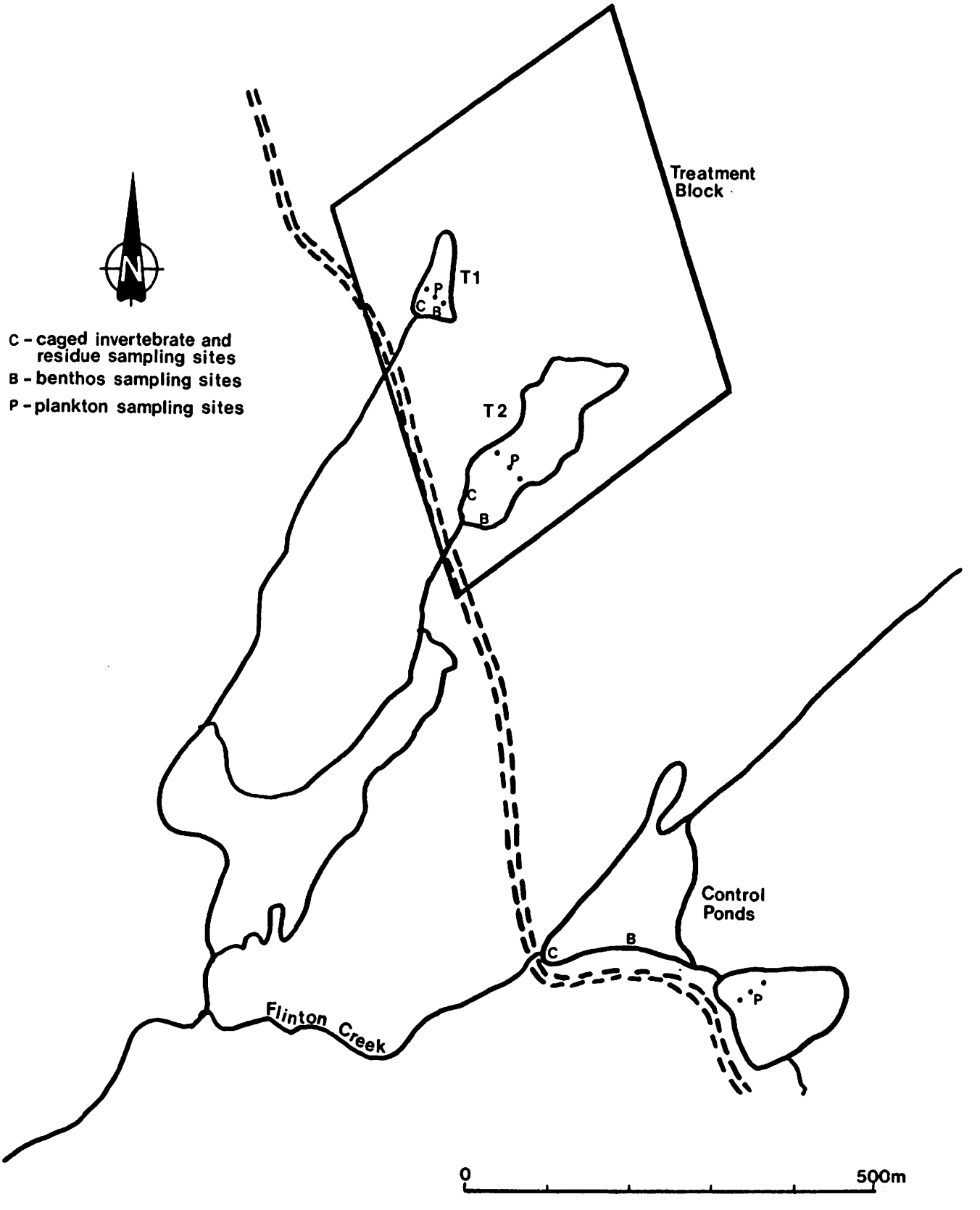

Figure 1. Treatment and control sites for the Dimilin aquatic impact studies.

Table 1

Physical and chemical characteristics of the study ponds based on data from zooplankton sampling sites

	Treatment Pond T1	Treatment Pond T2	Control Pond C
Area (ha)	0.32	1.4	1.0
Mean depth (m)	1.3	0.8	0.9
Maximum depth (m)	1.5	1.0	1.2
pH	5.8	6.2	6.2
Turbidity (JTU)	60	12	33
Total alkalinity (mg/L $CaCO_3$)	6.8	6.0	19.9
Water temperature (°C) Surface-Bottom			
8 June	25-12	23-21	22-16
9 July	24-14	27-25	26-21
12 Aug.	23-18	24-22	24-18
23 Sept.	14-13	14-13	13-12

a greater degree of thermal stratification throughout the spring and early summer. It was also consistently more turbid than the other ponds. Treatment pond 2, the largest and shallowest pond, never showed more than a 2°C difference in the temperatures of its surface and bottom waters throughout the entire study. The untreated control pond used for zooplankton studies was intermediate between the two treatment ponds in size, depth, degree of thermal stratification and turbidity. This pond was unique among the others in terms of the extensive degree to which floating aquatic vegetation covered its surface by mid-summer.

METHODS

Spray application

Dimilin was applied to the 25 ha pond study blocks between 0640 and 0700 EDT on 5 June 1986. Application was made by a Piper Pawnee

Brave equipped with four Micronair® AU4000 atomizers calibrated to emit 10 L spray mix/ha while flying 45 m swaths. Aircraft speed was 160 km/h with spray height about 20 m above the canopy. Spray was applied starting at the south edge of the block flying towards the north so as to minimize risk of extending the spray in the direction of the control pond due to late shut-off. Both treatment ponds were directly sprayed, receiving the full impact of numerous passes flown perpendicular to their long axis. Mean windspeed and direction over the application period measured at a height of 6 m with a portable recording weather station averaged 7.2 km/h from the north east. Air temperature was 13.6°C, relative humidity 85% and cloud cover almost total at the time of spraying. The spray mix consisted of 96.2% water, 2.8% DIMILIN 25 W and 1.0% Rhodamine® B dye by weight giving an application rate of 70 g active ingredient/ha.

Sampling of Substrates for Residue Analysis

Water, sediment, aquatic plants and fish were collected from the sampling sites in the control and spray area ponds prior to the spray application to confirm the absence of Dimilin and to determine the presence of any naturally occurring compounds which might interfere with the residue analyses. Following the spray application, various substrates were sampled at 1, 3, 6, 12 h, 1, 2, 3, 5, 7, 10, 15, 20, and 30 d postspray according to the following sampling procedures.

Water: From each site, water was sampled by dipping a clean wide-mouthed 1L-Teflon bottle about 1 cm below the water surface allowing approximately 300 mL of water to flow into the container without stirring up and entraining bottom sediment. The process was

repeated in triplicate at various locations to obtain representative samples each consisting of approximately 900 mL of pooled pond water. Each bottle was then tightly sealed with the Teflon screw cap, labelled and immediately stored in a cooler with ice packs at 0°C and transported to the field laboratory where it was kept frozen at -20°C until analysis.

Sediment: Pond sediment samples in triplicate were taken from the same locations as the water samples. At each sampling site, a clean wide-mouthed amber coloured 500 mL glass jar was lowered to the bottom and 1 cm of undisturbed surface sediment was scooped by gently moving the jar around until it became half-filled with sediment. The bottle was tightly sealed with a Teflon-lined screw cap, brought to the surface and decanted to remove all the water. The sample was then labelled and further processed as described above.

Aquatic Plants: Manna grass *(Glyceria borealis)* was sampled from around the same vicinity as where the pond water samples were collected. Whole plants were up-rooted, rinsed with water to remove any adhering sediment or debris and then tightly squeezed to remove excess water. The plants were then wrapped in aluminum foil, placed in polyethylene bags, labelled and processed as above.

Fish: Caged creek chub *[Semotilus atromaculatus* (Mitchill)] purchased at a local bait store were sampled from control and spray ponds prior to the spray application and at 1 and 3 d postspray intervals. The creek chub (mean wt. 14 ± 2 g, mean length 13 ± 1 cm) were housed in conical shaped cages (20 cm dia. x 80 cm length) constructed of 13 mm mesh aluminum screening with 10 fish per cage to form a composite sample

at each sampling time per pond. At each sampling period, one cage was removed and the fish euthanized. The composite fish sample was then wrapped in aluminum foil, labelled and stored for analysis as described above.

Analytical Procedures

The analytical methods used in the present study, e.g. extraction, cleanup and analysis of substrates, are the modified methods of Diprima *et al.* (1978) and Duphar B. V. (1982, 1985). The derivitization methods reported in these sources were not required for the various substrates studied since no co-extractive impurities were found to interfere with the HPLC analyses.

Water: The water samples were allowed to thaw and 300 mL aliquots of each sample were extracted separately three times, each time using 75 mL of pesticide grade dichloromethane. The pooled organic phase of each sample was dried by passing through a column of anhydrous Na_2SO_4, flash evaporated gently to dryness and reconstituted in HPLC grade acetonitrile. The CH_3CN solution was then concentrated to a volume of 1 mL in a stream of dry N_2 (Meyer N-evap) and passed through a 0.45 µm filter (Millipore) for HPLC analysis without any further cleanup.

Sediment: The thawed sediment samples were filtered under suction to remove excess water. Ten gram aliquots of each sediment in triplicate were refluxed with 15 mL distilled water and 150 mL pesticide grade CH_3CN for 30 minutes. The cooled mixture was filtered through a Whatman #1 filter paper and then flash evaporated to approximately 10 mL to remove the acetonitrile. The concentrated extract was brought up to

a volume of 100 mL with distilled H_2O and partitioned three times with 50 mL of pesticide grade hexane. The pooled hexane was then flash evaporated gently to dryness and reconstituted in 3 mL of pesticide grade dichloromethane and 25 mL of pesticide grade petroleum ether (pet. ether) for column cleanup.

The column used for cleanup was prepared by filling a chromatographic tube (40 cm length x 10 mm ID, fitted with a sintered glass frit and 100 mL reservoir) with 25 cm Florisil (60 - 100 mesh, 5.5% deactivated with H_2O) and topping off with a glass wool plug. The column was pre-washed with 100 mL pet. ether and the flow rate was adjusted to 2 drops/second by applying gentle suction. After pre-wash, the sample in CH_2Cl_2/pet. ether was added to the column followed by 25 mL of pet. ether rinses. The column was eluted successively with 45 mL of pet. ether, 30 mL of acetone/pet. ether (1:9) and finally with 10 mL of acetone/pet. ether (1:4). All eluates were discarded. The diflubenzuron was then eluted from the column with a further addition of 50 mL acetone/pet. ether (1:4). The eluate was collected and flash evaporated gently to dryness. The residue was then taken up in 10 mL of HPLC grade CH_3CN to give a sample concentration of 1 g sediment/1 mL CH_3CN for HPLC analysis.

Aquatic Plants: The thawed aquatic plants were rinsed well in distilled H_2O and the excess water was squeezed out. The plants were then dried between folds of absorbent paper and chopped into fine pieces. The plant tissues (triplicate of 10 g) were then extracted gently by macerating in 2 x 50 mL of pesticide grade CH_2Cl_2 for 5 minutes using a Polytron® (PT-20). The plant extracts corresponding to

each sample were filtered through glass wool to remove particulate matter. They were pooled and flash evaporated just to dryness. The sample was then taken in 3 mL CH_2Cl_2 and 25 mL pet. ether for column cleanup as described above.

Fish: After the creek chub samples were allowed to thaw, four to six fish from each composite sample were chopped into small pieces using a sharp knife and mixed thoroughly, following the removal of all internal organs. Ten gram aliquots in triplicate of cut up fish with 20 g of Na_2SO_4 were homogenized with 3 x 50 mL CH_2Cl_2 in a Polytron® (Type PT-20) for 3 minutes and the supernatant extract was filtered through a column of Na_2SO_4. The pooled extract of each sample was then flash evaporated to dryness and reconstituted in 3 mL CH_2Cl_2 and 25 mL pet. ether for column cleanup as described under sediment.

HPLC Analysis: Diflubenzuron residues present in the final extracts were analysed using a Hewlett-Packard Model 1084B high performance liquid chromatograph equipped with an automatic sampling system (HP 79842A), variable wavelength detector (HP 79875A) and a HP Model 79850B integrator. The column used was a HP:RP-8 [20 cm x 4.6 mm ID, containing MOS (methyl octyl silyl)-Hypersil, 10 µm pore size] with a mobile phase of 50:50 CH_3CN/H_2O at a flow rate of 1.0 mL/min. The variable wave-length detector was assigned a signal of 254:430 nm (sample:reference) and the solvent and column temperatures maintained at 40°C. The retention time of the pesticide at these conditions was 10.4 minutes.

Detector response was calibrated daily with analytical standard prepared in acetonitrile. Quantitation of the samples was based upon

the peak heights obtained from injections of the cleaned extracts compared to those of the external standard injections. Each value recorded in Tables 2, 3, 5, 6 and 7 is the average of triplicate analyses for each substrate at each site location along with the appropriate standard deviation (SD). Results are not corrected for extraction efficiency.

Table 2. Percent recovery of diflubenzuron from some aquatic substrates after fortification.

	% recovery		
Substrate	1.0 ppm	0.5 ppm	0.1 ppm
Sediment	90 ± 5	86 ± 7	86 ± 9
Manna grass	97 ± 3	98 ± 4	92 ± 7
Creek chub	94 ± 4	87 ± 1	91 ± 6
	1.0 ppb	0.5 ppb	0.1 ppb
Pond water	93 ± 4	86 ± 5	84 ± 8

Samples of sediment, aquatic plants and fish sampled prior to the spray application or from the control pond were fortified in triplicate with 1.0, 0.5 and 0.1 ppm (µg/g) levels of diflubenzuron while pond water was spiked with 1.0, 0.5 and 0.1 ppb (µg/L) levels. Each sample was extracted and analysed according to the methods described above to determine the extraction efficiency. The percent recovery levels and standard deviations found for each substrate are presented in Table 2. The minimum detection limit (MDL) of the pesticide was 0.1 ppb for water and 0.1 ppm (fresh weight) for the other substrates. None of the cleaned extracts of unfortified control

substrates showed any interference in the HPLC analysis corresponding to diflubenzuron.

Biological sampling

Zooplankton: Zooplankton samples were collected from the study ponds with a Schindler-Patalas plankton trap (Schindler 1969). The trap was lowered to just below the pond surface where the trap doors were allowed to close capturing a 12 L sample of water. This was then strained through the collection bucket which was fitted with NITEX® monofilament bolting cloth with 64 μ mesh openings. The collection bucket's rubber drain plug was then removed and the concentrated zooplankton sample was rinsed into a jug and preserved by adding enough formalin to give a 4% formaldehyde solution.

Nine Schindler-Patalas samples were taken from each study pond on 10 sampling periods: 5 and 2 days before and 1, 3, 5, 9, 21, 34, 68 and 110 days after treatment. On each occasion three samples were taken from each of three stations (east, mid and west) across the pond. Depths at each station were:

	East	Mid	West
T1	1.1 m	1.5 m	1.4 m
T2	0.7 m	0.8 m	1.0 m
Control	0.8 m	1.2 m	0.8 m

The asymetric depth profiles across the two treatment ponds reflect the presence of rock ridges along their west shorelines.

Zooplankton samples were counted in a gridded dish using a dissecting microscope. When large numbers of organisms were present, total numbers were extrapolated from counts of one grid from each of the six rows on the dish (eg. 6 of a possible 36 grids). The grids selected

were chosen by first numbers appearing in lists of random numbers. Numbers of phantom midge larvae, *Chaoborus* sp., present in samples were based on scans of the entire dish. No attempt was made to identify Cladocera beyond the family level because of the large numbers of samples and individuals. In the absence of species specific life history and ecology data from similar pond habitats, identifications to the species level would not likely clarify the nature of the effects seen. Larval stages (nauplii) of copepods were identified separate from later stages, but no attempt was made to distinguish between copepodid stages and sexually mature non-moulting adults. Rotifers were present in large numbers in all zooplankton samples, but no attempt was made to count or identify them except to note dramatic increases when apparent.

Caged invertebrates: Three different aquatic invertebrates were held in cages in the study ponds for an eleven day period to look for Dimilin induced mortality. Juvenile scuds or fresh-water shrimp (Amphipoda), water boatmen nymphs (Hemiptera: Corixidae) and phantom midge larvae, *Chaoborus* sp. (Diptera: Chaoboride) were collected from treatment pond 1 on 2 June by sweep netting along the shoreline. The organisms were separated from vegetation and debris in the field laboratory, and the next day (3 June) were placed in groups of ten in floating cages in the three study ponds. Each cage consisted of a round 1 L white plastic container with the bottom and two 5x10 cm windows on opposite sides cut out and covered with fine mesh cloth screening. Five cages of each type of organism were inserted in holes in a large sheet of styrofoam which was then moored at the shoreline of the pond.

When the cages were checked on 4 June (one day prior to treatment) survival of amphipods and phantom midge larvae was found to be

reasonable, with only 3.3 and 2.7% mortality recorded. Water boatmen survival was found, however, to be very poor with 17.3% mortality observed. Dead amphipods and phantom midge larvae were replaced with live individuals, but no attempt was made to restock water boatmen cages. Thereafter mortality was assessed daily and dead individuals were removed without replacement.

On 7 June, two days after treatment, groups of phantom midge larvae and pupae which had been collected before treatment and reared in the field laboratory were caged in the study ponds in the same fashion to look for residual activity of diflubenzuron residues persisting to this point. Due to limited numbers of organisms available, three groups of 10 (T2), 8 (T1) and 5 (Control) larvae and one group of 5 pupae (all ponds) were used for this aspect of the study.

Benthos: Benthic invertebrate populations were assessed by sweep sampling from selected portions of shoreline in the study ponds 4 days before and 3, 9, 21, 34, 68 and 110 days after treatment. Sweep samples were taken using a long-handled D-frame net which was 29 cm wide and had an 800 μ mesh. The net was extended out from the shore of the pond toward the center a distance equal to the length of the handle plus the net (1.5 m) and then drawn back along the bottom to the shoreline. Five samples were collected from each pond on each sampling date and preserved immediately with formaldehyde. Organisms were later sorted, counted and identified in the laboratory.

RESULTS AND DISCUSSION

Fate of Dimilin in a pond environment

Residues in pond water: The concentrations (ppb) of diflubenzuron found in the top 1 cm of water collected at intervals of time from the ponds are given in Table 3 and represented in Figure 2. From the data, it is apparent that T-1, the smaller pond received a higher initial concentration (13.82 ppb) of the chemical than T-2, the larger pond (5.90 ppb). The initial dissipation of diflubenzuron in T-1 was quite rapid (DT_{50} = 0.4 days), and after a period of 2 days the chemical persisted at levels only slightly higher than those observed in T-2. The probable cause for this faster rate of dissipation in T-1 than in T-2 is that dilution effects in T-1 would be more noticeable due to

Table 3. Average concentrations of diflubenzuron in pond water collected at intervals of time following aerial spray application

Time after application	Average (n=3) concentration (ppb) of diflubenzuron	
	T-1	T-2
Prespray	N.D.	N.D.
1 h	13.82 ± 1.17	5.90 ± 0.21
3	9.67 ± 0.81	5.87 ± 0.90
6	5.99 ± 0.43	6.09 ± 0.63
12	6.28 ± 0.99	4.22 ± 0.21
1 d	4.31 ± 0.46	2.76 ± 0.20
2	3.36 ± 0.23	2.06 ± 0.32
3	1.84 ± 0.47	1.40 ± 0.28
5	0.63 ± 0.32	0.44 ± 0.15
7	0.47 ± 0.40	0.23 ± 0.06
10	1.02 ± 0.62	0.45 ± 0.18
15	0.22 ± 0.07	0.11 ± 0.05
20	N.D.	N.D.
30	N.D.	N.D.

N.D. - Not detected; detection limit 0.10 ppb

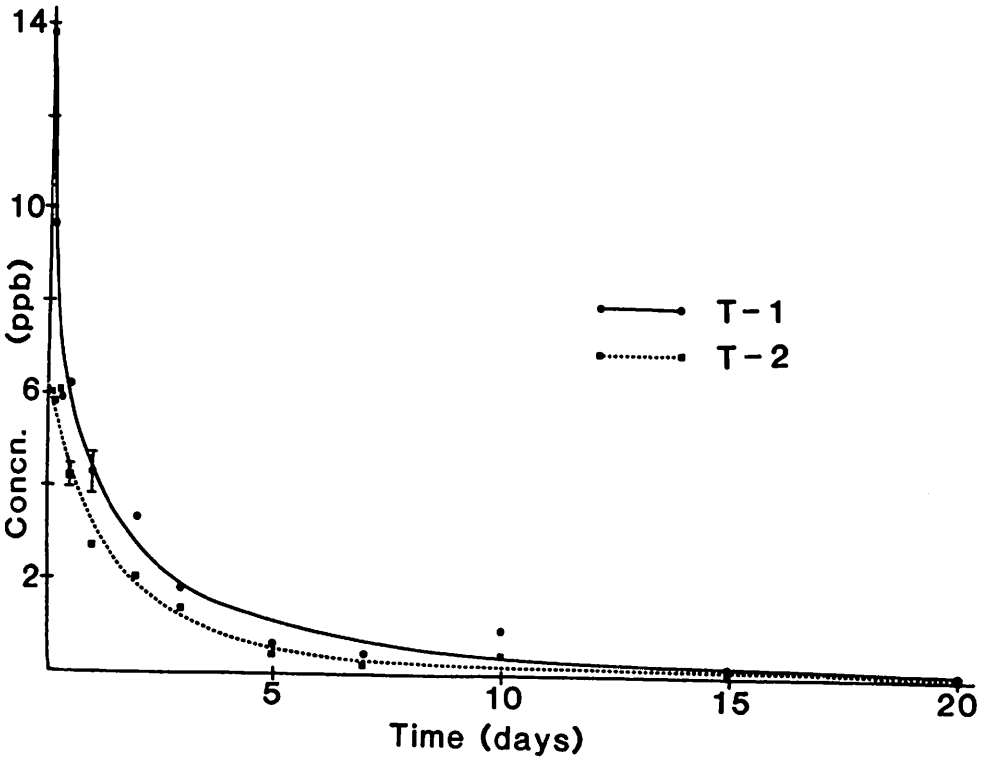

Figure 2. Diflubenzuron residues in pond water collected from the treated ponds.

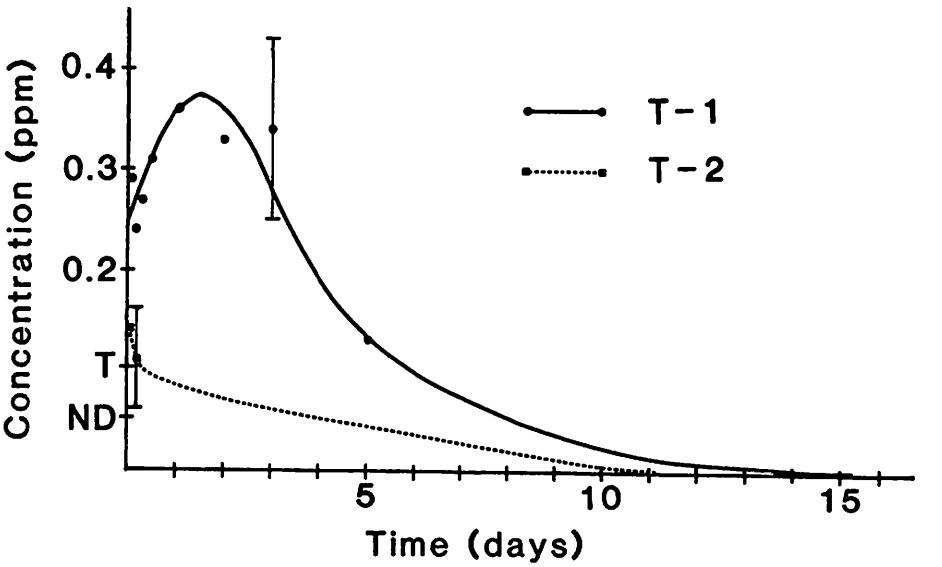

Figure 3. Diflubenzuron residues in manna grass collected from the treated ponds.

its greater average depth (T-1 = 1.30 m; T-2 = 0.80 m, Table 1). Schaefer and Dupras (1976) and Mian and Mulla (1982) found that Dimilin, when applied as a wettable powder, undergoes rapid homogeneous distribution in simulated pond environments, supporting our present hypothesis.

Another factor which could have enhanced the dilution effect is that the chemical has a tendency to adsorb onto particulate matter and since T-1 had a higher turbidity (60 JTU) than T-2 (12 JTU), even distribution would be more rapidly carried out by the suspended particles. Also, degradation by microbial organisms would be more prominent in T-1's turbid waters (Mian and Mulla, 1982).

Conversely, the higher levels of diflubenzuron noticed in T-1 over time could also be explained due to the turbid nature of the water. Since the chemical would be adsorbed onto the suspended particles, it would be less available to hydrolytic and photolytic degradations as well as the fact that photolysis would be less eminent in deeper and murkier waters. However, the chemical degraded to below the detectable level (0.1 ppb) within 20 days in both ponds. The rates of disappearance of the chemical appear to be similar to the observations noted earlier by Apperson *et al.* (1978) in ponds treated by a hand sprayer.

The dissipation of diflubenzuron in the pond waters appears to follow an exponential decay pattern, according to equations (1) to (6):

$$Y = Be^{-Ct} \qquad (1)$$

$$\log (Y) = \log (B) - (C/2.303)\, t \qquad (2)$$

$$Y = B \text{ (when } t = 0) \qquad (3)$$

$$Y = 0 \text{ (when } t = \infty) \qquad (4)$$

$$DT_{50} = (2.303 \log 2)/C \quad (5)$$

$$DT_{90} = (2.303 \log 10)/C \quad (6)$$

In the above equations, 'B' represents the percent of A.I. concentrations decayed and 'C' is the dissipation rate constant (the rapidity with which the residues are lost, i.e., the greater the value of 'C', the faster the decay).

Non-linear regression analysis of the data in Table 3 yielded the numerical constants presented in Table 4 which gives DT_{50} and DT_{90} values of 0.4 and 1.3 d and 1.4 and 4.2 d respectively for T-1 and T-2. Similar degradation patterns were found for some forestry substrates studied by Sundaram (1986).

Table 4. Decay characteristics of pond water residues of diflubenzuron following its aerial application and regression coefficients B and C of the exponential decay equation $Y = Be^{-Ct}$.

Sample site	B[a]	C[b]	DT_{50}[c] (days)	DT_{90}[d] (days)	R^2[e] (%)
T-1	100.0	0.0702	0.4	1.4	85.9
T-2	100.0	0.0228	1.3	4.2	97.2

[a] Percent of a.i. concn. decayed
[b] Decay constant
[c] Dissipation time for 50% of the initial concn.
[d] Dissipation time for 90% of the initial concn.
[e] Coefficient of determination

Residues in Sediment: The concentrations of diflubenzuron observed in the pond sediments collected at intervals of time are given in Table 5. From the data, it appears that the chemical does not accumulate or persist in sediment to any great extent as was demonstrated in the study by Apperson *et al.* (1978) where no residues were found in the sediments of diflubenzuron treated ponds and lakes. How-

ever, the values obtained from the small pond show that sediment could possibly act as a sink for the chemical (max. concn. = 0.24 ppm at 1 d) but the concentrations found are just above the detectable limits and consequently, any relationship derived could not be adequately justified. Also, the values obtained for T-2 do not illustrate a similar tendancy (max. concn. = 0.16 ppm at 3 h) but again the chemical existed only at or near trace levels (0.05 - 0.10 ppm) and is very short-lived. Non-detectable levels were reached within 3 days (post spray) in T-2 and 5 days in T-1. Therefore, no reliable determination of the DT_{50} of DT_{90} values could be derived due to the transient nature of the data obtained.

Table 5. Average concentrations of diflubenzuron in sediments collected at intervals of time following aerial spray application

Time after application	Average (n=3) concentration (ppm) of diflubenzuron	
	T-1	T-2
Prespray	N.D.	N.D.
1 h	N.D.	N.D.
3	T	0.16 ± 0.06
6	0.13 ± 0.05	T
12	0.11 ± 0.04	T
1 d	0.24 ± 0.08	T
2	0.12 ± 0.06	0.14 ± 0.04
3	T	N.D.
5	N.D.	N.D.
7	N.D.	N.D.

N.D. - Not detected; detection limit 0.10 ppm
T - Trace; 0.05 - 0.10 ppm

Residues in aquatic plants: The residue levels of diflubenzuron in manna grass collected at intervals of time are given in Table 6 and shown in Figure 3. The maximum concentration found was 0.36 ppm in T-1 at 1 day. The data from T-1 indicate that

Table 6. Average concentrations of diflubenzuron in an aquatic plant (Manna Grass)* collected at intervals of time following aerial spray application

Time after application	Average (n=3) concentration (ppm) of diflubenzuron	
	T-1	T-2
Prespray	N.D.	N.D.
1 h	0.29 ± 0.11	0.14 ± 0.06
3	0.24 ± 0.08	0.11 ± 0.05
6	0.27 ± 0.13	T
12	0.31 ± 0.09	T
1 d	0.36 ± 0.08	T
2	0.33 ± 0.07	T
3	0.34 ± 0.09	T
5	0.13 ± 0.04	T
7	T	N.D.
10	N.D.	N.D.
15	N.D.	N.A.

* - Manna grass, *Glyceria borealis*
N.A. - Not analysed
N.D. - Not detected; detection limit 0.10 ppm
T - Trace; 0.05 - 0.10 ppm

manna grass acted as a sink for the chemical by gradually taking up the chemical and then releasing it over a period of time. The residue levels found in manna grass from T-2 were at or near trace levels (0.05 - 0.10 ppm) which were too low to discern any similar conclusion. The chemical dissipated from the plants to non-detectable levels at 7 days and 10 days postspray for T-2 and T-1 respectively. Work done by other authors (Booth and Ferrell 1977, Metcal *et al.* 1975) show that diflubenzuron is degraded in aquatic vegetation over time.

Residues in fish: The concentrations of diflubenzuron in creek chub collected at intervals of time are given in Table 7. The chemical apparently was not taken up by the fish to any great extent as only the 1 day samples showed detectable levels. The residue levels were 0.11 ppm in fish from T-2 compared to the trace levels found in specimens from T-1. Since creek chub are a bottom feeding fish, uptake

Table 7. Average concentrations of diflubenzuron in caged creek chub* from ponds after aerial spray application.

Time after application	Number of fish analysed		Avg tail length (mm)		Avg body mass (g)		Avg residue (ppm)	
	T-1	T-2	T-1	T-2	T-1	T-2	T-1	T-2
Prespray	6	7	128	132	13.1	14.7	N.D.	N.D.
1 d	5	6	136	123	12.9	15.2	T	0.11 ± 0.07
3	5	4	129	117	14.2	13.9	N.D.	N.D.

* Creek chub, *Semotilus atromaculatus* (Mitchill); all internal organs of the fish were removed and the remaining tissues were analysed for diflubenzuron.
N.D. - Not detected; detection limit 0.05 ppm on wet wt. basis.
T - Trace; 0.05 - 0.10 ppm.

of the chemical was probably obtained from the pond sediment which may account for the low levels observed. Mian and Mulla (1982) also showed that diflubenzuron is not accumulated by fish species. No mortality was observed for any of the treated fish studied during the course of the experiment.

Biological effects of Dimilin in a pond environment

Zooplankton: Pre-treatment samples revealed some substantial differences in the composition of zooplankton populations in the three study ponds (Table 8, Appendix 1). Daphnidae were present in similar numbers in all three ponds, but were the only significant component of the Cladoceran fauna in T-1 whereas Bosminidae were also an important component in T-2 and the control pond. Bosminidae were somewhat less abundant than Daphnidae in T-2, but were 5 to 10 times more numerous in the control pond. Phantom midge larvae, *Chaoborus* sp. (Diptera: Chaoboridae) were only abundant in T-1 samples. Differences between zooplankton populations at the three stations within each study

Table 8. Zooplankton catches from diflubenzuron treated and an untreated control pond near Kaladar, Ontario, May-September 1986. Expressed as mean and standard deviation of nine 12 L Shindler-Patalas trap samples per pond

Date		31 May	3 June	6 June	8 June	10 June	14-15 June*	26 June	9 July	12 Aug.	23 Sept.
Days before or after treatment		-5	-2	+1	+3	+5	+9	+21	+34	+68	+110
Treatment Pond 1 (T-1)											
Cladocera:	Daphnidae	634±441	340± 157	149± 107	6±7	0	0	0	0	0.2±0.6	235±218
:	Chydoridae	0.7±1.0	0.2± 0.4	0.3± 1.0	0.1±0.3	0	0	0.1±0.3	0	0.1±0.3	0
Copepoda:	nauplii	788±312	353±161	410± 123	0.3±0.7	0	0	11± 7	68± 26	280±196	400±370
:	copepodids and adults	234± 70	289± 97	221± 76	9± 5	11± 9	7± 5	16± 10	10± 5	92± 44	78± 69
Diptera:	Chaoboridae	19± 9	50± 8	8± 5	21± 9	13± 7	21± 9	4± 3	0.1±0.3	0.4±0.7	1.6±0.7
Treatment Pond 2 (T-2)											
Cladocera:	Daphnidae	203±141	205± 58	142± 122	1± 2	0	0	0	0	991±151	648±304
:	Bosminidae	77± 21	128±129	40± 19	6± 11	0.1±0.3	1± 2	0.3±1.0	0	775±453	26± 24
Copepoda:	nauplii	776±134	1203±206	516± 172	43± 23	1± 2	89± 37	342±206	75± 48	741±401	152± 57
:	copeodids and adults	421±216	453±140	372± 100	212± 59	78± 49	119± 70	211±116	109± 47	451±144	481±192
Diptera:	Chaoboridae	0	0.1±0.3	0	0	0	0.1± 0.3	0	0	0.1±0.3	0
Untreated Control Pond (C)											
Cladocera:	Daphnidae	364±156	405± 138	217± 104	1171± 283	592± 194	110± 64	8± 8	5± 6	2± 3	7± 17
:	Bosminidae	1674±921	4053±1263	3547±1591	15740±5670	5382±2698	5643±1576	31± 26	45± 56	62± 50	3± 3
Copepoda:	nauplii	1582±523	1159± 489	597± 125	288± 74	265± 117	228± 77	1347±675	436±354	191± 76	604±186
:	copepodids and adults	1574±383	1555± 625	918± 597	2364±708	1221± 308	1393± 632	65± 42	272±175	29± 20	31± 25
Diptera:	Chaoboridae	0.4±1.0	0.7± 1.3	0.1± 0.3	7± 7	22± 14	0	0.2±0.7	0.1±0.3	0.3±0.5	0

* T-1 and C sampled 14 June p.m., T-2 sampled 15 June a.m.

Table 9. Percentage of sampling dates (N=10) when significant* differences in numbers of organisms were found in zooplankton samples from three stations within study ponds.

	T-1	T-2	C
Cladocera: Daphnidae	20	10	40
Bosminidae	-	0	30
Copepoda: nauplii	20	30	20
copepodids and adults	10	10	60

*$p<0.05$

pond were tested with one way ANOVA accepting significance at $P<0.05$. The raw data were log (X+1) transformed to approach normality before testing for differences. Results of the tests are indicated in the tables of Appendix 1 and summarized in Table 8.

The results of the ANOVA tests show that in both treatment ponds significant differences between numbers of organisms at the three stations sampled (east, mid, west) were detected in only 12 to 16% of the possible cases, and in no more than 30% of the sample periods for any group of organism (copepod nauplii in T-2). Considerably more variability was found between the three stations within the control pond. In 37.5% of the cases, numbers of organisms were significantly different, and copepodid and adult copepod numbers were significantly different on 60% of the sampling dates. There were, however, few indications that any one station in the control pond had consistently higher or lower numbers of any group of organism. In light of the results of these analyses it was felt that the impact of Dimilin on zooplankton could be assessed by comparisons of pooled catches from all three stations in each of the study ponds.

The Dimilin application had a dramatic effect on zooplankton populations in the two treated ponds (Table 8, Fig. 4). The effects in T-1 appeared to be somewhat greater and more prolonged in duration than

in T-2. Cladocera virtually disappeared from samples from both ponds by the fifth day after spraying, not re-appearing in T-2 until August and T-1 until September. Copepods were somewhat less affected than cladocerans in both ponds. Nauplii disappeared from T-1 samples by 5 days post-spray, re-appearing in small numbers by 21 days post-spray. Small numbers of adult copepodids persisted in T-1 samples throughout the post-spray period. Substantially less impact on copepods was evident in T-2. Nauplii numbers declined to very low levels by 5 days post-spray but rapidly increased over the next two weeks. Copepodid and adult copepods in T-2 were only moderately depressed after the treatment. A further difference in zooplankton response to treatment in the two ponds was a dramatic increase in rotifers, primarily *Kellicottia* and *Polyarthra*, which was readily noticeable in T-1 samples 9 and 21 days post-spray but never observed in T-2. Similar dramatic increases in rotifer populations after crustacean mortality due to organophosphate insecticide treatments have been reported by Hurlbert et al. (1972).

Zooplankton populations in the control pond showed a pattern of increase to high numbers followed by a subsequent decline in the two weeks after the treated ponds were sprayed. Following this, zooplankton numbers in the control pond, especially cladoceran populations, fell to and remained at quite low levels. This is a normal pattern for cladocera in pond situations, where small numbers of females survive overwinter or hatch from resting eggs. As water temperatures increase, rapid parthenogenetic reproduction occurs to give large populations, which then wane to small numbers during the summer (Pennak, 1978). A second population pulse may occur in the fall, but Pennak reports that

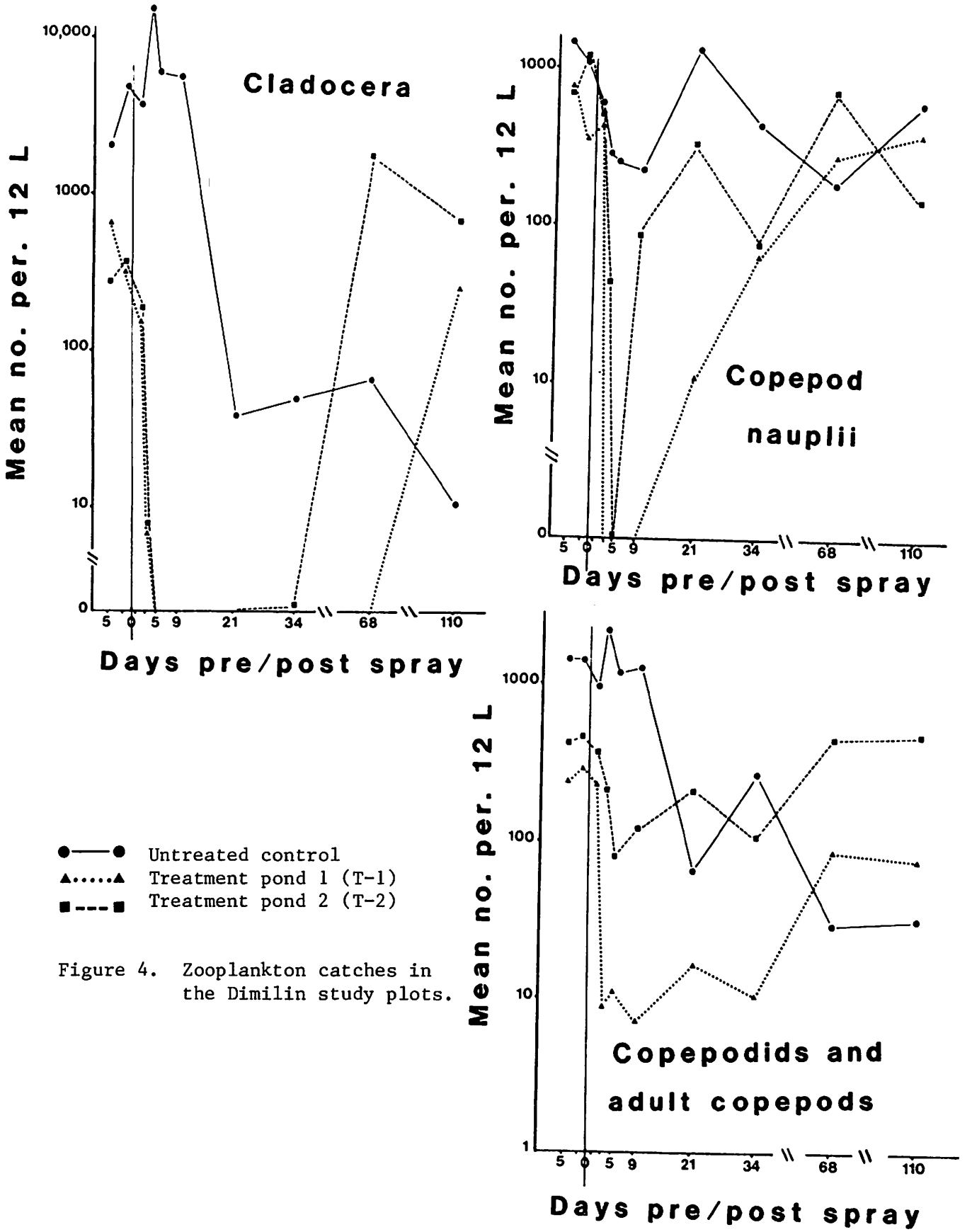

Figure 4. Zooplankton catches in the Dimilin study plots.

not only is this unpredictable, but the same species may show different population curves in two adjacent water bodies in the same year or in the same pond from year to year. In light of this, it is difficult to evaluate at what point and to what extent recovery of zooplankton in the treated ponds took place. The natural depression of numbers in mid-summer may have contributed to the prolonged periods when cladocera were virtually absent from T-1 and T-2 samples.

The influence of the Dimilin impacts observed on the reproductive dynamics of cladocera in the treated ponds was not determined during this study. The impacts apparently occurred prior to the period of intensive parthenogenetic female reproduction at the end of which males and sexual females generally appear and produce fertilized eggs which can overwinter as ephippia (Pennak, 1978). Sexual reproduction can also take place by the same means following an autumn pulse in cladoceran populations, so a return of reasonably large numbers of cladocerans to the treatment ponds by the end of the summer may have provided sufficient over-wintering forms to ensure normal populations in the spring of 1987.

The source of the cladocera which re-established populations in the treated ponds is another unanswered question. They may have originated from resting eggs, from migrants from unaffected water bodies or from surviving individuals occupying other areas of the ponds. One observation which supports the third possibility was the presence of Daphnidae in readily observable numbers in shoreline sweeps taken on 12 August from T-1. Cladocera were still virtually absent from the zooplankton samples collected at this time, suggesting a recolonization of

shoreline areas before reappearance in the deeper portions of the pond. Such an occurrence has been noted in a lake where cladocerans disappeared after treatment with a synthetic pyrethroid insecticide and reappeared in numbers at a shoreline sampling station a month prior to reappearing at a mid lake station (Kingsbury, 1976).

Numbers of phantom midge larvae, *Chaoborus* sp., captured in zooplankton samples in T-1 declined gradually over the study period. During the immediate post-spray period many of the caged *Chaoborus* larvae were pupating and emerging. Recruitment of a new generation also appeared to be occurring at this time, judging from the sudden appearance of large numbers of very small *Chaoborus* larvae in zooplankton samples from the control pond on 8 and 10 June. For some unknown reason, these new recruits to the control pond largely disappeared by their lowest levels in zooplankton catches from T-1, they were found in peak abundance in shoreline sweeps from that pond (Appendix 2). This suggests that *Chaoborus* larvae may not have been directly affected by toxic effects of Dimilin , but rather that they redistributed within the pond in response to the disappearance of their microcrustacean prey from mid-pond waters.

Numerous other field studies in lakes and ponds, mostly carried out in the Southwestern United States, have shown that Dimilin applications can adversely impact crustacean zooplankton, especially cladocerans (Ali and Mulla 1978 a and b, Apperson et al. 1978, Colwell and Schaefer 1980). These studies have also shown the lack of effects on rotifers. Recovery periods of from 2 weeks up to six months are reported by these authors for various organisms, with recovery appearing

to be somewhat faster in pond than lake situations. Helson and Surgeoner (1977) documented moderate to severe effects on cladocera and moderate effects on copepods in southwestern Ontario pools treated at 45 g AI/ha in mosquito control trials. The results of the current study confirm that Dimilin will impact on zooplankton in Canadian forest ponds in a similar manner.

Caged invertebrates: Interpretation of the results of caged invertebrate studies is confounded by the high levels of control mortality among all three groups of organisms. Although a longer period of acclimation may have reduced this problem, it is felt that the main difficulty was in providing organisms with suitable cage conditions for good survival and still being able to make daily observations on all individuals with minimal handling stress. In this study, it appeared that cage conditions were less than optimal although they did allow good observation conditions.

Post-spray mortality of caged *Chaoborus* was similar in all the study ponds (Table 10). A greater proportion of caged larvae pupated in the treated ponds than in the control pond over the caging period. Pupal mortality was high among all groups but highest in T-1. *Chaoborus* caged in the control pond and T-1 two days after Dimilin application experienced relatively little mortality over the next seven days (Table 11). Survival of *Chaoborus* set up at the same time in T-2 was much poorer.

There is little indication in these data of a substantial negative impact of the Dimilin application on *Chaoborus*.

Table 10. (A) Percent mortality and (B) developmental status at the end of the caging period of *Chaoborus* larvae caged before treatment in the diflubenzuron study ponds near Kaladar, Ontario, 5-14 June, 1986.

(A)	Spray Day	Days After Application 1	2	3	4	5	6	7	8	9
Control	0	12	28	48	54	56	58	58	60	62
T-1	6	10	24	42	48	48	54	60	62	64
T-2	2	6	16	39	47	53	59	65	67	67

(B)	Number of larvae caged	Percentage of larvae who: Survive as larvae	Die as larvae	Pupate	Percentage of pupae who: Survive as pupae	Die as pupae	Emerge as adults
Control	50	32	36	32	6	81	12
T-1	50	36	20	44	0	100	0
T-2	51	27	29	43	0	86	13

Table 11. (A) Percent mortality and (B) developmental status at the end of the caging period of *Chaoborus* caged two days after treatment in the diflubenzuron study ponds near Kaladar, Ontario, 5-14 June, 1986.

(A) Days after application	3	4	5	6	7	8	9
Days after caging	1	2	3	4	5	6	7
Control	10	10	15	15	15	15	15
T-1	0	0	3	10	10	10	13
T-2	23	28	43	48	57	71	71

(B)	No. of larvae caged	No. of pupae caged	Percentage of larvae who: Survive as larvae	Die as larvae	Pupate	Percentage of pupae who: Survive as larvae	Die as larvae	Emerge as adults
Control	15	5	73	7	20	25	25	50
T-1	25	5	84	12	4	0	17	83
T-2	30	5	23	47	30	0	79	21

The mortality rate of caged amphipods in the two treatment ponds was about twice that of the control for the first four or five days after treatment (Table 12). Beyond this time relatively little mortality occurred in any of the groups. This suggests that for a short period after treatment Dimilin residues caused some amphipod mortality. Mortality of caged water boatmen nymphs was very high over the two days between set up and spray application, suggesting that cage conditions were quite unsuitable for this organism. Similar mortality patterns were observed among treated and control groups for the first few post-sprays, following which mortality was heavier in the treatment ponds (Table 13). This suggests some effect of Dimilin on these organisms, but the high control mortality makes this a weakly supported conclusion.

Table 12. Percent mortality of amphipods caged before treatment in the diflubenzuron study ponds near Kaladar, Ontario. 5-14 June, 1986.

	Spray Day	Days After Application 1	2	3	4	5	6	7	8	9
Control	2	6	20	30	34	38	40	40	40	40
T-1	2	12	28	34	70	78	80	80	80	82
T-2	2	18	44	50	70	76	82	84	84	86

Benthos: Sweep net catches from the three study ponds are presented in Appendix 2. All ponds displayed a diverse fauna with larval and adult beetles (Coleoptera), dragonfly and damselfly nymphs (Odonata), water boatmen (Hemiptera: Corixidae) and midge larvae (Diptera: Chironomidae) well represented. Mayfly nymphs (Ephemeroptera), caddisfly larvae (Trichoptera), phantom midge larvae (Diptera:

Table 13. Percent mortality* of immature water boatmen caged before treatment in the diflubenzuron study ponds near Kaladar, Ontario. 5-14 June, 1986.

	Spray Day	Days After Application 1	2	3	4	5	6	7	8	9
Control	14	9	23	40	40	49	49	49	49	53
T-1	14	12	21	40	46	60	74	81	84	84
T-2	58	24	33	57	76	86	86	86	86	90

*Post-spray values refer to percent mortality of individuals surviving on spray day.

Chaoboridae) and amphipods (Amphipoda) tended to be more unevenly distributed, occurring in large numbers in some ponds and being much less abundant in others.

The overall effect of the Dimilin treatment on aquatic invertebrates was not great. Table 14 presents sweep net catches and the results of t-tests for significant population changes for some of the more abundant or apparently affected organisms. The strongest indication of impact appears to be on water boatmen nymphs (Corixids), especially in T-1. No effect was evident on adult water boatmen (genus *Sigara)*, as would be expected because they have completed their development. Significant declines were noted for some Odonata genera in T-2, but these do not seem to indicate Dimilin impacts because numbers rapidly return to higher levels or are coincident with declines in the control pond. Significant declines in numbers of the mayfly nymph *Caenis* in T-1 and immature notonectids in T-2 occur at the same time these groups show significant declines in the control pond, suggesting they are not related to the treatment. *Chaoborus* and amphipod numbers in T-1

Table 14. Sweep net catch numbers (x±S.D.; N=5) for selected aquatic invertebrates from treatment (T-1, T-2) and control (C) ponds. Asterisks indicate significant (P < 0.05) reductions in benthos density between specified sample days.

Taxa	Site	Days before or after application							t-tests of log (n+1) transformed numbers between specified sample days
		- 5	+3	+9	+21	+34	+68	+110	
Ephemeroptera									
Caenis nymphs	T-1	7.2± 6.2	5.6± 4.3	0.4± 0.6	0	0	0	3.4± 4.4	day 3 vs day 9 P = 0.035*
	T-2	21.2±26.2	20.0±15.5	5.6± 5.5	0.2± 0.4	0	1.0± 1.0	14.8±11.6	day 3 vs day 9 P = 0.071
	C	64.2±38.7	13.2+ 7.3	2.0± 2.8	1.2± 1.6	1.0± 1.2	4.6± 2.7	64.8±59.4	day 3 vs day 9 P = 0.011*
Odonata									
Anisoptera	T-1	1.6± 1.1	1.0± 1.0	0.6± 0.6	0	0.2± 0.4	0.6± 0.9	1.0± 1.4	day 9 vs day 21 P = 0.070
Celithemus naiads	T-2	3.8± 2.2	4.6± 3.2	3.2± 1.8	0.8± 1.3	0.4± 0.5	3.0± 1.6	2.2± 1.9	day 9 vs day 21 P = 0.031*
	C	10.8±10.8	6.4± 4.8	2.2± 3.8	9.8± 4.5	3.6± 4.2	1.2± 1.3	2.4± 2.1	
Sympetrum naiads	T-2	18.2+18.1	9.0± 2.9	0.8± 1.1	0	0	0.6± 0.9	0	day 3 vs day 9 P = 0.002*
	C	1.4± 1.5	2.4± 2.7	0.2± 0.4	0	0	0	0	day 3 vs day 9 P = 0.066
Zygoptera	T-1	2.0± 2.5	1.8± 2.2	1.2± 1.0	0.2± 0.4	0	2.8± 1.5	7.2± 4.9	day 3 vs day 9 P = 0.85
Coenagrion naiads	T-2	8.6± 4.8	9.2± 4.3	1.4± 1.1	0.4± 0.6	0	5.4± 3.8	11.2± 6.9	day 3 vs day 9 P = 0.000*
	C	3.6± 1.5	2.0± 1.9	0	0.6± 0.5	1.6± 2.1	4.6± 2.2	22.2± 8.8	day 3 vs day 9 P = 0.072
Hemiptera									
Corixid nymphs	T-1	29.2±20.4	33.6±15.5	4.0± 2.8	0	0.6± 0.9	6.6± 2.5	0.2± 0.4	day 3 vs day 9 P = 0.000*
	T-2	1.2± 1.3	2.0± 2.5	0.2± 0.4	0	0.8± 0.8	0	0	day 3 vs day 9 P = 0.16
	C	1.2± 1.6	1.2± 1.1	0.4± 0.9	0.6± 0.9	1.6± 2.1	4.6± 2.2	22.2± 8.8	day 3 vs day 9 P = 0.80
Sigara adults	T-1	1.6± 1.3	6.0± 2.9	3.8± 2.2	3.6± 3.2	1.4± 1.5	1.0± 0.7	1.6± 1.5	
	T-2	0.4± 0.9	0	0	0.2± 0.4	0.2± 0.4	0	0	
	C	1.2± 1.8	0.2± 0.4	0.4± 0.5	0.2± 0.4	0.2± 0.4	0	0.8± 1.3	
Notonectid nymphs	T-2	9.8± 5.1	7.8± 6.3	0.2± 0.4	0.2± 0.4	0	0.8± 1.3	2.4± 1.3	day 3 vs day 9 P = 0.007*
	C	1.2± 1.3	2.4± 1.5	0	0.6± 1.3	0	0.6± 1.3	0	day 3 vs day 9 P = 0.019*
Notonecta adults	T-2	0	0	0.2± 0.4	0.2± 0.4	0	0.8± 1.3	2.4± 1.3	
	C	0.2± 0.4	0	0.2± 0.4	0.2± 0.4	0.4± 0.5	0.4± 0.5	0.2± 0.4	
Coleoptera									
Agabus larvae	T-1	2.8± 2.7	9.2± 4.8	0.2± 0.4	2.2± 1.3	1.8± 1.9	4.8± 2.8	0.2± 0.4	day 3 vs day 9 P = 0.001*
	T-2	2.4± 1.8	1.8± 0.8	0	0.6± 0.9	0	0	0	day 3 vs day 9 P = 0.002*
	C	0.2± 0.4	2.0± 2.1	0	0	0	0.2± 0.4	0.4± 0.5	day 3 vs day 9 P = 0.080
Diptera									
Chironomidae larvae	T-1	31.2±28.0	48.2±18.9	32.8±35.3	27.8+15.3	24.8±14.2	58.0±29.6	288.4±201.4	
	T-2	43.8±33.7	46.6±14.8	10.2± 7.0	15.6± 7.6	32.4± 9.7	12.0± 5.3	26.8±26.8	day 3 vs day 9 P = 0.033*
	C	86.4±41.9	35.8±17.2	18.2± 7.7	42.4+27.4	49.2±68.7	26.0± 9.0	68.2±41.3	day 3 vs day 9 P = 0.058
Chaoborus larvae	T-1	17.2± 6.9	15.0± 7.7	2.4± 2.8	6.4± 9.8	47.8±18.2	44.4±20.6	17.0± 4.2	
Amphipoda	T-1	4.2± 4.4	1.6± 1.9	9.2± 7.6	44.6±33.7	4.2± 4.1	2.6± 3.4	25.8± 9.2	
	T-2	0.8± 1.8	1.4± 1.7	0	0.4± 0.9	0	7.4±12.1	2.0± 2.3	
	C	0	0.4± 0.5	0	0.8± 0.8	0	2.8± 3.0	8.6± 5.5	

fluctuated erratically over the study period, but peak after treatment. Chironomid larvae remain abundant in all ponds throughout the study, although a brief significant decline is indicated in T-2.

Previous studies have indicated variable levels of impact of Dimilin on benthic organisms. Population reductions have been reported among mayfly nymphs (Mulla et al. 1975, Ali and Mulla 1978b), water boatmen nymphs (Farlow et al. 1978), *Chaoborus* larvae (Apperson et al. 1978), amphipods (Ali and Mulla 1978a and b), coleoptera larvae (Ali and Lord, 1980) and dragonfly nymphs (Steelman et al. 1975). In almost all cases, however, reductions are partial and temporary. All of these groups were quite well represented in the current study, but most showed few indications of being noticeably affected by the Dimilin treatment, with the exception of water boatmen nymphs.

CONCLUSIONS

The results of the residue analyses carried out during this study show that diflubenzuron residues are rapidly dissipated in various substrates of a pond environment following an aerial application of a DIMILIN 25 W formulation. The maximum residues found in water, sediment, aquatic plants and fish were 13.82 ppb (T-1 at 1 h), 0.24 ppm (T-1 at 1 d), 0.36 ppm (T-1 at 1 d) and 0.11 ppm (T-2 at 1 d), respectively. The rate of dissipation of the chemical was rapid in all substrates studied with non-detectable levels observed in 20 days for water, 5 days in sediment, 10 days in aquatic plants and 3 days in fish. No metabolic breakdown was examined in this study, but the major dissipation processes involved seemed to include dilution, hydrolysis, photolysis and microbial degradation.

The impacts on aquatic organisms seen in this study were similar to those previously reported when Dimilin has been directly applied to ponds and lakes. The greatest effect was on crustacean zooplankton, especially cladocerans, with only limited suggestions of effects on pond benthos. Recovery of populations of even the most severely affected organism (Daphnidae in the small pond) was well established by three months after treatment. Had any attempt been made to avoid directly overspraying the ponds, as would be the case in an operational pest control program, the impact on aquatic organisms would undoubtedly have been substantially reduced.

The results of this study suggest that Dimilin could be used for forest insect control programs in Canadian situations without causing major long-term disruptions to aquatic ecosystems. Even when direct overspray of forest ponds occurred, the resultant effects are substantially less than those documented by Gibbs et al. (1984) who conducted similar studies in ponds oversprayed with the carbamate insecticide carbaryl. They reported carbaryl residues persisting for over a year in pond water and sediment accompanied by long term impacts on pond benthos. Other pest control options such as *Bacillus thuringiensis* or viruses will pose less hazard than Dimilin to aquatic ecosystems, but may not be as effective against the target pest.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the substantial contributions of Pfizer C.&G. Inc., London, Ontario and Duphar B.V., Weesp, Holland, to the conduct of this study. Particular appreciation is extended to Ray Wellman, Pfizer's manager of technical services, for his support of the program and his excellent assistance in site selection and spray application. The cooperation of the property owner, Mr. Clayton Lessard, in allowing the use of his land for this experiment is gratefully acknowledged.

Nicholas Boyonoski, Johanna Broks, David Curry, John Hatherley, Karen Hyyrylainen, John Leung, Tarmo Poldmaa, Pat Sarrich and David Thomas assisted in the collection and analysis of samples and contributed greatly to the success of this project.

REFERENCES

Ali, A. and J. Lord. 1980. Impact of experimental insect growth regulators on some nontarget aquatic invertebrates. Mosq. News. 40:564-571.

Ali, A. and M.S. Mulla. 1978a. Effects of chironomid larvicides and diflubenzuron on nontarget invertebrates in residential-recreational lakes. Environ. Entomol. 7:21-27.

Ali, A. and M.S. Mulla. 1978b. Impact of the insect growth regulator diflubenzuron on invertebrates in a residential-recreational lake. Arch. Environm. Contam. Toxicol. 7:483-491.

Apperson, C.S., C.H. Schaefer, A.E. Colwell, G.H. Werner, N.L. Anderson, E.F. Dupras Jr. and D.R. Longanecker. 1978. Effects of diflubenzuron on *Chaoborus astictopus* and nontarget organisms and persistence of diflubenzuron in lentic habitats. J. Econ. Entomol. 71:521-527.

Booth, G.M. and D. Ferrell. 1977. Degradation of Dimilin by aquatic foodwebs. In: Pesticides in Aquatic Environments. M.A.Q. Khan (ed.), p. 221-243. Plenum Press, New York.

Colwell, A.E. and C.H. Schaefer. 1980. Diets of *Ectalurus nebulosus* and *Pomoxis nigromaculatus* altered by diflubenzuron. Can. J. Fish Aquat. Sci. 37:632-639.

Diprima, S.J., Cannizzaro, R.D., Roger, J.-C. and Ferrell, C.D. 1978. Analysis of diflubenzuron residues in environmental samples by high-pressure liquid chromatography. *Jour. Agric. Food Chem. 26(4):* 968-971.

DUPHAR B.V. Liquid chromatographic methods for the determination of diflubenzuron. *Tech. Notes*, L3-86-3 (1982), L3-86-12 (1985) and L3-86-18 (1985). DUPHAR B.V.' Weesp, Holland.

Farlow, J.E., T.P. Breaud, C.D. Steelman and P.E. Schilling. 1978. Effects of the insect growth regulator diflubenzuron on non-target aquatic populations in a Louisiana intermediate marsh. Environ. Entomol. 7:199-204.

Gibbs, K.E., T.M. Mingo and D.L. Courtemanch. 1984. Persistence of carbaryl (Sevin-4-Oil) in woodland ponds and its effects on pond macroinvertebrates following forest spraying. Can. Ent. 116: 203-213.

Helson, B.V. and G.A. Surgeoner. 1977. Effects of Dimilin 1G on certain non-target invertebrates in natural pools at a dosage of 44.8 g ai/ha, 1977 research. Dept. of Environmental Biology, University of Guelph. Unpublished report. 9 pp.

Hurlbert, S.H., M.S. Mulla and H.R. Willson. 1972. Effects of an organophosphorus insecticide on the phytoplankton, zooplankton and insect populations of fresh-water ponds. Ecol. Monogr. 42:269-299.

Kingsbury, P.D. 1976. Studies of the impact of aerial applications of the synthetic pyrethroid NRDC-143 on aquatic ecosystems. Chemical Control Research Institute Information Report CC-X-127, Canadian Forestry Service, Ottawa, Ont. 111 p.

Metcalf, R.L., P.Y. Lu and S. Bowlus. 1975. Degradation and environmental fate of 1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl) urea. J. Agric. Food Chem. 23:359-364.

Mian, L.S. and Mulla, M.S. 1982. Biological and environmental dynamics of insect growth regulators (IGRs) as used against Diptera of public health importance. Vol. 84, pp. 27-112. *In:* Gunther, F.A. and Gunther, J.D. (eds.), *Residue Reviews*. Springer-Verlag, New York.

Mulla, M.S., G. Majori and H.A. Darwazeh. 1975. Effects of the insect growth regulator Dimilin or TH-6040 on mosquitoes and some nontarget organisms. Mosq. News. 35:211-216.

Pennak, R.W. 1978. Fresh-water invertebrates of the United States. 2nd Edition. John Wiley & Sons Inc., New York. 803 p.

Schaefer, C.H. and Dupras, E.F. Jr. 1976. Factors affecting the stability of Dimilin in water and the persistence of Dimilin in field waters. *Jour. Agric. Food Chem. 24:* 733-739.

Schindler, D.W. 1969. Two useful devices for vertical plankton and water sampling. J. Fish. Res. Bd. Canada 26: 1948-1955.

Steelman, C.D., J.E. Farlow, T.P. Breaud and P.E. Schilling. 1975. Effects of growth regulators on *Psorophora columbiae* (Dyar and Knab) and nontarget aquatic insect species in rice fields. Mosq. News. 35:67-76.

Sundaram, K.M.S. 1986. Persistence and degradation of diflubenzuron in conifer foliage, forest litter and soil following simulated aerial application. *Gov't. Canada, Can. For. Serv., For. Pest Mngmt. Inst., Inf. Rept.* FPM-X-74, 19 p.

APPENDIX 1

Zooplankton catches from
the diflubenzuron treated
and control ponds.
Near Kaladar, Ontario
May-September, 1986

Appendix 1 Table 1

Zooplankton catches from three stations in the untreated control pond (C) near Kaladar, Ontario, May-September 1986. Expressed as mean and standard deviation of three 12 L Shindler-Patalas trap samples per station

Date		31 May	3 June	6 June	8 June	10 June	14 June	26 June	9 July	12 Aug.	23 Sept.
Days before or after treatment		-5	-2	+1	+3	+5	+9	+21	+34	+68	+110
East Station											
Cladocera:	Daphnidae	360±113	412± 175	193± 86	975± 238	743± 164	152± 26 ●	0 ●	2± 4 ●	0.3±0.6	0 ●
:	Bosminidae	2669±923 ●	3433± 574	3975±1347	11517±5394	2570±1235 ●	7234±1523	7± 9	99± 68 ●	36±14	2± 4
Copepoda:	nauplii	1392± 23 ●	1027± 270	529± 62	259± 77	253± 101	262± 54	692±78 ●	227± 42	121±36	558±178
:	copepodids and adults	1825±271	2073± 346 ●	592± 222 ●	2876± 885	1552± 242 ●	2148± 236 ●	21±17 ●	143± 69	11± 4 ●	25± 25
Diptera:	Chaoboridae	1± 2	0	0	4± 4	13± 1	0	0	0	0.7±0.6	0
Mid Station											
Cladocera:	Daphnidae	509±146	375± 77	315± 91	1472± 229	433± 134	44± 16 ●	10± 4 ●	1± 1 ●	4± 4	0 ●
:	Bosminidae	989± 69 ●	5111±1757	4691±1397	17291±3536	7049±2820 ●	4613±1050	38± 33	6± 3 ●	38± 28	4± 3
Copepoda:	nauplii	1113±103 ●	1639± 576	643± 210	300± 24	263± 151	179± 22	1264±191 ●	324±292	197± 48	741±245
:	copepodids and adults	1484±336	1739± 472 ●	1658± 282 ●	1789± 430	1132± 159 ●	1273± 202 ●	77± 37 ●	260±151	29± 11 ●	44± 35
Diptera:	Chaoboridae	0	0.7±0.6	0.3±0.6	2± 1	35± 16	0	0	0	0	0
West Station											
Cladocera:	Daphnidae	223± 45	428± 193	142± 63	1065± 36	602± 182	135± 72 ●	14± 10 ●	13± 3 ●	1± 1	21± 27 ●
:	Bosminidae	1364±449 ●	3616± 653	1975± 647	18412±6776	6528±1281 ●	5083± 810	47± 12	30± 26 ●	112± 58	2± 4
Copepoda:	nauplii	2242±211 ●	812± 44	620± 62	304± 116	280± 146	241± 121	2085±554 ●	757±420	254± 77	514± 55
:	copepodids and adults	1414±505	853± 165 ●	503± 240 ●	2425± 374	979± 181 ●	759± 152 ●	95± 31 ●	414±200	47± 23 ●	25± 17
Diptera:	Chaoboridae	0.3±0.6	1± 2	0	15± 8	19± 11	0	1± 1	0.3±0.6	0.3±0.6	0

● indicates significant ($p < 0.05$) differences exist between the numbers of this organism found at the three sampling stations on this date.

Appendix 1 Table 2

Zooplankton catches from three stations in diflubenzuron treatment pond 1 (T-1) near Kaladar, Ontario, May-September 1986. Expressed as mean and standard deviation of three 12 L Shindler-Patalas trap samples per station

Date		31 May	3 June	6 June	8 June	10 June	14 June	26 June	9 July	12 Aug.	23 Sept.
Days before or after treatment		-5	-2	+1	+3	+5	+9	+21	+34	+68	+110
East Station											
Cladocera:	Daphnidae	1003±623	187± 83	241±134 ●	1±1	0	0	0	0	0	183±114 ●
:	Chydoridae	1± 2	0	0	0	0	0	0	0	0	0
Copepoda:	nauplii	739±133 ●	346± 99	432± 21	0	0	0	12± 4	65±24	475±191 ●	608±491
:	copepodids and adults	281± 73	262± 50	281± 75	6±3	21±4	6± 4	20±14	9± 6	105± 31	67± 27 ●
Diptera:	Chaoboridae	20± 4	44± 5	10± 3	21±4	22±2	20±12	2± 3	0	0	1± 1
Mid Station											
Cladocera:	Daphnidae	402±215	380±134	151± 52 ●	5±6	0	0	0	0	1± 1	58± 57 ●
:	Chydoridae	0.3±0.6	0.3±0.6	1± 2	0.3±0.6	0	0	0.3±0.6	0	0.3±0.6	0
Copepoda:	nauplii	518± 76 ●	260±224	501± 90	0.7±1.2	0	0	6± 8	49±23	258±102 ●	61± 32
:	copepodids and adults	210± 12	239±118	232± 27	12±8	9±8	5± 2	19± 8	11± 1	114± 61	16± 10 ●
Diptera:	Chaoboridae	23± 10	56± 9	10± 6	27±13	7±1	20± 9	6± 2	0	1± 1	2±0
West Station											
Cladocera:	Daphnidae	497±175	453±132	57± 10 ●	12± 9	0	0	0	0	0	465±209 ●
:	Chydoridae	0.7±0.6	0.3±0.6	0	0	0	0	0	0	0	0
Copepoda:	nauplii	1108±315 ●	452±127	296±141	0.3±0.6	0	0	14± 8	89±20	106± 64 ●	531±206
:	copepodids and adults	212± 97	367± 84	149± 60	8±4	3±2	11± 8	9± 6	10± 8	58± 23	151± 65 ●
Diptera:	Chaoboridae	17± 5	51± 7	4± 2	16± 8	12±2	23± 9	4± 2	0.3±0.6	0	1± 1

● indicates significant ($p < 0.05$) differences exist between the numbers of this organism found at the three sampling stations on this date.

Appendix 1 Table 3

Zooplankton catches from three stations in diflubenzuron treatment pond 2 (T-2) near Kaladar, Ontario, May-September 1986. Expressed as mean and standard deviation of three 12 L Shindler-Patalas trap samples per station

Date		31 May	3 June	6 June	8 June	10 June	15 June	26 June	9 July	12 Aug.	23 Sept.
Days before or after treatment		-5	-2	+1	+3	+5	+10	+21	+34	+68	+110
East Station											
Cladocera:	Daphnidae	257±106	273± 34●	91± 16	0.7±0.6	0	0	0	0	1129± 36	455±277
:	Bosminidae	91± 20	61± 21	44± 10	0.3±0.6	0	0	0	0	483±312	8± 14
Copepoda:	nauplii	807±100	1002±116	471± 84●	28± 9	0	114±24●	238±77	25±8 ●	497±110	99± 50
:	copepodids and adults	537±102●	373± 65	418± 97	162± 74	57±23	106±52	111±30	85±25	535± 84	423± 96
Diptera:	Chaoboridae	0	0.3±0.6	0	0	0	0	0	0	0	0
Mid Station											
Cladocera:	Daphnidae	255±206	170± 29●	142±201	2± 2	0	0	0	0	872±139	697±426*
:	Bosminidae	72± 20	209±156	33± 11	4± 4	0	0	1± 2	0	796±575	15± 13*
Copepoda:	nauplii	761±146	1323±114	699±152●	49± 34	2± 3	50±18●	247±59	129±28●	587±197	156± 40*
:	copepodids and adults	565±151●	562±188	331± 95	233± 19	87±25	77± 3	281±94	143±63	471± 63	394±204*
Diptera:	Chaoboridae	0	0	0	0	0	0.3±0.6	0	0	0.3±0.6	0*
West Station											
Cladocera:	Daphnidae	97± 8	174± 33●	182±126	0.7±0.6	0	0	0	0	970±141	809±242
:	Bosminidae	69± 24	115±158	43± 32	14± 19	0.3±0.6	4±2	0	0	1046±393	50± 15
Copepoda:	nauplii	761±196	1283±228	377± 76●	52± 21	1± 1	104±33●	540±268	70±15●	1139±480	201± 19
:	copepodids and adults	161± 45●	424±100	368±126	241± 47	91±87	173±98	241±143	101±40	347±212	596±259
Diptera:	Chaoboridae	0	0	0	0	0	0	0	0	0	0

* Sample size of two at Mid Station on 23 Sept. due to loss of one sample (preservative not added).

● indicates significant ($p < 0.05$) differences exist between the numbers of this organism found at the three sampling stations on this date.

APPENDIX 2

Benthos sweep net catches
from the diflubenzuron
treated and control ponds
near Kaladar, Ontario
June-September, 1986

Appendix 2 Table 1

Benthic invertebrates in sweep net catches from the untreated control pond (C) near Kaladar, Ontario. June-September 1986. Expressed as mean and standard deviation in five sweep samples.

Date	1 June	8 June	14 June	26 June	9 July	12 Aug.	23 Sept.
Days before or after treatment	-4	+3	+9	+21	+34	+68	+110
Ephemeroptera: *Caenis*	64.2±38.7	13.2± 7.3	2.0± 2.8	1.2± 1.6	1.0± 1.2	4.6± 2.7	64.8±59.4
Odonata: Anisoptera: *Anax*	-	-	-	0.2± 0.4	-	-	-
Boyeria	-	0.2± 0.4	-	-	-	-	-
Celithemus	10.8±10.8	6.4± 4.8	2.2± 3.8	9.8± 4.5	3.6± 4.2	1.2± 1.3	2.4± 2.1
Cordulia	0.8± 0.8	-	0.2± 0.4	-	0.8± 1.3	0.2± 0.4	-
Ladona	-	-	-	-	-	0.2± 0.4	-
Sympetrum	1.4± 1.5	2.4± 2.7	0.2± 0.4	-	-	-	-
Zygoptera: *Coenagrion*	3.6± 1.5	2.0± 1.9	-	0.6± 0.5	1.6± 2.1	4.6± 2.2	22.2± 8.8
Lestes	0.2± 0.4	1.0± 1.7	0.2± 0.4	0.6± 0.9	-	-	-
Hemiptera: Pleidae: *Neoplea*	-	-	-	-	-	1.4± 3.1	1.4± 3.1
Corixidae: Nymphs	1.2± 1.6	1.2± 1.1	0.4± 0.9	0.6± 0.9	3.6± 2.9	-	-
Sigara - A	1.2± 1.8	0.2± 0.4	0.4± 0.5	0.2± 0.4	0.2± 0.4	-	0.8± 1.3
Notonectidae: Nymphs	1.2± 1.3	2.4± 1.5	-	0.6± 1.3	-	0.6± 1.3	-
Notonecta - A	0.2± 0.4	-	0.2± 0.4	0.2± 0.4	0.4± 0.5	0.4± 0.5	0.2± 0.4
Trichoptera: *Nemotaulius*	-	-	-	-	-	-	5.0± 5.2
Oecetis	1.2± 1.1	0.6± 1.3	-	-	-	-	0.4± 0.5
Platycentropus	0.2± 0.4	-	-	-	-	-	27.2±38.5
Coleoptera: *Agabus* - L	0.2± 0.4	2.0± 2.1	-	-	-	0.2± 0.4	0.4± 0.5
Dytiscidae - A	-	-	-	-	0.4± 0.5	0.2± 0.4	-
Graphoderus - L	-	0.2± 0.4	-	-	-	-	-
Gyrinus - L	-	-	-	-	0.2± 0.4	-	-
Haliplus - L	0.2± 0.4	-	-	-	0.2± 0.4	-	0.2± 0.4
- A	0.4± 0.5	-	-	-	-	-	0.6± 0.9
Tropisternus - L	1.6± 1.3	2.4± 1.5	-	0.2± 0.4	-	-	-
Diptera: Chaoboridae: *Chaoborus*	-	-	-	0.2± 0.4	-	-	-
Ceratopogonidae - L	0.2± 0.4	-	-	-	-	-	-
Chironomidae - L	86.4±41.9	35.8±17.2	18.2± 7.7	42.4±27.4	49.2±68.7	26.0± 9.0	68.2±41.3
Amphipoda	-	0.4± 0.5	-	0.8± 0.8	-	2.8± 3.0	8.6± 5.5
Gastropoda	0.8± 1.3	5.6± 5.8	0.6± 0.5	3.4± 4.6	6.2± 3.1	12.4±27.7	23.8±14.3
Pelecypoda	-	-	-	-	-	0.2± 0.4	3.2± 3.5
Oligochaeta	-	-	25.2±28.8	-	0.8± 1.8	-	1.2± 1.6
Hydracarina	-	-	-	-	-	0.2± 0.4	-

L - larva P - pupa A - adult

Appendix 2 Table 2

Benthic invertebrates in sweep net catches from diflubenzuron treatment pond 1 (T-1) near Kaladar, Ontario. June-September 1986. Expressed as mean and standard deviation in five sweep samples

Date	1 June	8 June	14 June	26 June	9 July	12 Aug.	23 Sept.
Days before or after treatment	-4	+3	+9	+21	+34	+68	+110
Ephemeroptera: *Baetis*	0.2± 0.4	-	-	-	-	0.6± 1.3	0.4± 0.6
Caenis	7.2± 6.3	5.6± 4.3	0.4± 0.6	-	-	-	3.4± 4.4
Callibaetis	-	-	-	-	0.2± 0.4	2.2± 1.9	-
Odonata: Anisoptera: *Anax*	-	-	-	-	0.2± 0.4	0.2± 0.4	-
Celithemus	1.6± 1.1	1.0± 1.0	0.6± 0.6	-	0.2± 0.4	0.6± 0.9	1.0± 1.4
Cordulia	0.2± 0.4	-	-	0.4± 0.6	-	-	1.0± 2.2
Erythrodiplax	0.2± 0.4	-	-	-	-	-	-
Ladona	-	-	0.4± 0.9	-	-	-	1.6± 2.1
Sympetrum	-	0.2± 0.4	-	-	-	0.2± 0.4	-
Zygoptera: *Coenagrion*	2.0± 2.5	1.8± 2.2	1.2± 1.0	0.2± 0.4	-	2.8± 1.5	7.2± 4.9
Lestes	0.2± 0.4	0.6± 1.3	-	-	-	-	0.2± 0.4
Hemiptera: Corixidae: Nymphs	29.2±20.4	33.6±15.5	4.0± 2.8	-	0.6± 0.9	6.6± 2.5	0.2± 0.4
Sigara - A	1.6± 1.3	6.0± 2.9	3.8± 2.2	3.6± 3.2	1.4± 1.5	1.0± 0.7	1.6± 1.5
Notonectidae: Nymphs	0.4± 0.9	0.8± 1.3	0.4± 0.6	0.2± 0.4	-	0.4± 0.6	-
Notonecta - A	-	0.8± 1.3	0.2± 0.4	0.2± 0.4	0.2± 0.4	-	0.8± 1.1
Trichoptera: *Cernotina*	0.2± 0.4	-	-	-	-	-	-
Oecetis	-	-	-	-	-	0.8± 1.1	0.6± 0.2
Nemotaulius	-	-	-	-	-	-	2.4± 4.3
Coleoptera: *Agabus* - L	2.8± 2.7	9.2± 4.8	0.2± 0.4	2.2± 1.3	1.8± 1.9	4.8± 2.8	0.2± 0.4
Berosus - L	-	0.8± 1.3	-	-	-	-	-
- A	1.4± 1.3	0.2± 0.4	-	-	-	-	-
Coptotomus - L	0.2± 0.4	1.2± 2.7	0.6± 0.5	0.4± 0.6	0.8± 0.8	1.2± 0.8	-
Dinentes - L	4.2± 5.1	3.6± 3.6	5.2± 3.8	5.0± 2.3	7.4± 4.0	0.2± 0.4	-
Dytiscidae - A	-	0.2± 0.4	1.8± 0.8	1.2± 1.1	0.8± 1.1	1.6± 1.5	0.2± 0.4
Graphoderus - L	0.2± 0.4	0.8± 1.8	-	-	0.2± 0.4	-	-
Haliplus - L	-	-	0.2± 0.4	-	-	-	0.4± 0.6
- A	0.2± 0.4	-	0.6± 0.5	-	0.4± 0.6	-	-
Hydrocanthus - A	-	0.2± 0.4	-	-	-	-	-
Rhantus - L	1.6± 3.1	-	-	-	-	-	-
Tropisternus - L	-	0.4± 0.6	-	-	-	-	-
Diptera: Chaoboridae: *Chaoborus* - L	17.2± 6.9	15.0± 7.7	2.4± 2.8	6.4± 9.8	47.8±18.2	44.4±20.6	17.0± 4.2
- P	1.2± 1.3	0.2± 0.4	-	-	2.6± 2.5	3.6± 3.6	-
Chironomidae - L	31.2±28.0	48.2±18.9	32.8±35.3	27.8±15.3	24.8±14.2	58.0±29.6	288.4±201.4
Athericidae: *Atherix* - L	-	-	-	-	0.2± 0.4	-	-
Amphipoda	4.2± 4.4	1.6± 1.9	9.2± 7.6	44.6±33.7	4.2± 4.1	2.6± 3.4	25.8± 9.2
Gastropoda	0.2± 0.4	0.2± 0.4	-	-	-	-	0.4± 0.6
Oligochaeta	-	-	1.2± 2.2	8.6± 8.6	5.8± 5.4	-	4.4± 3.6

L - larva P - pupa A - adult

Appendix 2 Table 3

Benthic invertebrates in sweep net catches from diflubenzuron treatment pond 2 (T-2) near Kaladar, Ontario. June-September 1986. Expressed as mean and standard deviation in five sweep samples

Date	1 June	8 June	14 June	26 June	9 July	12 Aug.	23 Sept.
Days before or after treatment	-4	+3	+9	+21	+34	+68	+110
Ephemeroptera: *Baetis*	-	-	-	-	-	-	0.2± 0.4
Caenis	21.2±26.2	20.0±15.5	5.6±5.5	0.2±0.4	-	1.0± 1.0	14.8±11.6
Odonata: Anisoptera: *Anax*	-	-	-	-	-	-	0.2± 0.4
Celithemus	3.8± 2.2	4.6± 3.2	3.2±1.8	0.8±1.3	0.4±0.5	3.0± 1.6	2.2± 1.9
Cordulia	0.6± 1.3	-	2.8±2.7	0.2±0.4	0.8±0.8	0.2± 0.4	0.2± 0.4
Ladona	-	-	-	-	-	-	1.8± 1.6
Leucorrhinia	0.2± 0.4	-	0.4±0.5	-	-	-	-
Somatochlora	-	-	-	-	-	0.2± 0.4	-
Sympetrum	18.2±18.1	9.0± 2.9	0.8±1.1	-	-	0.6± 0.9	-
Zygoptera: *Coenagrion*	8.6± 4.8	9.2± 4.3	1.4±1.1	0.4±0.6	-	5.4± 3.8	11.2± 6.9
Lestes	0.2± 0.4	0.4± 0.9	-	-	-	-	-
Hemiptera: Pleidae: *Neoplea*	0.2± 0.4	-	-	-	-	-	-
Corixidae: Nymphs	1.2± 1.3	2.0± 2.5	0.2±0.4	-	0.8±0.8	-	-
Sigara - A	0.4± 0.9	-	-	0.2±0.4	0.2±0.4	-	-
Notonectidae: Nymphs	9.8± 5.1	7.8± 6.3	0.2±0.4	0.2±0.4	0.2±0.4	-	-
Notonecta - A	-	-	0.2±0.4	0.2±0.4	-	0.8± 1.3	2.4± 1.3
Trichoptera: *Oecetis*	-	0.4± 0.9	0.2±0.4	-	0.2±0.4	-	0.6± 0.9
Coleoptera: *Agabus* - L	2.4± 1.8	1.8± 0.8	-	0.6±0.9	-	-	-
Agabinus - L	0.4± 0.9	-	-	-	-	-	-
Agabetes - A	0.2± 0.4	-	-	-	-	-	-
Dineutes - L	-	-	-	-	-	-	0.2± 0.4
Dytiscidae - A	-	0.2± 0.4	-	-	-	0.4± 0.9	0.4± 0.5
Graphoderus - L	0.6± 0.5	-	0.2±0.4	-	0.2±0.4	-	-
- A	0.2± 0.4	-	-	-	-	-	-
Haliplus - L	-	-	0.2±0.4	0.2±0.4	-	0.2± 0.4	1.2± 1.6
- A	0.4± 0.6	0.2± 0.4	-	-	-	-	-
Hydaticus - A	0.4± 0.9	-	-	-	-	-	-
Tropisternus - L	-	1.4± 1.5	-	-	-	-	-
Diptera: Chaoboridae: *Chaoborus* - L	-	-	-	-	-	0.4± 0.6	-
Ceratopogonidae: L	-	0.4± 0.6	-	-	0.6±0.5	-	-
Chironomidae - L	43.8±33.7	46.6±14.8	10.2±7.0	15.6±7.6	32.4±9.7	12.0± 5.3	26.8±26.8
Amphipoda	0.8± 1.8	1.4± 1.7	-	0.4±0.9	-	7.4±12.1	2.0± 2.3
Gastropoda	-	-	-	2.4±3.1	0.8±1.8	-	6.2± 6.3
Pelecypoda	-	-	1.0±0.7	0.2±0.4	1.6±1.3	-	0.4± 0.9
Oligochaeta	-	-	1.2±2.7	6.6±8.2	1.2±1.6	-	2.8± 4.1
Hydracarina	1.0± 2.2	-	0.4±0.9	0.4±0.9	0.8±1.3	-	-

L - larva A - adult