AQUATIC PROGRAMS AND CONTAMINANTS CONTROL
ENVIRONMENTAL PROTECTION SERVICE
PACIFIC REGION

IMPACT OF INSECT GROWTH REGULATORS ON AQUATIC ORGANISMS CO-EXISTING WITH MOSQUITO LARVAE

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by

M.T.K. Wan

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ABSTRACT

The impact of insect growth regulators Altosid and Dimilin on selected non-target organisms was determined during experimental applications for mosquito control in British Columbia. Residues in water, persistence in the environment, and effects on aquatic crustaceans and insects of the two growth inhibitors were monitored.

Altosid was effective against mosquitoes and appeared harmless to marsh carbs, crustaceans, and fish at the Tsawwassen salt marshes. Dimilin treatments at Fort Langley, B.C., suppressed mosquito populations and reduced the population of zooplankton and certain non-target insects. Residues of these growth regulators were relatively persistent in the environment.

The implications of these findings, and the effectiveness of insect growth regulators as mosquito control agents and their environmental consequences are discussed.

RESUME

Des applications expérimentales, effectuées en Colombie-Britannique pour lutter contre les moustiques, avaient pour but de déterminer l'impact des régulateurs de croissance, l'Altoside et la Dimiline, sur les organismes non visés. On a également mesuré les résidus dans l'eau, la rémanance dans l'environnement ainsi que les effets de ces deux inhibiteurs de croissance sur les crustacés et insectes aquatiques.

D'Altoside s'est révélée efficace contre les moustiques et, par ailleurs, inoffensive pour les crabes, crustacés et poissons des salants de Tsawwassen. Dimiline à Fort Langley (C.-B.) n'a pas qu'enrayé la population de moustiques mais aussi réduit les populations de zooplancton et d'insectes non visés. On a trouvé que les résidus de ces deux régulateurs de croissance étaient relativement rémanants dans l'environnement.

On commente les implications des résultats, l'efficacité de ces régulateurs de croissance pour lutter contre les insectes ainsi que les conséquences environnementales qu'ils entraînent.

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CONCLUSIONS

Insect growth regulators (IGRs) can be used as effective mosquito control agents in British Columbia. Their effects on non-target organisms depend on the type of inhibitors.

IGR such as Altosid can be effective against mosquitoes with little or no harmful effects to crabs, zooplanktonic crustaceans, and fish in lentic waters. IGR having moderately long residual action such as certain formulations of Dimilin not only arrested mosquito development but also appeared to reduce the population of zooplankton and suppress the emergence of non-target insects belonging to the same Order as mosquitoes.

IGRs are generally much less toxic to birds, fish and small mammals than organophosphorus insecticides presently used for mosquito control in British Columbia. Their role in mosquito control programs should be actively investigated.

Efforts should also be undertaken to determine and understand more fully the persistence, sublethal effects and bioaccumulation of IGRs and their metabolites on fish and fish food organisms in the aquatic environment of British Columbia before being advocated for general use in mosquito larviciding programs.

1 INTRODUCTION

In the populated areas of British Columbia, chemical mosquito abatement is conducted because of the biting nuisance and the potential problem of mosquitos becoming vectors of human disease.

Many organochlorine and organophosphorus compounds have been used to control mosquitoes. Several of these materials are now environmentally unacceptable because of their harmful effects to birds, fish and other non-target organisms. A group of new compounds, the Insect Growth Regulators (IGRs) have recently appeared and may be more environmentally acceptable.

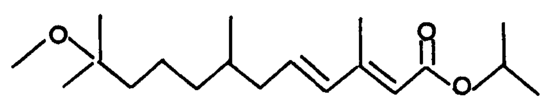
IGRs are mimics of insect hormones which are responsible for the control of various growth processes. When applied to immature stages, IGRs produce morphogenetic effects detrimental to insect development. IGRs are selective and only very minute quantities of the material are required to produce the desired effects. Because of these qualities, IGRs may be more environmentally acceptable for the control of insect pests.

In 1976 and 1978 field applications were conducted by the British Columbia Department of Agriculture and Simon Fraser Health Unit to assess the effectiveness of two IGRs - Altosid and Dimilin (TH 6040) for the control of mosquitoes. The Environmental Protection Service investigated the impact of these two compounds on selected non-target organisms co-existing with mosquito larvae, particularly the crustaceans and aquatic insects. The persistence of Altosid and Dimilin in the aquatic environment was also monitored.

2 LITERATURE REVIEW

2.1 Altosid[1]

Altosid is the trade name of the growth regulator, methoprene[2], which is also known as Entocon TM[3], and Zoecon ZR-515[4]. It is a juvenile hormone analogue synthesized from 2,4-dienoic esters, with the following structural formula (Durham et al., 1975).:



(Isopropyl (2E, 4E)-11 methoxy-3, 7, 11-trimethyl-2,4-dodecadienoate)

Altosid is very suspectible to microbial and photolytic degradation under most natural environmental conditions (Schooley et al, 1975; Swern, 1970). Under field conditions, technical and emulsion concentration formulation of ZR-515 have a half life of approximately two hours, but residues of a "slow release" formulation can be detected in water up to 24 hours after application (Pawson et al, 1972, Schaefer and Dupras, 1973).

Altosid, in very low concentrations, prevents the emergence of adults from pupae (Robins, 1972, Schneiderman, 1972, Staal, 1975). The effects may be expressed by the occurrence of larval-pupal or pupal-adult intermediates, defective reproductive organs, or abnormal embryogenesis. (Henrick et al, 1973).

Few field studies have been conducted on the impact of Altosid on non-target organisms. One simulated study, using normal dosages for mosquito larvae control detected no adverse effect on zooplankton and aquatic insects, although the emergence of Chironomidae (midges), Ephydridae (shoreflies, brineflies), and Psychodidae (mothflies), was reduced (Grandoni et al, 1976, Muir and Takahashi, 1973, 1974).

1,2,3,4 - Zoecon Corporation, 1975 California Avenue, Palo Alto, California 94304.

Another work, however, reported that repeated treatments of Altosid produced mortality in the early and late instars of the mayfly (Callibaetis pacificus Seman), and also reduced 84 percent of the larval dytiscid beetle (Laccopilus sp) biomass during one season (Norland and Mulla, 1975). Little or no effect on non-target aquatic organisms was detected after an aerial treatment of a pond with Altosid at the rate of 2.34 oz. active ingredient (a.i)/acre (Wan and Wilson, 1975).

Altosid is relatively non-toxic to birds and mammals, (Anon., 1973). The oral and acute LD_{50} for Mallard duck and Bobwhite quail are greater than 10,000 mg/kg. In rat and rabbit the oral and dermal LD_{50} are 34,600 mg/kg and 3,500 mg/kg respectively.

Altosid toxicity to fish varies according to species. Blue gill, trout and catfish, and largemouth bass have LC_{50} values of 4.62, 3.30, and 100 ppm respectively. However, in a recent static bioassay study with juvenile fish, McKague and Pridmore (1978) reported the 96 hr. LC_{50} for Rainbow trout as 106 ppm and for Coho salmon as 86 ppm.

Little is known of the sublethal effects of Altosid to fish. One laboratory study reported that fish exposed to this IGR become lethargic (Madder and Lockhart, 1978).

Recent studies indicate that insects can develop resistance to Altosid, (Schneiderman, 1972; Vinson an Plapp, Jr., 1974). One report stated that organophosphate and carbamate resistant strains of the common housefly (Musca domestica) exhibited cross resistance to Altosid (Cerf and Georghiou, 1974a).

2.2 Dimilin[5]

Dimilin is also known as ENT-29054[6], OMS 1804[7], PH 6040[8] and TH 6040[9]. It is an insect growth regulator which interferes with the formation of the insects cuticule during moulting

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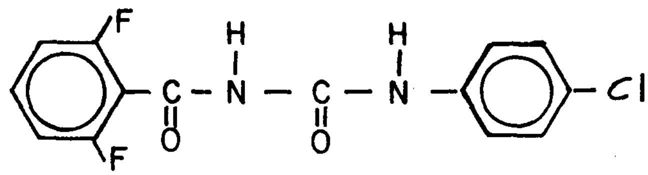
5,6,7,8,9 - Thompson-Hayward Chemical Company, 5200 Speaker Road, Kansas City, Kansas 66110.

and is a phenylurea substitute with the following structural formula, (Thompson-Hayward Chemical Company, 1974):



1-(4-chlorophenyl)-3-(2,6-diflourobenzoyl)-urea

Metcalf et al, (1975) reported that Dimilin does not appear to be susceptible to microbial and photolytic degradation. Other studies, however, found that the breakdown of this IGR depends to a large extent on microbial activity.

Dimilin in soil has a "half-life" of approximately two months (Nimmo and Wilde, 1975). The size of Dimilin carrier particles presumably affect their persistence in soil; residues on large particles persist longer than small ones. Studies found that residues on particles larger than ten microns were detectable more than 40 weeks while those residues on particles in the two micron range disappeared within two weeks (Anon., 1976).

Schaeffer and Dupras (1973) showed that the half-life of Dimilin in water is less than twenty-four hours and to be related to pH, temperature, and suspended organic matter. As in soil, particle size affects the time of degradation of Dimilin in water (Anon., 1976).

Dimilin, in very low concentrations, inhibits chitin synthesis, and prevents the formulation of cuticle (Cerf and Georghiou, 1974a). It induces abnormal endocuticular deposition and abortive moulting in juvenile insects in contrast to the direct toxic effects on the insect nervous system of conventional insecticides (Post and Vincent, 1973). Juvenile insects are most susceptible to Dimilin.

Feeding experiments have shown that Dimilin can act as a chemosterilant when ingested by female insects prior to or during oviposition. The viable embryos apparently develop normally but the young larvae fail to emerge or die shortly following emergence (Muira et al, 1975, Thompson-Hayward Chemical Company, 1974).

Dimilin has a wide range of biological activity and acts primarily as stomach poison (Mulden and Gijswijt, 1973). Although the main mode of entry is through ingestion, sucking organisms escape its effect because it is not a plant systemic.

Investigations into the impact of Dimilin on non-target organisms have been conducted in Laboratory and small scale field experiments. These studies indicate that a rate between 0.023 to 0.056 kg ai/ha (0.02 to 0.05 lb ai/ac), Dimilin reduced the population of the following organisms, (Muira and Takahasi, 1975; Mulla *et al*, 1975; Anon., 1976):

Cladocera (*Daphnia* sp)
Copepoda (*Cyclops* sp)
Ephemereptera nymps (*Callibaetis* spp.)
Chironomid larvae (*Goeldichironomus holoprasinus* Goeldi)
Corixid nymphs
Notonectid nymphs

However, the population of these organisms recovered within a period of 7-12 days after chemical treatment. Tadpole shrimp (*Triops longicaudatus* LeConte), clam shrimp (*Eulimnadia* spp.) and cladocera (*Daphnia* and *Moina* spp.) were susceptible to Dimilin at levels below 0.01 ppm under laboratory conditions (Muira and Takahashi, 1974).

Cunningham (1976) demonstrated that Dimilin reduced the reproductive lifespan of adult brine shrimp (*Artemia salin*) at levels between 2 to 10 ppb. In the same study immature shrimp exposed to concentrations greater than 10 ppb died within 3 days. Muira and Takahashi (1974) showed that adult aquatic beetles (*Laccophilus* spp., *Hydrophilus triangularis* Say, and *Tropisternus laterailis* Fab) were also sensitive to Dimilin. Spiders (*Pardos* spp. and *Lycosa* spp.), however, showed no apparent effects to this IGR, (Muira and Takahashi, 1974).

In acute toxicity bioassays, Dimilin (technical) or (W-25)[1] was found to be very toxic to certain crustaceans, but not to fish, birds and mammals, (Anon., 1976):

Crustacean 48 hour LC_{50}	
Waterfleas	1.55 ppb (W-25)
Glass shrimp	45 ppb (W-25)
Fish 96-hour LC_{50}	
Bluegill sunfish	135 ppm (technical)
Bluegill sunfish	660 ppm (W-25)
Rainbow trout	140 ppm (technical)
Rainbow trout	240 ppm (W-25)
Avian Oral LD_{50}	
Mallard duck	5000 mg/kg (technical)
Bobwhite quail	5000 mg/kg (technical)
Mammal Acute Oral LD_{50}	
Mouse	4 640 mg/kg (technical)
Rat	4 640 mg/kg (technical)
Rat	10 000 mg/kg (technical)

Little information is available on the effects of Dimilin degradation products on fish. One study reported that a metabolite of this IGR is quite toxic to fish. Of the three major breakdown products, 4-chlorphenyl urea, 3,6-diflurobenzoic acid, and 4-chloraniline, the latter compound is the most toxic to fish (Julin and Sanders, 1978). Julin and Sanders (1978) cite the following examples of 4-chloroaniline toxicity to fish:

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1 - 25% wettable powder

	96 hour LC_{50}
Bluegill	2.4 (1.8 - 3.2)
Fathead Minnow	12 (7 - 18)
Rainbow Trout	14 (11 - 16)
Channel Catfish	23 (18 - 29)

Booth et al, (1975a, 1976) demonstrated that under simulated conditions Dimilin uptake but not bioaccumulation occurred in fish. However, two recent reports indicated that Dimilin is accumulated in fish tissues at concentrations approximately 80 times those in surrounding water within a period of three weeks (Apperson et al, 1978; Schaefer et al, 1979). To date there has been no published investigation on the accumulation of Dimilin metabolites on fish tissues.

Little is known of the sublethal effects of Dimilin on fish. One study showed that prolonged exposure (10 months) of Fathead Minnows to Dimilin (6 to 100 ppb) produced no physiological and reproductive effects on the fish nor its offspring (Anon., 1976). Other study reported that fish will avoid water contaminated with sublethal concentrations of Dimilin, (Granett, 1978).

A recent study shows that insecticide resistant strains of insects possess high levels of cross-resistance to Dimilin, indicating that this compound is also subject to the risk of resistance development, (Cerf and Georghiou, 1974a, 1974b).

3 MATERIALS AND METHODS

3.1 Experimental Design

Altosid was applied to estuarine salt marshes of Tsawwassen, approximately 30 km southwest of Vancouver, British Columbia, (Figure 1). The plots consisted of six stagnant tidal pools totalling approximately 225 m^2 in area. The depth of water varied from 15 cm to 30 cm and the rate of treatment was approximately 0.032 kg ai/ha (0.028 lbs ai/acre). Impact and residue studies were conducted in these pools.

Dimilin was applied to two areas east of Vancouver: (1) Fort Langley, and (2) Port Coquitlam. The study areas at Fort Langley were approximately 5 km east of that town on the south bank of the Fraser River, adjacent to the Canadian National Railway (Figure 2). Three separate plots totalling 40 ha were aerially treated with Dimilin at 2 rates, 0.023 kg ai/ha (0.02 lb ai/acre) and 0.046 kg ai/ha (0.04 lb ai/acre). The control plot (approximately 2 ha) was situated approximately 3 km west of the treated plots. These plots were situated in tidal flood plains of the Fraser River, hence the depth of water varied according to the flooding conditions of the river. Impact studies were conducted only in the 0.046 kg ai/ha plot.

The study plots at Port Coquitlam were located in an old garbage dump site between Kingsway Avenue and Lougheed Highway (Figure 3). The treated pond was approximately 625 m^2 in area and Dimilin was applied twice by hand treatment at the rate of 0.034 kg ai/ha (0.03 lb ai/ac). The control pond (100 m^2 in area) was nearby, separated from the Dimilin pond by a dyke. Only Dimilin residue studies were conducted at this site.

3.2 Application of Insect Growth Regulators

Altosid was formulated as liquid micro-encapsulated (slow release) emulsion and was applied with a hand sprayer between 1300 and 1400 hours on May 17, 1976.

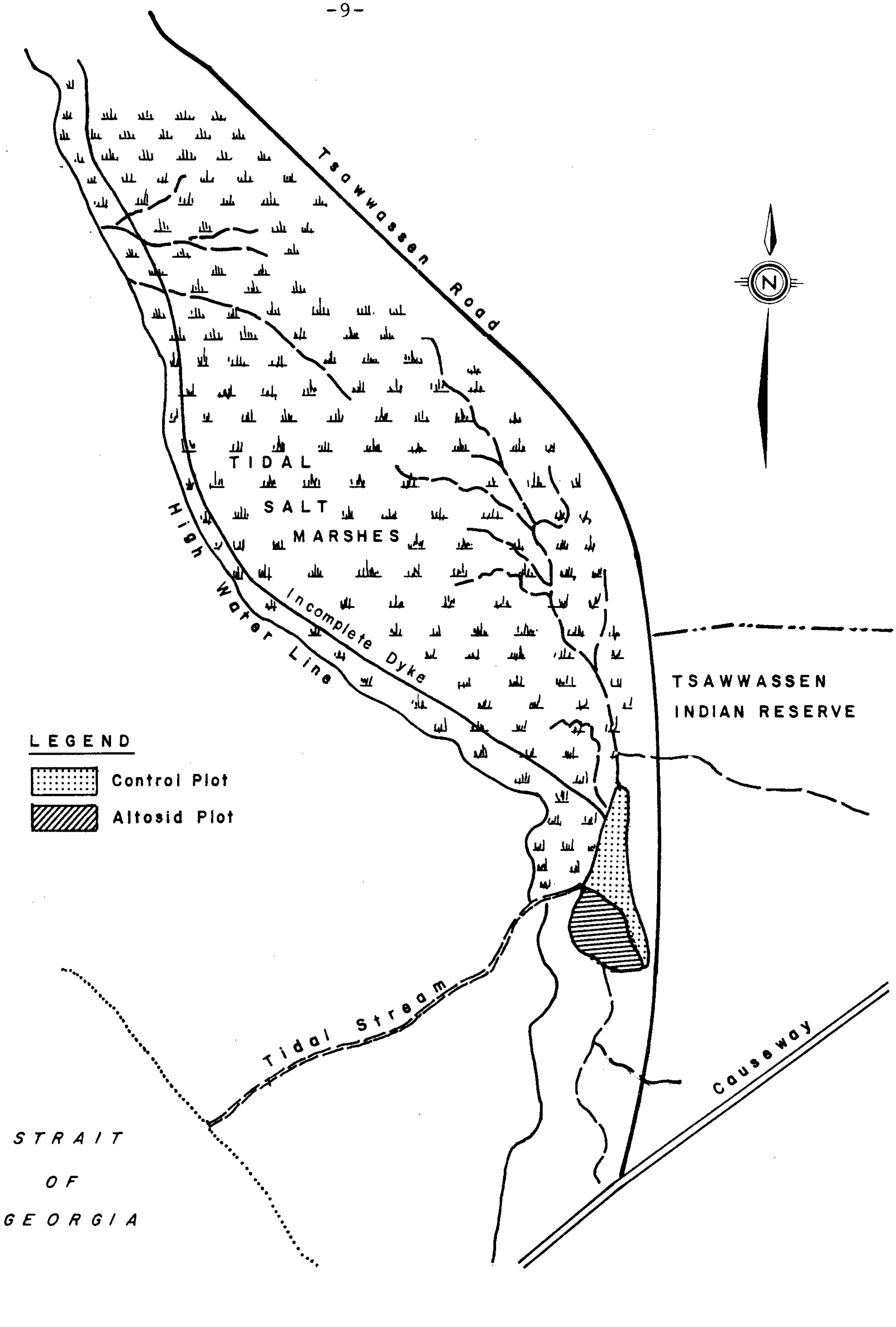

FIGURE I LOCATION OF ALTOSID STUDY PLOTS

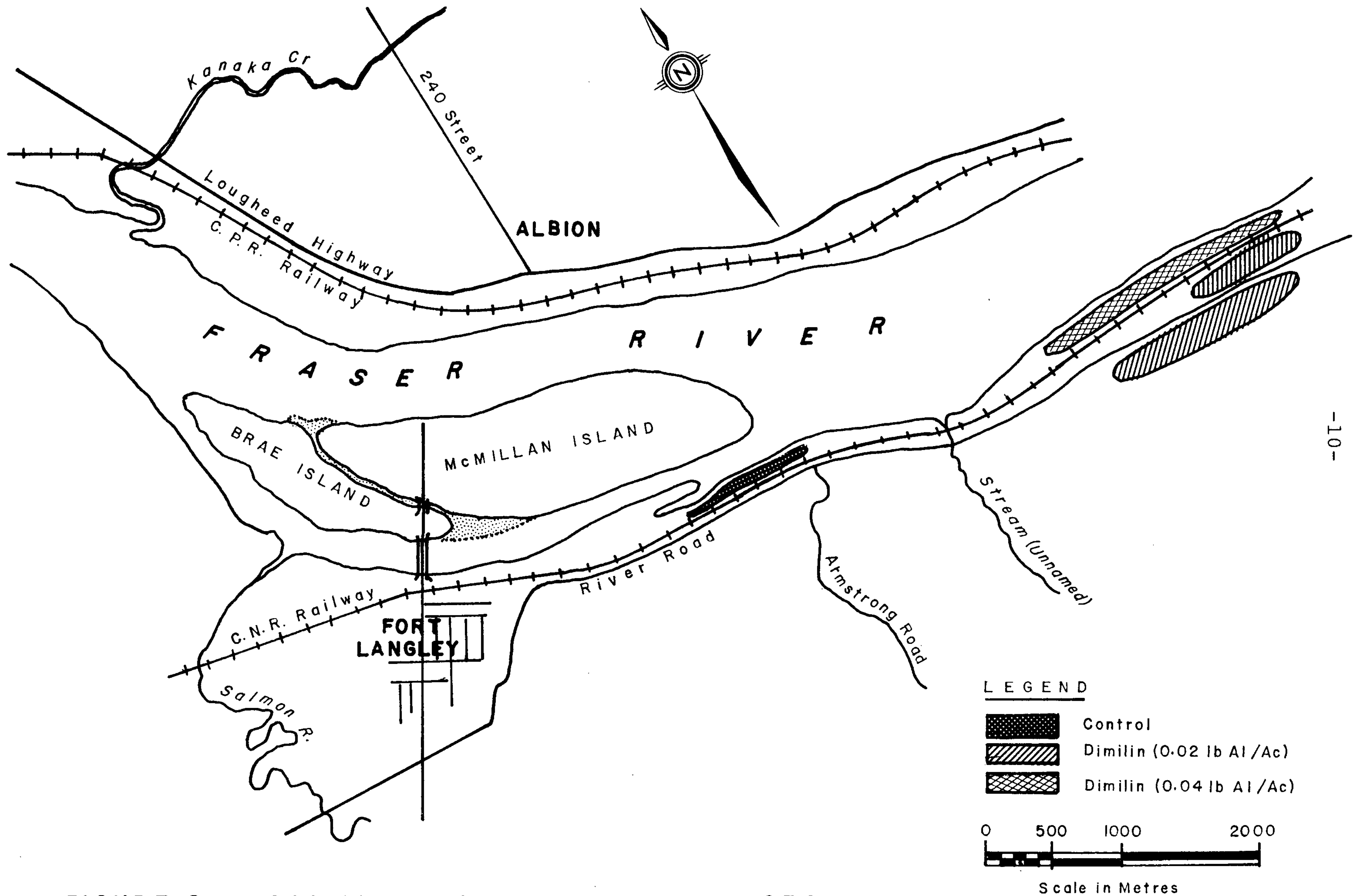

FIGURE 2 LOCATION OF DIMILIN STUDY PLOTS

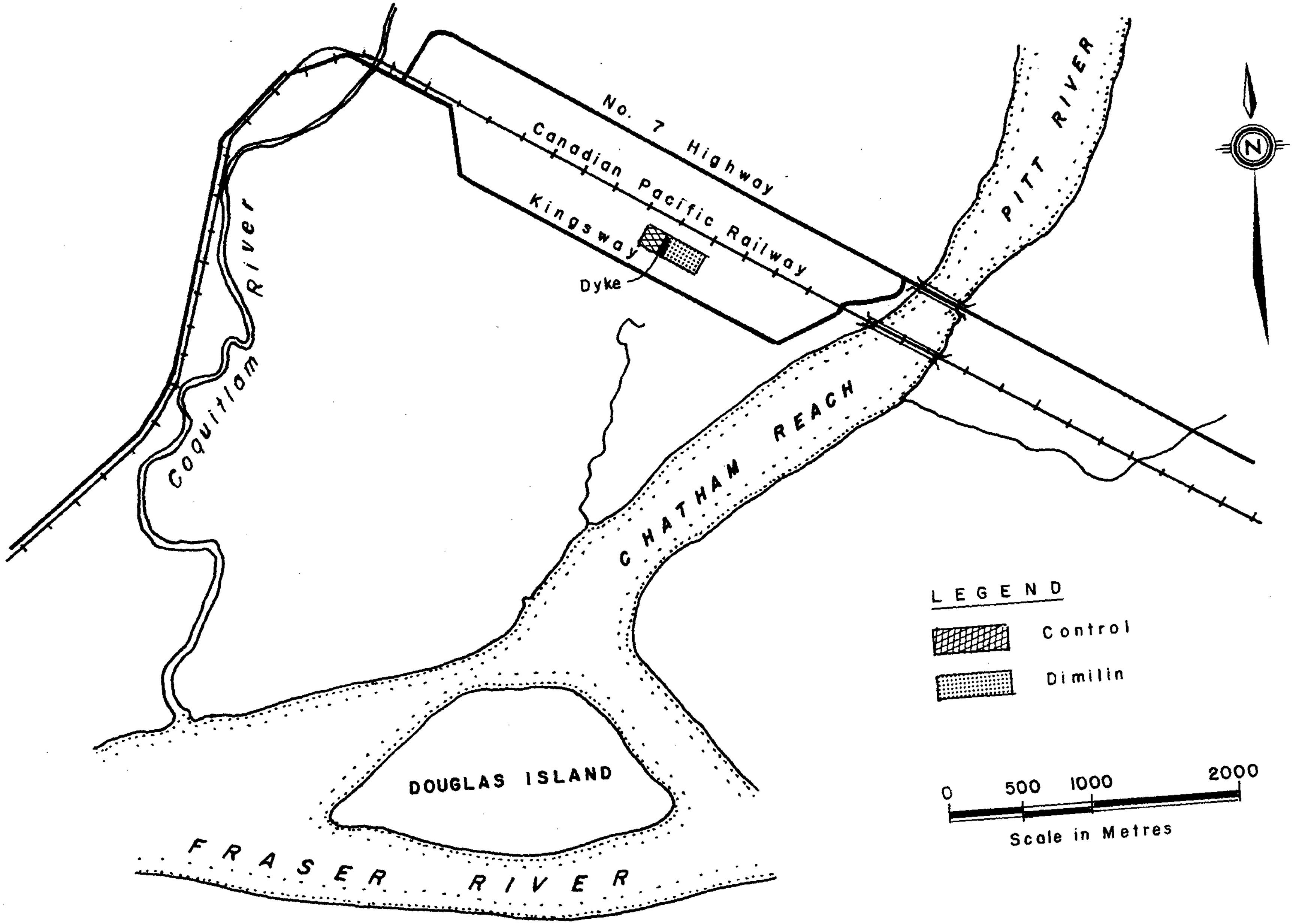

FIGURE 3 LOCATION OF DIMILIN STUDY PLOTS

Dimilin granules were applied with an agricultural spray aircraft[10] between 0615 and 0645 hours on June 29, 1976 at Fort Langley, and by hand treatment between 1100 and 1200 hours on June 29, 1978 at Port Coquitlam.

Other application details relating to these two experimental programs are given in Appendix 1.

3.3 Observations and Sampling

Water chemistry and temperature measurements, and biological sampling were conducted during the following periods:

Altosid

1. Pre-treatment, May 12-17, 1976 (6 days)
2. Post-treatment, May 18-24, 1976 (8 days)

Dimilin

Fort Langley (impact and residue studies)

1. Pre-treatment, June 16-24 (9 days)
2. Post-treatment, June 29-August 10, 1976 (43 days)

Port Coquitlam (Residue studies only)

1. Pre-treatment, June 29, 1978 (1 day)
2. Post-treatment, February 14, 1979 (234 days)

3.3.1 Water Chemistry and Physical Studies. Water temperatures were recorded once a day between 1100 - 1300 hours. Dissolved oxygen (DO), pH, sulfide concentrations and salinity of water were also measured daily. Appropriate Hach[11] kits are used to measure the pH and DO. In Port Coquitlam, only DO, pH and water temperatures were recorded.

3.3.2 Biological Sampling. Salt marsh invertebrates in the Altosid study were sampled with 800 ml dippers. In the Dimilin study, aquatic organisms were collected with emergence cases, an Ekman dredge and dippers.

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10 - Cessna Agwagen

11 - Hach Chemical Company, Ames, Iowa, 50010, U.S.A.

3.3.2.1 Emergence cages. The emergence cages were designed to trap newly emerged adult aquatic insects. The pyrimidal cages were made of plexiglass with aluminum supporting frames, and designed to enclose a water surface of 0.2 m^2 as described by Wan and Wilson, (1975).

Eight cages were placed in each plot (control vs. Dimilin-treated). They were spaced approximately 10 to 20 m apart and the edge of all cages was set about 0.3-0.6 cm below the water surface to prevent insect escape. The height of these cages in relation to the variation of water level was adjusted by using 'clipped' aluminium rods.

Newly emerged insects were trapped in a removable plexiglass cylinder on top of each cage. A short stem glass funnel (stem diameter 15 mm) was placed inside the cylinder to prevent insects from flying back into the main part of the cage. The insects were immobilized with 70% alcohol, and collected in labelled bottles daily. The cages were in place from June 17, 1976 to August 10, 1976.

3.3.2.2 Ekman dredge. An Ekman dredge (0.15m x 0.15m) was used to collect bottom invertebrate samples. Five grab samples were taken at random in each plot. The samples were pooled into a 125 mesh sieve and carefully washed to remove mud, silt, and organic debris. The benthic and mud organisms were retained in labelled bottles and preserved in 70% alcohol. Sediment samples at the Fort Langley study areas were also collected by using the Ekman dredge.

3.3.2.3 Dippers. Aluminium metal pots were used for the daily collection of aquatic invertebrates from submerged grasses and sedges. The pots were 12 cm diameter with a depth of 7 cm and equipped with a 2 m long handle.

Fifty dips were taken along two randomly selected 25m transects. All dips were pooled and strained through a 125 mesh sieve. After the required number of dips were taken, the samples were retained in labelled bottles and preserved in 70% alcohol.

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12 - Hach Chemical Company, Ames, Iowa, 50010, U.S.A.

All biological samples were taken daily between 1100 - 1400 hours.

3.3.3 Residue Sampling. Water samples were collected in 450 ml (16 fluid oz.) amber glass bottles. All bottles were rinsed with redistilled acetone and petroleum ether prior to use. Duplicate samples were taken from the treated plots before insecticide applications to determine the possible presence of naturally occurring compounds that may interfere with Altosid and Dimilin analyses. The following sampling regimes were used:

1. Altosid - prespray, 0, 1/2, 1, 2 hours, and 1, 2, 3, and 4 days post-spray.

2. Dimilin
   A. Fort Langley - pre-spray 0, 1, 4 hours, 1, 2, 4, 8, 16, 31 and 71 days post-treatment.
   B. Port Coquitlam - pre-spray 0, 4 hours, 1, 2, 4, 8, 75 (second application), 77, 78, 80, 88, 95, 109, 142, 170 and 234 days post-treatment.

In the Altosid samples 60 ml of acetone solvent were added to each 360 ml of water sample to prevent residue degradation. Approximately 50 ml of dichloromethane solvent were added to each Dimilin water sample. All samples were immediately frozen with dry ice en route to the laboratory.

The Dimilin sediment sampling regime was identical to the Dimilin water sampling program. The sediment samples were preserved with 10 ml of dichloromethane and then frozen on dry ice.

3.4 Residue Analyses

All residue analyses were done by the Pacific Region Water Quality Laboratory, Inland Waters Directorate of Environment Canada.

3.4.1 Extraction and Clean-up. Altosid residues were extracted from the water samples with hexane. The crude extract was then dried over anhydrous Na_2SO_4, concentrated and cleaned-up on a florisil column. The column eluates were concentrated in a rotary evaporator at 40°C, taken up in 25 ml of redistilled hexane and the clean-up extract analysed by gas liquid chromatography.

Dimilin residues were extracted from water samples in a similar manner, but they were derivatized in ethyl acetate before clean-up on florisil. Dimilin residues were extracted from sediments with a 1:1 acetone-hexane v/v mixture.

3.4.2 Gas Liquid Chromatography. GLC analysis was done on a Microtek MT-220[13] equipped with a ^{63}Ni electron capture detector. A 180 cm x 0.6 cm I.D. glass column was used. For Altosid analyses the column was packed with 3% OV-101 on Chromosorb W "H.P." 80/100 mesh. A mixture of 2% OV-101 and 3% OV-210 on similar chromosorb and mesh size was used for Dimilin analyses.

Nitrogen was used as the carrier gas at the rate of 82 ml/min over an oven temperature of 200°C for Altosid and at the rate of 65 ml/min at 150°C for Dimilin. Known amounts of Altosid and Dimilin standards were injected into the gas chromatograph to prepare standard peak height curves for the quantification of residues. The limit of detection for Altosid and Dimilin in water was 2 ppb and 0.10 ppb respectively. The detection limit fo Dimilin in sediment was 1 ppb.

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13 - Texas Instrument Limited, Austin, Texas, U.S.A.

3.5 Sample and Data Analyses

Invertebrate samples were counted and identified using the following keys: Borror and DeLong (1970), Pennak (1953) and Usinger, (1968). Genus and species identification of Diptera specimens were conducted by the Biosystematics Research Institute, Central Experimental Farm, Department of Agriculture, Ottawa.

A Hewlett-Packard computer Model 9830A[14] (with automatic plotter) was used for all calculations.

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14 - Hewlett-Packard Calculator Products Div., Box 301, Loveland, Col., 80537, U.S.A.

4 RESULTS AND DISCUSSION

4.1 Water Chemistry and Physical Parameters

Appendices II - IV summarize measurements of dissolved oxygen (DO), pH, salinity, air and water temperatures, depths of water, turbidity and sulfide content of the study plots of Tsawwassen and Fort Langley.

At Tsawwassen, the water chemistry and temperatures of the control and Altosid plots were comparable. DO in both varied from 8-12 ppm with a salinity averaging 21 o/oo. The water temperature of both fluctuated from 13-22.5 C, with pH varying from 7.0 - 8.4. The sulfide content in both was 1 mg/l.

At Fort Langley, the water chemistry and temperature of the Control and Dimilin plot varied considerably (Table 1). The pH, DO and depth of water were significantly higher in the control than in the Dimilin plot. The consistently greater depth in the control was due to flooding from Fraser River (Figure 4).

The salinity for both Dimilin treated and control plots was below detection before and after treatment. Suspended solids in the control increased for one day during flooding, but otherwise remained similar to the Dimilin Plot (10 mg/l non-filterable residue).

At Port Coquitlam, although the pH of water remained acidic (pH 5.5) during the period of study, there was a considerable variation of DO in the ponds (2.4 - 12 ppm).

4.2 Residues in Water and Sediments

Altosid and Dimilin residues measured in water and sediments are presented in Tables 2, 3, and 4.

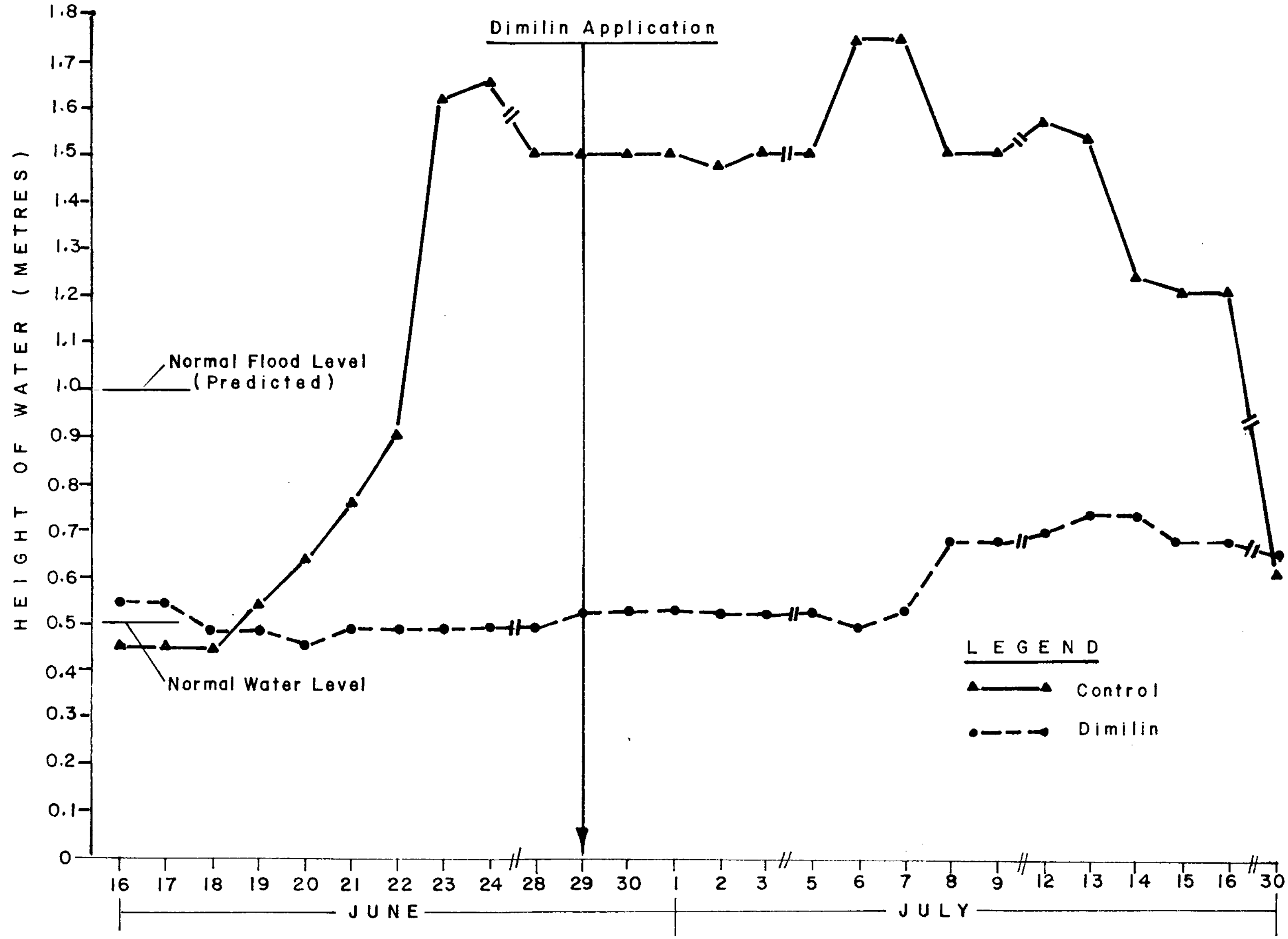

FIGURE 4 WATER LEVEL OF CONTROL AND DIMILIN PLOT AT FORT LANGLEY, B.C.

TABLE 1 WATER CHEMISTRY, TEMPERATURE AND DEPTH OF FORT LANGLEY PLOTS

Parameters	Control Mean ± S.E. (range)	Dimilin Mean ± S.E. (range)	Level of Significance
pH	6.7 ± 0.1 (6.3-6.9)	5.7 ± 0.1 (5.5-6.1)	0.001
DO (ppm)	6.2 ± 0.3 (4.0-8.0)	3.2 ± 0.2 (1.4-5.0)	0.001
T°C	16.3 ± 0.4 (12 - 20)	17.9 ± 0.4 (15 - 22)	0.020
Depth (m)	1.23 ± 0.09 (0.45-1.74)	0.54 ± 0.03 (0.45-0.72)	0.001

TABLE 2 ALTOSID RESIDUES IN TSWWASSEN SALT MARSH WATER

Sampling Time	Control (ppb)*	Treated (ppb)*
Pre-spray	L 2	L 2
0 Hours	L 2	10
0.5	L 2	7
1	L 2	11
2	L 2	13
24	L 2	14
48	L 2	L 2
72	L 2	18
96	L 2	17

*Limit of detection = 2 ppb.
L = less than

TABLE 3 DIMILIN RESIDUES IN SOIL AND WATER - FORT LANGLEY FLOOD PLAIN

Sampling Time	Control (ppb)*		Treated (ppb)*	
	Sediment	Water	Sediment	Water
Pre-spray	L 1.0	L 0.10	L 1.0	L 0.10
0 Hours	-	-	L 1.0	0.50
1	-	-	1.0	0.10
4	-	-	5.6	0.10
1 day	L 1.0	L 0.10	-	0.48
2	-	-	3.2	0.36
4	-	-	2.2	1.80
8	-	-	-	1.80
16	-	-	-	0.55
31	-	-	3.5	0.34
71	-	-	1.3	0.24

*Limit of detection: sediment = 1 ppb
water = 0.10 ppb
L = less than

TABLE 4 DIMILIN RESIDUES IN SOIL AND WATER - PORT COQUITLAM PONDS

Sampling Time	Control (ppb)* Sediment	Control (ppb)* Water	Treated (ppb)* Sediment	Treated (ppb)* Water
Pre-spray	L 1	L 0.10	L 1	0.10
4 Hours	L 1	L 0.10	110	5.9
1 (day)	L 1	L 0.10	80	3.7
2	-	-	220	2.8
4	-	-	90	3.5
8	-	-	80	8.5
75 (second spray)	L 1	L 0.10	70	0.10
75 (4 hours)	L 1	L 0.10	L 1	4.8
77 (day)	-	-	L 1	2.8
78			90	0.7
80			20	L 0.10
88			20	L 0.10
95			60	L 0.10
109			11	L 0.10
142			20	L 0.10
170			32	L 0.10
234	10	0.5	L 1	L 0.10

*Limit of detection: sediment = 1 ppb
water = 0.10 ppb
L = less than

4.2.1 Altosid residues. Altosid, a derivative of 2,4-dienoic ester, is unstable to heat, light, and microbial activity and should degrade rapidly in the field. It is expected to have short residual action and cause minimum disruptions in the environment. Schaefer and Dupras (1973) indicated that the slow release formulation of Altosid persisted only one day in water. Our residue studies with a similar material at the Tsawwassen salt marshes indicated that this hormone persisted longer than 24 hours. Altosid residues (SR-10 formulation) were detected in water for 4 days after application. Fourteen ppb of the hormone was detected one day after treatment, but decreased to 2 ppb 24 hours later. This drop in concentration was probably due to dilution from rain and high tide which increased the volume of water in the salt marsh tidal pools. Altosid concentration peaked at 18 ppb on the third day following treatment. The delayed attainment of maximum concentration probably resulted from the on-going release of thhe micro-encapsulated formulation, and a subsequent low tide and high evaporation rate that reduced the volume of water in the pools, thereby concentrating the hormone. Although Altosid was applied at a rate of 20 ppb in 15 cm water, an average concentration of 12 ppb was found and this was effective in arresting mosquito pupation.

4.2.2 Dimilin residues. Dimilin is stable to heat, light and depending on formulation can persist in sediments and water for an extended period of time. Our studies with a granular formulation of Dimilin concurred with the findings of Nimmo and de Wilde (1975), in that this material can persist in sediment-water systems for several months.

At Fort Langley, Dimilin was found in the water for 71 days following an aerial application. The concentration of this IGR increased slowly and reached a peak of 1.8 ppb eight days after treatment. Thereafter it decreased slowly, but was still detected at 0.24 ppb two months later. In the sediments, Dimilin was detected at 5.66 ppb four hours after application, but residues decreased to only 1.3 ppb, two months after treatment.

At Port Coquitlam, Dimilin was detected in water for 8 days after the first application and 3 days following the second treatment. In the sediments, Dimilin residues persisted for a period of 170 days.

A prescribed dosage of 0.046 kg ai/ha at Fort Langley should produce 15 ppb in 30 cm water, but an average concentration of only 0.90 ppb was found during the first month of application. A similar situation occurred in Port Coquitlam plots, where the application rate was 0.034 kg ai/a which should general 11 ppb Dimilin in water. Although the residue levels detected in water were higher than Fort Langley plots at 4.1 ppb and 2.8 ppb following the first and second application respectively, they were also lower than the expected concentration. This low concentration was due in part to the low solubility of Dimilin in water - (220 ppb at 20°C, Anon., 1974), and to the slow release adhesive used in the experimental formulation (Thompson-Hayward Chemical Co., 1977, personal communication).

In sediments, an average concentration of 2.6 ppb Dimilin was obtained after the first month of treatment at Fort Langley. This level decreased to 1.3 ppb in the second month. Higher levels of Dimilin residues, however, were detected in the pond at Port Coquitlam. The average concentration of Dimmilin residues two months after the first application was 108 ppb and this decreased to 34.3 ppb during the last three months even after a second Dimilin application.

The higher levels of Dimilin in sediments at Port Coquitlam were related to the frequency and probably the method of application. This study plot was treated twice by hand application, while the plot at Fort Langley was aerially sprayed once. Some Dimilin granules were expected to have missed the target area via drift during aerial application.

4.3 Biological Observations. Invertebrates colected included the major Orders of zooplankton, aquatic insects, mites, and snails. All arthropods collected from the emergence cages were adults while those from the Ekman dredge samples were mainly immature stages. Dip samples contained a mixture of adult and larva stages.

4.3.1 Emergence of Insects in the Dimilin study. All insects trapped in the emergence cages were from the Order Diptera.

Daily insect emergence trends in the control and Dimilin treated plot are illustrated in Figure 5. A lower rate of insect emergence is evident in the Dimilin plot after hormone application, (i.e., from June 30 until July 21, 1976). Lower daily insect emergence (calculated on a 5-day average) was also found in the Dimilin plot from July 22 to August 10, 1976. During that period a consistently higher rate of insect emergence occurred in the control.

The mean daily rate of emergence of all insects in the control remained basically unchanged during the period of investigation (i.e., pre-spray versus post-spray time period comparison). In the Dimilin plot, however, significantly lower rates of emergence as compared to the control and pre-spray levels in the treatment plot were found for all insects, but particularly muscidae and 6 genera of tendipedidae, after hormone applications (see Appendix VII for probability).

4.3.2 Dip Organisms. The groups of aquatic organisms caught with a dipper are shown in Appendix VI. They were mainly zooplanktonic crustaceans, insects, mites, fish, marsh crabs, clams and snails.

4.3.2.1 Altosid treatment at Tsawwassen. Salt marsh organisms from dip samples were chiefly planktonic crustaceans, which accounted for approximately 93% of the total population. The others comprised water mites, marsh crabs, fish and mosquito larvae and pupae.

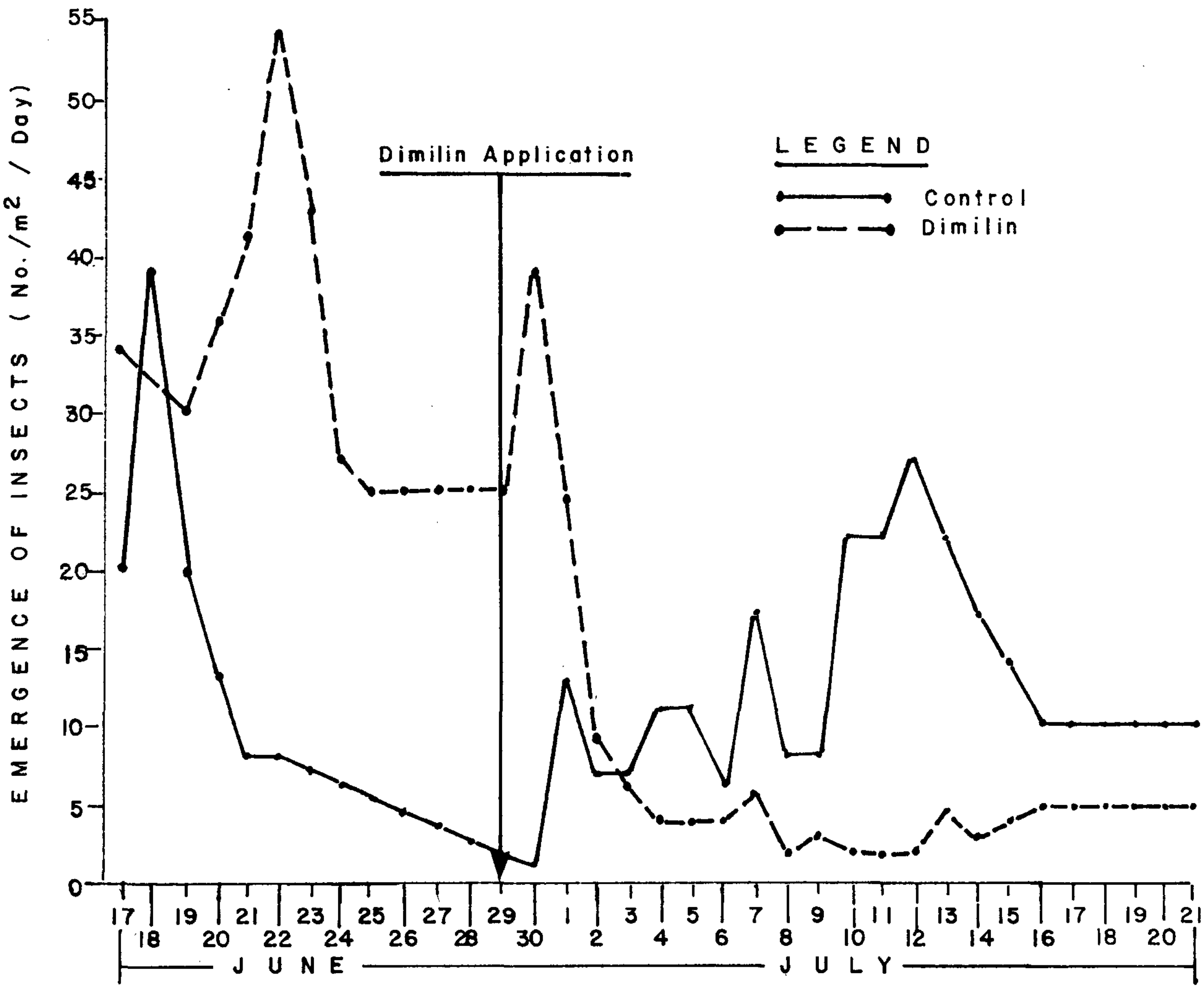

FIGURE 5 RATE OF EMERGENCE OF INSECTS IN CONTROL AND DIMILIN-TREATED STATIONS

Daily changes in number of amphipods, copepods, and mosquito larvae are illustrated in Figure 6. The changes in mean daily numbers caught before and after Altosid application are presented in Appendix VIII.

Harmful effects to crustaceans, fish, and marsh crabs were not detected after the application of Altosid to the salt marshes. No mortality or visible changes in behaviour were observed in fish (mostly sticklebacks, Gasterostea sp), and marsh crabs.

Significantly lower numbers of mosquito larvae (P=0.10, n= 12), however, were found in the control, but not in the Altosid plot during the post treatment period (May 18 to May 25, 1976). This reduction in number of mosquito larvae was attributed to pupation. In the Altosid plot, the unchanged numbers of the larvae during the same period may have resulted from the hormone arresting pupation.

Although the pupal population was comparable during the pre-spray period for both the control and Altosid plots, the control had a significantly higher pupal count (P=0.05, n - 12), than the Altosid plot during the post spray period.

4.3.2.2 Dimilin Treatment at Fort Langley. The organisms of the Fraser River flood plain caught in dip samples were crustaceans, insects, water mites, snails and clams. The daily changes in numbers of Amphipods, Cladocera, Copepoda, and Ostracoda are illustrated in Figures 7 and 8. Changes in mean numbers before and after Dimilin application are presented in Appendix IX.

Dimilin appeared to depress populations of Cladocera for about 5 days, but this crustacean recovered partially two weeks after treatment. It did not seem to have any effect on Copepoda and Ostracoda.

Significant reductions (see Appendix IX for probability), of water beetles and zooplankton also occurred in the control during the post-spray period (June 30 - July 16). However, this reduction was attributed to the unexpected high tide and flood of the Fraser River, which continually flooded the area for about 10 days.

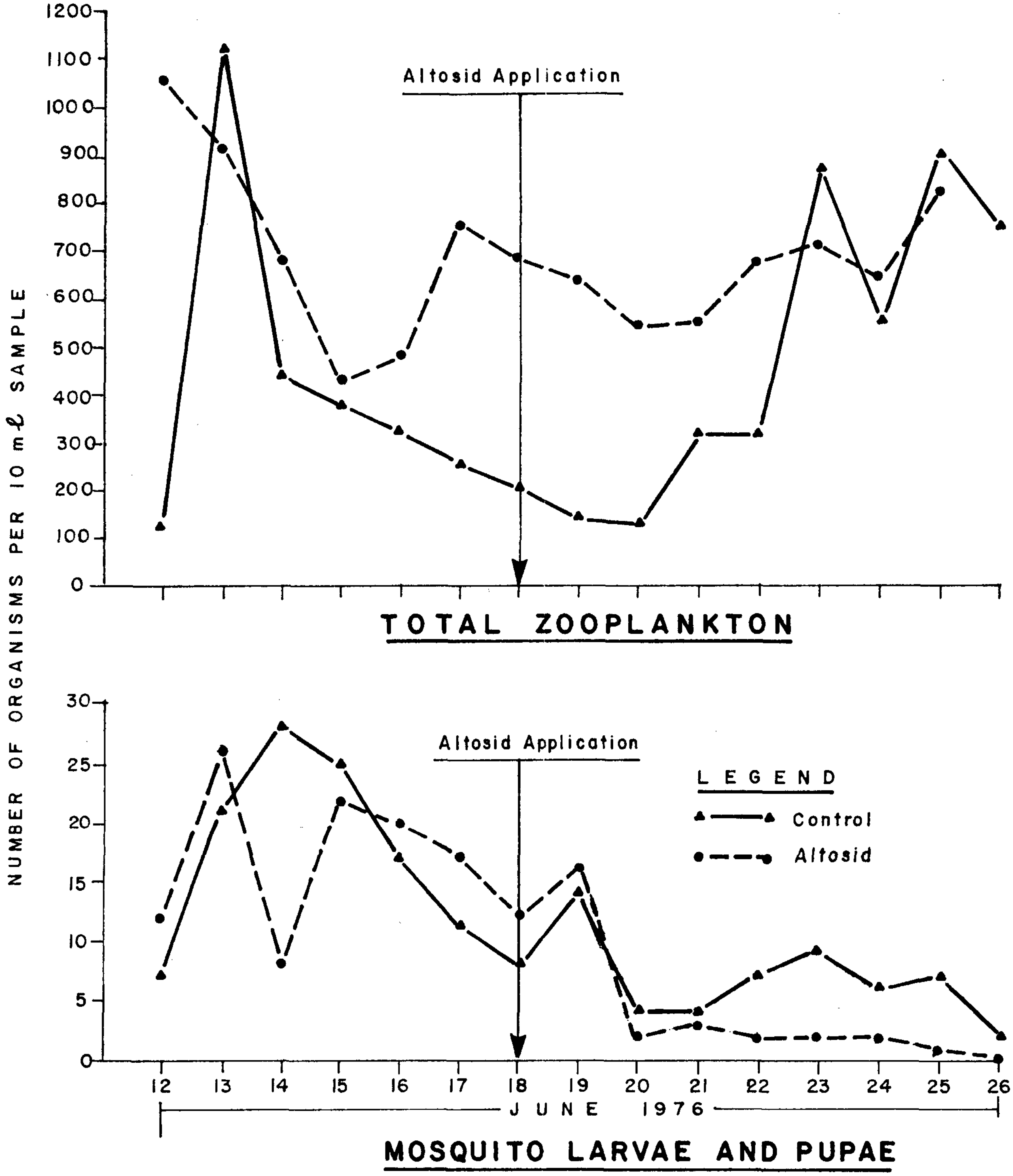

FIGURE 6 CHANGES IN NUMBERS OF ZOOPLANKTON AND IMMATURE MOSQUITOES IN CONTROL AND ALTOSID-TREATED PLOTS

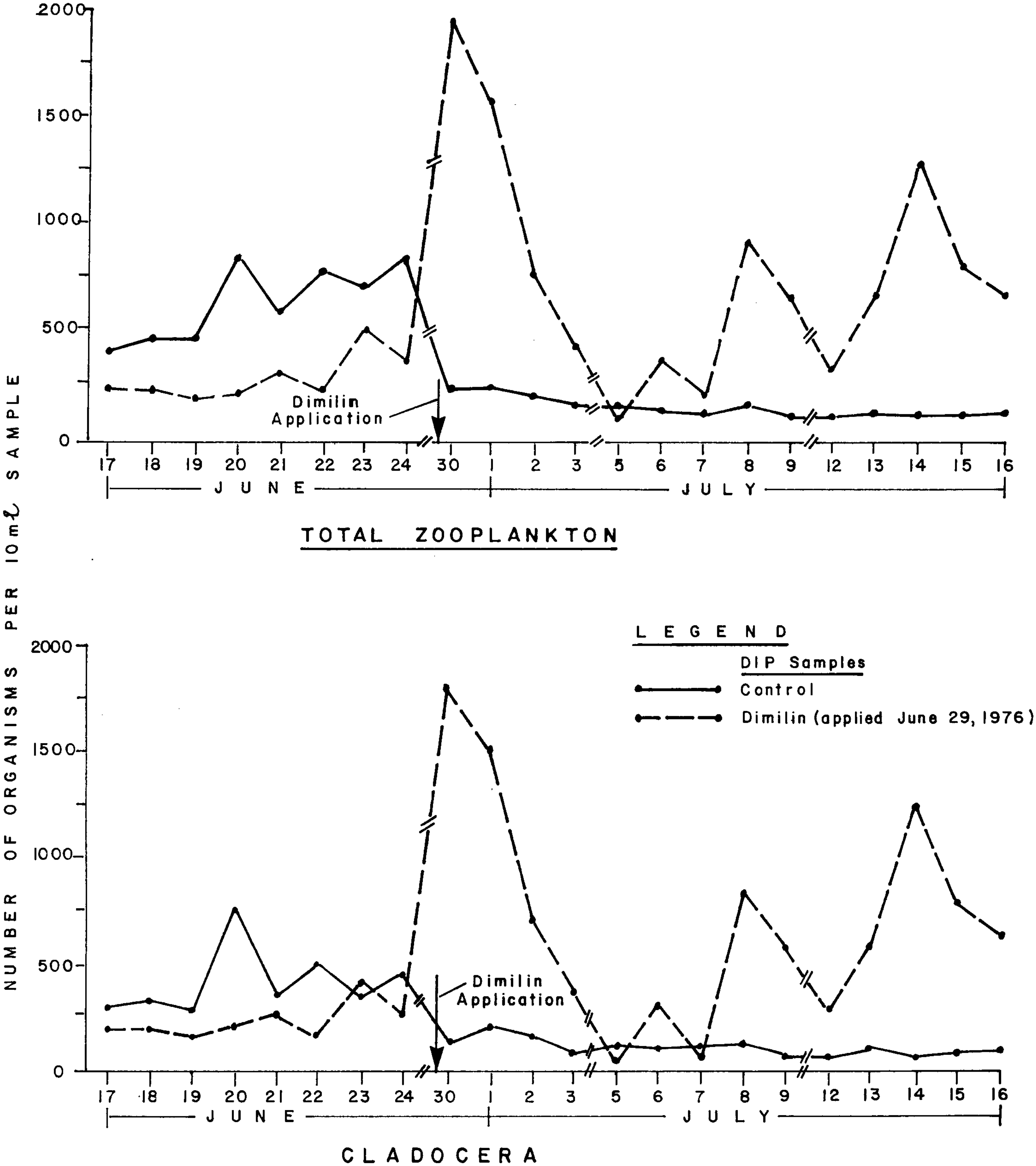

FIGURE 7 CHANGES IN ZOOPLANKTON NUMBERS IN CONTROL AND DIMILIN - TREATED PLOTS

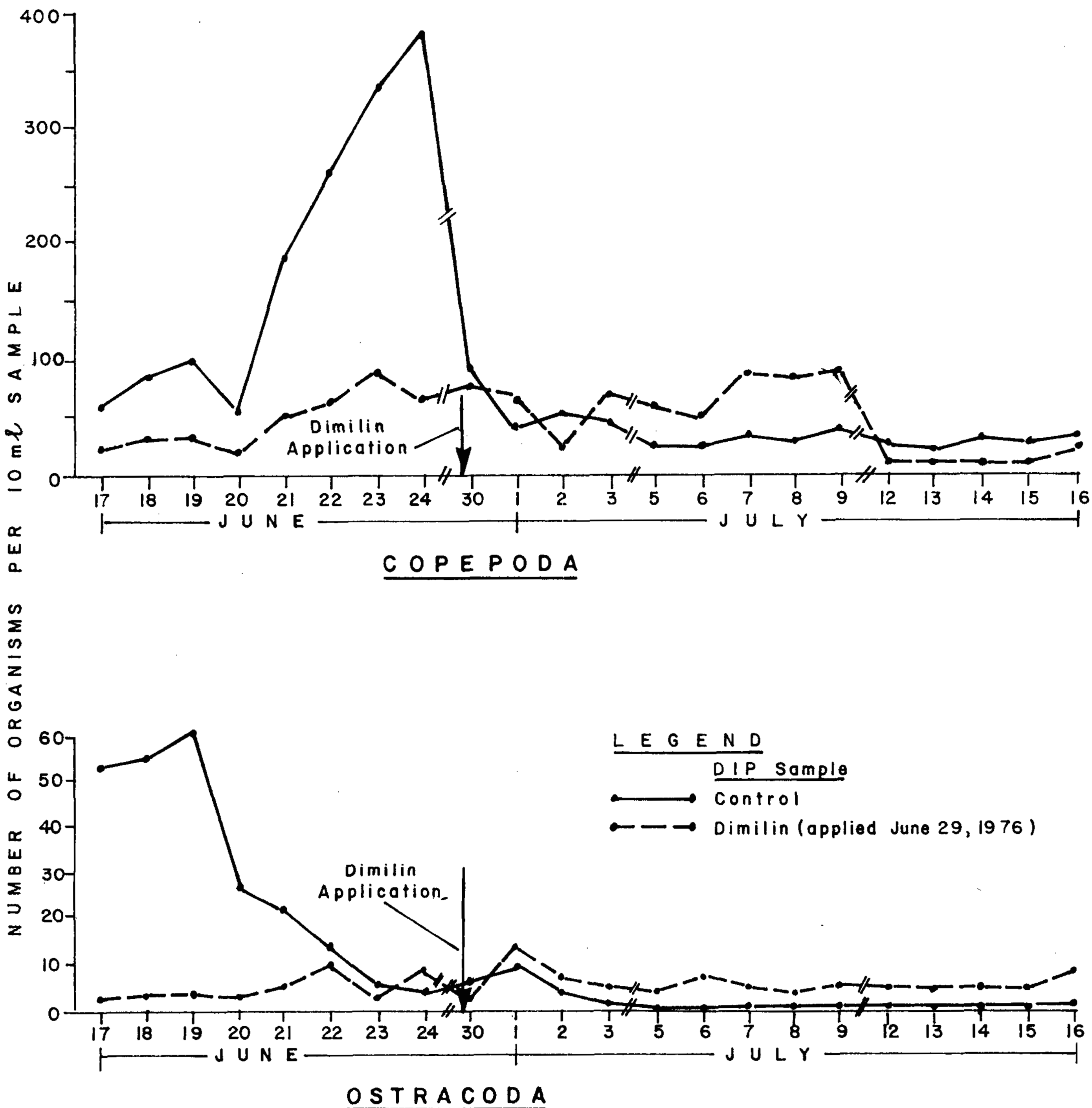

FIGURE 8 CHANGES IN ZOOPLANKTON NUMBERS IN CONTROL AND DIMILIN-TREATED PLOTS

In the Dimilin plot, significant post treatment increases in number of organisms were recorded for most groups, with the exception of the mosquito larvae, water bugs and mites.

4.3.3 Dredge Organisms - Fort Langley Dimilin Treatment. Organisms collected from the Ekman dredge samples included larvae of several Diptera, snails, clams, and some crustaceans. The changes in numbers before and after Dimilin application for the different groups of invertebrates are presented in Appendix X.

A significant decrease in Tendipedidae in the Dimilin plot after hormone application was evident, although a similar change also occurred in the control for the same period. This reduction was probably due to natural causes, such as pupation. A significant decrease in Cladocera and Ostracoda were also found in the Dimilin plot after treatment.

4.4 General Implications of IGRs

The results of these studies indicate that the effect of IGRs on non-target organisms in the aquatic environment depends on the type and characteristics of the inhibitor. Despite the apparent persistence of Altosid in water beyond four days following an application, there appeared to be no adverse effects on amphipods, copepods, fish and marsh crabs.

IGR such as Dimilin that persist in the sediments for an extended period of several months is expected to have longer residual action and this could have some effects on fish food organisms. A concentration of 0.3 - 0.9 ppb in water and 1.3 - 1.6 ppb in sediments appeared to be effective in inhibiting the emergence of mosquitoes as well as several non-target insects co-existing in the same aquatic environment. In the present study a sustained suppression of adult emergence of six genera of Tendipedidae was observed for more than one month, and the population did not recover for the season.

Tendipedidae (chironomidae) are important fish food organisms, (Borror and DeLong, 1971). Prolonged reduction in the emergence of this insect in flood plains, marshes, may have some effect on fish and other organisms dependent on this group of insects for food. It may result in the reduction in numbers of mating and breeding adult insects. This, in turn, could result in reduction of egg laying or larval hatching during the following season. Consequently, a food shortage for fish may be induced in the flood plains and marshes, which are important natural nurseries of many fish.

Another important consideration regarding the use of IGR such as Dimilin for larviciding is its possible effect on the chitin formation of non-target moulting crustaceans. Dimilin inhibits chitin formation in arthropods regardless of age and stage of development. Many female crustaceans must complete a moult before mating can occur (Barnes, 1968), and may be as vulnerable to chitin inhibitors in the adult as the juvenile stage.

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APPENDICES

APPENDIX I

APPLICATION DETAILS

APPENDIX I APPLICATION DETAILS

	Altosid	Dimilin	
	Tsawwassen	Fort Langley	Port Coquitlam
Weather	Sunny afternoon	Clear morning	Clear morning
Air temperature	20°C	15°C	18°C
Wind	8 km p.h.	5 km p.h.	-
Aircraft/other specifications			
(a) Aircraft	-	Fixed wing, Cessna Agwagen	-
(b) Flight speed	-	160 km p.h.	-
(c) Type of sprayer	Hand gun	Modified combine	Hand shaker
(d) Capacity	1 - 2 litres	450 kg per load	0.5 litres
(e) Height	-	18 m above ground	1 m above ground
(f) Rate	0.046 kg ai/ha	4.6 kg 1% granule/ha	0.034 kg ai/ha
Plot details			
(a) Location	Tsawwassen	5 km east of Fort Langley	15 km east of Vancouver
(b) Dimension	-	0.5 km long, 100 m wide	-
(c) total area	225 m	5 ha	625 m

APPENDIX II

WATER CHEMISTRY OF CONTROL AND ALTOSID PLOTS - TSAWWASSEN, B.C.

APPENDIX II WATER CHEMISTRY OF CONTROL AND ALTOSID PLOTS - TSAWWASSEN, B.C.

Date (1976)	DO (ppm)	pH	Salinity (0/00)	Water T°C	Sulfide mg/l
			Control Plot		
May 12	-	-	-	-	-
13	-	-	-	-	-
14	12	7.6	21	19	1
15	-	-	-	22.5	-
16	12	7.6	21	18	1
17	11	8.0	-	13	-
18	11	8.1	-	18.5	-
19	-	-	20.5	15	-
20	10	8.4	-	20.5	1
21	9	7.0	-	22.5	-
22	-	-	-	-	-
23	8	7.2	20.5	23	1
24	-	-	-	18	-
25	-	-	-	-	-
			Altosid Treatment Area		
May 12	-	-	-	-	-
13	-	-	-	-	-
14	12	7.7	21	20	1
15	-	-	-	20.5	-
16	12	7.4	21	18	1
17	11	8.0	-	13	-
18	11	8.0	-	19.5	-
19	-	-	20.5	15	-
20	10	8.2	-	20.5	1
21	9	7.3	-	22.5	-
22	-	-	-	18.5	-
23	8	7.3	20.5	23	1
24	-	-	-	18	-
25	-	-	-	-	-

APPENDIX III

WATER CHEMISTRY OF CONTROL PLOT
(FORT LANGLEY)

APPENDIX III WATER CHEMISTRY OF DIMILIN STUDY - CONTROL PLOT (FORT LANGLEY)

Date (1976)	Time of recording (hrs)	pH	DO (ppm)	Depth of water (m)	Salinity (0/00)	Suspended Solids (mg/l NFR)	Temperature (°C) Air	Temperature (°C) Water
June 16	1350	6.7	5	0.45	0.05	10	18	14
17	1100	6.7	5	0.45	-	-	20	15
18	1300	6.6	5	0.45	0.05	10	26	18
19	1325 (flooding)	6.7	4	0.54	-	-	21	18
20	1310	6.6	4	0.63	-	10	24	18
21	1230	6.3	4	0.75	-	-	22	16
22	1210	6.5	7	0.90	-	10	22	15
23	1225	6.6	8	1.62	0.05	21	15 (rain)	12
24	1230	6.7	7	1.65	-	10	15	14
28	1355	6.9	8	1.50	-	-	23	18
29	1215	6.9	7	1.50	-	-	22	18
30	1350	6.8	6	1.50	-	-	19	18
July 1	1640	6.7	8	1.50	0.05	10	19	18
2	1340	6.8	7	1.47	-	-	18	19
3	1340	6.7	6	1.50	-	-	20	17
5	1430	6.6	5	1.50	-	-	27	20
6	1400	6.7	7	1.74	-	-	22	20
7	1410	6.8	5	1.74	-	-	19	20
8	1345	6.8	7	1.50	0.05	10	16 (rain)	17
9	1300	6.8	7	1.50	-	-	16	16
12	1300	6.7	7	1.56	-	-	19	16
13	1400	6.7	7	1.53	-	-	19	16
14	1415	6.7	6	1.23	-	-	22	17
15	1400	6.9	8	1.20	-	-	26	19
16	1215	6.9	6	1.20	-	-	26	20
30	-	-	-	0.60	-	-	-	-

APPENDIX IV

WATER CHEMISTRY OF DIMILIN TREATED PLOT
(FORT LANGLEY)

APPENDIX IV WATER CHEMISTRY OF DIMILIN TREATED PLOT (FORT LANGLEY)

Date (1976)	Time of recording (hrs)	pH	DO (ppm)	Depth of water (m)	Salinity (0/00)	Suspended Solids (mg/l NFR)	Temperature (°C) Air	Water Water
June 16	1240	5.8	5.0	0.54	0.05	10	17	15
17	1240	5.8	5.0	0.54	-	-	20	19
18	1427	5.8	3.0	0.48	0.05	10	25	22
19	1455	5.7	2.5	0.48	-	-	23	21
20	1435	5.7	2.3	0.45	-	10	22	20
21	1420	5.7	2.0	0.48	-	-	22	22
22	1345	5.6	2.0	0.48	-	10	20	19
23	1330	5.7	1.4	0.48	0.05	10	14 (rain)	16
24	1330	5.7	1.6	0.48	-	10	15	16
28	1430	5.9	1.4	0.48	-	-	25	20
29	1130	5.9	2.0	0.51	-	-	19	18
30	1135	6.1	1.8	0.51	-	-	22	18
July 1	1135	5.8	3.2	0.51	0.05	10	18	18
2	1155	5.8	3.8	0.51	-	-	20	17
3	1135	5.8	4.0	0.51	-	-	18	16
5	1245	6.2	5.0	0.51	-	-	26	19
6	1245	6.0	5.0	0.48	-	-	22	21
7	1200	6.1	5.0	0.51	-	-	21	20
8	1200	5.5	3.0	0.66	0.05	10	16 (rain)	17
9	1200	5.5	3.0	0.66	-	-	16	16
12	1200	5.5	3.2	0.69	-	-	20	18
13	1300	5.5	2.8	0.72	-	-	19	19
14	1300	5.5	2.6	0.72	-	-	22	19
15	1200	5.7	3.6	0.69	-	-	24	19
16	1115	5.5	5.0	0.69	-	-	25	20
30	1030	5.8	3.2	0.60	-	-	24	17

APPENDIX V

WATER CHEMISTRY OF DIMILIN STUDY PLOTS
(PORT COQUITLAM)

APPENDIX V WATER CHEMISTRY OF DIMILIN STUDY PLOTS - PORT COQUITLAM

Date (1978)	Time of Recording	CONTROL				DIMILIN TREATED PLOT			
		pH	DO (ppm)	Temperature °C. Air	Temperature °C. Water	pH	DO (ppm)	Temperature °C. Air	Temperature °C. Water
June 29	0900	5.5	6	19	18	5.5	6	18	18
	1100	5.5	7	18	19	5.5	6	18	19
	1400	5.5	7	19	19	5.5	6	18	19
June 30	1300	5.5	8	20	19	5.5	7	20	20
July 01	1300	5.5	11	27	23	5.5	12	28	26
03	1300	5.5	8	21	21	5.5	9	23	22
07	1300	5.5	11	24	24	5.5	10	26	28
September 11	1100	5.5	5	14	8	5.5	5	15	8
	1400	5.5	5	15	8	5.5	5	15	8
12	1300	5.5.	2.4	20	18	5.5	2.8	20	17
14	1300	5.5	4	18	15	5.5	4	18	15
16	1330	5.5	5	11	13	5.5	6	12	13
18	1300	5.5	7	17	14	5.5	7	18	14
25	1345	5.5	8	22	15	5.5	8	21	15
October 11	1300	5.5	6	15	13	5.5	6	15	13
November 15	1300	5.5	6	6	4	5.5	6	6	4
December 12	1300	5.5	6	5	4	5.5	6	5	4
(1979)									
February 14	1300	5.5	8	5	1	5.5	9	5	1

APPENDIX VI

PRESENCE OF AQUATIC ORGANISMS AT TSAWWASSEN AND
FORT LANGLEY, TREATMENT AND CONTROL PLOT

APPENDIX VI PRESENCE OF AQUATIC ORGANISMS AT TSAWWASSEN AND FORT LANGLEY, TREATMENT AND CONTROL PLOT

Aquatic Organisms	Tsawwassen		Fort Langley					
	Control	Altosid	Control			Dimilin		
	(Dips)	(Dips)	(Emg cg)	(Dips)	(Ekm)	(Emg.cg)	(Dips)	Ekm)
ANNELIDA								
Order I	-	-	-	+	+	-	+	+
ARACHNIDA								
Hydracarina	+	+	-	+	+	-	+	+
CRUSTACEA								
Amphipoda	+	+	-	+	+	-	-	-
Cladocera	-	-	-	+	+	-	+	+
Copepoda	+	+	-	+	+	-	+	+
Ostracoda	-	-	-	+	+	-	+	+
DECAPODA								
Order I	+	+	-	-	-	-	-	-
GASTROPODA								
Order I	-	-	-	+	+	-	+	+
Order II	-	-	-	+	+	-	+	+
INSECTA								
Coleoptera								
F. Dytiscidae	-	-	-	+	+	-	+	+
F. Hydrophilidae	-	-	-	-	-	-	+	+
Diptera								
F. Anthomyiidae	-	-	-	-	-	+	-	-
F. Cecidomyiidae	-	-	+	-	-	-	-	-
F. Ceratopogonidae	-	-	+	-	-	+	-	-
F. Chaoboridae	-	-	+	-	+	-	-	-
F. Culicidae	+	+	+	+	-	+	+	-
F. Dolichopodidae	-	-	+	-	-	-	-	-
F. Empididae	-	-	+	-	-	-	-	+
F. Muscidae	-	-	+	+	-	+	+	+
F. Tendipedidae	+	+	+	+	+	+	+	+
Chironomus sp.	-	-	+	-	-	+	-	-
Psectrotanypus sp.	-	-	+	-	-	+	-	-
Tanytarsus sp.	-	-	+	-	-	+	-	-
Micropsectia sp.	-	-	+	-	-	+	-	-
Corynoneura sp.	-	-	+	-	-	+	-	-
Psectrecladius sp.	-	-	+	-	-	+	-	-
Chaoborus sp.	-	-	-	-	-	+		-
F. Unknown I	+	+	-	-	-	-	-	-
F. Unknown II	-	-	-	+	-	-	+	-

APPENDIX VI PRESENCE OF AQUATIC ORGANISMS AT TSAWWASSEN AND FORT LANGLEY, TREATMENT AND CONTROL PLOT (continued)

Aquatic Organisms	Tsawwassen		Fort Langley					
	Control	Altosid	Control			Dimilin		
	(Dips)	(Dips)	(Emg cg)	(Dips)	(Ekm)	(Emg.cg)	(Dips)	Ekm)
Ephemeronoptera								
F. Baetidae	-	-	-	+	-	-	+	-
Hemiptera								
F. Gerridae	-	-	-	-	-	-	-	-
F. Notodontidae	+	+	-	+	-	-	+	-
Odonata	-	-	-	+	+	-	+	+
PELECYPODA								
Order I	-	-	-	+	+	-	+	+
PISCES								
Order I	+	+	-	+	-	-	+	-
Total No. organisms	9	9	12	18	13	12	18	14

\+ present
\- absent, or not caught

APPENDIX VII

CHANGES IN DAILY RATE OF INSECT EMERGENCE BEFORE AND AFTER DIMILIN TREATMENT

APPENDIX VII CHANGES IN DAILY RATE OF INSECT EMERGENCE BEFORE AND AFTER DIMILIN TREATMENT

Insecta	Control			Dimilin		
	Pre-spray (June 17-June 22)	Post-spray (June 30-July 16)	Level of Significance	Pre-spray (June 17-June 22)	Post-spray (June 30-July 16)	Level of Significance
Culicidae	0.4 ± 0.3 (0-2)	0.5 ± 0.1 (0-2)	N.S	not caught	not caught	-
Empedidae	0.2 ± 0.1 (0-1)	0.1 ± 0.09(0-2)	N.S.	not caught	not caught	-
Muscidae	0.2 ± 0.1 (0-1)	0.5 ± 0.2 (0-2)	N.S.	1.7 ± 0.4 (0-4)	0.8 ± 0.2 (0-3)	0.05
Tendipedidae	18.0 ± 5.2(8-39)	13.1 ± 1.8(1-27)	N.S.	31.4 ± 2.4 (25-54)	7.1 ± 2.4(2-39)	0.001
<u>Chironomus (C) Maturus</u>	1.8 ± 0.5 (1-4)	1.4 ± 0.3 (1-4)	N.S.	6.1 ± 1.0 (3-10)	2.4 ± 0.4 (1-7)	0.01
<u>Psectrotanypus dyari</u>	not caught	not caught	-	8.2 ± 1.0 (4-11)	1.6 ± 1.1(0-16)	0.01
<u>Tanytarsus sp.</u>	2.0 ± 0.4 (1-3)	2.3 ± 0.5 (0-6)	N.S.	7.9 ± 1.8 (2-24)	0.7 ± 0.3 (0-4)	0.01
<u>Micropsectra sp.</u>	1.2 ± 0.4 (1-3)	1.9 ± 0.5 (0-7)	N.S.	2.7 ± 0.7 (1-5)	0.8 ± 0.2 (0-3)	0.02
<u>Corynoneura sp.</u>	11.5 ± 5.2(2-33)	6.9 ± 1.4(0-19)	N.S.	4.8 ± 1.1 (0-9)	1.3 ± 0.7 (0-9)	0.02
<u>Psectrecladius sp.</u>	1.5 ± 0.5 (0-3)	1.2 ± 0.2 (0-3)	N.S.	1.4 ± 0.3 (0-3)	0.04± 0.04(0-1)	0.01
<u>Chaoborus sp.</u>	not caught	not caught	-	0.2 ± 0.1 (0-2)	0.1 ± 0.07(0-1)	N.S.
All Insects	18.8 ± 5.3 (8-40)	14.2 ± 1.8(1-27)	N.S.	33.1 ± 2.5 (26-57)	8.0 ± 2.5(4-41)	0.01

Mean number/M /day ± S.E. (range); N.S. = non-significant change.

APPENDIX VIII

CHANGES IN INVERTEBRATE NUMBERS OF DIP SAMPLES BEFORE AND AFTER ALTOSID APPLICATION

APPENDIX VIII CHANGES IN INVERTEBRATE NUMBERS OF DIP SAMPLES BEFORE AND AFTER ALTOSID APPLICATION

Invertebrates	Control			Altosid		
	Pre-spray (May 5 - May 17)	Post-spray (May 18 - May 25)	Level of Significance	Pre-spray (May 5 - May 17)	Post-spray (May 18 - May 25)	Level of Significance
Amphipoda	1.7 ± 0.4 (1-3)	2.4 ± 0.7 (1-6)	N.S.	1.5 ± 0.4 (1-3)	0.8 ± 0.3 (0-2)	N.S.
Copepoda	439.7 ± 157.6 (116-1118)	463.9 ± 111.5 (131-907)	N.S.	715.2 ± 111.7 (420-1072)	656.5 ± 35.1 (533-823)	N.S.
Culicidae						
(larvae)	23.5 ± 9.2(2-63)	4.2 ± 1.3(2-11)	0.10	16.5 ± 2.8(7-23)	12.5 ± 1.8(1-14)	N.S.
(pupae)	1.7 ± 0.4 (1-3)	2.6 ± 0.7 (1-7)	N.S.	1.0 ± 0.5 (0-3)	0.5 ± 0.4 (0-2)	N.S.
(total)	25.1 ± 10.1(1-65)	6.8 ± 1.2(2-14)	0.05	17.5 ± 3.0(12-26)	13.0 ± 2.1(1-16)	N.S.
Diptera						
(unidentified)	0.7 ± 0.2 (0-2)	1.0 ± 0.2 (0-2)	N.S.	0.8 ± 0.1 (0-1)	1.5 ± 0.5 (0-4)	N.S.
Hydracarina	8.3 ± 3.1(4-19)	7.9 ± 0.8(5.12)	N.S.	4.8 ± 1.3(2-10)	3.3 ± 0.4 (2-5)	N.S.
Pisces						
(Gasterosteus spp)	0.1 ± 0.1 (0-2)	0.8 ± 0.5 (0-2)	0.05	0.5 ± 0.4 (0-3)	not caught	-

Mean daily sample ± S.E. (range); N.S. = non-significant change.

APPENDIX IX

CHANGES IN INVERTEBRATE NUMBERS BEFORE AND AFTER DIMILIN APPLICATION AS DETERMINED FROM DIP SAMPLES

APPENDIX IX CHANGES IN INVERTEBRATE NUMBERS BEFORE AND AFTER DIMILIN APPLICATION AS DETERMINED FROM DIP SAMPLES

Invertebrates	Control			Dimilin		
	Pre-spray (June 17-June 24) Mean ± S.E. (range)	Post-spray (June 30-July 16) Mean ± S.E. (range)	Level of Significance	Pre-spray (June 17-June 24) Mean ± S.E. (range)	Post-spray (June 30-July 16) Mean ± S.E. (range)	Level of Significance
Amphipoda	7.9 ± 2.6(0-17)	48.4 ± 9.0(13-113)	0.001	not caught	not caught	-
Coleiptera						
F. Dyfiscidae	3.3 ± 0.8 (1-6)	0.4 ± 0.2 (0-2)	0.01	4.5 ± 1.3 (1-7)	3.2 ± 1.1 (1-15)	N.S.
F. Hydrophilidae	not caught	not caught	-	1.1 ± 0.2 (0-2)	2.0 ± 0.4 (0-6)	N.S.
Diptera						
F. Culicidae						
(larvae)	3.0 ± 1.0 (0-6)	not caught	-	4.9 ± 0.9 (2-9)	0.9 ± 0.2 (0-2)	0.001
(pupae)	not caught	not caught	-	1.3 ± 0.4 (0-3)	2.3 ± 0.6 (0-4)	N.S.
(total)	3.0 ± 1.0 (0-6)	-	-	6.2 ± 1.0 (3-12)	3.2 ± 0.6 (2-10)	0.02
F. Tendipedidae						
(larvae)	3.1 ± 2.3(0-17)	not caught	-	8.0 ± 1.3 (7-11)	8.9 ± 2.9 (1-42)	N.S.
(unidentified)	1.8 ± 0.5 (1-4)	0.4 ± 0.2 (0-1)	0.05	3.1 ± 0.7 (1-6)	1.9 ± 0.3 (0-4)	N.S.
Ephemeroptera	not caught	1.9 ± 0.5 (0-5)	-	0.3 ± 0.2 (0-1)	0.2 ± 0.1 (0-1)	N.S.
Grastropoda						
F. unidentified I	9.5 ± 1.0(7-15)	20.6 ± 2.3(12-32)	0.001	5.1 ± 1.2 (1-10)	17.7 ± 4.1 (3-53)	0.01
F. unidentified II	1.9 ± 0.7 (0-4)	1.9 ± 0.5 (0-4)	N.S.	1.0 ± 0.2 (0-2)	7.1 ± 1.8 (2-25)	0.01
Hemiptera						
F. Notonectidae	0.4 ± 0.2 (0-1)	0.4 ± 0.2 (0-2)	N.S.	5.5 ± 1.1 (2-8)	2.6 ± 0.4 (1-5)	0.05
Hydracarina	not caught	not caught	-	1.6 ± 1.0 (0-7)	0.1 ± 0.08(0-2)	0.20
Odonata	1.0 ± 0.4 (0-2)	0.9 ± 0.5 (0-7)	N.S.	not caught	not caught	-
Pelecypoda	8.4 ± 5.2(0-41)	0.2 ± 0.1 (0-1)	N.S.	not caught	not caught	-
Zooplankton						
(total)	613.6 ± 68.4 (390-827)	127.7 ± 13.2 (78-221)	0.001	263.8 ± 38.3 (194-480)	744.4 ± 146.8 (88-1939)	0.01
Cladocera	403.6 ± 59.3 (278-744)	90.8 ± 9.6 (42-171)	0.001	213.1 ± 30.9 (134-382)	691.0 ± 146.4 (71-1848)	0.01
Copepoda	181.0 ± 48.8 (51-382)	35.6 ± 5.0 (18-53)	0.01	45.5 ± 9.8 (19-89)	48.1 ± 9.2 (2-89)	N.S.
Osteracoda	29.0 ± 8.7(3-61)	1.3 ± 0.7 (0-9)	0.01	2.9 ± 1.0 (1-9)	5.2 ± 0.8 (2-12)	N.S.

n = number of pooled 50 dip samples; * = significant change; N.S. = non-significant change;
; 1 = increase; 1 = decrease

APPENDIX X

CHANGES IN INVERTEBRATE NUMBERS BEFORE AND AFTER DIMILIN APPLICATION AS DETERMINED FROM EKMAN DREDGE SAMPLES

APPENDIX X CHANGES IN INVERTEBRATE NUMBERS BEFORE AND AFTER DIMILIN APPLICATION AS DETERMINED FROM EKMAN DREDGE SAMPLES

Invertebrates	Control			Dimilin		
	Pre-spray (June 17-June 23) Mean ± S.E. (range)	Post-spray (June 30-July 16) Mean ± S.E. (range)	Probability 't' test (n=20)	Pre-spray (June 17-June 23) Mean ± S.E. (range)	Post-spray (June 30-July 16) Mean ± S.E. (range)	Probability 't' test (n=19)
Annelida	1.4 ± 1.0 (0-7)	0.9 ± 0.2 (0-2)	N.S.	0.6 ± 0.2 (0-1)	0.6 ± 0.2 (0-2)	N.S.
Coleoptera						
F. Dytiscidae	1.3 ± 0.3 (0-2)	not caught	-	0.3 ± 0.2 (0-1)	not caught	-
Diptera (total)	15.0 ± 4.3 (1-31)	1.6 ± 0.3 (0-4)	0.05	9.6 ± 1.7 (5-18)	3.9 ± 0.8 (1-8)	0.01
F. Tendipedidae I	12.1 ± 4.3 (1-31)	1.4 ± 0.3 (1-4)	0.05	2.4 ± 1.0 (0-7)	1.1 ± 0.3 (0-3)	N.S.
F. Tendipididae II	not caught	not caught	-	7.1 ± 1.1 (4-11)	2.8 ± 0.6 (0-6)	0.01
F. unidentified	2.9 ± 2.5 (0-20)	3.6 ± 1.4 (0-10)	N.S.	not caught	not caught	-
Gastropoda						
F. unidentified	3.1 ± 0.9 (1-7)	2.0 ± 0.6 (0-7)	N.S.	1.0 ± 0.2 (0-1)	0.7 ± 0.2 (0-2)	N.S.
F. unidentified	1.4 ± 0.4 (0-3)	0.2 ± 0.11 (0-1)	0.01	0.9 ± 0.4 (0-2)	0.4 ± 0.2 (0-2)	N.S.
Pelecypoda	7.1 ± 2.5 (0-16)	0.4 ± 0.2 (0-3)	0.05	not caught	not caught	-
Zooplankton (total)	46.1 ± 11.3 (5-89)	33.4 ± 7.0 (10-74)	N.S.	34.9 ± 5.5 (18-50)	26.1 ± 4.9 (3-54)	N.S.
Amphipoda	2.4 ± 0.6 (1-5)	1.9 ± 0.4 (0-5)	N.S.	not caught	not caught	-
Cladocera	17.6 ± 4.7 (1-29)	11.4 ± 3.8 (2-45)	N.S.	15.6 ± 5.2 (4-41)	6.4 ± 2.2 (0-23)	0.20
Copepoda	21.9 ± 8.6 (2-63)	17.5 ± 3.8 (4-53)	N.S.	7.5 ± 2.0 (2-17)	16.1 ± 3.4 (3-45)	0.05
Ostracoda	4.3 ± 1.1 (1-7)	2.0 ± 0.3 (0-4)	N.S.	11.8 ± 2.6 (3-21)	3.6 ± 0.9 (0-12)	0.01

n = number of pooled (5x) dredge samples; * = significant change; N.S. = Non-significant change;