Impact of *Bacillus thuringiensis* var. *israelensis* in dosages used for black fly (Simuliidae) control, against target and non-target organisms in the Torch River, Saskatchewan.

by

David Kenneth Burton

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Masters of Science
in
Department of Entomology

Winnipeg, Manitoba

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BY

DAVID KENNETH BURTON

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTERS OF SCIENCE

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ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my advisor, Dr. M.M. Galloway for her support throughout this project. I would also like to thank Dr. T.D. Galloway for his encouragement and suggestions. I would also like to thank Dr. W.L. Lockhart for reviewing this thesis.

Thanks also to Michele Quaglia and Steve Miele whose technical assistance made this project possible. This research was supported by a Operating Grant #2024 and a Special Services Contract #ISU81-00126 from Agriculture Canada to the Canada Biting Fly Centre. BTI was supplied and technical assistants were funded by Zoecon-Sandoz, Inc. and Abbott Laboratories.

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DEDICATION

In memory of my mother Hilda Burton who remains a constant source of support, encouragement and inspiration.

To Bill and Lorraine Burton without whose financial support and encouragement I would never have finished.

To Michele whose love has made it all worth while.

Chapter I

INTRODUCTION

Bacillus thuringiensis Berliner is a spore-forming bacterium that produces one or more crystals of toxic protein (delta-endotoxin) with each spore. *B. thuringiensis* has been divided into 24 serotypes on the basis of the flagellar antigens (H-antigens) of the vegetative cells. Serotypes can be further subdivided into biotypes with the use of antigenic sub-factors and phenotypic characterists (Norris 1964; Krywienczyk and Fast 1980; Sekijima and Ono 1982). Based on the classification of deBarjac and Bonnefoi (1962) 27 biotypes have been found and are now considered varieties or serovars (deBarjac 1982).

The pathogenicity of most varieties of *B. thuringiensis* is specific to insects of the order Lepidoptera. These varieties have been used to control agricultural and forestry caterpillar pests (Forsberg *et al.* 1976).

Several varieties of *B. thuringiensis* have shown some pathogenicity against insects of the order Diptera (Hall *et al.* 1977; Lacey and Mulla 1977; Lacey *et al.* 1978; Lacey and Federici 1979; Panbangred *et al.* 1979; Ignoffo *et al.* 1980, 1981a; Padua *et al.* 1980; Larget and deBarjac 1981c;

Finney and Harding 1982; Samasanti et al. 1982). Another species, B. sphaericus is also pathogenic to Diptera (Ramoska et al. 1977; Mulligan et al. 1978,1980; Myers and Yousten 1978,1981; Wraight et al. 1978,1981; Ramoska and Pacey 1979; Davidson 1981,1982; Davidson et al. 1981; Wickermesinghe and Mendis 1981; Lacey and Singer 1982; Wan and Jin-Ru 1982; Yousten and Davidson 1982). Dosages of B. sphaericus and other varieties of B. thuringiensis necessary to cause pathogenicity in dipteran larvae are too high for these bacteria to be useful as biological control agents.

Goldberg and Margalit (1977) isolated a spore-forming B. thuringiensis from mosquito breeding sites in the Negev, Israel. This isolate demonstrated a higher pathogenicity for species of Aedes, Culex, Anopheles and Uranotaenia than any other variety of B. thuringiensis or B. sphaericus previously tested. LC50 determinations against the species ranged closer to those normally associated with chemical insecticides (Goldberg and Margalit 1977). Undeen and Nagel (1978) tested the same isolate against species of Simulium and obtained similar results. deBarjac (1978a) identified this isolate as a new serotype (H-14) and designated it variety israelensis.

The discovery of B. thuringiensis var. israelensis (BTI), serotype H-14, has resulted in a renewed interest in the biological control of pest and vector species of Culicidae and Simuliidae.

Chapter II

LITERATURE REVIEW

Evaluation of Bacillus thuringiensis var. israelensis in the control of black flies (Simuliidae) and mosquitos (Culicidae).

2.1 CLASSIFICATION AND DISTRIBUTION

The formal classification of BTI and synonymy is given below.

Kingdom Procaryote, Division II: The Bacteria

Family Bacillaceae

Bacillus thuringiensis serotype H-14 deBarjac

Bacillus thuringiensis var. israelensis deBarjac

ONR 60A (Goldberg and Margalit 1977)

WHO/CCBC accession number 1884

WHO/CCBC accession number 1897

BTI has been found occurring naturally in the Mediterranean region, Africa, South East Asia, Europe and North America (deBarjac in Anonymous 1979).

2.2 BIOLOGICAL CHARACTERISTICS

B. thuringiensis is composed of aerobic, gram-positive endospore-forming rods that may produce a crystalline protein body (delta-endotoxin) in the vegetative cell during endospore formation.

As many as five different toxic components produced during the growth cycle of *B. thuringiensis* have been identified (Forsberg *et al.* 1976). These are the vegetative cell, exoenzymes, exotoxins, endotoxins,and endospores. Endospores and endotoxins are released into the medium by lysis of the sporangia at the end of sporulation. These endospores germinate to yield rod-shaped vegetative cells which undergo multiplication. Hydrolytic enzymes (exoenzymes) are secreted by the vegetative cells to enhance degradation and absorption of nutrients from the medium. Some varieties also secrete beta- or alpha-exotoxins during the multiplication stage. After a period of vigorous vegetative growth, the cells may develop into sporangia. Each sporangium produces a heat resistant endospore. In most varieties of *B. thuringiensis* a crystalline protein body (delta-endotoxin) is also produced (Forsberg *et al.* 1976). The toxic

components and the amount of each toxin produced depends on the variety of B. thuringiensis, the strain of the variety and the culture conditions (Conner and Hansen 1967; Dulmage 1971; Dulmage and Rhodes 1971; Dulmage and deBarjac 1973).

Commercial formulations of B. thuringiensis have been primarily based on strains which do not produce the alpha- or beta-exotoxins. These exotoxins are heat stable and act as ATP analogs. They have some toxic effects on birds and mammals, especially when injected. As well exotoxins when ingested have been shown to have a broad spectrum effect on invertebrates (Forsberg et al. 1976). Tests on Anagasta kuhniella (Zeller)(Lepidoptera) have shown that BTI does not produce the heat stable alpha- or beta-exotoxins (deBarjac 1978b).

By studing the pathogenicity of isolated crystals and of spore free or crystal free mutants of BTI, Larget and de-Barjac (1981b) showed that the crystalline protein body is the only component toxic to culicid larvae. Similar results were obtained for simuliid larvae when treated with B. thuringiensis kurstaki and B. thuringiensis kenyae (Lacey et al. 1978).

BTI is morphologically different from other B. thuringiensis serotypes. The crystalline protein body of BTI is irregular in shape and size, while most other serotypes of B. thuringiensis produce similar sized diamond-shaped pro-

tein crystals (deBarjac 1978a; Charles and deBarjac 1982). Serologically BTI crystals are distinct from crystals of B. thuringiensis serotypes toxic to Lepidoptera (Guillet and deBarjac 1979; Krywienczyk and Fast 1980; Wie et al. 1982). BTI crystals differ from other B. thuringiensis crystals in amino acid composition (Guillet and deBarjac 1979; Tyrell et al. 1979; Larget and deBarjac 1981b).

The high pathogenicity of BTI to Diptera is related to the special characteristics of its crystalline protein endotoxin (Larget and deBarjac 1981a,b). The crystal protein of BTI is a protoxin of a single subunit of approximately 134,000 daltons molecular weight. This protoxin is rapidly converted to 26,000 dalton toxic subunits in the presence of high pH and suitable enzymes in the gut of a susceptible host (Tyrell et al. 1979,1981; Huber et al. 1981; Aronson et al. 1982). Alkaline pH may not be neccessary to convert protoxin into smaller toxic subunits (Klowden et al. 1983). The narrow spectrum of activity of the delta-endotoxin of BTI is probably due to the absence in most invertebrates of the enzymic system transforming the protoxin into the toxic subunits.

Charles and deBarjac (1981a,b) studied the histopathology in Aedes aegypti (L). larvae treated with the protoxin of BTI. Proteolysis of the protoxin occurs shortly after ingestion and the delta-endotoxin is released into the ante-

rior midgut. Shortly after the beginning of intoxication the midgut epithelial cells swell and the apical microvilli of the epithelium decrease in size, widen and disappear. There is enlargement of the intra- and inter-cellular spaces in the basal region of the cells. Rough endoplasmic reticula become disintegrated and form spherical structures which increase in size during intoxication. The Golgi apparatus within the cardia cells produces secretory vesicles and the peritrophic membrane assumes an abnormal configuration. The histopathological changes progress from the basal to the apical area of the cell. Mosquito larvae show severe histological changes within the cells of the gastric caeca and posterior stomach, as well as in the cardia and anterior stomach epithelium (Charles and deBarjac 1981a,b). These histological changes are similar to those observed for lepidopteran larvae treated with other serotypes of B. thuringiensis (Ramakrishnan and Tiawari 1967; Sutter and Raun 1967). Lacey and Federici (1979) studied the histopathology in Simulium vittatum Zett. treated with B. thuringiensis kurstaki and B. thuringiensis kenyae. They observed histological changes in the gastric caecae and posterior stomach. No significant pathological change occurred in the anterior stomach. The absence of significant pathological change in the anterior stomach may be due to a lack of absorption of delta-endotoxin in this region of the midgut (Lacey and Federici 1979).

The toxic action of the delta-endotoxin results in paralysis of the gut. The development of paralysis results from an increase in the alkalinity of the haemolymph. Paralysis is the result of damage to the gut epithelium and the subsequent equilibration between the highly buffered midgut and the relatively poorly buffered haemolymph (Heimpel and Angus 1959; Nishiitsutsuji-Uwo *et al*. 1979). Faust *et al*. (1974) showed that the delta-endotoxin of *B. thuringiensis* uncouples oxidative phosphorylation by stimulating mitochondrial oxygen uptake and inhibiting ATP production. Loss of ATP production leads to metabolic imbalance and disintergration of the gut epithelium. This inturn leads to an upset in the ion transport system and the resulting paralysis. Both the lepidopteran and dipteran active endotoxins of *B. thuringiensis* act as ionophores. However, the lepidopteran active toxins influence cation transport where as the dipteran active toxins influence anion transport (Schnell and Nickerson 1983).

2.3 STANDARDIZATION

Black fly and mosquito larvae treated with BTI are not killed by the development of spores but through the direct action of activated delta-endotoxin (Lacey and Federici 1979; Charles and deBarjac 1981b). The amount of delta-endotoxin in a *B. thuringiensis* preparation has no direct relationship to the number of spores (Dulmage 1971). The pro-

portion of spores to crystals of a B. thuringiensis preparation varies with the Bacillus strain used and the medium in which it is produced. Therefore the viable spore count is not a realistic measure of the insecticidal activity of a Bacillus preparation. The easiest way to express the dosage is in terms of mg of formulation/litre or in ppm. Because formulations can differ in potency a standard (IPS-78) of BTI was developed in 1978, by the International Reference Centre on B. thuringiensis. This standard was assigned the arbritrary titre of 1000 International Toxic Units (ITU)/mg for fourth instar Ae. aegypti larvae. Aedes aegypti larvae were chosen as the test insect because of their high susceptibility to BTI and for their relative ease of rearing in the laboratory.

Dame et al. (1981) compared the toxicity of five industrial formulations of BTI with that of the standard reference formulation (IPS-78). Three wettable powder formulations produced by Abbott Laboratories (ABG-6406-125, ABG-6478-199 and ABG-6406-122) had ITU values of 651, 567 and 286, respectively. A wettable powder formulation produced by Biochem (666-PM-50) had a toxicity of 3482 ITU. The semi-liquid formulation SAN-402-I-WDC (Zoecon-Sandoz Inc.) had a toxicity of 688 ITU.

An experimental formulation produced by Biochem Products (R.153-78) has been shown to have a toxicity of 3000 ITU (Guillet and deBarjac 1979).

The toxicity of preparations ABG-6406-125, ABG-6108D (Abbott Laboratories) and SAN-402-I-WDC bioassayed against fourth instar Ae. aegypti larvae were 500, 1000 and 600 ITU, respectively, when compared to IPS-78 (Ignoffo et al. 1982).

A standard bioassay method has been developed for the evaluation of the insecticidal formulations derived from BTI (Hall and Arakawa 1980). This bioassay is based on the comparison of the larval mortality resulting from the reference preparation with that from cultures, preparations or formulations of unknown potency. Another laboratory protocol has been developed for the bioassay and efficacy testing of BTI against black flies (Lacey et al. 1982b).

A rapid, inexpensive alternative to the bioassay has recently been developed using immunochemical technology (Wie et al. 1982). An enzyme-linked immunosorbent assay is used to detect and quantitate the parasporal crystal endotoxins of Bacillus thuringiensis. The measurement of antigens with antibodies has been shown to correlate well with insect toxicity to Bacillus thuringiensis kurstaki (Andrews et al. 1980). The enzyme-linked immunosorbent assay also provides a method to determine the dispersal and persistence of insecticides formulated with B. thuringiensis.

2.4 STABILITY AND FIELD PERSISTENCE

The stability of BTI to various environmental factors has been studied by various authors. Mulligan et al. (1980) reported that exposure of BTI (SAN-402-I-WDC) to sunlight, 35C or pH values between 4.3 and 10.0 did not effect insecticidal activity. Ignoffo et al. (1981b) reported that 30C, 5% salinity and pH values between 4.0 and 10.0 did not effect larvicidal activity of BTI (ABG-6406-18). However, 24 hour exposure to simulated sunlight deactivated BTI.

Ignoffo et al. (1982) tested the high temperature sensitivity of four formulations of BTI. The half life of SAN-402-I-WDC, ABG-6108D and ABG-6108 at 50C was 18.0 , 7.2 and 6.0 days, respectively. After 28 days of exposure to 50C all four formulations had lost their larvicidal activity.

Ignoffo et al. (1983) tested the effect of temperature and water on the insecticidal activity of BTI (ABG-6108). Both aqueous and dry preparations of BTI were exposed for 500 days at 5C, 30C and 50C. There was no loss of activity in either the dry or aqueous preparations after 500 days at 5C. At 30C the aqueous preparation was stable while the dry preparation had a half life of 52 days. At 50C loss of insecticidal activity occurred after 28 and 208 days for the dry and aqueous preparations, respectively.

In the field the presence of suspended material in the water column effects the persistence and effectiveness of BTI. Van Essen and Hembree (1982) tested the effects of suspended and dissolved soil constituents on the residual larvicidal activity of four formulations of BTI (ABG-6406-125, ABG-6478-194, SAN-402-I-WDC and Biochem LRB-676) in large outdoor artificial containers. All four formulations were tested at a rate of 0.9 ppm against 3rd instar Ae. aegypti larvae and effectiveness decreased with time although one formulation (Biochem LRB-676) was still effective (80% control) 4 days post treatment. Similar results were reported by Ignoffo et al. (1981b) and Ramoska et al. (1982b). Adsorption of BTI onto soil particles may effectively remove the delta-endotoxin from the feeding zone of mosquito larvae. Sediments treated with BTI showed effective insecticidal activity once dried and reflooded (Larget 1981).

2.5 PRODUCTION AND FORMULATION

BTI is produced by submerged liquid fermentation. It can be grown on nutrient agar media (Goldberg and Margalit 1977), trypose blood agar media (Undeen and Nagel 1978), sporulation media containing yeast autolysate and on a medium with casaminoacids and sodium citrate (Vankova et al. 1978).

BTI used for the preparation of the standard reference formulation (IPS-78) is cultivated on the following medium at 30C for 35 to 40 hours: wheat flour (15g), glucose (10g), peptone (5g), yeast extract (5g), KH_2PO_4 x $7H_2O$ (0.1g), $MgSO_4$ x $7H_2O$ (0.5g), NaCl (3g), $FeSO_4$ (0.1g) and water. The spore crystal mixture is precipitated with acetone and collected by centrifugation (deBarjac 1979).

On a commercial scale, production is carried out in $1x10^5$ litre fermenters using a medium of brewers yeast, cornflour and supplemented tap water (Couch and Ross 1980). BTI is marketed by three companies in North America (Abbott Laboratories as Vectobac ; Zoecon-Sandoz, Inc. as Teknar and Biochem Products as Bactimos) and experimental preparations have been produced by industrial groups in Western Europe, China and the USSR (Gaugler and Finney 1982). BTI is presently available as a primary powder, a wettable powder, an aqueous concentrate, an emulsion and a granular formulation.

2.6 EFFICACY ON TARGET ORGANISMS

2.6.1 Black Flies (Simuliidae)

The potential of BTI as a black fly control agent has been investigated both in the laboratory and in the field.

Laboratory tests of BTI have demonstrated its high potential as a biological control agent of black flies.

Undeen and Nagel (1978) tested BTI (ONR-60A Goldberg) against Simulium verecundum Stone and Jamnback, Simulium vittatum Zetterstedt, Cnephia ornithophila Davies,Peterson and Wood, Stegopterna mutata (Malloch) and Prosimulium mixtum Syme and Davies. None of the species tested differed significantly in their susceptibility to dosages of $5x10^4$ cells/ml for 30 min. Differences in susceptibility were noticed after 24 hours.

Undeen and Berl (1979) investigated the susceptibility of Simulium damnosum s.l. Theobald. to BTI. There was a large difference in the effectiveness of the standard reference formulation (84% mortality after a 1 min exposure to $5x10^6$ spores/ml) and a freshly produced aqueous suspension (99% mortality after 1 min exposure to $1x10^5$ spores/ml).

Weiser and Vankova (1978) tested a 10^9 spore/ml dosage of BTI against 3rd and 4th instar Eusimulium latipes Roubaud and Odagmia ornata Enderlein. High mortality was observed in both species, however tests were carried out in stagnant, unaerated water.

Frommer et al. (1980) treated S. vittatum larvae with a primary powder formulation (ABG-6406-125) in the laboratory. Black fly larvae were exposed to BTI for 15,30,60,90, 120 min and 24 hours. A sharp decrease in LC50 (0.81 to 0.32 ppm) and LC90 (1.71 to 0.86 ppm) values occurred when exposure times were increased from 30 to 60 min. Changes in

LC50 and LC90 values were minimal when exposure times were expanded beyond 60 min.

Molloy et al. (1981) tested various instars of S. vittatum and S. verecundum with two primary powder formulations (R.153-78 and ABG-6331-17). Mortality was consistently higher among early instar Simulium larvae . Simulium verecundum larvae were significantly more susceptible than S. vittatum larvae. Mortality of S. vittatum larvae treated at 20C was twice that recorded at 10C. R.153-78 was 3 to 4 times more toxic than ABG-6331-17 even though both powders contained equal cell concentrations.

Gaugler and Molloy (1980) reported that the efficacy of BTI could be either reduced or enhanced by disrupting normal feeding behaviour. Feeding was inhibited by exposing S. vittatum larvae to high concentrations of suspended food particles. When feeding was inhibited before exposure to BTI larval mortality was reduced to an insignificant level. When feeding was inhibited after BTI exposure mortality was increased by nearly 90% because the delta-endotoxin was retained longer in the larval gut. Similar results were obtained by Molloy et al. (1981).

Field evaluations conducted with BTI are in agreement with laboratory findings, showing a high toxicity to a wide range of black fly species under diverse conditions.

A primary powder formulation (R.153-78) was evaluated against S. damnosum s.l. in a small river in southern Ivory Coast, West Africa (Guillet and deBarjac 1979; Guillet and Escaffre 1979). A dosage of 0.2mg/l for 10 min resulted in total larval mortality immediately downstream of the treatment point within 24 hours.

Field trials were conducted in small Newfoundland streams against larvae of S. venustum Say, S. verecundum, S. tuberosum (Lundstrom), St. mutata, P. mixtum and C. ornithophila (Undeen and Colbo 1980). Four streams having different temperatures (3-22C), flow rates (200-3400 l/min) and species composition were treated with a locally produced culture of BTI. Depending on the water temperature, between 54% and 100% mortality was observed directly downstream of the treatment point. Treatment of streams with lower water temperatures resulted in lower black fly mortality . The distance of significant larval mortality (>80%) downstream was positively correlated with stream discharge.

Frommer et al. (1981a,b) treated a stream (18,269 l/min, 16-19C) in Tennessee with BTI (ABG-6406-125) at a dosage of 3.1 ppm/35 min. for control of S. vittatum larvae. Although spore concentrations were the highest near the treatment site no reduction of black fly larvae was noted immediately downstream. At the most distant site (312m), where spore concentrations were the lowest, the highest mean mortality (40%) was recorded. When the same stream was

treated at a dosage of 1.55 ppm/70 min better results were obtained, however efficacy was still the highest at the furthest downstream site. A third trial was conducted in the same stream when dense aquatic vegetation was present. Stream discharge had increased to 23,900 l/min (Frommer et al. 1981c). A BTI formulation (ABG-6478-194) twice as potent was applied at a dosage of 3.1 ppm/35 min. Mortality at the nearest site was increased to 65%, however efficacy was still highest at the furthest downstream site. Increased efficacy immediately downstream was attributed to the presence of dense aquatic vegetation retarding the downstream movement of BTI and increasing spore dispersal (Frommer et al. 1981d). While the parasporal crystals are the active ingredient in BTI it was assumed that the physical carry and distribution of crystals and spores were similar.

Molloy and Jamnback (1981) treated a small stream in New York, with BTI (R.153-78) at a dosage of 0.5 mg/l for 15 min., to control S. vittatum, S. verecundum, S. tuberosum, S. pugetense (Dyar and Shannon), S. parnassum Malloch, Prosimulium sp. and St. mutata. Stream discharge was 770 l/min and water temperature was 13C. Reductions in populations of black fly larvae of 96, 86, 53 and 11% were obtained at 20, 86, 130 and 705m downstream, respectively. Low mortality at the furthest downstream sites was attributed to low stream discharge, low water temperature and to numerous pools along the course of the stream where the BTI may have settled out of suspension.

Undeen et al. (1981) tested the efficacy of BTI against five species of Simulium in three small mountain streams in Guatemala. A locally produced aqueous suspension of BTI was applied at a dosage of $2x10^5$ spores/ml for 1 min into a stream having a discharge of 10 l/min. Ninety-two percent mortality of black fly larvae was obtained for the first 14m downstream. In a second larger stream the same aqueous suspension was applied at a dosage of $2x10^5$ spores/ml for 1 min at two sites and $1x10^5$ spores/ml for 1 min at three sites. These sites where at 25m intervals along the stream. Stream discharge at the first two sites was 210 l/min and 840 l/min at the three further downtream sites. Larval mortality was approximately 85% over the 125m portion of the stream. A third stream was treated with BTI (SAN-402-I-WDC) at a dosage of $1x10^6$ spores/ml for 10 min. Stream discharge was calculated as 156 l/min. Larval mortality of 100% was obtained for the first 25m downstream with 13% mortality below this point.

Lacey et al. (1982a) carried out a large scale field trial in the Marahoue River, Ivory Coast (discharge $457m^3$/sec; water temperature 26.8C) against temephos resistant Simulium populations. BTI (SAN-401-I-WDC) applied at a dosage of 1.5 mg/l for 10 min resulted in complete removal of S. damnosum s.l. larvae for 19km and partial reduction for an additional 15km. Other Simulium species were less susceptible, being completely removed for only 4km and partially removed for an additional 16km.

Chilcott et al. (1983) tested two formulations of BTI against Austrosimulium larvae in three New Zealand streams. A dosage of 10 ppm/15 min of SAN-402-I-WDC applied to the Waitati river (118 l/sec. ; 13C) resulted in 100% mortality of black fly larvae for the first 292m downstream. A dosage of 2 ppm/15 min of SAN-402-I-WDC applied to Donald Hill Stream (56.2 l/sec. ; 19C) resulted in 100% mortality of black fly larvae for 544m downstream. Application of R.153-78 at a rate of .2 mg/l for 15 min to Lindsay Stream (28 l/sec. ; 10.5C) resulted in 100% mortality of black fly larvae immediately downstream. No significant mortality was observed at sites 120,200 and 580m downstream.

Pistrang and Burger (1984) applied BTI (SAN-402-I-WDC) at a rate of 10 ppm for 1 min to a small stream in New Hampshire (13,500 l/min. ; 13.6-16.4C) in an attempt to control five species of Simuliidae. Larval mortality of 100% was obtained for up to 1300m downtream.

BTI exhibits high toxicity to a wide range of black fly species. Instar (Guillet and deBarjac 1979; Guillet and Escaffre 1979; Molloy et al. 1981; Molloy and Jamnback 1981), water temperature (Molloy et al. 1981; Molloy and Jamnback 1981), black fly species composition (Undeen and Nagel 1978; Molloy et al. 1981; Lacey et al. 1982b) and the presence of aquatic vegetation (Frommer et al. 1981a,b,c,d) can effect the efficacy of BTI against black flies. Downstream carry which is directly correlated with stream discharge is

the most important factor affecting larval black fly mortality in stream trials. To date significant downstream carry has been obtained only in high discharge rivers (Lacey et al. 1982a). Formulations are needed which keep the toxic delta-endotoxin of BTI suspended in the water column for longer distances. Reduced settling rates are obtained with materials having smaller particle size. However, Guillet and Escaffre (1979) have reported a direct correlation between particle size and toxicity. Further research is needed to identify the particle size providing the optimal balance of both downstream carry and efficacy.

2.6.2 Mosquitos (Culicidae)

The potential of BTI as a mosquito control agent has been investigated both in the laboratory and in the field. The first laboratory investigators reported effective control of larvae of Aedes aegypti, Anopheles sergentii (Theobald), Culex pipiens (L.), Cx. univitattus Theobald and Uranotaenia unguiculata Edwards (Goldberg and Margalit 1977). Aedes, Culex and Uranotaenia larvae were more susceptible to BTI (ED50 of 600-3000 spores/ml) than Anopheles larvae (ED50 of 50,000 spores/ml). The susceptibility of Aedes, Culex and Anopheles species to various formulations of BTI has been compared in numerous laboratory and field studies (deBarjac 1978b; Sinegre et al. 1979; Tyrell et al. 1979; Mulla et al. 1980; Dame et al. 1981; Larget and deBarjac

1981c; Lebrun and Vlayen 1981; Lacey and Singer 1982; McLaughlin et al. 1982). Anopheles larvae are 10 to 20 times less susceptible to BTI due to their surface feeding behaviour. Nugud and White (1982) tested three formulations of BTI (IPS-78, AGB-6406, SAN-402-I-WDC) against larvae of An. arabiensis Patton. A higher efficacy was obtained for SAN-402-I-WDC because of its reduced tendency to sediment out of the water column.

Garcia and DesRochers (1979) tested a locally produced culture of BTI in the laboratory against six species of mosquito from California (Ae. dorsalis (Meigen), Ae. sierrensis (Ludlow), Cx. pipens

Cx. tarsalis Coquillett, Culiseta incidens (Thomson) and Cs. inornata (Williston)). A mortality of 100% was obtained at a dosage of between 10^4 and 10^5 cells/ml for Aedes and Culex species. Only 40% mortality of Culiseta larvae was obtained at a dosage of 10^4 cells/ml. Additional tests were conducted in water from natural breeding sites and run simultaneously indoors and outdoors. Neither differences in water quality nor full sun exposure significantly diminished the activity of BTI at a dosage of between 10^5-10^6 cells/ml. At a dosage of 10^4 cells/ml there was significantly higher mortality in the outdoor trials. The higher temperature of the outdoor tests (high of 31C and a low of 14.5C) compared to the constant indoor temperature (22C) may have accounted for the observed differences.

Sinegre et al. (1980b) tested BTI (R.153-78) in the laboratory against larvae of Ae. caspius (Pallas), Ae. detritus (Haliday), Cx. pipiens and Coquillettidia. LC90 values for Aedes larvae were between 0.06 and 0.08 mg/l. LC90 values for Cx. pipiens larvae were between .02 and .07 mg/l. Coquillettidia larvae were more susceptible to BTI when not attached to a vegetative support (LC90 of 2.5 mg/l compared to 11 mg/l when attached).

Schnetter et al. (1981) reported LC90 values of between 0.1 and 0.18 mg/l for larvae of Ae. vexans (Meigen), Ae. cantans (Meigen), Ae. sticticus (Meigen), Ae. rusticus (Rossi) and Cx. pipiens treated with a locally produced BTI preparation. Early instar larvae were more susceptible than later instars. Early instar mosquito larvae are generally more susceptible to BTI (Sinegre et al. 1979; Hembree et al. 1980; Luthy et al. 1980; Mulla et al. 1980; Mulligan et al. 1980; Van Essen and Hembree 1980; Dame et al. 1981).

A LC50 of between $1x10^3$ and $1x10^4$ spores/ml was obtained for larvae of Cx. tarsalis and Cx. pipiens when treated with SAN-402-I-WDC (Garcia and DesRochers 1980).

Ignoffo et al. (1980) reported 100% mortality of Ae. aegypti larvae when exposed for one hour to 90 mg/l of BTI (ABG-6108).

Mulligan et al. (1980) tested BTI (SAN-402-I-WDC) against larvae of Cx. quinquefasciatus Say under different laboratory conditions of water quality and temperature. A mortality of 100% was reported for larvae treated in tap water with a dosage of 0.1 mg/l. Autoclaved sewage effluent and raw sewage effluent reduced larval mortality to 65% and 3%, respectively. Water quality, especially large amounts of organic matter, reduces the efficacy of BTI against mosquito larvae (Ignoffo et al. 1981a,b; Purcell 1981; Ramoska et al. 1982b; Van Essen and Hembree 1982). This loss of activity is attributed to inactivation of the delta-endotoxin or binding of the endotoxin with organic materials (Ignoffo et al. 1981a; Van Essen and Hembree 1982). NaCl concentration of up to 20% has little effect on larvicidal activity of BTI (Garcia and DesRochers 1979, 1980; Sinegre et al. 1979; Purcell 1981; Goettell et al. 1982a).

Mulligan et al. (1980) found temperature to have only a slight effect on larvical activity with 82, 85 and 92% mortality of Cx. quinquefasciatus larvae at 10C, 20C and 35C, respectively. Sinegre et al. (1979) found high temperature to increase larvicidal activity and attributed this to higher metabolism and feeding of mosquito larvae at higher temperatures. A similar response to increased temperature was also reported by Mulla et al. (1980) and Wraight et al. (1982). Luthy et al. (1980) reported good control of Aedes larvae at temperatures as low as 4C. Some Aedes larvae are adapted to feeding and growth at low temperature.

DeMaio et al. (1981) tested BTI (ABG-6108) against larvae of the treehole mosquito Ae. triseriatus (Say) in the laboratory. Greater than 80% mortality was reported at concentrations of 1.0 ppm.

Ali et al. (1981) found that the addition of food resulted in a decreased efficacy of BTI against Cx. quinquefasciatus larvae due to competition between the food and endotoxin. Ignoffo et al. (1981a,b) found that the presence of food or BTI-killed mosquito larvae increased both the rate and extent of mortality in Cx. quinquefasciatus and Ae. aegypti larvae.

Lacey and Lacey (1981) tested the larvicidal activity of BTI (IPS-78) in laboratory experiments against mosquitos of the Central Amazon Basin. High mortalities (92-95%) were reported for larvae of Cx. quinquefasciatus, Cx. mollis Dyar and Knab and Limatus flavisetosus Castro with dosages between .2 and .3 mg/l. Only 63% mortality was observed for larvae of Li. durhami Theobald and Trichoprosopon digittatum (Rondani) at a dosage of 0.1 mg/l. No mortality was observed for Cx. (Carrollia) sp. larvae at a dosage of 0.2 mg/l. The lower susceptibility of this species is thought to be due to differences in feeding behaviour.

Sinegre et al. (1980b) applied BTI (R.153-78) to small field plots in France. A dosage of 0.1 mg/l and 0.4 mg/l resulted in 100% mortality of Ae. detritus and Cx. pipiens larvae, respectively.

deBarjac et al. (1980) reported between 88 and 100% mortality of Culex spp. larvae when exposed to 0.2 mg/l of BTI in small freshwater ponds in France.

Engler et al. (1980) and Krieg et al. (1980) treated flooded ditches in West Germany with a locally produced preparation of BTI. A dosage of 10^4-10^5 spores/ml resulted in 100% mortality of Ae. cantans, Ae. rusticus and Cx. morsitans (Theobald) larvae.

Garcia and DesRochers (1980) tested BTI (SAN-402-I-WDC) against Ae. dorsalis and Cx. tarsalis larvae in salt and brackish Californian marshes. A dosage of 1 kg/ha resulted in 85% mortality. Merriam and Axtell (1983) obtained 100% mortality of Ae. taeniorhychus Wied. larvae with a dosage of 1 kg/ha of BTI (ABG-6108) applied to a Californian salt marsh.

Hembree et al. (1980) applied BTI (ABG-6406) to small rice plots in Arkansas to control Psorophora columbiae (Dyar and Knab). Larval mortality was greater than 75% when BTI was applied at dosages greater than 4.4 mg/ml . Similar results were obtained by Stark and Meisch (1983) for P. columbiae and An. quadrimaculatus Say in Kansas rice plots. Application of BTI (ABG-6108) at a dosage of 0.5-1.0 mg/ha resulted in 97-100% mortality of mosquito larvae. McLaughlin and Billodeaux (1983) reported between 91-99% mortality of P. columbiae in Louisiana rice fields with BTI (ABG-6108) applied at a dosage between 0.25 and 0.60 kg/ha.

Mulla et al. (1980) reported between 90-100% mortality of Ae. nigromaculis (Ludlow), Cx. tarsalis, Cx. peus Speiser and P. columbiae larvae with dosages of BTI between 0.12-2.25 kg/ha in irrigated pastures. Ramoska et al. (1982a) reported good control of Ae. dorsalis, Ae. nigromaculis, Ae. vexans and Ae. melanimon Dyar with dosages of BTI between 1.1-1.6 l/ha when applied to a pasture in Montana.

Schnetter et al. (1981) reported 100% mortality of Ae. vexans larvae in a 0.5 ha ditch in West Germany when a locally produced formulation of BTI was applied at a dosage of 0.30 kg/ha. Greater than 80% control of Cx. quinquefasciatus larvae was obtained with a dosage of 0.6 g/m^3 of BTI (Dulmage HD500/R179) when applied to roadside ditches (McLaughlin and Fukuda 1982).

Davidson et al. (1981) and Sebastien and Brust (1981) used 1m^2 artificial ponds to test the efficacy of BTI to various species of mosquito. Davidson et al. (1981) reported a 95% reduction in larval populations of Cx. annulirostris Skuse and Cx. quinquefasciatus with a 1 kg/ha dosage of BTI (ABG-22874). An. annulipes Walker larvae were controlled at a dosage of 2 kg/ha. Sebastien and Brust (1981) reported between 95-100% reduction in larval populations of Cx. restuans Theobald and Ae. vexans when they applied BTI (ABG-6108) at a dosage of 3.2 mg/l. Similar results were obtained for BTI (Biochem 660-PM50) applied at a dosage of 0.8 mg/l. Culex restuans populations in Kansas were also

successfully reduced by BTI (ABG-6108) when applied to tire habitats (Ramoska et al. 1981).

DeMaio et al. (1981) tested BTI (ABG-6108) against Ae. triseriatus in treehole and tire habitats in Indiana. At a dosage of 1.0 ppm, greater than 93% control was observed in tire habitats. Treehole habitats showed similar but lower mosquito mortalities. Purcell (1981) obtained 99% mortality of Ae. triseriatus larvae with a 1.7 mg/l dosage of BTI (Biochem 660-PM50) applied to water holes in Florida.

In Oregon a 73 to 99% reduction in the larval populations of Cx. peus and Cx. pipiens was obtained with dosages of BTI (ABG-6108) between 0.4-1.63 kg/ha applied to a heavily polluted log pond (Eldridge and Callicrate 1982).

Goettel et al. (1982b) conducted field evaluations of BTI (SAN-402-I-WDC) against Ae. vigilax (Skuse) and Cx. sitiens Wied. in brackish water habitats in Fiji. Dosages between 1.251-2.001 l/ha resulted in 82-93% mortality of both species after 48 hours.

Lacey and Dame (1982) tested the effect of BTI (IPS-78) on the predatory mosquito Toxorhynchites rutilis rutilis (Coquill.) and discussed the possible use of Toxorhynchites mosquitos and BTI in integrated pest management (IPM) programs. No mortality of 4th instar Toxoryhnchites larvae was observed when exposed to 10 ppm of BTI in the absence of

prey. Exposure to 1.0, 5.0 and 10.0 ppm of BTI in the presence of excess prey (20 Ae. aegypti larvae/ Toxorhynchites larvae) resulted in 23, 62 and 95% mortality of Toxorhynchites larvae, respectively. A 1 ppm dosage of BTI posed little threat to 4th instar Toxorhynchites larvae in the presence of moderate numbers of prey. Dosages of BTI that could have severe effects on 4th instar larvae of Toxorhynchites would be too high to be practical in an IPM program.

Stewart et al. (1983) tested BTI against Cx. tarsalis in rice fields stocked with the mosquito fish Gambusia affinis Baird and Girard. The best control was obtained in a field treated with 1.1 kg/ha of BTI and 0.28 kg/ha of Gambusia affinis. One treatment with BTI resulted in season long control of Cx. tarsalis in those fields also treated with the mosquito fish.

Guillet (Anonymous 1979) tested BTI in conjunction with chemical insecticides against Ae. aegypti larvae. A synergic effect of about 1.8-1.9 times was obtained with chlorophoxim and temephos. Permethrin and decamethrin decreased the effectiveness of BTI by 0.7-0.8 times. Permethrin and decamethrin are pyrethroid compounds which disturb feeding of mosquito larvae. This decreased feeding may have resulted in a decrease in the efficacy of BTI.

Sun et al. (1980) tested the possibility of cross-resistance to BTI of Cx. quinquefasciatus and An. albimanus

larvae variously resistant to organophosphorous, carbamate, chlorinated hydrocarbon and pyrethroid insecticides. No significant difference between insecticide-susceptible and -resistant strains of either species to BTI was observed.

Georghiou and Vasquez (1981) tested the potential of Cx. quinquefasciatus to develop resistance towards BTI. Only application of extremely high selective pressure (99.97%) for 16 generations resulted in a significant tolerance to BTI.

Klowden et al. (1983) tested the toxicity of BTI to adult Ae. aegypti. Adult females were killed by the parasporal crystals of BTI (ONR-60A) when introduced into the insect midgut as an enema. LD50 for adult females was 0.21 ug/mg of mosquito wet weight compared to 0.018 ug/mg for larval mosquitos.

BTI is effective against a wide range of mosquito species under varied environmental conditions. Successful control of mosquito larvae using BTI has been obtained in a variety of habitats including freshwater ponds, ditches, salt marshes, rice plots, irrigated pastures, treeholes and artificial containers. Good control of mosquito populations may be attainable with IPM programs using BTI in conjunction with other biological control agents of mosquitos.

Both instar and feeding behaviour of mosquitos have been found to affect susceptibility. Environmental factors such as temperature, water quality and availability of food can affect BTI efficacy. The most critical factor affecting larval mosquito mortality seems to be the organic content of the water. Large amounts of organic matter can reduce the efficacy of BTI by increasing the settling rate of the delta-endotoxin and through feeding competition. The resulting decrease in larval mosquito uptake of BTI in turn results in a lower efficacy. In organically rich aquatic environments, mosquito control may only be obtained with very large dosages of BTI. BTI is currently one of the safest and most effective control agents available for the pest management of mosquito larvae.

2.6.3 Other Invertebrates

The high toxicity of BTI to mosquito and black fly larvae has resulted in tests of its efficacy against other important dipteran pests and vectors of diseases.

Larget and deBarjac (1981a,b) tested BTI in the laboratory for control of Culicoides sp. Second and third instar larvae were kept for 12 days in culture media containing 0.4 to 4000 mg of BTI (IPS-78). Mortality was slightly higher when larvae were exposed to 40, 400 and 4000 mg/l than in untreated controls. However, larval mortality did not in-

crease with increased BTI dosage suggesting that mortality was not due to the action of BTI. Lacey and Kline (1983) tested BTI againt larvae of Culicoides spp. and Leptoconops spp. in the laboratory. Both genera utilized bacteria in their larval diet however none of the larvae tested were adversely affected by BTI.

Second instar larvae of Drosophila were reared on nutrient substrate containing from 0.4 to 400 mg of BTI (IPS-78) (Larget and deBarjac 1981a,b). No mortality was observed when larvae were exposed to BTI at these concentrations.

deBarjac et al. (1981) demonstrated the pathogenicity of BTI against Phlebotomus papatasi Scopoli and Lutzomyia longipalpis Lutz and Neiva both vectors of leishmaniasis.

Exposure of 2nd instar larvae of Musca domestica L. to 100 and 400 mg of BTI resulted in no significant mortality (Larget and deBarjac 1981a,b). Vankova (1981) tested a locally produced culture of BTI containing $30x10^{10}$ spores/g against larvae of Musca domestica. The LD50 was $2.2x10^{3}$ mg BTI/kg of medium. A mortality of 21% was obtained for adult flies fed a concentration of 500 g BTI/l of a mixture of sugar and dried milk.

Van der Geest and deBarjac (1982) tested BTI against adult tsetse fly Glossina pallidipes Austen. A dosage of 20

mg/ml resulted in 20 and 30% mortality. Pure preparations of crystal endotoxin produced no mortality. Mortality was apparently caused by penetration of growing bacteria through the gut wall into the haemolymph.

The activity of BTI against pest species of Lepidoptera has also been tested. deBarjac (1978a) and Tyrell et al. (1979) reported that BTI was ineffective against larvae of Anagasta kuehniella (Zeller), Manduca sexta (L.), Plutella xylostella (L.) and Spodopterna litura (F.). Ignoffo et al. (1981a) found that BTI was toxic to the lepidopterans Trichopluisa ni (Huber), Heliothis zea (Boddie) and H. virecens (F.). Insecticidal activity against these lepidopterans was similar to that of early commercial formulations of Bacillus thuringiensis galleriae (Shvetova) and Bacillus thuringiensis thuringiensis (Heimpel and Angus 1959).

2.7 EFFECT ON NON-TARGET ORGANISMS

2.7.1 Invertebrates

Numerous laboratory and field studies have been conducted to test the effect of BTI on non-target invertebrates in mosquito and black fly breeding habitats.

BTI has a low toxicity to a wide range of invertebrates at dosages as much as 200 times those used for mosquito and black fly control (Garcia et al. 1980; Sinegre et al. 1980a; Lebrun and Vlayen 1981; Schnetter et al. 1981).

significant mortalities at dosages close to those used for mosquito and black fly control have been recorded only for other nematoceran Diptera.

Garcia et al. (1980) obtained 100% mortality of Dixa sp. larvae with a dosage of 10^6 spore/ml of a locally produced formulation of BTI. Dosages of 10^4, 10^5 and 10^6 spores/ml resulted in 15, 50 and 100% mortality of chironomid larvae, respectively. When treated at dosages of 10^6 and 10^7 spores/ml for four days larvae of Palpomyia sp. (Ceratopogonidae) showed 42 and 100% mortality , respectively. No mortality was observed for larvae of Ephydra sp. (Ephydridae) exposed to a dosage of 10^6 spores/ml for 30 days.

Sinegre et al. (1980a) tested BTI (R.153-78) against Chironomus sp. and Chaoborus sp. (Chaoboridae). Dosages of 0.040, 0.155,0.600 and 2.500 mg/l for 24 hours resulted in 8, 92, 100 and 100% mortality of Chironomus sp. larvae, respectively. No mortality was observed for Chaoborus sp. larvae at dosages as high as 2.5 mg/l for 48 hours.

Ali et al. (1981) tested four formulations of BTI (IPS-78,R.153-78,ABG-6108 and SAN-402-I-WDC) against four species of Chironomidae in laboratory experiments. LC90 values for Glyptotendipes paripes Edwards larvae were 13.14, 9.84,32.38 and 23.59 ppm after 48 hours for IPS-78, R.153-78, ABG-6108 and SAN-402-I-WDC, respectively. LC90 values for Chironomus decorus Johannsen larvae were 8.61,

4.56, 30.76 and 26.98, for C. crassicaudatus Malloch were 10.56, 4.92, 47.02 and 28.36 and for Tanytarsus sp. were 11.38, 6.34 and 22.61. Chironomid larvae were 13 to 75 times less susceptible to BTI then larvae of Ae. aegypti or Cx. quinquefasciatus.

Larget and deBarjac (1981b) tested BTI (IPS-78) in the laboratory against Chironomus plumosus (L.). The LC50 ranged between 0.2 and 0.4 mg/l for 48 hours. Lebrun and Vlayen (1981) reported a LC50 value of 0.28 mg/l after 48 hours for C. plumosus when exposed to BTI (R.153-78).

Schnetter et al. (1981) obtained 100% mortality of Chironomus sp. and Smittia sp. larvae with dosages of 1.6, 16 and 160 mg/l of a locally produced culture of BTI. No mortality was observed for larvae of Chaoborus sp., Mochlonyx sp. and Eristalomya sp.

Results from field tests with BTI agree with laboratory results in the specificity of BTI to nematoceran Diptera. No mortality in non-target invertebrates, with the exception of nematoceran Diptera, has been recorded from tests of BTI in mosquito breeding habitats (Engler et al. 1980; Garcia et al. 1980; Miura et al. 1980; Purcell 1981; Schnetter et al. 1981; Sebastien and Brust 1981; Mulla et al. 1982). No significant mortality in non-target invertebrates, including nematoceran Diptera, has been recorded in black fly river treatments with BTI (Dejoux 1979; Colbo and Undeen

1980; Yameogo 1980; Molloy and Jamnback 1981; Lacey et al. 1982a; Chilcott et al. 1983).

Chironomid midges, though non-biters, often occur in large swarms, creating nuisance and economic problems that necessitate the initiation of control measures (Mulla 1974). BTI has been used in attempts to control nuisance midge populations. Schaefer (1979) obtained 76% control of Chironomus sp. larvae in a 12 ha field in California by aerial application of 1.12 kg of BTI (SAN-402-I-WDC). Ali (1981) tested (ABG-6108) against nuisance chironomids in experimental ponds and a golf course pond in Florida. Dosages of 0.25, 0.50, 0.75, 1.00 and 2.50 ppm applied to experimental ponds resulted in 18-43, 23-61, 36-61, 52-72 and 53-88% reduction of total chironomid larvae, respectively.

The toxicity of BTI to chironomid larvae in mosquito breeding habitats is relatively low when compared with its toxicity to mosquito larvae (Ali et al. 1981). However, the relative susceptibility of chironomid species associated with black fly larvae has not yet been determined. BTI is likely toxic to some species of lotic chironomids, however a lack of significant mortality in stream tests of BTI (Colbo and Undeen 1980; Molloy and Jamnback 1981; and this study) suggests that chironomid populations are unlikely to be seriously disrupted by the dosages used in black fly control programs.

2.7.2 Vertebrates

Because BTI is applied to water sources to control mosquitos and black flies it has been tested for possible effects on aquatic vertebrates. Garcia et al. (1980) tested a locally produced formulation of BTI against three species of fish and three species of amphibian. No mortality of the mosquito fish, the rain water killifish, the stickleback, frogs, toads or newts were repoarted at dosages of 10^7 spores/ml of BTI. Lebrun and Vlayen (1981) tested BTI (R.153-78) against the fish Tilapia sp. No mortality was observed for dosages of up to 100 ppm. A dosage of 4000 ppm resulted in 50% mortality . This was 2000 times the dosage required to obtain 50% mortality of Cx. quinquefasciatus larvae. Schnetter et al. (1982) have stated that toxins produced by BTI affect tadpoles of the genus Xenopus. This toxicity is not associated with the crystalline delta-endotoxin and does not involve histological changes in the intestinal epithelium of the tadpole. The toxic factors are associated with the vegetative and sporulating cells and are not affected by temperatures of up to 60C. The toxic factors are destroyed by autoclaving.

Before BTI can be used as a vector control agent its potential as a hazard to man and other mammals must be evaluated. deBarjac et al. (1980) reported a lack of toxicity or pathogenicity in mice, rats, rabbits and quinea pigs ino-

culated with BTI at a dosage of 10^7-10^8 bacteria/animal. Shadduck (1980) injected BTI intracerebrally, intraperitoneally and orally into rats. Harmful effects were noted only when more than 10^6 bacteria were injected directly into the brains of weanling rats. Bacterial replication was not detected in any mammalian tissue *in vivo*. Dosages of 10^{11}-10^{12} bacteria fed to mice and rats did not result in any clinical or histopathological abnormalities. Over two generations, mice fed BTI showed no change in weight gains, mortality or fertility (Schnetter *et al*. 1981). Thomas and Ellar (1982) studied the effects of alkali activated BTI endotoxin on mammalian cells *in vitro*. Mouse fibroblasts, primary pig lymphocytes and mouse epithelial cells were susceptible to the alkali activated endotoxin. The activated endotoxin was also haemolytic for rat, mouse, horse and human erythrocytes. None of these *in vitro* effects were observed when the various mammalian cell types were exposed to unactivated BTI protoxin.

Chapter III

MANUSCRIPT

Impact of Bacillus thuringiensis var. israelensis in dosages used for black fly control, against target and non-target organisms in the Torch River, Saskatchewan.

3.1 ABSTRACT

Drift and benthic densities of aquatic organisms were measured prior to and following two applications of BTI to the Torch River, Saskatchewan. A 14.5 and 20.2 fold increase in the drift density of Simulium spp. occurred following application of BTI in June and August 1982, respectively. Following BTI application an increase in drift density of chironomid larvae in June and a decrease in benthic density of chironomid larvae in August were observed. However, no change in the benthic and drift densities of any non-target organism can be attributed solely to BTI application.

3.2 INTRODUCTION

Bacillus thuringiensis H-14 deBarjac is an effective black fly larvicide, both in laboratory studies (Undeen and Nagel 1978; Undeen and Berl 1979; Frommer *et al.* 1980; Molloy *et al.* 1981; Molloy and Jamnback 1981) and in the field (Guillet and deBarjac 1979; Undeen and Colbo 1980; Frommer *et al.* 1981a,b; Molloy and Jamnback 1981; Undeen *et al.* 1981; Lacey *et al.* 1982).

Recent studies have demonstrated that BTI has little or no effect on non-target organisms associated with black fly larvae (Dejoux 1979; Colbo and Undeen 1980; Molloy and Jamnback 1981; Chilcott *et al.* 1983; Pistrang and Burger 1984). However, this conclusion is based on investigations which emphasized the efficacy of BTI against black fly larvae rather than its impact on non-target organisms.

A study was conducted to assess BTI as a control agent for pest species of black flies under Canadian prairie river conditions. As part of this study drift of target and drift and benthic density of non-target organisms were rigorously measured to determine the environmental impact of BTI.

3.3 STUDY AREA

The Torch River drains Candle Lake, situated in central Saskatchewan, and flows east for approximately 150 km before emptying into the Saskatchewan River (Fig. 1).

Four factors made the Torch River desirable for BTI trials:

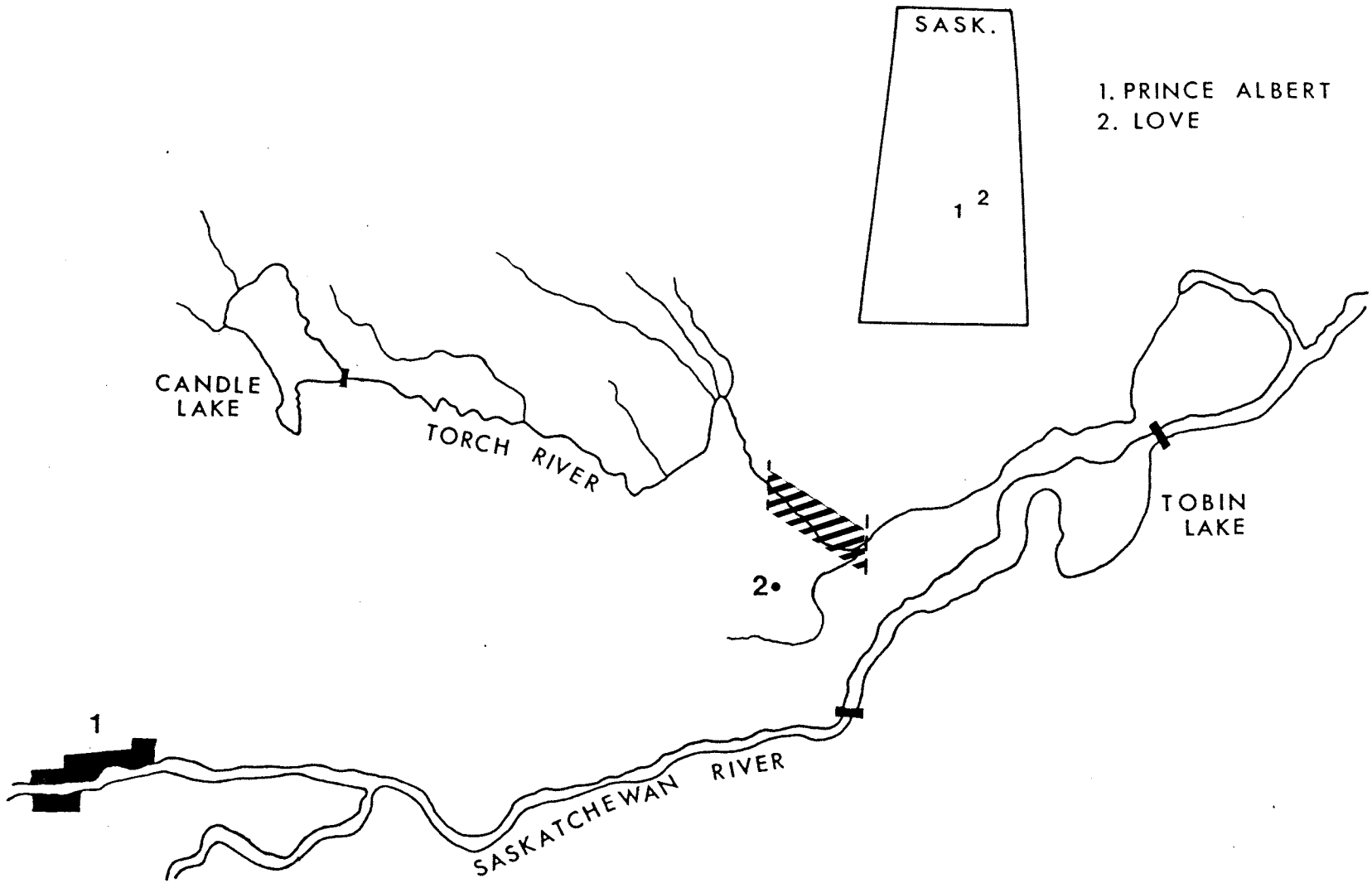
1. The river supports large populations of Simulium luggeri Nicholson and Mickel the major black fly pest of livestock in Saskatchewan (Fredeen 1977). In addition the larval black fly population is composed of other Simulium species which are minor pests of man, livestock and wildlife.

2. Macroinvertebrate diversity and population levels were suitable for assessing the impact of BTI on non-target organisms.

3. The Torch River is considerably smaller than the Saskatchewan River. Procedures for sampling lotic fauna are easier to carry out and more reliable in smaller rivers. Preliminary field evaluations of BTI should be carried out to establish its efficacy against pest and non-target species before trials are attempted in major rivers.

4. The Torch River is not a potable water source. Federal restrictions prohibit the experimental use of any agent in a water source used for human consumption.

Fig. 1. **Location of Torch River in Saskatchewan.** Stipled area refers to study site.


SASK.
1
2
1. PRINCE ALBERT
2. LOVE
CANDLE LAKE
TORCH RIVER
TOBIN LAKE
2
1
SASKATCHEWAN RIVER

The study site selected was a 11.1 km stretch approximately 75 km downstream of Candle Lake (Fig. 2). The dominant riparian vegetation consisted of trembling aspen (Populus tremuloides Michx.), jack pine (Pinus banksiana Lamb.), white birch (Betula papyrifera Marsh) and white spruce (Picea glauca (Moench) Voss). The area surrounding the river was under cultivation, except for approximately 0.5 km on each side of the river. Width of the river during the study was 35m to 45m depending on river discharge, which fluctuated seasonally. The river consisted of shallow riffles with a mean depth of 40cm, and a few deeper pool areas. River substrate in the riffles was composed of cobble sized stones and large boulders.

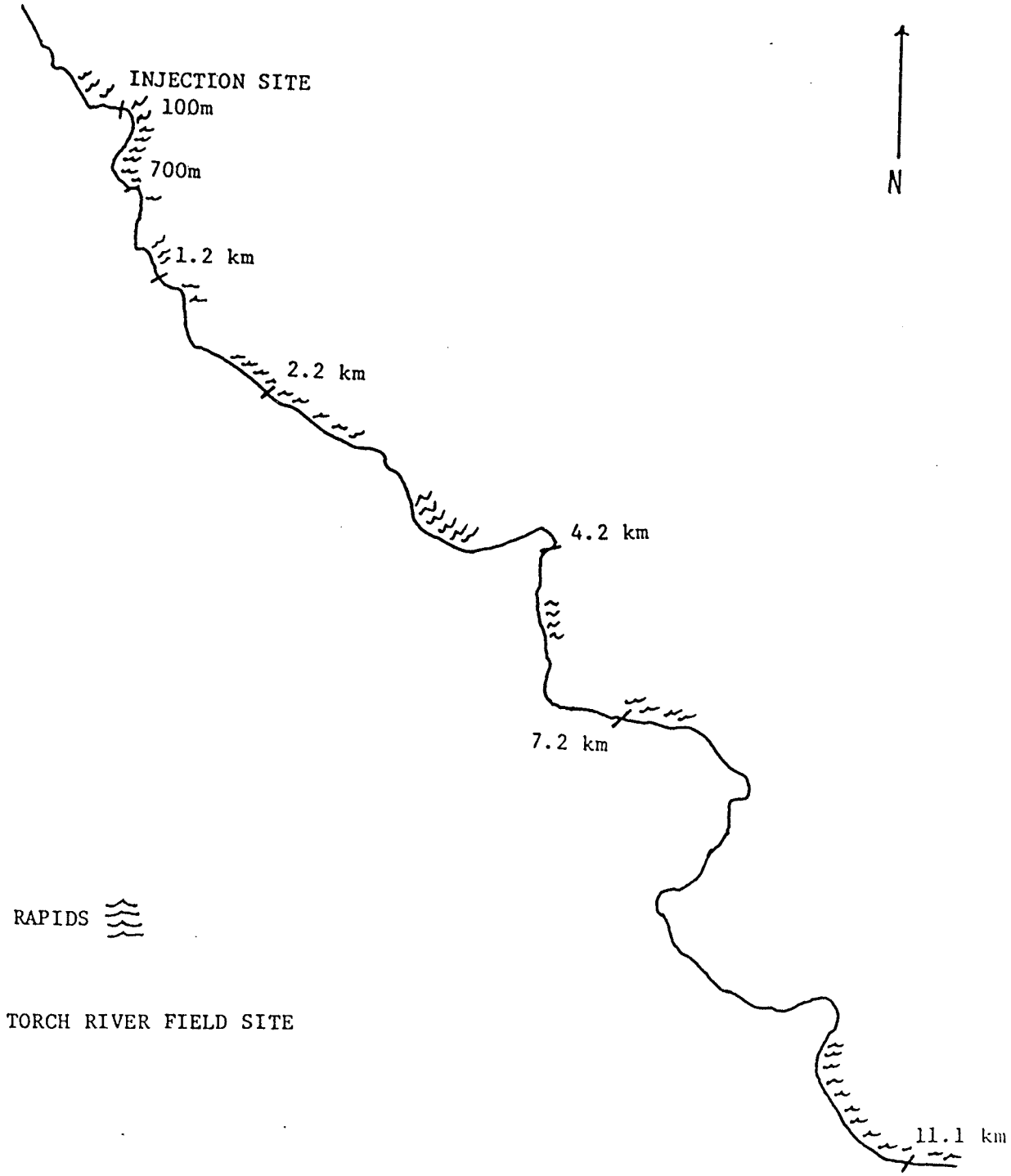
3.4 MATERIALS AND METHODS

3.4.1 Application of BTI

A trial was conducted in 1981 in order to test the methods used for application and monitoring the effect of BTI. Two formulations of BTI were applied to the Torch River in 1982 and their impact was assessed using methods devised in 1981. All formulations were applied to the river using calibrated CP3 knapsack sprayers. Applicators where positioned on a transect across the river and BTI was applied at a pre-set dosage.

Fig. 2. Torch River field site, Saskatchewan.
Stipled area in Fig. 1.

TORCH RIVER, SASKATCHEWAN



3.4.2 Measurement of Enviromental Parameters

Water velocity mesurements used to calculate river discharge and drift densities were made using a Price 625 pygmy flow meter.

Water temperature and pH levels were determined using a Fisher Model 640 Acumet mini pH/mv meter.

Other parameters recorded were sunrise, sunset and cloud cover.

3.4.3 Drift Samplers

Modified bomb drift samplers (Appendix A)(Burton and Flannagan 1976) were used to monitor changes in the drift of aquatic organisms. Modifications involved slight dimensional changes and elimination of stabilizing fins. Samples were taken at four hour intervals and stored in 95% ethanol. Samples were taken for 24 hours before and after each BTI application.

Two sets of four drift samplers were positioned along the river. One set was positioned in a riffle area 100m upstream of the application point and served as a control. The other set was positioned in a riffle area 250m downstream of the application point. Drift samplers were positioned in mid-stream at mid-depth between two metal rods anchored into the river substrate. Positioning of drift

samplers was dictated by availablility of suitable substrate and adequate water flow.

Water velocity measurements were made directly upstream of each drift sampler at each daylight sampling period. Mean water velocity was calculated for each drift sampler over the 48 hour sampling period (Table 1).

Drift samples were subsampled in the laboratory using the Imhoff Cone technique of Wrona et al. (1982)(Appendix B). Aquatic organisms from subsamples were sorted and identified with 10x magnification . Drift rates were standardized to numbers of organisms/200m^3 (drift density), using mean water velocity data, to allow comparison between samplers and sites (Elliott 1970).

One way analysis of variance (ANOVA) was used on ln(x+1) transformed data to compare pre- and post-application results.

3.4.4 Benthic Samples

Samples were taken 48 hours before and after BTI application at three sites along the river. The first site located 75m upstream of the application point served as a control. Two additional sites were located 200m and 800m downstream of the application point. At each sampling period 10 rocks were handpicked into a 500um mesh net held downstream. Rocks were selected at 1m intervals from the central 10m of a transect across the river at each site.

Table 1. Position of and waterflow into bomb drift samplers in the Torch River, Saskatchewan.

U - 100m upstream (control) drift samplers

D - 250m downstream drift samplers

p - distance of sampler from south bank (m)

d - depth of river at mouth of drift sampler (cm)

$\bar{v}$ - mean velocity at mouth of drift sampler (m^2/sec)

Drift sampler	p	June 17-19		Aug. 5-7	
		d	$\bar{v}$	d	$\bar{v}$
U_1	23.0	70	.753	60	.714
U_2	21.0	62	.795	64	.746
U_3	18.0	65	.784	67	.735
U_4	16.0	74	.778	68	.726
D_1	30.0	63	.455	55	.389
D_2	26.5	68	.689	55	.685
D_3	23.0	72	.801	65	.777
D_4	19.5	65	.332	55	.206

Subsequent samples were taken from transects 1m upstream from the previous transect. Organisms were removed from the net and rock and stored in 95% ethanol. Samples were sorted and identified in the laboratory with 10x magnification.

The surface area of each rock was estimated using the plastic wrap technique of Doeg and Lake (1981)(Appendix C). For comparison, samples were standardized to number of organisms/500cm^2 of surface area. ANOVA on ln(x+1) transformed data was used to compare pre- and post-application results.

3.4.5 Insect Emergence

Meter-square emergence traps were used to monitor the emergence of aquatic macroinvertebrates during each BTI trial. One trap was positioned 80m upstream and another 180m downstream from the application point. Each trap was emptied daily for four days prior to and following each BTI application. Emergence traps were unreliable and emergence data was complimented with data collected from daily sweep net samples along the river shoreline.

3.5 RESULTS

3.5.1 Application of BTI

Dosage and application times used for the 1981 and 1982 BTI trials were those recommended by the manufacturer (Table 2)(Galloway et al. in prep.). Potency ratings given in Table 2 are those supplied by the manufacture. The Teknar EC formulation has been reported to have a potency of only 600-700 ITU (Dame et al. 1981; Ignoffo et al. 1982) as compared to 1500 ITU supplied by the manufacturer.

Downstream carry (Table 2) is based on greater than 50% mortality of black fly larvae on artificial substrates 24-48 hours after treatment (Galloway et al. in prep.).

3.5.2 Measurement of Environmental Parameters

River discharge, pH and water temperature during each of the BTI trials are listed in Table 2. Little change was observed in pH or water temperature during and between trials. There was a large difference in river discharge between the two August trials however, both trials in 1982 had similar river discharge.

Cloud cover was minimal during all trials and sunrise and sunset were approximately at 4.30 hours and 21.30 hours during the June trial and 5.30 hours and 20.45 hours during the August trials.

Table 2. Summary of physical, environmental and chemical parameters during BTI trials, in the Torch River, Saskatchewan.

1 - based on >50% mortality of black fly larvae attached to artificial substrates (Galloway *et al.* in prep.).

	1981 Preliminary Trial	1982 Trials	
	Aug. 19	June 18	Aug. 6
Product	Teknar(R) EC	Teknar(R) EC	Vectobac(R) WP
Manufacturer's potency (A.a. I.T.U./mg)	1500	1500	2000
Dosage (ppm)	3.75	3.5	2.0
Dosage time (min)	20	15	15
River discharge (m^3/sec)	2.5	12.0	11.0
pH	8.4	8.4-8.5	8.6
Temp (^oC)	19-20	16-17	19-20
Downstream carry[1]	>700 m	>4.2 km	>11.1 km

3.5.3 Drift

More than 192 taxa were recovered in drift samples during June and August 1982 (Appendix D).

In June six taxa comprised 92% of the total non-target faunal drift. Of all the non-target organisms collected Baetis spp. larvae comprised 40%, water mites 16%, chironomids 15%, ephemerellids 10%, ostracods 7% and white sucker fry 4% (Table 3). In June 66% of the non-target fauna collected in drift samplers were scrapers, 16% predators, 7% filter-feeders and 2% shredders.

In August seven taxa comprised 90% of the total non-target faunal drift. Of all the non-target organisms collected Baetis spp. larvae comprised 41%, Tricorythodes minutus Traver larvae 16%, water mites 9%, chironomids 9%, Nectopysche diarina (Ross) larvae 5.4%, Hydropysche spp. larvae 4.5%, Triaenodes frontalis Banks larvae 2.7% and Caenis pr. simulans McDunnough larvae 2.4% (Table 4). In August 72% of the non-target fauna collected in drift samplers were scrapers, 9% predators, 9% shredders and 6% filter-feeders.

After being sorted and identified, drift samples were combined into 24 hour pre- and post-treatment samples for each taxon.

Table 3. Mean drift density (number/200m^3) of aquatic organisms 24 hours pre- and post-application of BTI in June 1982, to the Torch River, Saskatchewan.

*** - significant at the .001 confidence level.

( ) - standard error

[ ] - number of genera

Taxa	Upstream Control				Downstream Treatment			
	June 17-18		June 18-19	F (ANOVA)	June 17-18		June 18-19	F (ANOVA)
Class Crustacea								
Subclass Ostracoda								
Order Podocopa [3]	276.8	(93.9)	276.1	(27.1) NS	397.0	(78.5)	409.5	(48.6) NS
Class Arachnida								
Order Acari [>20]	640.5	(151.5)	736.5	(92.8) NS	898.8	(49.8)	1040.7	(37.3) NS
Class Insecta								
Order Ephemeroptera								
Fam Baetidae								
Baetis spp.	1724.6	(396.6)	1556.8	(203.3) NS	3005.2	(475.8)	2497.9	(228.8) NS
Fam Heptageniidae [3]	12.0	(4.5)	12.5	(1.8) NS	17.8	(5.9)	26.1	(3.5) NS
Fam Ephemerellidae [2]	302.3	(67.7)	384.4	(55.3) NS	680.2	(65.8)	782.1	(119.3) NS
Fam Baetiscidae								
Baetisca laurentina	3.1	(1.3)	0.7	(0.6) NS	0.4	(0.4)	1.4	(0.8) NS
Fam Ephemeridae								
Ephemera simulans	7.3	(3.8)	16.5	(4.8) NS	27.4	(5.1)	29.7	(8.4) NS
Order Odonata								
Fam Gomphidae								
Ophiogomphus colubrinus	27.0	(7.7)	27.0	(2.4) NS	49.1	(6.1)	43.1	(7.7) NS

Continued .....

Taxa	Upstream Control					Downstream Treatment				
	June 17-18		June 18-19		F (ANOVA)	June 17-18		June 18-19		F (ANOVA)
Fam Calopterygidae										
Calopteryx aequabile	1.5	(6)	1.5	(0.6)	NS	5.7	(4.2)	0.0	0.0)	NS
Order Plecoptera										
Fam Pteronarcyidae										
Pteronarcys dorsata	30.6	(6.7)	34.4	(2.4)	NS	50.7	(19.9)	24.9	(6.5)	NS
Fam Chloroperlidae										
Hastaperla brevis	15.7	(6.7)	16.2	(5.1)	NS	97.4	(14.9)	98.1	(17.5)	NS
Other Plecoptera [3]	2.0	(0.7)	0.6	(0.6)	NS	2.2	(2.2)	3.5	(1.3)	NS
Order Hemiptera										
Fam Corixidae										
Sigara spp.	10.4	(3.8)	13.7	(4.8)	NS	27.2	(9.7)	14.3	(3.5)	NS
Order Trichoptera										
Fam Hydropsychidae										
Cheumatopsyche nr. *campyla*	4.5	(2.9)	8.9	(5.2)	NS	28.0	(1.2)	30.7	(11.8)	NS
Hydropsyche spp.	11.2	(2.3)	12.2	(4.0)	NS	41.8	(17.4)	39.0	(4.1)	NS
Fam Hydroptilidae										
Hydroptila consimilis	26.6	(9.0)	25.0	(5.5)	NS	39.2	(14.4)	56.4	(21.0)	NS
H. spatulata	18.9	(3.7)	23.8	(3.5)	NS	40.5	(8.1)	42.3	(16.1)	NS

Continued .....

Taxa	Upstream Control					Downstream Treatment				
	June 17-18		June 18-19		F (ANOVA)	June 17-18		June 18-19		F (ANOVA)
Fam Leptoceridae										
Triaenodes frontalis	52.6	(9.6)	66.8	(7.1)	NS	89.0	(9.2)	123.0	(17.4)	NS
Other Trichoptera [7]	63.9	(22.7)	88.0	(5.7)	NS	79.1	(15.2)	108.0	(28.0)	NS
Order Coleoptera										
Fam Elmidae										
Optioservus fastiditus	13.3	(5.7)	16.6	(3.2)	NS	25.3	(3.0)	36.8	(11.0)	NS
Order Diptera										
Fam Simuliidae										
Simulium spp.	154.1	(25.7)	128.3	(12.8)	NS	161.7	(8.8)	2368.0	(298.8)	***
Fam Chironomidae [>30]	619.6	(97.4)	578.4	(38.9)	NS	907.3	(185.0)	1308.8	(251.8)	NS
Other Diptera [2]	24.3	(3.1)	22.2	(5.7)	NS	40.9	(12.5)	46.0	(1.5)	NS
Class Osteichthyes										
Order Cypriniformes										
Fam Catostomidae										
Catostomus commersonni	145.3	(18.9)	164.3	(34.8)	NS	280.1	(84.3)	291.6	(65.9)	NS

Table 4. Mean drift density (number/200m^3) of aquatic organisms 24 hours pre- and post-application of BTI in August 1982, to the Torch River, Saskatchewan.

*** - significant at the .001 confidence level.

( ) - standard error.

[ ] - number of genera.

Taxa	Upstream (Control) Aug. 5-6		Upstream (Control) Aug. 6-7		F (ANOVA)	250 m Downstream Aug. 5-6		250 m Downstream Aug. 6-7		F (ANOVA)
Class Crustacea										
Subclass Ostracoda										
Order Podocopa [3]	63.8	(7.8)	50.7	(1.9)	NS	52.5	(13.5)	56.5	(12.8)	NS
Class Arachnida										
Order Acari [>30]	294.5	(15.6)	214.4	(11.3)	NS	367.9	(87.9)	341.2	(58.1)	NS
Class Insecta										
Order Ephemeroptera										
Fam Baetidae										
Baetis spp.	1171.8	(100.9)	892.1	(101.5)	NS	1708.1	(113.7)	1808.9	(181.4)	NS
Fam Heptageniidae [3]	17.8	(5.4)	19.2	(4.0)	NS	42.1	(4.9)	56.3	(9.8)	NS
Fam Tricorythidae										
Tricorythodes minutus	380.6	(71.3)	285.5	(38.1)	NS	873.0	(158.6)	846.2	(137.8)	NS
Fam Caenidae										
Caenis pr. *simulans*	101.7	(14.2)	59.1	(9.5)	NS	62.6	(34.3)	64.5	(24.1)	NS
Fam Ephemeridae										
Ephemera simulans	16.5	(2.4)	19.2	(4.0)	NS	37.1	(5.1)	19.8	(9.0)	NS
Other Ephemeroptera [3]	5.1	(2.0)	3.7	(2.6)	NS	1.3	(1.3)	2.6	(1.5)	NS

Continued .....

Taxa	Upstream (Control) Aug. 5-6		Upstream (Control) Aug. 6-7		F (ANOVA)	250 m Downstream Aug. 5-6		250 m Downstream Aug. 6-7		F (ANOVA)
Order Odonata										
Fam Gomphidae										
Ophiogomphus colubrinus	6.7	(4.4)	2.3	(0.8)	NS	23.8	(5.6)	18.3	(1.1)	NS
Order Plecoptera										
Fam Pteronarcyidae										
Pteronarcys dorsata	20.1	(2.7)	17.4	(4.4)	NS	59.3	(21.3)	48.1	(10.6)	NS
Other Plecoptera [2]	3.2	(2.4)	1.6	(0.6)	NS	9.8	(4.4)	8.2	(4.0)	NS
Order Hemiptera										
Fam Corixidae										
Sigara spp.	52.9	(14.0)	30.6	(8.8)	NS	46.6	(22.2)	36.8	(17.3)	NS
Order Trichoptera										
Fam Psychomyiidae										
Psychomyia flavida	9.5	(3.6)	8.5	(4.0)	NS	18.0	(7.3)	21.9	(7.3)	NS
Fam Hydropsychidae										
Hydropsyche spp.	101.1	(12.4)	92.8	(15.7)	NS	215.7	(56.1)	225.5	(51.1)	NS
Fam Hydroptilidae [3]	6.3	(1.4)	3.3	(0.7)	NS	36.3	(15.3)	32.6	(6.4)	NS
Fam Brachycentridae [2]	17.5	(4.1)	9.1	(3.4)	NS	62.1	(28.9)	79.8	(24.5)	NS

Continued .....

Taxa	Upstream (Control) Aug. 5-6		Upstream (Control) Aug. 6-7		F (ANOVA)	250 m Downstream Aug. 5-6		250 m Downstream Aug. 6-7		F (ANOVA)
Fam Leptoceridae										
Nectopsyche diarina	100.6	(11.2)	99.6	(10.2)	NS	325.0	(84.1)	257.4	(62.7)	NS
Triaenodes frontalis	92.2	(18.1)	79.3	(19.9)	NS	62.6	(15.9)	88.3	(19.7)	NS
Other Trichoptera [8]	12.1	(3.8)	12.4	(5.7)	NS	35.6	(7.8)	47.0	(7.2)	NS
Order Coleoptera [6]	2.1	(1.3)	1.5	(1.2)	NS	6.4	(2.1)	1.0	(0.7)	NS
Order Diptera										
Fam Simuliidae										
Simulium spp.	69.9	(13.4)	70.2	(11.4)	NS	72.7	(20.1)	1817.7	(469.7)	***
Fam Chironomidae [>30]	231.9	(43.6)	177.3	(28.8)	NS	415.2	(129.4)	440.1	(135.2)	NS
Other Diptera [5]	4.2	(2.5)	3.4	(0.5)	NS	3.8	(1.3)	5.0	(2.2)	NS
Class Osteichtyhes										
Order Cypriniformes										
Fam Catastomidae										
Catostomus comersonni	24.3	(4.4)	20.5	(5.1)	NS	22.0	(7.7)	23.7	(11.5)	NS

During the June 1982 trial no significant difference (ANOVA) between 24 hour pre- and post-treatment drift densities in either the control or treatment drift samples was observed for 58 genera of aquatic insects, more than 20 genera of aquatic mites, 3 genera of ostracods and 1 genus of fish (Table 3). A significant difference was observed for Simulium spp. larvae with a 14.5 fold increase in drift density in the downstream drift samplers following BTI application.

During the August 1982 trial no significant difference between 24 hour pre- and post-treatment drift densities in either the control or treatment drift samples was observed for 71 genera of aquatic insects, more than 20 genera of aquatic mites, 3 genera of ostracods and 1 genus of fish (Table 4). A significant difference was observed for Simulium spp. larvae with a 20.2 fold increase in the downstream drift density following BTI application.

The five numerically dominant taxa in the drift samples of each 1982 trial were analysized using 4 hour drift density data.

In the June trial Baetis spp. (Fig. 3) and Ephemerellidae (Fig. 4) larvae exhibited a nocturnal drift periodicity in both the control and downstream sites. Neither group showed a significant change in drift density following application of BTI.

Fig. 3. Mean (±s.e.) drift density of *Baetis* spp. larvae during the June 1982 BTI trial, in the Torch River.

A - control site

B - 250m downstream site

↓ - time of BTI application

\\\ - night sampling period

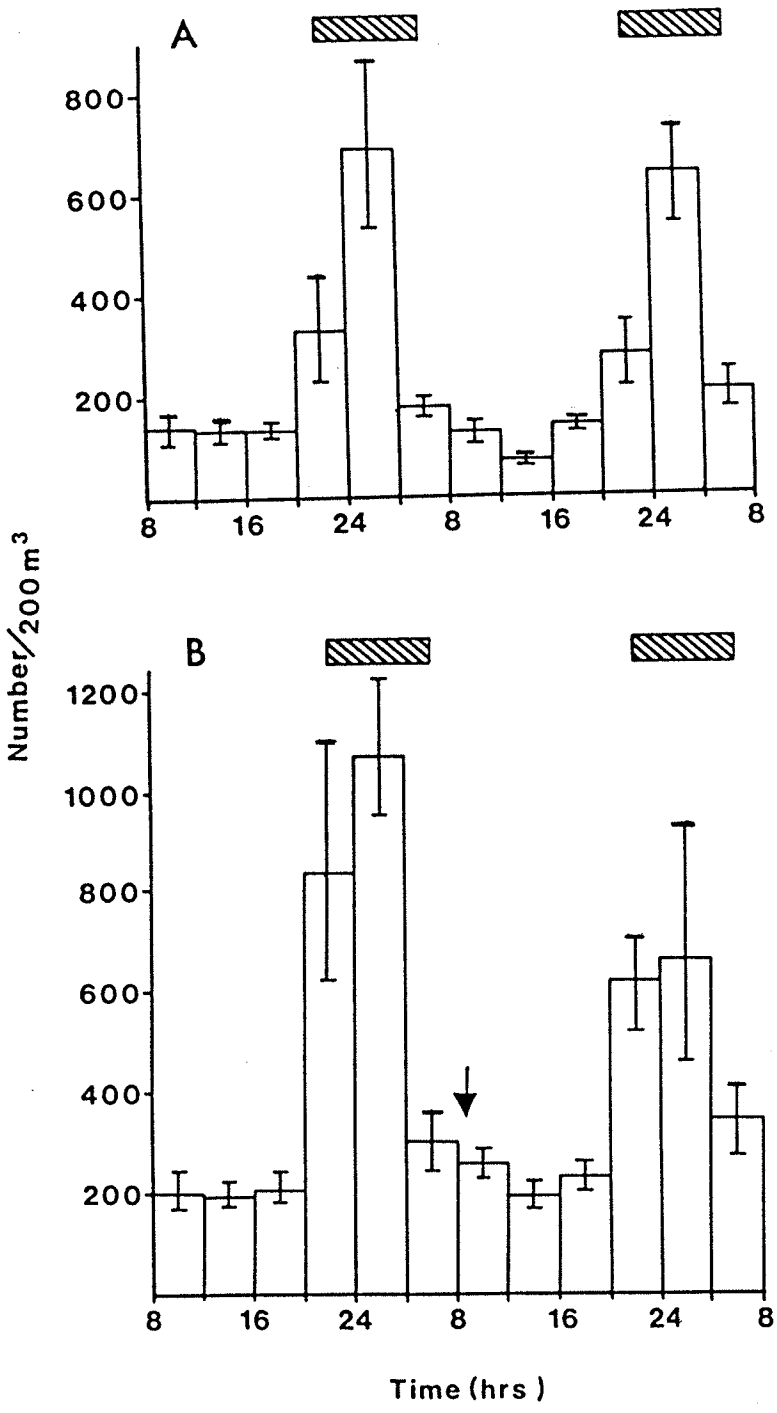
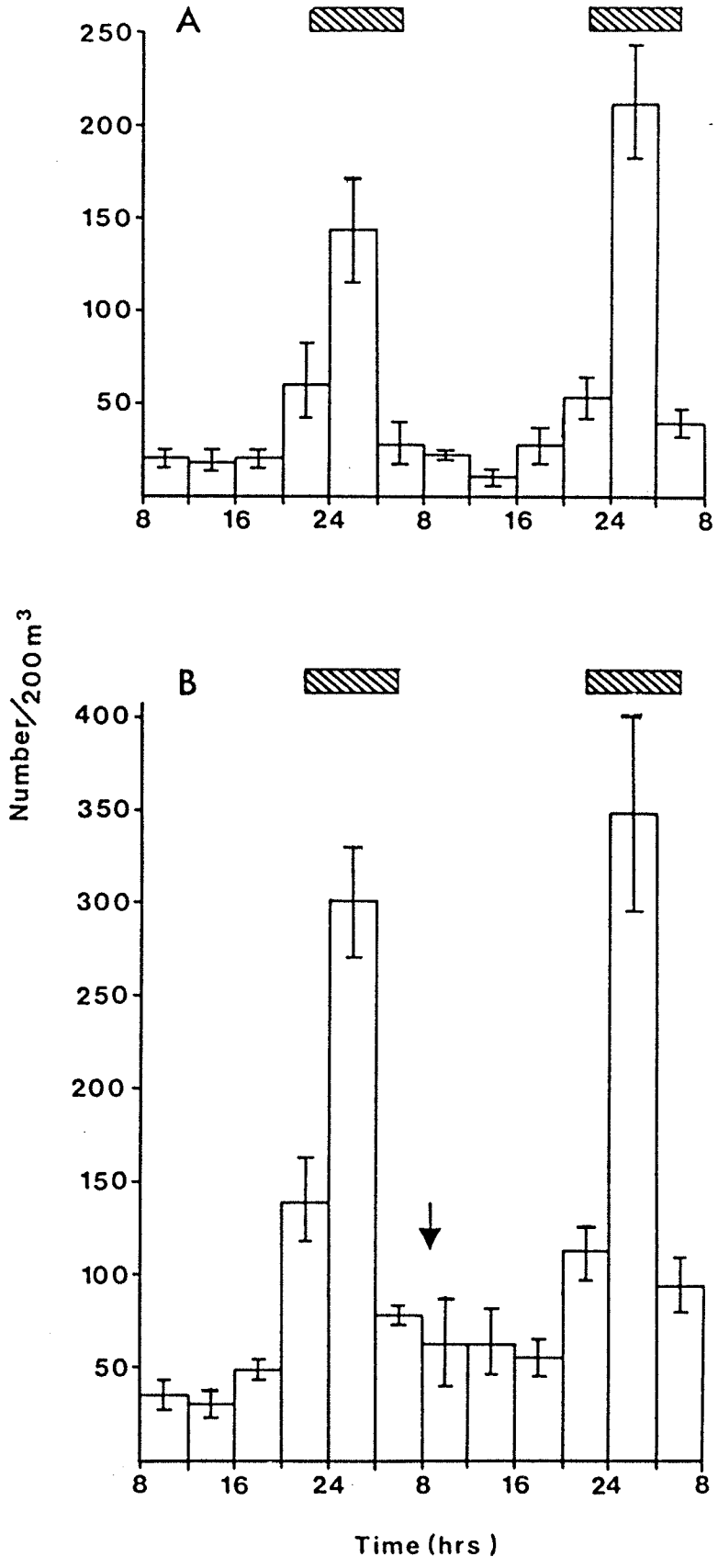

A
800
600
400
200
8
16
24
8
16
24
8
B
1200
1000
800
600
400
200
8
16
24
8
16
24
8
Number/200 m³
Time (hrs)

Fig. 4. Mean (±s.e.) drift density of Ephemerellidae larvae during the June 1982 BTI trial, in the Torch River, Saskatchewan. Legend as in Fig. 3.


A
250
200
150
100
50
8
16
24
8
16
24
8
B
Number/200 m³
400
350
300
250
200
150
100
50
8
16
24
8
16
24
8
Time (hrs)

In the August trial Baetis spp. (Fig. 5) and Tricorythodes minutus (Fig. 6) larvae exhibited a nocturnal drift periodicity in both the control and downstream drift sites. Again, no significant change in drift densities was observed following application of BTI.

Chironomid larvae showed no significant diurnal or nocturnal periodicity in either the June (Fig. 7) or August (Fig. 8) 1982 drift samples. Although an increase in the drift density of chironomid larvae was observed following the June application of BTI, this increase was not significant. A similar increase was not observed following the August application of BTI.

A diurnal increase in drift of Acari was observed between 16-20 hours in the control and treatment sites during both the June (Fig. 9) and August (Fig. 10) 1982 trials. No change in drift densities was observed following either the June or August BTI applications.

Simulium spp. larvae exhibited a nocturnal drift periodicity in both the control and treatment drift sites during both the June (Fig. 11) and August (Fig. 12) 1982 trials. A significant increase in drift densities was observed for up to 16 hours following BTI application at the treatment drift site in both June (Fig. 11) and August (Fig. 12) 1982 trials.

Fig. 5. Mean (±s.e.) drift density of *Baetis* spp. larvae during the August 1982 BTI trial , in the Torch River, Saskatchewan. Legend as in Fig. 3.
Sampling period between 24-4 hours on August 6 at Control site (A) was improperly sampled and is omitted.

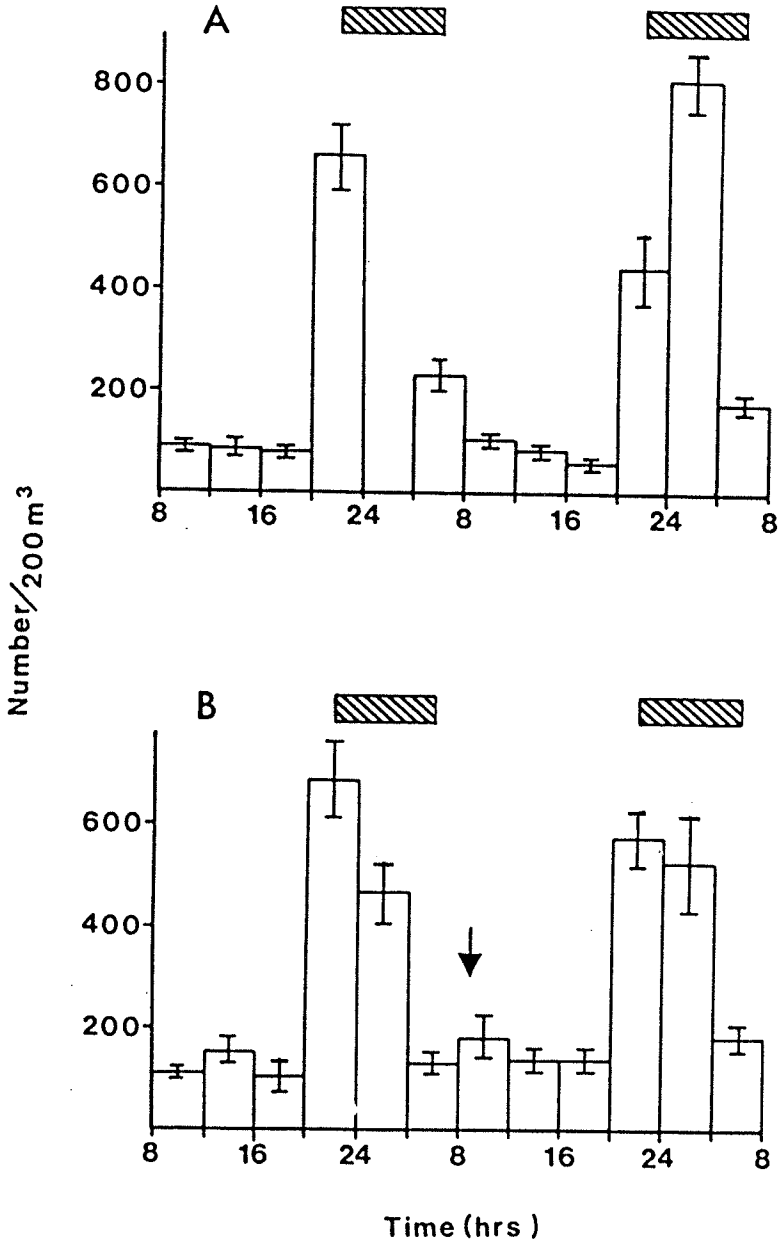

A
800
600
400
200
8
16
24
8
16
24
8
B
600
400
200
8
16
24
8
16
24
8
Number/200 m³
Time (hrs)

Fig. 6. Mean (±s.e.) drift density of *Tricorythodes minutus* larvae during the August 1982 BTI trial, in the Torch River, Saskatchewan.

Legend as in Fig. 3 and 5.

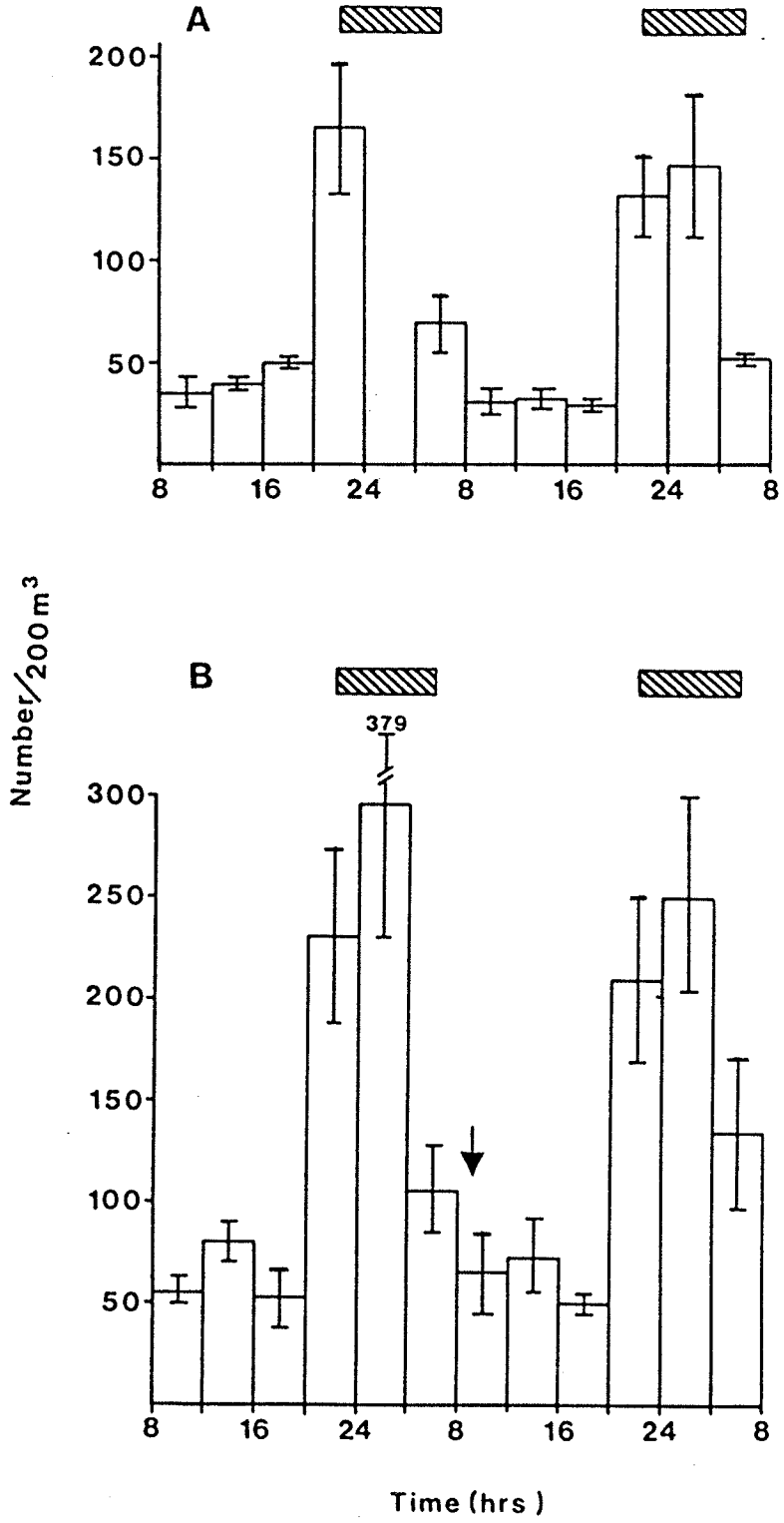

A
200
150
100
50
8
16
24
8
16
24
8
B
379
300
250
200
150
100
50
Number/200m³
8
16
24
8
16
24
8
Time (hrs)

Fig. 7. Mean (±s.e.) drift density of Chironomidae larvae during the June 1982 BTI trial , in the Torch River, Saskatchewan.

Legend as in Fig. 3.

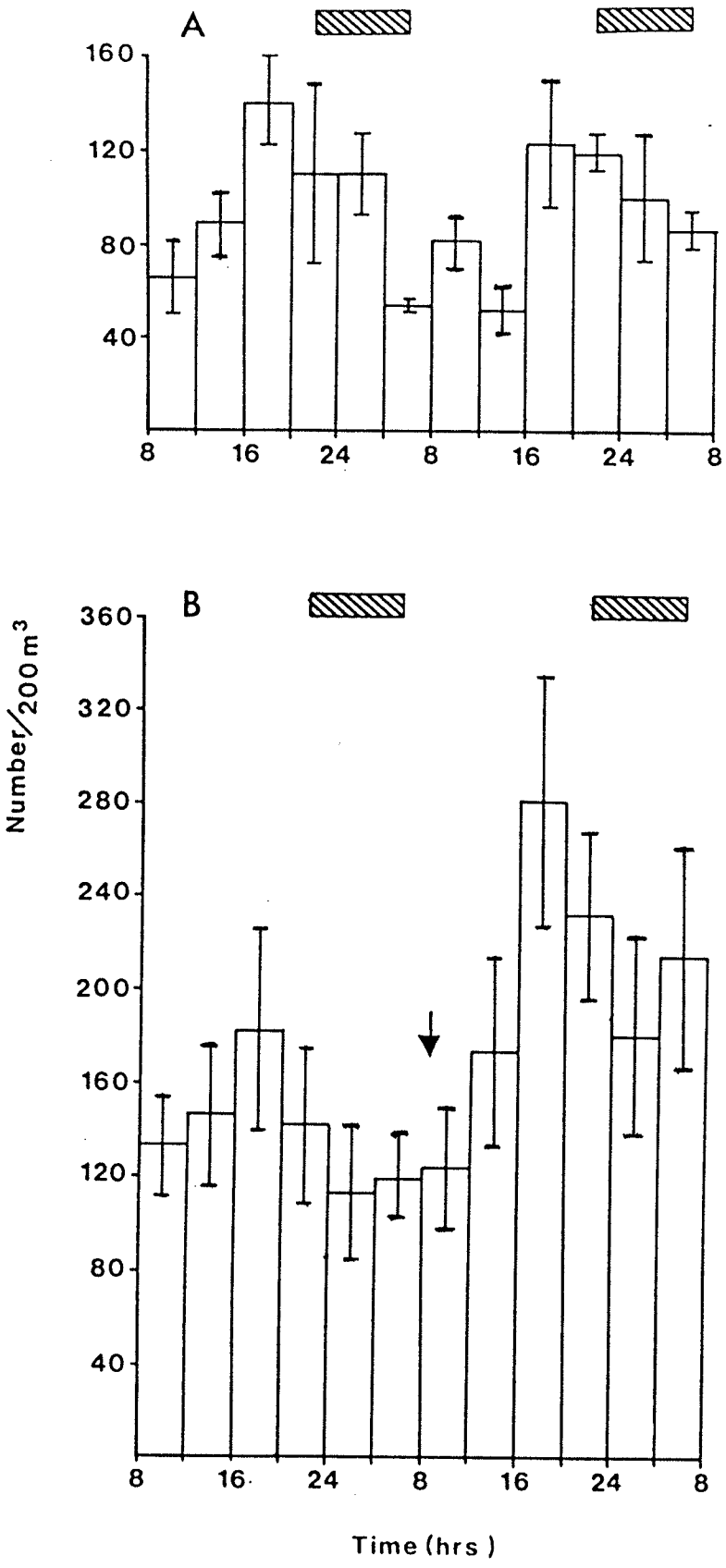

A
160
120
80
40
8
16
24
8
16
24
8
B
360
320
280
240
200
160
120
80
40
Number/200 m³
8
16
24
8
16
24
8
Time (hrs)

Fig. 8. Mean (±s.e.) drift density of Chironomidae larvae during the August 1982 BTI trial , in the Torch River, Saskatchewan. Legend as in Fig. 3 and 5.

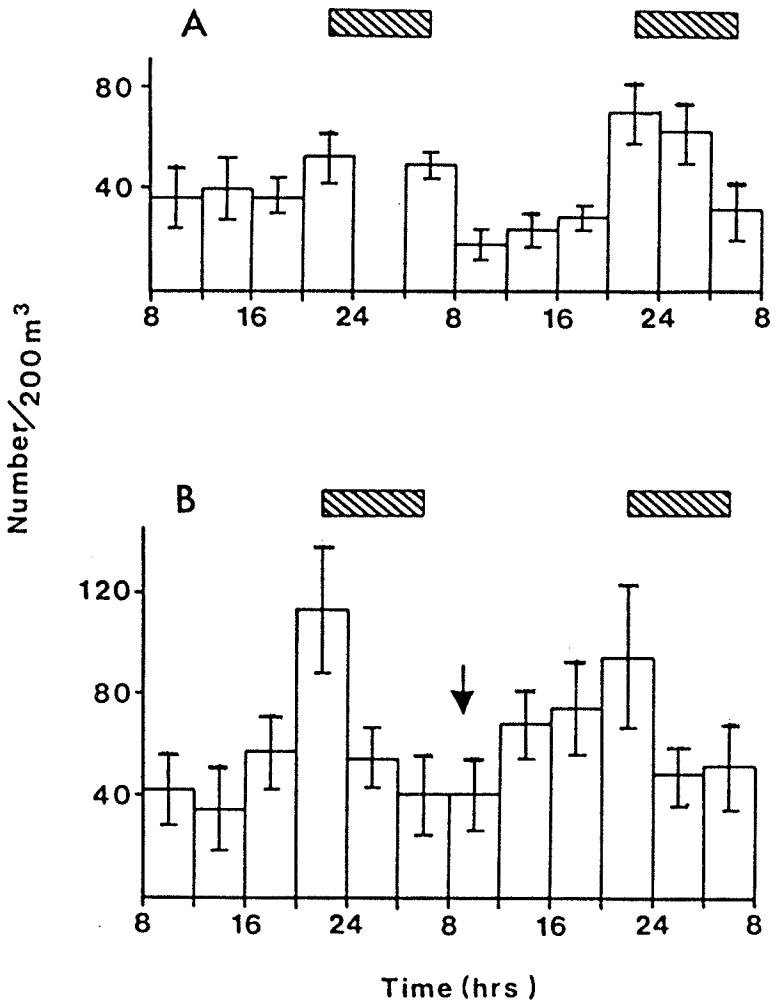

A
80
40
8
16
24
8
16
24
8
B
120
80
40
8
16
24
8
16
24
8
Number/200 m³
Time (hrs)

Fig. 9. Mean (±s.e.) drift density of Acari during the June 1982 BTI trial, in the Torch River, Saskatchewan. Legend as in Fig. 3.

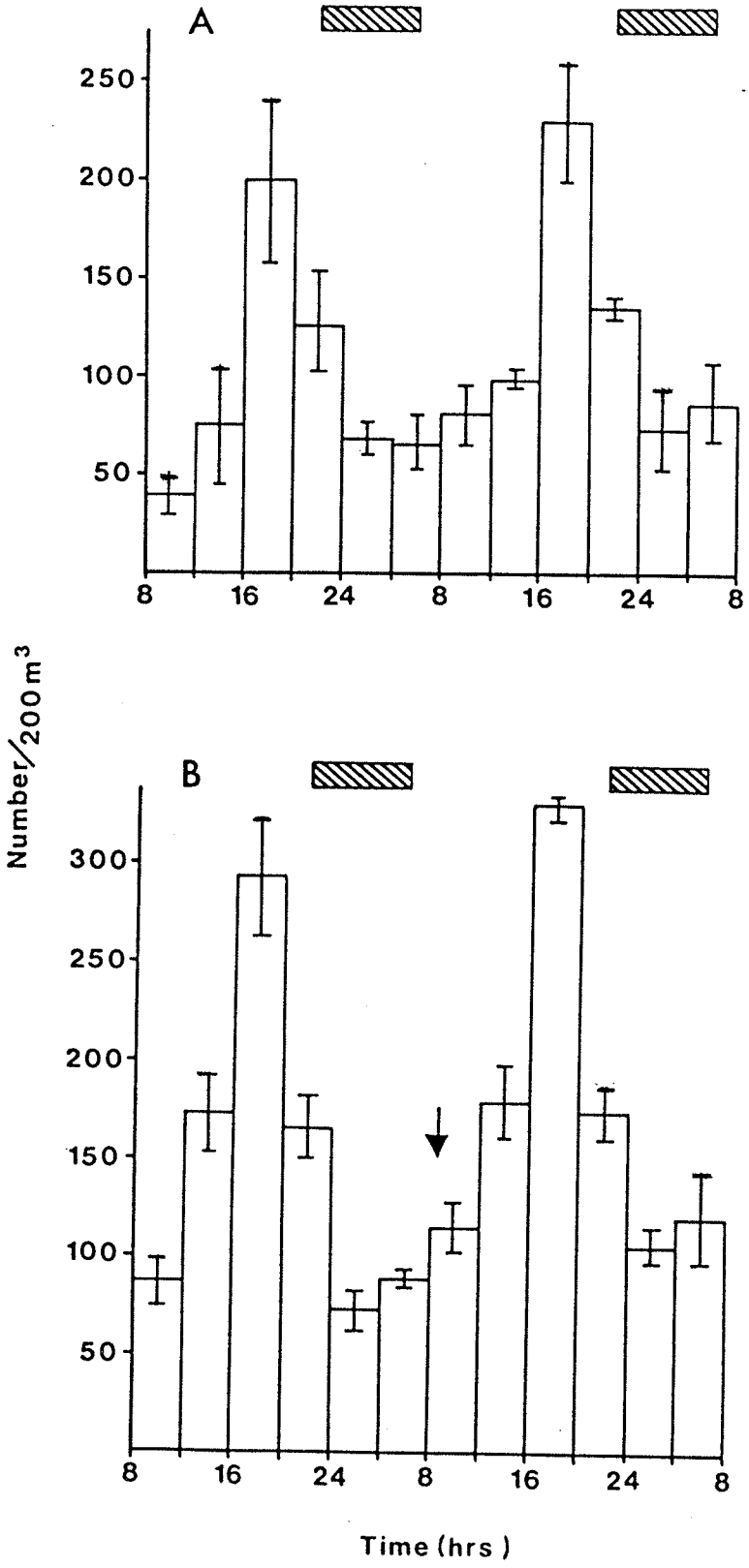

A
250
200
150
100
50
8
16
24
8
16
24
8
B
Number/200 m³
300
250
200
150
100
50
8
16
24
8
16
24
8
Time (hrs)

Fig. 10. Mean (±s.e.) drift density of Acari during the August 1982 BTI trial, in the Torch River, Saskatchewan. Legend as in Fig. 3 and 5.

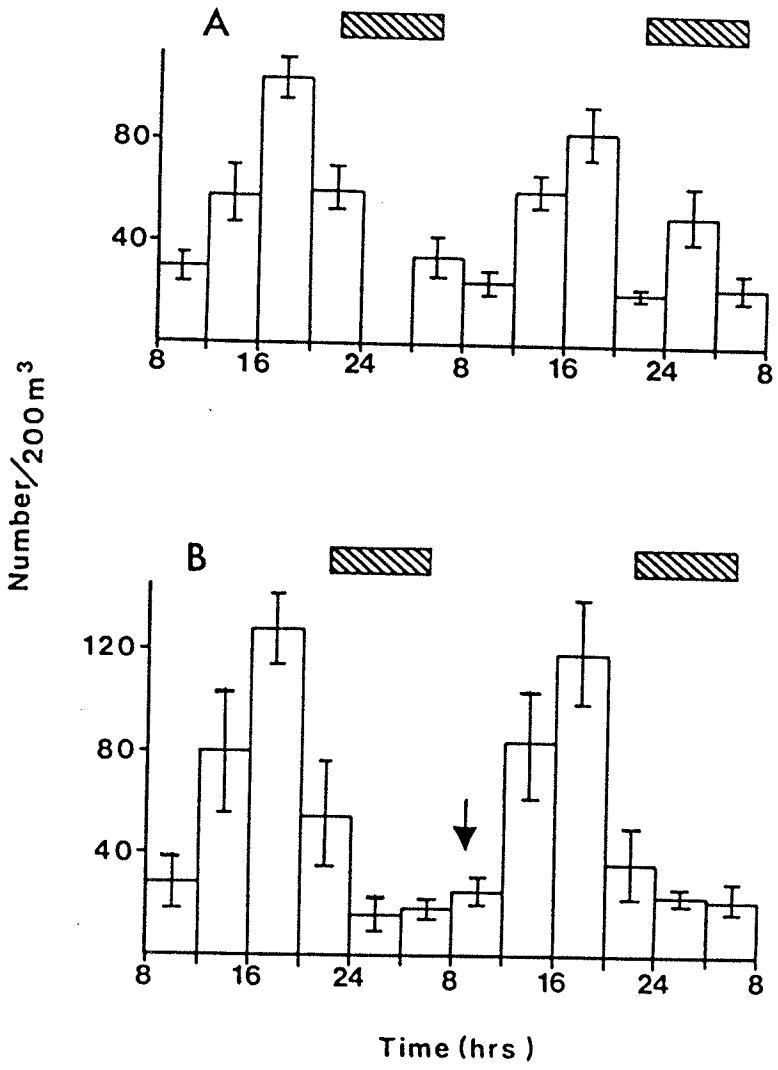

A
80
40
8
16
24
8
16
24
8
B
120
80
40
8
16
24
8
16
24
8
Number/200 m³
Time (hrs)

Fig. 11. Mean (±s.e.) drift density of *Simulium* spp. larvae during the June 1982 BTI trial, in the Torch River, Saskatchewan.

Legend as in Fig. 3.

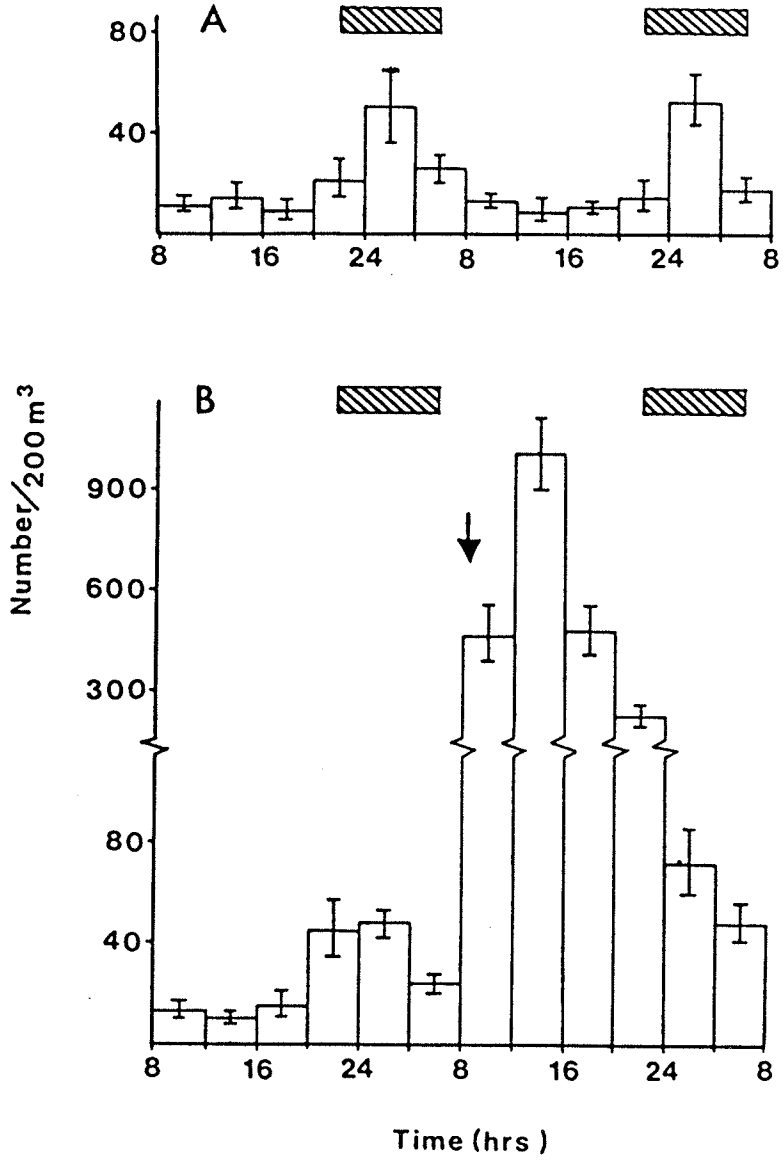
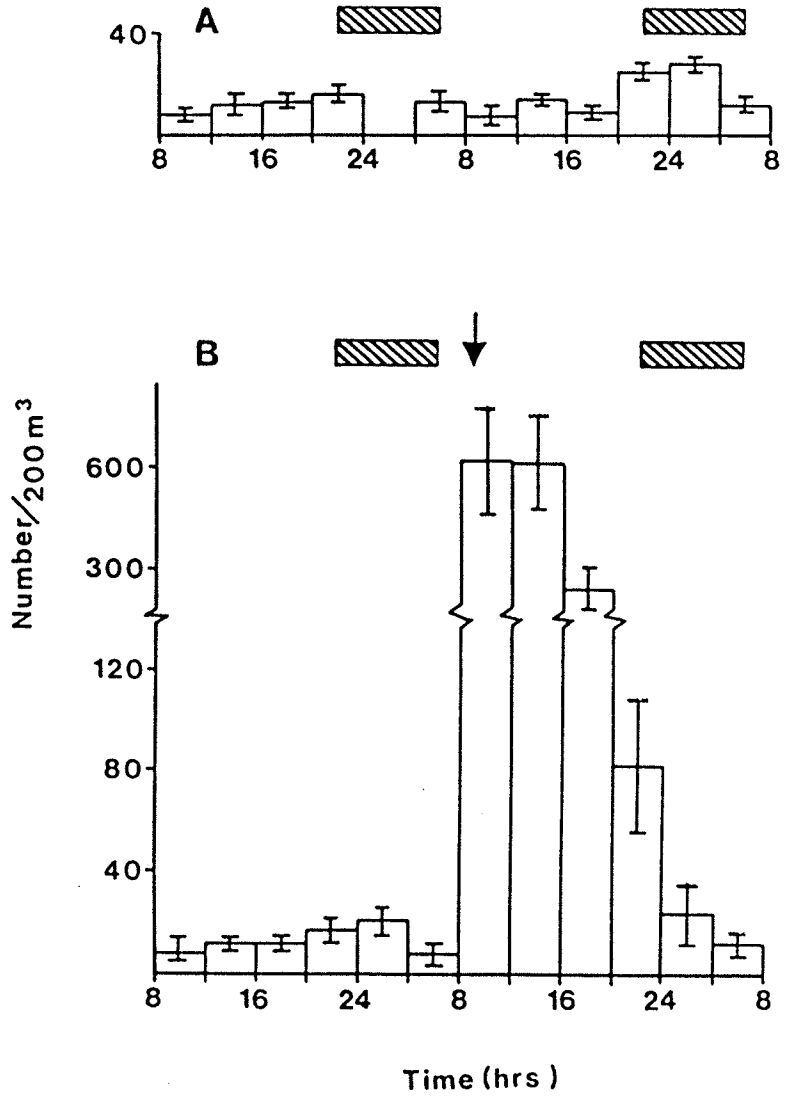

A
80
40
8
16
24
8
16
24
8
B
Number/200 m³
900
600
300
80
40
8
16
24
8
16
24
8
Time (hrs)

Fig. 12. Mean (±s.e.) drift density of *Simulium* spp. larvae during the August 1982 BTI trial, in the Torch River, Saskatchewan.

Legend as in Fig. 3 and 5.


A
40
8
16
24
8
16
24
8
B
Number/200m³
600
300
120
80
40
8
16
24
8
16
24
8
Time (hrs)

3.5.4 Benthic Invertebrates

During the June 1982 trial benthic samples contained 46 genera of aquatic invertebrates (Table 5)(Appendix D). Four taxa comprised 91% of the total benthic fauna. Chironomid larvae comprised 67% of the benthic fauna, Baetis spp. larvae 9%, Hydroptila spp. larvae 9%, and ephemerellid larvae 6%. Of the benthic fauna collected in June 94% were scrapers, 3% filter-feeders, 2% predators and 1% shredders.

A significant decrease in benthic density of Baetis spp. larvae was observed at the 800m downstream site 24 hours following the June application of BTI (Table 5).

During the Auqust 1982 trial a similar number of genera, but different composition of aquatic invertebrates was observed in benthic samples (Table 6)(Appendix D). Eight taxa comprised 90% of the total benthic fauna. Chironomid larvae comprised 36% of the benthic fauna, Tricorythodes minutus larvae 18%, Hydropysche spp. larvae 11%, Baetis spp. larvae 9%, Psychomyia flavida (Hagen) larvae and pupae 8%, other Ephemeroptera 3.1%, Chimarra nr. socia Hagen larvae 2.5% and Protoptila sp. larvae 2.4% (Table 6). Of the benthic fauna collected in August 82% were scrapers, 15% filter-feeders, 2% predators and 1% shredders.

Table 5. Mean benthic density (number/500cm^2) of aquatic invertebrates before (June 17) and after (June 19) the June 1982 application of BTI to the Torch River.

[ ] - number of genera.

*** - significant at the .001 confidence level.

( ) - standard error.

Taxon	Upstream (Control)			200 m Downstream			800 m Downstream		
	June 17	June 19	F (ANOVA)	June 17	June 19	F (ANOVA)	June 17	June 19	F (ANOVA)
Class Arachnida									
Order Acari									
Fam Hygrobatidae									
Hygrobates neocalliger	2.57(0.80)	0.91(0.36)	NS	2.45(1.04)	1.91(0.73)	NS	1.92(1.22)	1.32(0.72)	NS
Class Insecta									
Order Ephemeroptera									
Fam Baetidae									
Baetis spp.	19.58(3.52)	10.28(2.71)	NS	9.76(2.58)	12.19(2.27)	NS	20.19(2.52)	7.99(1.70)	***
Fam Ephemerellidae [2]	11.26(5.55)	6.24(1.15)	NS	9.91(1.72)	8.76(1.56)	NS	5.46(1.98)	6.23(2.63)	NS
Other Ephemeroptera [5]	1.18(0.58)	0.47(0.31)	NS	0.74(0.43)	0.82(0.41)	NS	1.80(1.25)	0.43(0.22)	NS
Order Odonata									
Fam Gomphidae									
Ophiogomphus colubrinus	0.36(0.25)	0.33(0.25)	NS	0.56(0.24)	0.66(0.39)	NS	0.71(0.24)	-	NS
Order Plecoptera									
Fam Pteronarcyidae									
Pteronarcys dorsata	2.21(1.28)	0.46(0.35)	NS	0.72(0.26)	0.26(0.18)	NS	0.14(0.14)	1.00(0.80)	NS
Fam Perlidae									
Acroneuria lycorias	0.17(0.17)	0.48(0.35)	NS	0.36(0.24)	0.12(0.12)	NS	-	-	NS

Continued .....

Taxon	Upstream (Control)			200 m Downstream			800 m Downstream		
	June 17	June 19	F (ANOVA)	June 17	June 19	F (ANOVA)	June 17	June 19	F (ANOVA)
Fam Chloroperlidae									
Hastaperla brevis	0.44(0.30)	-	NS	-	-	NS	0.15(0.15)	-	NS
Order Trichoptera									
Fam Philopotamidae									
Chimarra nr. *socia*	0.18(0.18)	-	NS	0.19(0.19)	-	NS	0.17(0.17)	0.23(0.23)	NS
Fam Psychomyiidae									
Psychomyia flavida	3.00(1.51)	0.98(0.36)	NS	1.77(1.03)	0.94(0.55)	NS	0.72(0.50)	0.29(0.20)	NS
Fam Hydropsychidae									
Cheumatopsyche sp.	2.54(0.70)	1.74(0.67)	NS	3.33(1.25)	2.18(0.87)	NS	4.54(1.34)	4.44(1.59)	NS
Hydropsyche spp.	0.70(0.70)	0.16(0.16)	NS	1.98(0.83)	1.25(0.72)	NS	2.22(1.24)	1.39(0.66)	NS
Fam Glossosomatidae									
Protoptila sp.	0.19(0.19)	0.33(0.22)	NS	3.58(1.21)	2.60(0.85)	NS	0.30(0.30)	0.92(0.43)	NS
Fam Hydroptilidae									
Hydroptila consimilis	23.22(4.14)	16.77(4.33)	NS	12.64(2.63)	8.50(1.62)	NS	5.13(1.24)	5.22(1.26)	NS
H. spatulata	1.75(0.89)	0.53(0.40)	NS	1.82(0.36)	1.47(0.54)	NS	1.47(0.54)	1.07(0.33)	NS
Other Trichoptera [4]	0.49(0.34)	0.51(0.27)	NS	1.02(0.38)	0.91(0.46)	NS	0.37(0.19)	0.28(0.19)	NS

Continued .....

Taxon	Upstream (Control)			200 m Downstream			800 m Downstream		
	June 17	June 19	F (ANOVA)	June 17	June 19	F (ANOVA)	June 17	June 19	F (ANOVA)
Order Diptera									
Fam Tipulidae									
Antocha sp.	1.99(0.72)	0.83(0.40)	NS	1.69(0.47)	1.64(0.87)	NS	0.63(0.33)	0.78(0.39)	NS
Fam Simuliidae									
Simulium spp.	0.22(0.22)	0.14(0.14)	NS	0.14(0.14)	0.32(0.32)	NS	-	-	NS
Fam Chironomidae [>20]	156.98(30.09)	76.19(13.75)	NS	60.89(11.53)	53.03(8.54)	NS	155.88(38.18)	79.06(23.06)	NS
Fam Athericidae									
Atherix variegata	1.06(0.49)	0.86(0.39)	NS	-	0.12(0.12)	NS	-	0.16(0.16)	NS

Table 6. Mean benthic density (number/500cm^2) of aquatic invertebrates before (Aug. 4) and after (Aug. 8) the August 1982 application of BTI to the Torch River.

[ ] - number of genera.

*** - significant at the .001 confidence level

( ) - standard error.

Taxon	Upstream (Control)			200 m Downstream			800 m Downstream		
	Aug. 4	Aug. 8	F (ANOVA)	Aug. 4	Aug. 8	F (ANOVA)	Aug. 4	Aug. 8	F (ANOVA)
Class Insecta									
Order Ephemeroptera									
Fam Baetidae									
Baetis spp.	11.10(3.20)	6.94(1.37)	NS	6.97(1.47)	5.54(1.93)	NS	6.64(2.34)	6.71(1.96)	NS
Fam Tricorythidae									
Tricorythodes minutus	8.53(2.13)	9.27(2.12)	NS	37.31(10.43)	16.32(3.68)	NS	11.57(1.58)	4.32(1.43)	***
Other Ephemeroptera [5]	3.46(0.89)	2.57(0.59)	NS	3.13(0.77)	2.88(0.89)	NS	1.03(0.38)	1.95(0.88)	NS
Order Odonata									
Fam Gomphidae									
Ophiogomphus colubrinus	0.42(0.29)	0.46(0.32)	NS	1.26(0.41)	1.04(0.47)	NS	0.50(0.26)	0.72(0.39)	NS
Order Plecoptera									
Fam Pteronarcyidae									
Pteronarcys dorsata	0.19(0.19)	0.99(0.61)	NS	0.91(0.62)	-	NS	0.16(0.16)	0.22(0.22)	NS
Fam Perlidae									
Acroneuria lycorias	0.39(0.26)	0.24(0.24)	NS	-	0.20(0.20)	NS	-	0.73(0.39)	NS
Fam Perlodidae									
Isoperla transmarina	0.17(0.17)	0.43(0.29)	NS	-	0.21(0.21)	NS	0.17(0.17)	-	NS

Continued.....

Taxon	Upstream (Control)			200 m Downstream			800 m Downstream		
	Aug. 4	Aug. 8	F (ANOVA)	Aug. 4	Aug. 8	F (ANOVA)	Aug. 4	Aug. 8	F (ANOVA)
Order Trichoptera									
Fam Philopotamidae									
Chimarra nr. *socia*	4.30(1.82)	1.09(0.61)	NS	1.36(0.53)	1.33(0.89)	NS	2.25(1.10)	1.74(0.94)	NS
Fam Psychomyiidae									
Psychomyia flavida	6.66(1.83)	5.27(1.09)	NS	4.65(1.23)	7.18(2.29)	NS	5.73(1.53)	11.19(2.66)	NS
Fam Polycentropodidae									
Neureclipsis bimaculata	0.65(0.43)	0.69(0.38)	NS	0.66(0.38)	1.45(0.66)	NS	0.65(0.39)	0.73(0.50)	NS
Fam Hydropsychidae									
Hydropsyche spp.	19.39(13.51)	3.89(0.95)	NS	5.99(2.08)	6.98(3.26)	NS	9.62(3.73)	7.57(2.97)	NS
Fam Glossosomatidae									
Protoptila sp.	0.89(0.38)	2.81(2.81)	NS	1.42(0.67)	3.02(1.27)	NS	1.66(0.68)	1.88(1.19)	NS
Fam Hydroptilidae									
Hydroptila spp.	1.59(0.48)	0.16(0.16)	NS	0.32(0.32)	1.55(0.92)	NS	0.18(0.18)	0.16(0.16)	NS
Mayatrichia ayama	0.84(0.45)	0.67(0.34)	NS	1.11(0.61)	1.01(0.60)	NS	0.49(0.49)	1.12(0.68)	NS
Other Trichoptera [4]	-	1.08(0.37)	NS	1.06(0.64)	1.51(0.67)	NS	0.29(0.20)	0.84(0.61)	NS
Order Diptera									
Fam Tipulidae									
Antocha sp.	1.60(0.51)	1.88(0.66)	NS	0.86(0.38)	1.16(0.63)	NS	1.16(0.67)	1.14(0.59)	NS

Continued .....

Taxon	Upstream (Control)			200 m Downstream			800 m Downstream		
	Aug. 4	Aug. 8	F (ANOVA)	Aug. 4	Aug. 8	F (ANOVA)	Aug. 4	Aug. 8	F (ANOVA)
Fam Simuliidae									
Simulium spp.	4.78(4.42)	-	NS	-	-	NS	0.17(0.17)	-	NS
Fam Chironomidae [>20]	42.11(6.05)	25.97(2.31)	NS	30.12(5.04)	11.29(2.00)	***	38.93(6.49)	25.62(4.11)	NS
Fam Athericidae									
Atherix variegata	1.13(0.64)	0.71(0.47)	NS	-	-	NS	-	0.29(0.29)	NS
Fam Empididae									
Hemerodromia sp.	0.51(0.35)	0.24(0.24)	NS	-	0.18(0.18)	NS	-	-	NS

Following application of BTI a significant decrease in the benthic density of chironomid larvae and Tricorythodes minutus larvae was observed at the 200m and 800m downstream sites, respectively.

No significant numbers of black fly larvae were found in benthic samples before or after application of BTI in either of the trials. Black fly larvae densities were monitored using artificial substrates (Galloway et al. in prep.).

3.5.5 Insect Emergence

Qualitative data on insect emergence during BTI trials was obtained through analysis of drift samples, sweep net samples and emergence traps. During the June 1982 BTI trial adults of Baetis spp., Ephemerella sp., Hastaperla brevis, (Banks) and Ophiogomphus colubrinus Selys were abundant. During the August 1982 BTI trial adults of Tricorythodes minutus, Mayatrichia ayama Mosely, and Cheumatopsyche campyla Ross were abundant.

3.6 DISCUSSION

A large increase in the drift density of black fly larvae was recorded following both BTI treatments. In contrast no significant increase in drift density was observed for over 190 non-target taxa.

There was also a large decrease in the number of live black fly larvae found on artificial substrates up to 11.1 km downstream (Galloway et al. in prep.). However, only small reductions in the benthic densities of Baetis spp. larvae, Tricorythodes minutus larvae and chironomid larvae were observed. Decreases in Baetis spp. larvae and Tricorythodes minutus larvae can be attributed to emergence as large numbers of adults were collected in emergence traps, sweep nets and drift samples during the June 1982 and August 1982 trials, respectively. A decrease in the density of benthic chironomid larvae during the August 1982 trial may have been the result of BTI application. No increase in the drift density of chironomid larvae was observed in the August 1982 trial, suggesting that the decrease in benthic density may have been due to benthic sampling variablity. In August benthic samples were collected 48 hours after drift sampling had ended. A significant increase in the drift density of chironomids may have been observed if drift sampling had been continued for an additional 24 hour period following BTI application. Changes in the drift and benthic densities of chironomid larvae may have been significant at the subfamily or generic levels. There was an increase in the drift density of Tvetenia and Eukiefferiella larvae after the June 1982 BTI trial.

In this study the use of both spatial and temporal controls as well as replicate sampling at each site allowed for

the establishment of the naturally occurring variability of both the benthos and drift. Thus changes in both the benthic and drift densities following BTI application could be compared for significance against their established natural occurring densities. Many of the previous studies on the impact of BTI on non-target organisms in black fly breeding habitats have failed to use both spatial and temporal controls and/or have used inadequate sample replication.

Dejoux (1979), Yameogo (1980) and Pistrang and Burger (1984) used invertebrate drift to test the effect of BTI on the fauna of small streams. All three studies recorded an increase in the drift of black fly larvae following BTI application.

Dejoux (1979) observed little change in the drift rate of non-target organisms, with only chironomid larvae showing any significant increase.

Pistrang and Burger (1984) recorded increases in the drift rate of Epeorus fragilis (Morgan), Baetis brunneicolor McDunnough, Parapsyche apicalis (Banks) and Pycnopysche divergens (Walker) following application of BTI. However, no spatial control or replication of drift samplers was established and therefore the statistical significance of these increases cannot be determined. Furthermore, adults of Baetis brunneicolor were emerging during the study and this may account for the apparent increased drift of this species.

Colbo and Undeen (1980), Molloy and Jamnback (1981), and Chilcott et al. (1983) all studied the effects of BTI on the benthic fauna of streams. All three studies reported no significant adverse effect of BTI on the non-target fauna, while significant mortality of black fly larvae was observed.

Colbo and Undeen (1980) recorded a decrease in the benthic density of larvae of Hydropysche sp. and Baetis sp. following a river treatment with BTI. Decrease in the population density of Hydropysche sp. larvae was attributed to pupation. Decrease in the population density of Baetis sp. larvae may have been due to sampling error as a similar decrease did not occur in subsequent trials.

Molloy and Jamnback (1981) recorded an increase in the benthic density of mayflies, caddisflies, stoneflies, chironomids, and elmids five days after treatment with BTI. However, as a result of the small sample size used, these increases may be more a reflection of sample variability then actual population increases.

Chilcott et al. (1983) reported no change in the population levels of mayflies, caddisflies, stoneflies, beetles, dixids, crane flies, chironomids or snails following various BTI trials in New Zealand. However, a very small sample size was used and no statistical analysis of non-target results was presented.

Future work on the impact of BTI on non-target organisms in streams should closely follow the protocol established by Undeen and Lacey (1982). Further work is needed on the impact of BTI on stream dwelling chironomids, especially at the species level.

Comparison of the results of this study with those of studies using chemical insecticides (Wallace et al. 1973; Helson and West 1978; Flannagan et al. 1979; Haufe et al. 1980a; Yasuno et al. 1981,1982a,b; Dejoux et Troubat 1982) demonstrates that BTI has significantly less effect on non-target organisms and is an environmentally safer black fly control agent. The use of BTI has less of an impact on other trophic levels. Therefore, we can expect less disruption of the predator-prey interactions within the lotic community.

Chapter IV

GENERAL DISCUSSION

BTI is an unique serotype of Bacillus thuringiensis. The morphological and physiological uniqueness of the delta-endotoxin has given BTI a high toxicity to nematoceran Diptera. This toxicity is increased in filter feeding Diptera, such as mosquitos, black flies and some dixids and chironomids, because they readily feed on bacteria-sized particles suspended in the water column.

The recent registration of BTI by the United States and Canada for mosquito control has resulted in the extensive use of BTI. Effective control of many mosquito species has been obtained both in the laboratory and in the field. Use of BTI in conjunction with chemical insecticides and other biological control agents could greatly decrease the use of chemical insecticides for mosquito control. Formulation research has a major role to play in extending the residual activity and increasing the efficacy of BTI in order to maximize its potential as a mosquito larvicide.

BTI is currently the only practical biological control agent available for black fly control. At present BTI is only used extensively as a black fly control agent by the

World Health Organization's Onchocerciasis Control Programme in West Africa. The target species Simulium damnosum s.l. has become resistant to most currently used chemical black fly larvicides. Simulium damnosum is highly susceptible to BTI and breeds in large rivers which ensure good carry, allowing for the effective use of BTI. Formulations are needed which remain suspended in the water column over longer distances in flowing systems in order for BTI to compete with present chemical black fly larvicides. A potentially large market exists for BTI in Central America for control of onchocerciasis and in North America for control of agricultural pest and nuisance black fly species.

An examination of the literature shows that the results of previous studies cannot be closely compared because of the dissimilar procedures used both in laboratory and field evaluations. Standardization of these methods to facilitate data comparison would be advantageous. Problems especially arise in comparing the use of BTI products in which the potency is unknown, not reported or inaccurately reported. Before any BTI product is used it should be standardized against the currently available reference preparations (IPS-78) in the laboratory, preferably against the target species to be tested in the field. In addition, standardized field procedures, which have been developed for the evaluation of BTI in black fly breeding habitats (Undeen and Lacey 1982), should be followed to enhance comparison of research studies.

Black flies and mosquitos are two of the most important vectors of medical and veterinary disease in the world. In addition, black flies and mosquitos are two of the most economically important pests of both humans and livestock in the world. The high susceptiblity of many black fly and mosquito species to BTI has resulted in a renewed interest in their biological control. Certainly BTI is far safer than any chemical insecticide currently used for black fly or mosquito control. This is expected since strains of Bacillus thuringiensis have been used for 20 years against agricultural and forest pests without evidence of toxic effects to man or the environment (Forsberg et al. 1976). The use of BTI provides a way to decrease the use of potentially environmentally hazardous chemical insecticides in aquatic systems for black fly and mosquito control.

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Appendix A

DRIFT SAMPLING

Drift is defined as the downstream movement of organisms in a river current (Waters 1965, 1972). Macroinvertebrate drift has been divided into three types. Catastrophic drift occurs in response to a physical or chemical change to the environment. This change may take the form of a flood, addition of a pollutant or pesticide or disturbances caused by terrestrial animals. Behavioural drift occurs at a consistent period of the day or night presumably as a behavioural response to such factors as predation, feeding preferences, crowding, pre-emergence activities or enviromental cues. Behavioural drift usually exhibits a 24 hour cycling (diel periodicity). Constant drift refers to the low numbers of organisms continuously represented in the drift and has no continuous diel periodicity.

In this study a modified Burton and Flannagan (1976) bomb drift sampler (Fig. 13) was used to measure possible changes in drift following treatment of the Torch River, Saskatchewan with BTI for black fly control. Modifications involved slight dimensional changes and removal of stabilizing fins. This sampler is a much more efficient drift sampler when compared to conventional conical drift net samplers (Burton and Flannagan 1976; Flannagan et al. 1979).

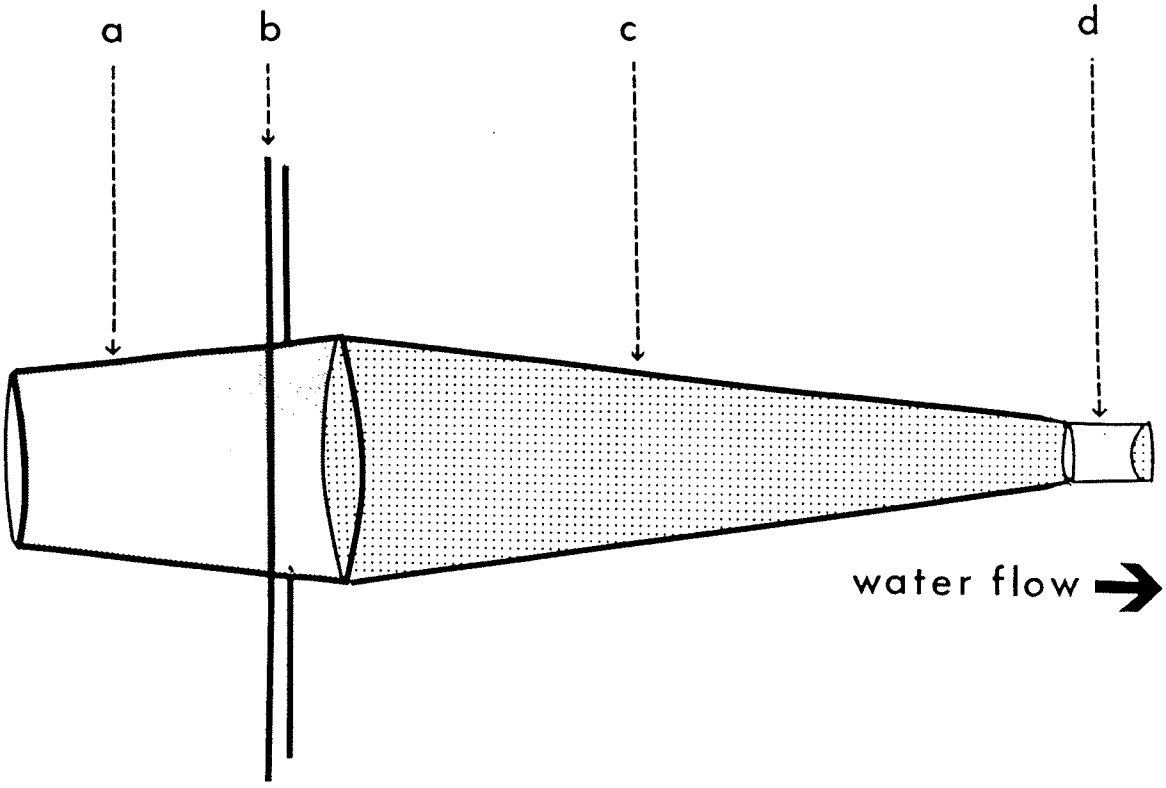
Fig. 13. Modified Burton and Flannagan (1976) bomb drift sampler.

a - steel cone (15cm diameter upstream aperture)

b - iron support rods

c - 500 µm nylon mesh netting

d - removable plastic jar


a
b
c
d
water flow

Numerous studies have shown catastrophic drift of macroinvertebrates following the application of pesticides to river systems (Hatfield 1969; Wallace et al. 1973; Wallace and Hynes 1975; Flannagan et al. 1979). The resulting catastrophic drift varies from low total numbers (Wallace et al. 1973) to hundreds of thousands of specimens (Flannagan et al. 1980) depending on the toxicant applied and on the chemical, biotic and hydrologic characteristics of the river treated. The fate of macroinvertebrates in the drift remains uncertain. It has been suggested that drifting macroinvertbrates may recover from sublethal concentratons of a toxicant and recolonize downstream (Fredeen 1975; Haufe et al. 1980a,b). Wallace et al. (1973) showed a low survival rate for drifting macroinvertebrates following river treatment with methoxychlor, Dursban and Abate . Those macroinvertebrates that do survive treament may be considered "ecologically dead" due to their displacement downstream (Muirhead-Thompson 1973; Flannagan et al. 1980).

The measure of catastrophic drift constitutes a valuable parameter of the degree of stress caused to the benthic commmunity in toxicant treated rivers. Drift sampling gives us a rapid view of the more susceptible organisms as species that are physiologically susceptible will be more likely to be contaminated, killed and swept into the drift.

Appendix B

IMHOFF CONE SUBSAMPLER

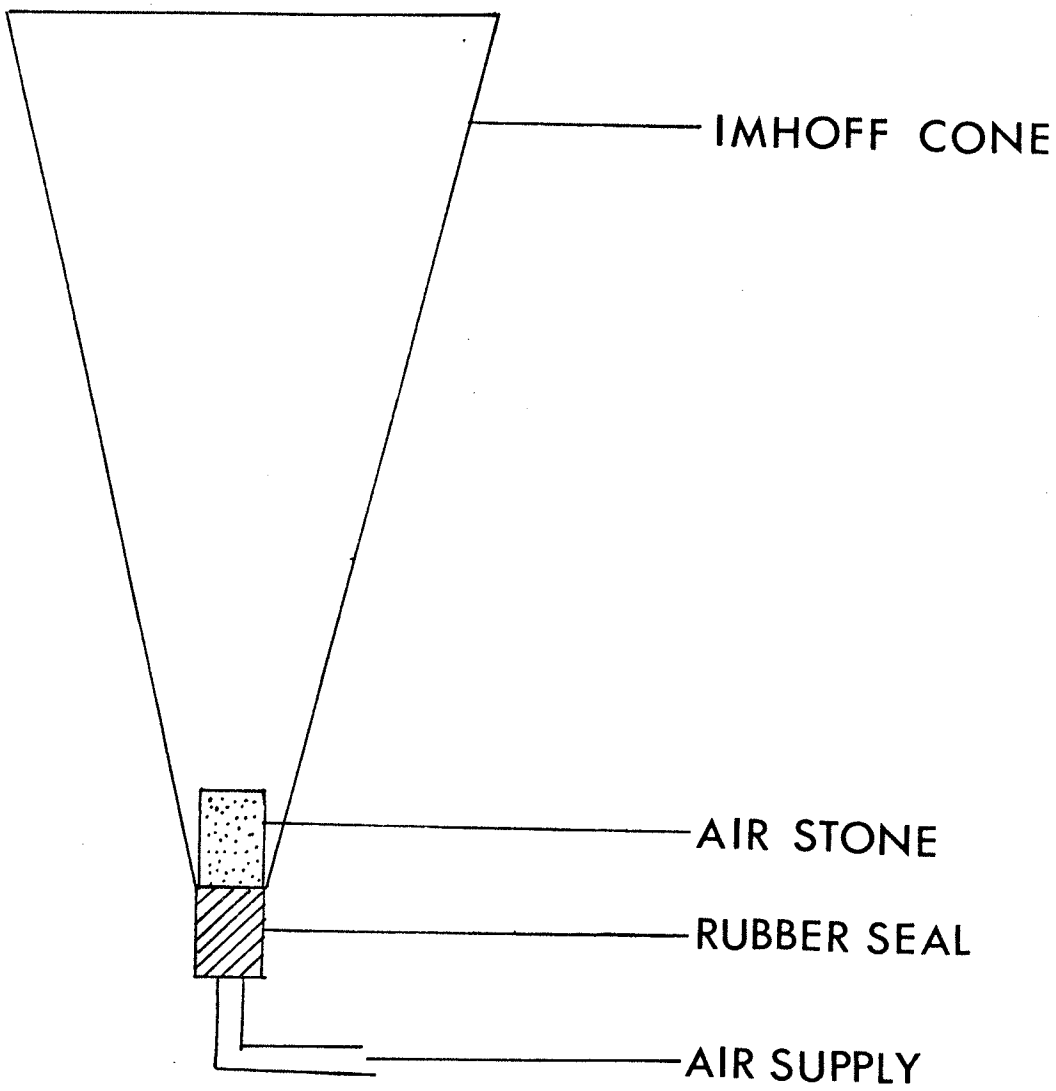
B.1 INTRODUCTION

The large amount of labour required to process lotic and lentic macroinvertebrate samples has stimulated the development of numerous subsampling techniques (Waters 1969). Optimally, subsampling should be performed with a minimum of effort while yielding the maximum amount of information. In addition the subsampler should be simple to operate, inexpensive and most importantly yield statistically reliable results. Wrona et al. (1982) described a simple, volumetric subsampler that fits these criteria.

B.2 MATERIALS AND METHODS

The subsampler consists of a glass Imhoff settling cone with an aquarium air stone, connected to a compressed air supply, sealed into its base (Fig. 14). Subsampling was accomplished by placing the sample into the cone and filling the apparatus to a total volume of 1.6 litres. Large masses of filamentous algae were cut into smaller pieces to enhance suspension and mixing. Following agitation for 2-3 min to ensure thorough mixing, ten 63 ml subsamples were removed.

Fig. 14. Imhoff cone subsampler used to subsample stream drift samples.


IMHOFF CONE
AIR STONE
RUBBER SEAL
AIR SUPPLY

The performance of the subsampler was tested using stream drift samples. To estimate the total number of organisms in each sample from subsample counts it is necessary to establish that the organisms are randomly distributed in the subsampler. This was accomplished by calculating the Index of Dispersion based on the variance to mean ratio of the subsample counts and tested for conformity to a Poisson series. The Index of Dispersion approximates a Chi-squared distribution giving the following relationship.

$$I=X^2=S^2(n-1)/\ x \quad \text{when } n<31$$

where I=Index of Dispersion, X^2 = Chi-squared statistic, n=number of subsampling units taken, S^2 = sample variance, x= sample mean with n-1 degrees of freedom (Elliott 1977).

Agreement to a Poisson series was checked by examining whether the calculated X^2 value occurred between the 0.05 significance levels of the theortical percentage points of the X^2 distribution (Elliott 1977). Precision of the estimate (+or- 95% confidence limits) of the total number of individuals per taxon in the original sample was determined using the relationship

$$D= t/\ n$$

where D= index of precision, t= appropriate value from the Student's t-distribution for a defined probability level (t=2.262 for n=10 at the .05 confidence level) and n=total number of individuals counted for a given taxon (Elliott 1977).

In seven drift samples the material remaining in the subsampler following removal of the 10 subsamples was sorted and identified in order to obtain the actual number of each taxon in the original sample. This allowed for the calculation of the exact % error of the subsampler method using the relationship.

$$\%error = (CT-RT)/RT \times 100$$

where CT= the estimated number of individuals of a given taxa from subsamples and RT= the actual number of individuals of a given taxon.

B.3 RESULTS AND DISCUSSION

Random mixing of organisms was consistently achieved with the subsampler (Table 7)(I values between 2.5 and 18.1: Table 8). As the subsample counts conformed to a Poisson series, the Poisson distribution and its related statistics were used as a suitable model for estimating total numbers and associated 95% confidence limits of each taxon in the samples.

Table 7. Numbers and percentages of Imhoff cone subsamples showing a random or contagious distribution of taxa.

	June			August		
Taxa	Random	Contagious	%	Random	Contagious	%
Class Crustacea						
Subclass						
Ostracoda						
Order Podocopa	86	4	.95	85	0	1.00
Class Arachnida						
Order Acari	85	5	.94	91	1	.99
Class Insecta						
Order Ephemeroptera						
Fam Baetidae						
Baetis spp.	89	1	.99	88	4	.96
Fam Tricorythidae						
Tricorythodes minutus	-	-	-	90	2	.98
Fam Ephemerellidae	89	1	.99	-	-	-
Order Diptera						
Fam Chironomidae	85	5	.94	89	3	.97
Fam Simuliidae						
Simulium spp.	87	3	.97	90	2	.98

Table 8. Precision of the Imhoff cone subsamples for estimating total number of a taxon in drift samples.

I - Index of dispersion

D - Index of precision

CT - the estimated number of individuals of a given taxon from subsamples

RT - the actual number of individuals of a given taxon

Taxon	Sample no.	Σx	I	% D	CT	RT	% error
Class Arachnida							
Order Acari	1	13	6.30	63	33.8	34	1
	2	21	16.60	49	54.6	38	44
	3	28	13.40	43	72.8	48	52
	4	11	8.10	68	28.6	36	21
	5	19	13.10	52	49.4	51	3
	6	48	14.70	33	124.8	120	4
	7	32	9.25	40	83.2	78	6
Class Insecta							
Order Ephemeroptera							
Fam Baetidae							
Baetis spp.	1	34	5.41	39	88.4	94	6
	2	46	10.08	33	119.6	117	2
	3	230	12.10	15	598.0	609	2
	4	47	14.10	33	122.2	132	7
	5	45	12.60	34	117.0	115	2
	6	52	7.20	31	135.2	148	9
	7	40	13.50	36	104.0	76	37
Fam Ephemerellidae	1	4	11.00	113	10.4	5	108
	2	7	11.60	86	18.2	14	30
	3	20	6.80	51	52.0	79	34
	4	6	4.00	92	15.6	17	8
	5	7	14.50	86	18.2	17	7
	6	10	4.00	72	26.0	23	13
	7	9	23.20	75	23.4	17	38

Continued .....

Taxon	Sample no.	Σx	I	% D	CT	RT	% error
Order Diptera							
Fam Chironomidae	1	12	14.70	65	31.2	36	13
	2	22	5.30	48	57.2	47	22
	3	32	15.10	40	83.2	72	16
	4	21	3.30	49	54.6	49	11
	5	11	11.80	68	28.6	30	5
	6	38	4.60	37	98.8	89	11
	7	37	7.60	37	96.2	77	25
Fam Simuliidae							
Simulium spp	1	9	3.30	75	23.4	18	30
	2	6	10.70	92	15.6	13	20
	3	17	13.10	55	44.2	52	15
	4	4	11.00	113	10.4	8	30
	5	4	11.00	113	10.4	8	30
	6	8	4.50	80	20.8	14	49
	7	3	13.67	131	7.8	8	3
Total Taxa	1	93	8.40	24	241.8	255	5
	2	242	4.52	15	629.2	610	3
	3	387	14.20	12	1006.2	1017	1
	4	156	6.80	18	405.6	435	7
	5	211	5.10	16	548.6	537	2
	6	344	6.90	12	894.4	876	2
	7	272	9.25	14	707.2	592	20

Once randomness is demonstrated as few as one subsampling unit is required for estimating the density and confidence limits of the organisms in the total sample. When the distribution of the organisms in a subsampler has been shown to follow a Poisson series, the degree of precision of the estimated total count for a given taxon is dependent upon the total number of individuals counted , rather then the number of subsampling units taken (Elliott 1977).

Hickley (1975) and Elliott (1977) have advocated that a sufficient number of subsamples be used to ensure a count of at least 100 individuals in order to receive +or- 20% precision at 95% confidence limits. Although, it would be desirable to achieve an abundance estimate within +or- 20% of the true count, this rule is restricted to samples in which a taxon is abundant and precludes the use of subsampling to samples where abundance estimates of both common and rare taxa are required.

Lund et al. (1958) have indicated that most ecological studies are concerned with changes in population abundances of greater than 100%, in which case an estimate of abundance to an accuracy of +or- 50% (a total count of only 16 at 95% confidence limits) would be sufficient to observe any trends.

Considering the above ideas and the time required to sort and identify each subsample, I determined that at most

ten subsamples from each sample could be analyzed. Abundant taxa were only counted in those subsample units required to achieve the desired degree of precision (>100 individuals counted) and ignored in subsequent subsamples. Quantification of the rarer taxa was based upon processing of all ten subsamples.

By applying this procedure, general trends between samples (Table 8) were ascertained despite some taxa displaying large error terms as a result of low counts.

Appendix C

BENTHIC SAMPLING IN LOTIC WATERS

Often a comparison of results from various investigations of benthos is not possible because of the wide range of procedures employed. The number of benthic samplers is nearly proportional to the number of investigations. These samplers have been grouped into five basic types (Macan 1958; Cummins 1962).

1. Hand Collection- This procedure involves the lifting of individual stones and removal of organisms. Usually a fine mesh net is held downstream when each stone is removed in order to capture any organisms that may be removed by the movement of the stone. In order for this method to yield quantitative data some accurate measurement of rock surface area must be made.

2. Artificial Substrates- Are devices placed in an ecosystem to study colonization by indigenous organisms (Cairns 1982). In aquatic ecosystems artificial substrates are usually placed in a stream and removed after a known period of time. Again some method must be used to ensure that no organisms are lost when substrates are removed. If artificial subrates are all the same no measurement of surface area is neccesary to obtain quantitative data.

3. Boxes and Cylinders- This procedure involves the enclosure of a given area by a box or cylinder and the subsequent removal of the animals contained within. The chief consideration when using these type of samplers is that they should fit closely to the substratum preventing the loss of organisms under the sampler.

4. Fixed Nets- A known area upstream is disturbed and the organisms dislodged from it are washed into a net. This category includes the Surber type square-foot sampler (Moffett 1936; Surber 1937) which is the most widely used sampling device for investigations of stream benthos.
The Surber sampler consists of two square frames which fold together for carrying and open out at right angles for use. In operation, one frame lies flat on the substratum and serves to mark out the area to be sampled while the other stands vertically downstream and supports a net into which the current washes specimens dislodged in the marked area. Again as with boxes and cylinders the chief consideration when using these samplers is that they must fit closely to the substatum to prevent loss of animals under the frame.

5. Push or Drag Nets- These samplers take the form of various types of scoops or shovels equipped with nets which retain the animals and large substrate materials. These samplers are inserted into the substrate and pushed upstream for a known distance.

All these samplers have their advantages and disadvantages depending on the parameter being measured and the physical nature of the stream being sampled.

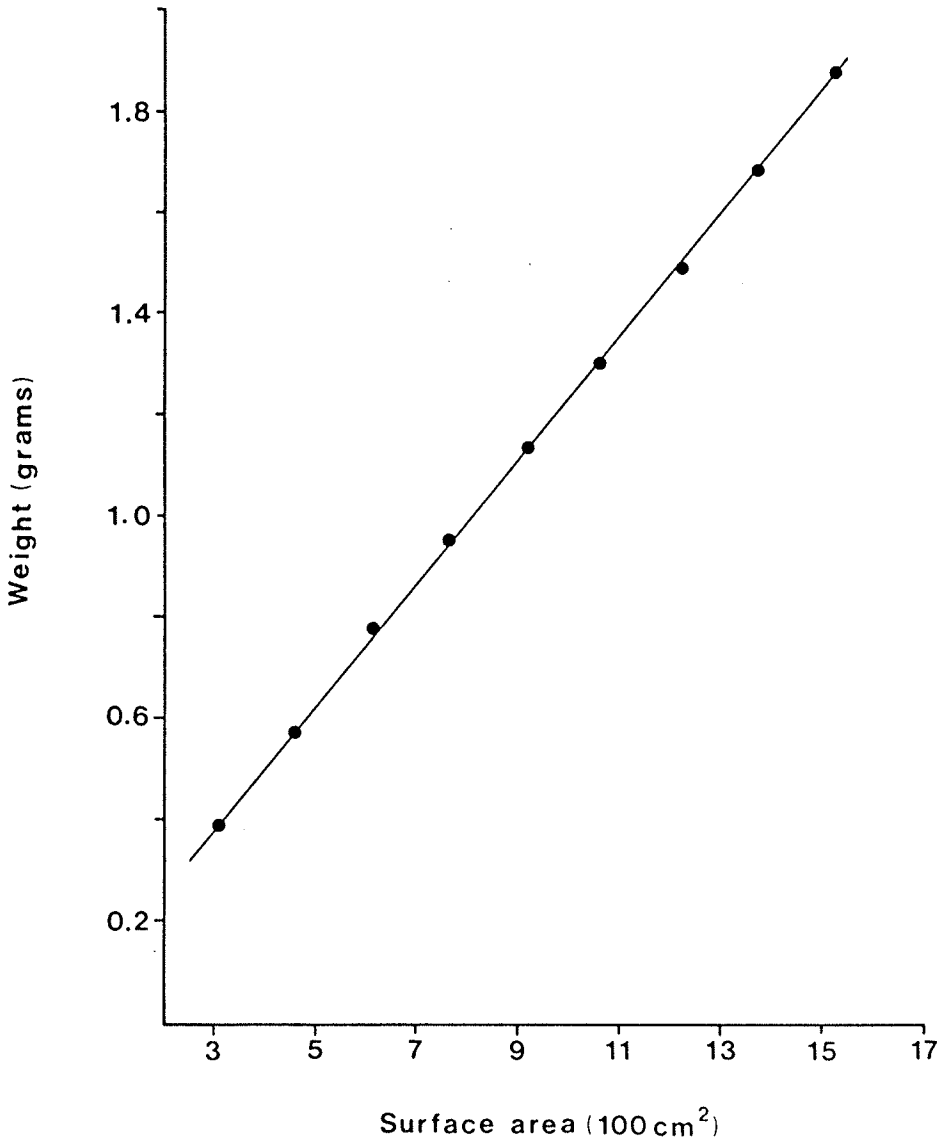
Because of the nature of the substrate in the Torch River push or drag nets, fixed nets and boxes and cylinders could not be used to obtain quantitative samples. In addition, fixed nets and box and cylinder samplers collect large amounts of abiotic material which results in an increased sorting time of each sample in the laboratory. Because of this increase in sorting time fewer samples can be processed and this small sample size can result in a low precision of the parameter being estimated (Elliott 1977; Resh 1979). The use of artificial substrates was not appropriate due to the long period of time required for colonization and stabilization of these substrates. Also artificial substrates are selective for those organisms that will colonize them and may not be a true representation of the naturally occurring benthic population.

Because of these difficulties in using artificial substrates, fixed nets , boxes, cylinders and push and drag nets, hand collection of natural substrates was considered the best method for obtaining benthic samples in the Torch River. Rocks of similar size were removed along a transect (Resh 1975) into a 500um mesh net held downstream. All macroinvertebrates were removed and stored in 95% ethanol.

The surface area of each rock was estimated using a plastic wrap technique described by Doeg and Lake (1981). Handiwrap (Dow Chemical Canada), a self-adhesive, thin film, plastic food wrap was moulded over the rock surface so as to conform closely with its shape, care being taken not to stretch the plastic. Creases were smoothed out and all areas of overlap cut off. Each piece of plastic was weighed. A conversion factor between mass and area was then calculated from the mass of known areas of plastic (Fig. 15). To test the reliability of a single measurement, the suface area of a single rock was evaluated ten times. All values fell into a narrow range with a standard deviation of only 5% of the mean.

Several other methods of measuring surface area have been described such as latex moulds (Calow 1972; Minshall and Minshall 1977), inked squares of known surface area (Kovalak 1978), aluminum foil (Shelley 1979) and mathematical formule's using length, width and height (Dall 1979). These methods have all proved to be unsuitable as either being inaccurate or too time consuming.

Fig. 15. Weight vs. surface area of plastic Handiwrap®.


1.8
1.4
1.0
0.6
0.2
Weight (grams)
3
5
7
9
11
13
15
17
Surface area (100 cm²)

The technique of Doeg and Lake (1981) allowed quantitative use of hand collected natural substrates. Therefore we were able to obtain an accurate estimate of the naturally occurring benthic population. The use of hand collected natural substrates enabled the processing of a larger sample size and enhanced the precision of the estimated benthic population density.

Appendix D

LIST OF INVERTEBRATES FOUND IN THE TORCH RIVER

Table 9 is a list of invertebrates collected using drift samplers, benthic samples and emergence traps from the Torch River during 1981-1982.

The following is a list of people who helped in identification and the specimens they identified. R.W. Davies - Hirudinea; L.D. Delorme - Ostracoda; J.C. Conroy and M.M. Quaglia - Acari; R.E. Roughley - Dytiscidae; I. Askevold - Chrysomelidae; K.W. Simpson - Stempellina montivaga ; D.R. Oliver - Brilla n.sp.; B. Bilyi - Larsia n.sp.

All other specimens were identified by the author. Specimens have been placed in the J.B. Wallis Museum, Department of Entomology, University of Manitoba or with the specialists that aided in identification.

Table 9. List of aquatic invertebrates found in the Torch River, Saskatchewan.

Taxa	Drift	Benthos	Emergence[1]
Phylum Annelida			
Class Hirudinea			
Order Rhynchobdellida			
Fam. Glossiphioniidae			
Placobdella ornata (Verrill)	+	-	-
Order Pharyngobdellidae			
Fam. Erpobdellidae			
Erpobella punctata (Leidy)	+	-	-
Phylum Arthropoda			
Class Crustacea			
Subclass Ostracoda			
Order Podocopa			
Fam. Cypridopsidae			
Cypridopsis vidua (Muller)	+	-	-
Fam. Cypridae			
Cypricercus reticulatus (Zaddach)	+	-	-
Fam. Cyclocyprididae			
Cyclocypris ampla Furtos	+	-	-
C. serena (Koch)	+	-	-
C. sharpel Furtos	+	-	-
Subclass Copepoda			
Order Branchiura			
Fam. Argulidae			
Argulus sp.	+	-	-
Subclass Malacostraca			
Order Amphipoda			
Fam. Talitridae			
Hyalella azteca (Saussure)	+	-	-
Order Decapoda			
Fam. Astacidae			
Orconectes sp.	+	-	-
Class Arachnida			+[2]
Order Acari			
Fam. Eylaidae			
Eylais extendens (Muller)	+	-	-
Fam. Hydrachnidae			
Hydrachna cruenta Muller	+	-	-
Fam. Homocaligidae			
Homocaligus muscorum Habeeb	+	-	-

Continued .....

Taxa	Drift	Benthos	Emergence
Fam. Limnocharidae			
Limnochares (Cyclothrix) americana Grube	+	-	-
Fam. Neoacaridae			
Neoacarus n. sp.	+	-	-
Fam. Hydroplantidae			
Protzia constans (Marshall)	+	-	-
Fam. Hydrodromidae			
Hydrodroma despiciens (Muller)	+	-	-
Fam. Sperchontidae			
Sperchonopsis sp.	+	-	-
Sperchon n. sp.	+	+	-
Fam. Lebertiidae			
Lebertia acadiensis Habeeb	+	-	-
L. distincta Marshall	+	-	-
L. needhami Marshall	+	-	-
L. quinquemaculosa Marshall	+	-	-
L. porosa (Thor)	+	-	-
L. n. sp. 1	+	-	-
L. n. sp. 2	+	-	-
L. n. sp. 3	+	-	-
Fam. Torrenticolidae			
Torrenticola amplexa tenuipalpis Lundblad	+	+	-
T. ellipsoidalis (Marshall)	+	+	-
T. indistincta (Marshall)	+	-	-
T. neoconnexa Habeeb	+	+	-
Fam. Limnesiidae			
Limnesia cornuta Wolcott	+	-	-
Fam. Hygrobatidae			
Atractides grouti Habeeb	+	-	-
A. nodipalpis americana (Marshall)	+	-	-
A. n. sp.	+	+	-
Hygrobates neocalliger Habeeb	+	+	-
H. americanus Habeeb	+	-	-
H. (Mixobates) uncatus Sokolow	+	-	-
Mesobates forcipatus Thor	+	-	-
Fam. Arrenuridae			
Arrenurus (Megaluracarus) aphelocercus Lavers	+	-	-
Fam. Aturidae			
Aturus deceptor	+	+	-
Ljania michiganensis Cook	+	-	-

Continued .....

Taxa	Drift	Benthos	Emergence
Fam. Mideopsidae			
Mideopsis americana Marshall	+	+	-
M. delicata (Habeeb)	+	-	-
M. borealis (Habeeb)	+	+	-
Fam. Oribatidae			
Ceratozetes minimus Sellnick	+	-	-
Hermannia sp.	+	-	-
Hydrozetes petrukevitchi Newell	+	-	-
Galumna sp.	+	-	-
Trimalaconothrus angulatus Willman	+	-	-
Class Insecta			
Order Ephemeroptera			
Fam. Siphlonuridae			
Isonychia sp.	+	+	-
Fam. Baetidae			
Baetis brunneicolor McDunnough	+	+	+
B. flavistigia McDunnough	+	+	+
B. hageni Eaton	+	+	+
B. pygmaeus (Hagen)	+	+	+
B. tricaudatus Dodds	+	+	+
Fam. Heptageniidae			
Heptagenia sp.	+	+	+
Nixe sp.	+	+	-
Stenonema vicarium (Walker)	+	+	+
Fam. Ephemerellidae			
Ephemerella n. sp.	+	+	+
Eurylophella temporalis (McDunnough)	+	+	+
Fam. Tricorythidae			
Tricorythodes minutus Traver	+	+	+
Fam. Caenidae			
Caenis simulans McDunnough	+	+	-
Fam. Baetiscidae			
Baetisca laurentina McDunnough	+	-	-
Fam. Leptophlebiidae			
Paraleptophlebia debilis (Walker)	+	-	-
P. temporalis (McDunnough)	+	-	-
Fam. Ephemeridae			
Ephemera simulans Walker	+	-	+
Fam. Polymitarcyidae			
Ephoron album (Say)	+	-	-
Order Odonata			
Fam. Gomphidae			
Ophiogomphus colubrinus Selys	+	+	+
Fam. Aeshnida			
Aeshna umbrosa umbrosa	+	+	+
Fam. Calopterygidae			
Calopteryx aequabile (Say)	+	+	+

Continued .....

Taxa	Drift	Benthos	Emergence
Order Plecoptera			
Fam. Nemouridae			
Amphinemura linda (Ricker)	-	+	-
Fam. Taeniopterygidae			
Taeniopteryx nivalis (Fitch)	+	-	-
Fam. Pteronarcyidae			
Pteronarcys dorsata (Say)	+	+	+
Fam. Perlidae			
Acroneuria lycorias (Newman)	+	+	+
Fam. Perlodidae			
Isogenoides frontalis (Newman)	+	-	-
Isoperla transmarina (Newman)	+	+	+
Fam. Chloroperlidae			
Hastaperla brevis (Banks)	+	+	+
Order Hemiptera			
Fam. Gerridae			
Gerris spp.	+	-	-
Fam. Corixidae			
Sigara decoratella (Hungerford)	+	-	-
S. lineata (Forster)	+	-	-
S. trilineata (Provancher)	+	-	-
Fam. Notonectidae			
Notonecta spp.	+	-	-
Fam. Saldidae			
Saldula opacula (Zetterstedt)	+	-	-
Order Megaloptera			
Fam. Sialidae			
Sialis velata Ross	+	-	+
Order Trichoptera			
Fam. Philopotamidae			
Chimarra socia Hagen	+	+	+
Fam. Psychomyiidae			
Psychomyia flavida Hagen	+	+	+
Fam. Polycentropodidae			
Neureclipsis bimaculata (Linnaeus)	+	+	-
Nyctiophylax moestus Banks	+	+	-

Continued .....

Taxa	Drift	Benthos	Emergence
Fam. Hydropsychidae			
Cheumatopsyche campyla Ross	+	+	+
Hydropsyche bifida Banks	+	+	+
H. bronta Ross	+	+	+
H. riola Denning	+	+	-
H. slossonae Banks	+	+	+
Hydropsyche spp.	+	+	+
Arctopsyche ladogensis (Kolenati)	-	+	-
Fam. Glossosomatidae			
Glossosoma sp.	+	+	-
Protoptila sp.	+	+	-
Fam. Hydroptilidae			
Hydroptila consimilis	+	+	+
H. spatulata Morton	+	+	+
H. waubesiana Betten	+	-	+
Hydroptila spp.	+	+	+
Neotrichia sp.	+	+	+
Mayatrichia ayama Mosely	+	+	+
Fam. Phryganeidae			
Phryganea cinerea Walker	+	-	+
Ptilostomis ocellifera (Walker)	-	+	-
Fam. Brachycentridae			
Brachycentrus americanus (Banks)	+	+	+
B. numerosus (Say)	+	+	+
Micrasema rusticum (Hagen)	+	+	+
Fam. Lepidostomatidae			
Lepidostoma sp.	+	+	-
Fam. Limnephilidae			
Anabolia sp.	-	+	-
Grammotaulius sp.	-	+	-
Nemotaulius sp.	+	-	-
Pycnopsyche guttifer (Walker)	-	+	-
Fam. Odontoceridae			
Nerophilus sp.	+	-	-
Fam. Helicopsychidae			
Helicopsyche borealis (Hagen)	-	+	-
Fam. Leptoceridae			
Ceraclea sp.	+	-	-
Nectopsyche diarina (Ross)	+	+	+
Oecetis avara (Banks)	+	+	+
O. disjuncta (Banks)	+	+	+
Triaenodes frontalis Banks	+	+	-
Order Lepidoptera			
Fam. Noctuidae			
Simyra sp.	+	-	-

Continued .....

Taxa	Drift	Benthos	Emergence
Order Coleoptera			
Fam. Gyrinidae			
Gyrinus confinis LeConte	+	-	-
Gyrinus spp.	+	-	-
Fam. Haliplidae			
Haliplus spp.	+	-	-
Fam. Dytiscidae			
Colymbetes paykulli Erichson	+	-	-
Colymbetes sp.	+	-	-
Deronectes (*Potamonectes*) *elegans* (Panzer)	+	-	-
Hygrotus sayi Balfour-Browne	+	-	-
Fam. Hydrophilidae			
Laccobius agilis	-	+	-
Fam. Staphylinidae	+	-	
Fam. Hydraenidae			
Hydraena circulata gr	+	+	-
Ochthebius sp.	-	+	-
Fam. Elmidae			
Optioservus fastiditus (LeConte)	+	+	-
Fam. Chrysomelidae			
Donacia hirticollis Kirby	+	-	+
Plateumaris germani (Mann.)	+	-	+
Fam. Curculionidae	+	-	+
Order Hymenoptera	+	-	+
Order Diptera			
Fam. Tipulidae			
Antocha sp.	+	+	+
Dicranota (*Dicranota*) *noveboracensis* Alexander	+	+	+
Erioptera (*Symplecta*) *cana* (Walker)	+	-	+
Fam. Ceratopogonidae			
Palpomyia sp.	+	-	-
Fam. Simuliidae			
Simulium luggeri (Nicholson and Michel)	+	-	+
S. tuberosum (Lundstrom)	+	-	-
S. vittatum Zetterstedt	+	-	-
Fam. Chironomidae			
Subfam. Tanypodinae			
Ablabesymia illinoensis (Malloch)	+	-	
A. mallochi (Walley)	+	+	
A. parajanta Roback	+	-	

Continued .....

Taxa	Drift	Benthos	Emergence
Labrundinia sp.	+	-	
Larsia n. sp.	+	-	
Natarsia sp.	+	-	
Nilotanypus sp.	+	+	
Procladius sp.	+	-	
Thienemannimyia sp.	+	+	
Conchapelopia sp.	+	+	
Subfam. Diamesinae			
Sympotthastia sp.	+	+	
Subfam. Orthocladinae			
Brillia n. sp.	+	+	
Corynoneura celeripes (Winnertz)	+	-	
C. taris Roback	+	-	
C. sp. 5 Oliver *et al.*	+	-	
Cricoptopus bicinctus (Meigen)	+	+	
C. laricomalis gr.	+	+	
C. trifascia gr.	+	+	
Eukiefferiella bavarica gr.	+	+	
E. claripennis gr.	+	+	
Epoicocladius flavens (Malloch)	+	-	
Lopescladius sp.	+	-	
Nanocladius sp.	+	-	
Orthrocladius (*Euorthocladius*) spp.	+	+	
O. (*Eudactylocladius*) spp.	+	+	
O. (*Orthocladius*) spp.	+	+	
O. (*Pogonocladius*) spp.	+	+	
Rheocricotopus sp.	+	+	
Thienemanniella xena	+	-	
Tvetenia discoloripes gr.	+	+	
Synorthocladius sp.	+	+	
Subfam. Chironominae			
Chironomus spp.	+	-	
Cryptochironomus fulvus gr.	+	-	
Endochironomus nigricans (Johannsen)	+	+	
Dicrotendipes neomodestus (Malloch)	+	+	
Microtendipes sp.	+	+	
Paratanytarsus sp.	+	-	
Pseudochironomus sp.	+	+	
Phaenopsectra sp.	+	-	
Polypedilum convictum (Walker)	+	+	
P. fallax gr.	+	+	
P. illinoense (Malloch)	+	+	
Micropsectra sp.	+	+	
Rheotanytarsus sp.	+	+	

Continued .....

Taxa	Drift	Benthos	Emergence
Stempellina nr. *montivaga*	+	+	
Stempellinella sp. 1 Oliver *et al.*	+	-	
Tanytarsus spp.	+	+	
Zavrelia gr.	+	-	
Fam. Dixidae			
Dixa sp.	-	+	-
Fam. Tabanidae			
Chrysops sp.	-	+	+
Fam. Athericidae			
Atherix variegata Walker	+	+	-
Fam. Empididae			
Hemerodromia sp.	+	+	-

[1]Includes invertebrates collected from emergence traps and sweep net samples.

[2]Larval mites attached to macroinvertebrates could not be identified.

[3]Adult Chironomidae were not identified.

Appendix E

LIST OF VERTEBRATES COLLECTED IN THE TORCH RIVER

Table 10 is a list of aquatic vertebrates collected in drift and benthic samples from the Torch River, Saskatchewan during 1981-1982.

All specimens where identified by the author. Reference specimens have been placed in the J.B. Wallis Museum, Department of Entomology, University of Manitoba.

Table 10. List of aquatic vertebrates collected in the Torch River.

Class Osteichthyes

Order Cypriniformes
Fam. Cyprinidae
Rhinichthys atratulus (Hermann)
Rhinichthys cataractae (Valenciennes)

Fam. Catostomidae
Catostomus comersonni (Lacépède)

Order Gasterosteiformes
Fam. Gasterosteidae
Culaea inconstans (Kirtland)