The Impact of Methoxychlor on Selected Non-Target Organisms in a Riffle of the Souris River, Manitoba

by

Robert John Sebastien

A thesis
presented to the University of Manitoba
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THE IMPACT OF METHOXYCHLOR ON SELECTED NON-TARGET ORGANISMS IN A RIFFLE OF THE SOURIS RIVER, MANITOBA

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ABSTRACT

A riffle on the Souris River, located at Bunclody Manitoba, was treated with methoxychlor on 15 July 1982 at a rate of 0.3 mg/litre for 15 min. The treatment caused an immediate catastrophic drift in all of the 16 species of invertebrates examined. 'Normal' behavioral drift densities were exceeded by many orders of magnitude. The catastrophic drift peak lasted from 4 to 24 h depending on the species involved, and was followed by a large decrease in drifting populations.

Individual taxa demonstrated varying abilities to recolonize artificial substrates following treatment. Species that had a high propensity to drift under natural conditions recolonized most rapidly e.g. (Baetis spp., and Caenis tardata). Taxa that required the longest period of time to recolonize were generally univoltine species that had a very low propensity to drift and a limited ability to disperse as adults (e.g. Psychomyia flavida). The impact of the methoxychlor treatment on some species was influenced by the phenology of those species. Emerging taxa that were in the pupal stage and apparently not affected by the pesticide, or that were in the terrestrial adult stage and thus not exposed to the pesticide in the river, acted as important sources for recolonization.

The methoxychlor treatment resulted in an immediate 3-fold increase in drift density of white sucker fry (Catostomus commersoni), and some were moribund in the pool downstream of the treatment riffle immediately following injection. A catastrophic drift of crayfish (Orconectes virilis) juveniles was also observed following injection. Juveniles of this species may be far more sensitive to methoxychlor than has been previously indicated for adults.

Species diversity in the drift was significantly reduced ($p<.05$) at the treatment site for six days following treatment. Species richness and total numbers in the drift were significantly reduced ($p<.05$) at the treatment site for at least 33 days following treatment. The methoxychlor injection significantly reduced ($p<.05$) species diversity on artificial substrates at the treatment site for 14 days. Species richness and total numbers on substrates were significantly lower ($p<.05$) at the treatment site for 4 and 8 days respectively following treatment. Species richness was a better indicator than diversity indices, such as the Shannon-Weaver and Simpson's, of biological change induced by the methoxychlor treatment.

Drift was restored only after recovery of the benthic standing crop as measured on artificial substrates. This suggests a density relationship of drift to benthic standing crop. Invertebrate drift is a more sensitive measure than benthic density of the impact of methoxychlor treatments on aquatic invertebrate communities.

DEDICATION

To my parents, Camille and Helen Sebastien, who are my best friends and have always supported me throughout my academic and athletic careers.

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Chapter I

INTRODUCTION

In some regions, blood-sucking black flies (Diptera:Simuliidae) are the most serious insect pests that affect both humans and livestock. Black flies can affect the health of man and animals in several ways: they are vectors of disease, their bites are injurious, and because of their great abundance and habit of landing and crawling about on the head, face and other parts of the body, their presence may be at times unbearable. The most spectacular outbreaks affecting livestock are those of Simulium arcticum Malloch from the Saskatchewan and Athabasca rivers. These outbreaks can become severe enough to kill cattle. In the period between 1944 and 1948, more than 1,300 cattle were killed in Saskatchewan alone by S. arcticum (Fredeen 1977).

In an effort to prevent damaging outbreaks of black flies, chemical larvicides are continuously under test. In the past chemicals have been the only significant means by which these pests have been controlled. Black fly larvae are particularly susceptible to control by toxic chemicals because of their highly restricted and specific breeding habits and their method of feeding in lotic (running) water.

When a chemical larvicide is applied to a stream or river, it is automatically dispersed by the current to sites below the point of treatment. Larvae may be killed by exposure to larvicides in three ways: 1) by ingestion of toxic particles, 2) by exposure of the cuticle to contact insecticides, or 3) indirectly, by starvation or anoxia, after being carried downstream into pools if forced to release from their site of attachment by the chemical (Jamnback 1976).

The earliest toxic chemical used for black fly control was DDT, which was initially considered most satisfactory because of its low cost, ease of application, greater "selectivity" to black fly larvae than to other members of the stream fauna, and its outstanding transport downstream. However, with the discovery of its significant effects on non-target organisms, its long-term stability in the environment and its biomagnification in the food chain, DDT was banned for general use in Canada in 1969.

Among the first chemicals to be tested in the field as a possible replacement for DDT was methoxychlor. Over the years, methoxychlor has probably been the most extensively used chemical in Canada for the control of black flies, and it is presently registered for the control of S. arcticum and S. luggeri Nicholson and Mickel in western Canada. In northeastern North America, where black fly larvae occur in numerous rapidly flowing streams in inaccessible mountainous

areas, methoxychlor is typically administered by aircraft at the rate of 4.51 litres (one gallon) of 15% methoxychlor solution plus 0.5% surfactant per flight mile (Jamnback 1969). The swath technique is utilized (streams are treated every 1/4 mile). Concentrations of about 0.008 mg/litre/5 min are effective for the control of black fly larvae.

In western Canada, methoxychlor is applied from the ground at a single location as an emulsion at a rate of 0.3 mg/litre/7.5-15.0 min. Effective control is obtained for long distances downstream from the point of application. Single 15 min injections of 0.30 mg/litre eliminated 98% of the black fly larvae for 64 km downstream, and 46% for a distance of 139 km in the Saskatchewan River (Fredeen 1974). More recently, single 7.5 min injections of 0.6 mg/litre methoxychlor into the North Saskatchewan River eliminated 100% of third to sixth instars of the larvae of S. arcticum at distances of 40 and 80 km downstream, 91% at 121 km, and 66% at 161 km. Ninety-six % of the larvae less than 1 mm long were removed from the 161 km site (Fredeen 1975). The majority of the methoxychlor apparently adsorbs to suspended solids which are then carried great distances downstream. Fredeen (1974,1975) suggested that adsorbed methoxychlor was selective to filter-feeding animals such as black fly larvae, acting as an internal poison subsequent to ingestion.

The impact of methoxychlor on non-target aquatic invertebrates has been a topic of much controversy and disagreement among investigators. Conflicting reports regarding the effect of methoxychlor treatments on invertebrate drift, and the length of time required for benthic recolonization, abound in the literature (e.g. Fredeen 1974, 1975, 1982; Haufe et al. 1980, Flannagan et al. 1979, Wallace and Hynes 1975).

The present study had the following major objectives: 1) to establish the length of time required for a community of non-target invertebrates inhabiting a riffle of the Souris River, Manitoba to recover relative to an untreated riffle site following an injection of methoxychlor at a rate of 0.3 mg/litre/15 min; 2.) to determine what taxa were most adversely affected by the methoxychlor treatment; 3) to determine the importance of invertebrate drift from untreated areas upstream in the recolonization of the treated riffle; 4) to determine the relationship between invertebrate drift and numbers in the benthos following methoxychlor treatment.

Chapter II

LITERATURE REVIEW

2.1 THE TOXICITY OF METHOXYCHLOR TO NON-TARGET AQUATIC INSECTS (LABORATORY STUDIES)

Fredeen (1972) evaluated the effect of methoxychlor against rheophilic species of Trichoptera larvae (Brachycentrus lateralis (Say), Hydropsyche morosa Hagen, and H. recurvata Banks). Larvae were exposed to concentrations of 0.02, 0.05, 0.075, 0.1, and 0.5 mg/litre for six h in glass aquaria. Small proportions of larvae affected by methoxychlor treatment recovered during an 18-h post-treatment observation period. The LC_{50} for H. morosa averaged 0.04 mg/litre. Methoxychlor was more toxic to Trichoptera larvae at 10° C than at 20° C.

Anderson and DeFoe (1980) exposed stoneflies and caddisflies to methoxychlor in a flowing-water test system for 28 days at concentrations of 0.15, 0.42, 1.30, 2.17, and 4.23 ug/litre. Behavioural changes in the caddisfly Brachycentrus americanus (Banks) and in the stonefly Pteronarcys dorsata Say were observed within four days at 2.2 ug/litre. The LC_{50} for the caddisfly Hydropsyche sp. was 2.9 ug/litre at 14 days and 1.3 ug/litre at 28 days. The stonefly P. dorsata did not die by the end of the

exposure period at the highest concentration tested (4.2 ug/litre). Thus, if the exposure time is increased, very low concentrations could cause significant reductions in the survival of some species.

Merna and Eisele (1973) performed acute and chronic continuous flow bioassays on a number of aquatic invertebrates. Ninety-six h TL_{50} values were calculated for the following aquatic insects: Stenonema interpunctatum (Say) - 1.96 ug/litre; Chironomus tentans Fabricius - 1.62 ug/litre; Cheumatopsyche sp. - 3.24 ug/litre. Chronic continuous-flow bioassays interpreted at 2-week intervals for up to 42 days yielded the following TL_{50} values : Stenonema terminatum (Walsh) - 0.41 ug/litre; S. interpunctatum - 0.47 ug/litre; Cheumatopsyche sp. - 0.71 ug/litre. Higher, or comparable emergence than the controls in most of the insect species was observed at low dosage levels (0.125 or 0.25 ug/litre). Slower growth was observed in the highest dosage aquaria for Stenonema and a general linear increase occurred as the dosage level was decreased. Mayflies and midges had a faster rate of growth at low methoxychlor levels (0.125 and 0.25 ug/litre) than in the control. The growth of Cheumatopsyche sp. was greater in the control than in all dosed samples in all cases. Markedly reduced growth occurred in all cases at the 0.5 ug/litre level, indicating there may be a threshold beyond which lower pesticide exposures do not inhibit growth. The

authors failed to give a reason for the apparent enhanced emergence and growth rate observed in most of the insect species at low dosage levels compared to controls.

Sanders and Cope (1968) conducted bioassays on naiads of three species of stoneflies to compare the relative acute toxicities of methoxychlor to other insecticides. The estimated LC_{50} values of methoxychlor for Pteronarcys californica Newport exposed at 15.5° C were 30 ug/litre after 24 h, 8.0 ug/litre after 48 h, and 1.4 ug/litre after 96 h. During the test period it appeared that larger, later-instar naiads were less susceptible to given concentrations of pesticides than were smaller, early instars of the same species.

Methoxychlor was very toxic to aquatic insects under both static and flowing-water laboratory conditions. The toxicity of methoxychlor increased with increasing exposure time for most organisms examined.

2.2 THE TOXICITY OF METHOXYCHLOR TO NON-TARGET AQUATIC INSECTS IN LOTIC ENVIRONMENTS (FIELD STUDIES)

2.2.1 Overview

Burdick et al. (1968) reported that a 20% solution of methoxychlor in fuel oil applied for 20 min in a small stream produced a heavy drift of mayflies and stoneflies and a smaller drift of caddisflies. Black flies comprised only 8.8% of the drift. A second preparation, a 24% methoxychlor

emulsifiable concentrate, was applied to a different portion of the stream and caused a reduction in total sample weight of non-target organisms of 54.9%, and an 11.9% reduction in number. A wettable powder appeared to produce much less effect on mayflies, stoneflies and caddisflies, but was also less effective against black fly larvae. A large number of insects were affected by these treatments, and drifted through the area, but there was no evidence that all, or most, of these insects later died. After the application, some of the drifting mayflies, stoneflies, and caddisflies were held in trays in stream water for periods up to 4.5 h with no observed mortality.

Wallace et al. (1973) observed that drift samples taken after a treatment of 0.075 mg/litre methoxychlor (emulsifiable concentrate) for 15 min into a small stream near Baie Comeau, Quebec contained very large numbers of Ephemeroptera, Trichoptera, and especially Plecoptera, as compared with pre-treatment samples. However the drift of chironomids was essentially unaltered. Most of the drifting invertebrates found in post-treatment drift net samplers were dead. Surber samples taken 24 h after treatment indicated that bottom densities were depleted, however, no species of non-target invertebrates were eradicated from the streams. The apparent rapid recolonization in the stream after treatments, in spite of the catastrophic drift which occurred, might have been due to invertebrates recolonizing

the upper layers of stream gravel from the hyporheic habitat. Recolonization may also have occurred from downstream drift. On several occasions following treatments, predaceous Plecoptera nymphs were observed eating Trichoptera larvae which had been affected by treatments. Exposure to the insecticide leading to drift, leading in turn to predation, and possibly further drift of predators could well extend the impact of such treatments. Delayed and sublethal effects among a wide spectrum of species, including fish, which might not otherwise have been directly affected by the initial pesticide exposure, could occur as a result of this phenomenon (Wallace et al. 1973).

An 11-year study of insecticide effects on stream insects was undertaken in the Adirondack Mountains of New York State by Burdick et al. (1974). The spray program was conducted by aircraft and was repeated two or three times annually over an area of 150 square miles. The area was originally sprayed with DDT for three years, then left untreated for two years, followed by six years of treatment with methoxychlor. Both DDT (as a 20% solution) and methoxychlor (as a 15% solution) had significant effects on Plecoptera, Ephemeroptera, Trichoptera and Diptera. Methoxychlor reduced the average daily standing crop by 21.3% compared with untreated streams for the treatment period. The treatment produced a slight decline in diversity and annual production, but recovery occurred at least by the next

season. The mean number of families within the untreated stream for the entire season was 11.7 compared with 9.6 for the methoxychlor-treated streams (Burdick et al. 1974).

Fredeen (1974) reported that single 15 min injections of 0.18 to 0.24 mg/litre methoxychlor as an emulsifiable concentrate into the Saskatchewan River removed 75 to 99% of the larvae of S. arcticum for distances up to 34 km downstream. Higher concentrations (0.30 mg/litre) eliminated about 98% of the larvae from rapids 64 km distant and an estimated 46% at a distance of 139 km. Non-target aquatic insects on artificial substrates were also adversely affected. Populations of Plecoptera were reduced by about the same percentage as populations of S. arcticum larvae. Ephemeroptera were less affected, followed by Trichoptera and Chironomidae, in that order. Larvae in gravel on the river bed were less affected than larvae attached to the surfaces of artificial substrates, and larvae in river-bed sand (mainly Chironomidae) were unharmed. Recolonization of the artificial substrates began immediately after each treatment. Trichoptera and Chironomidae larvae recolonized treated areas rapidly but the Simuliidae and Plecoptera were slow to recolonize. Populations of Chironomidae, Trichoptera and Ephemeroptera in many sites were completely restored within 7 to 14 days after a single treatment to a distance of 145 km downstream from the treatment site. Therefore, Fredeen (1974) concluded that 15 min injections

of 0.3 mg/litre methoxychlor should not permanently suppress aquatic insects at the family or order level in the Saskatchewan River.

Fredeen (1975) observed that a single 7.5-min injection of 0.6 mg/litre of methoxychlor into the North Saskatchewan River affected non-target organisms in the river up to a distance of 161 km. Plecoptera larvae (longer than 1 mm) declined by 5% at the control site and by 81, 100, 66, and 90%, respectively at sites 40, 80, 121, and 161 km downstream from the treatment site. Populations of Ephemeroptera larvae larger than 1 mm long were reduced by 41% to 89% in three of the treated sites. Chironomidae larvae increased at all sites except at the 161 km site where larvae larger than 1 mm were reduced by 21%. Larger trichopteran larvae declined at the untreated check site and at the 40 and 161 km treated sites but increased at the 80 and 121 km treated sites. The treated portion of the river was rapidly repopulated by insect larvae. In the four treatment sites, population densities of Chironomidae larvae, larger than 1 mm, equalled or surpassed the pre-treatment densities within 1 to 3 weeks. Similarly, Ephemeroptera, Trichoptera, and Plecoptera at the treatment sites equalled or surpassed the pre-treatment densities after 1-4, 1-7, and 4-5 weeks respectively. Populations of larvae smaller than 1 mm in length were generally restored more rapidly. The numbers of taxa that were encountered in

all four treated sites during the 10 weeks following the treatment greatly surpassed the numbers encountered before the treatment. None of the taxa present during the pre-treatment period disappeared and, in fact, most attained levels of abundance during these 10 post-treatment weeks that greatly surpassed those of the pre-treatment populations (except for Plecoptera which were comprised of about 99% Isoperla sp. at two treated sites). In all tests performed in the Saskatchewan River, methoxychlor was considerably less harmful to non-target insect larvae identified to the ordinal level (except Plecoptera) than to black fly larvae. Fredeen (1975) concluded that since methoxychlor adsorbed readily onto silt particles carried by the water, it presumably affected filter feeders such as black fly larvae more than non-filter feeders.

Wallace and Hynes (1975) treated two Ontario streams with a 15% methoxychlor solution applied by aircraft at a rate of 1 gal. (Imp.) (4.54 litres) per flight mile, and from the ground at an initial calculated rate of 0.075 mg/litre/15 min. Both the aerial and ground treatments produced a copious drift of aquatic insects in the streams. In this study, the impact of methoxychlor as a black fly larvicide was not confined to simuliid larvae. In both streams, simuliid larvae comprised only a small portion (less than 4% by weight) of the drift of insects after the treatments. Plecoptera appeared to be the group most severely affected

by both treatments. In both streams, most of the drift occurred within 150 min following treatment. Lower numbers of non-target organisms than before treatment were present in Surber samples, however, no taxa were eliminated from the streams. Catastrophic drift caused by methoxychlor certainly represented a loss to the total standing crop of insects in the streams.

Wallace et al. (1976) dosed the Chalk River, Ontario for 15 min with an oil solution of methoxychlor (0.79 ug/litre). Simuliid larvae comprised most of the invertebrate drift, together with only small numbers of Trichoptera (Cheumatopsyche sp. and Hydropsyche sp.), Ephemeroptera (Ephemerella sp.) and some Sialis sp. up to a distance of 770 m. The numbers of drifting simuliid larvae reached a peak 60 min after the beginning of the treatment at 275 m and 550 m, and somewhat later at 770 m from the point of application.

Eisele and Hartung (1976) treated a small stream continuously for over one year with methoxychlor at a rate of 0.2 ug/litre. Invertebrate populations were monitored using artificial substrates and bottom samples of riffle invertebrates. Most taxa exposed to the chemical exhibited a slight decrease in density on plate samplers at the time of application or shortly thereafter. Baetis spp. and plecopterans appeared to be the most affected by the treatment. Hydropsyche sparna Ross and Cheumatopsyche sp.

experienced initial reductions when exposed to methoxychlor but quickly recovered, even though the exposure was continuous. The methoxychlor treatment did not affect the aeshnid Boyeria vinosa (Say), chironomids, empidids, or elmid beetle adults and larvae. Invertebrate populations on both plate samplers and bottom samplers were negatively affected at the initiation of stream treatment. The effects were short-lived, however, and in both cases the density usually returned to pre-treatment levels within a month. There was no substantial change in species diversity in the community as a result of the treatment. It was concluded that a continuous low-level methoxychlor stress affected stream invertebrate populations, but not as much as extreme natural environmental stresses such as high current velocity resulting from flooding.

2.2.2 Critique of studies in western Canada

A number of studies concerning methoxychlor additions to large rivers in western Canada, for the purpose of black fly control, are considered most relevant to the work presented here. The following critical review will identify deficiencies in design and interpretation of data in these studies.

Flannagan et al. (1979, 1980) evaluated the effect of a single 0.3 mg/litre injection of methoxychlor into the Athabasca River, Alberta, on the non-target invertebrate

fauna in 1974. Four sampling stations were used in the study: I) upstream control, II) 200 m downstream of the treatment, III) 67 km downstream, and IV) 400 km downstream of treatment. Except for the chironomids at station IV, the mean densities of all taxa sampled using Ponar and modified Birge-Ekman grabs at the three treated stations were lower at the end of the sampling period (4 weeks) than just prior to treatment, whereas the reverse was true for the control station. Barbecue baskets filled with 5-7 cm diameter stones with a pan of fine sediment attached below the basket were used to simulate the rock-over-sand bottom common in the river. Invertebrate populations at station I essentially doubled over the sampling period while the populations at stations II and III decreased markedly over the same period with some post-treatment recovery at station II. Plecoptera and Ephemeroptera were the taxa most seriously affected; reductions at station III were more extreme than at station II. Some signs of recovery in Ephemeroptera, Plecoptera, and hydropsychid Trichoptera were evident at station II and in hydropsychids at station III. Chironomids were apparently unaffected at station II but showed a drastic decline with time at station III. The greatest long-term effect on non-target invertebrates was observed at station IV. It was suggested that sites nearest the injection could be recolonized by downstream drift and migration of adults from unaffected areas upstream, whereas the sites further downstream would require a much longer

period of time. Population levels were similar in 1975 at stations I and II compared to 1974. However, there were lower numbers of Plecoptera, Ephemeroptera, and Trichoptera at stations III and IV in 1975 compared to 1974. Thus a significant reduction in standing crop still remained at the two furthest downstream sampling sites one year after treatment.

Flannagan et al. identified organisms only to ordinal level in these studies, so effects at the genus or species levels are unknown. Pre- and post-treatment dates being compared were unspecified, making the data presented difficult to interpret. Also, the degree of variation in the grab samples is so large that only changes of several orders of magnitude could be detected. The criterion for recovery was based on benthic invertebrate populations at the treatment sites returning to control site levels. However, one treated site (station III) was very different from the control site in species composition before treatment. Since the control community does not appear to be the same as that sampled at station III prior to treatment, it may not be a suitable control at least for this site.

Catastrophic drift, especially of Ephemeroptera, Trichoptera and Plecoptera was recorded at stations III and IV, more or less simultaneous with the expected (calculated) arrival of methoxychlor. Drift continued at above normal

densities for 4 to 12 h, then dropped to below pre-treatment levels. However, post-treatment numbers of Trichoptera in the drift remained above pre-treatment levels throughout the remainder of the sampling period (4 days). The effect of the treatment on Trichoptera was perhaps more prolonged, or some Trichoptera were slower to react than other invertebrates. There were only small differences in the sensitivities among the various taxa to methoxychlor. The predaceous Plecoptera, detritus-feeding Ephemeroptera, and the filter-feeding animals all appeared to be affected by methoxychlor at about the same time. Consequently, methoxychlor may not necessarily be an internal poison (as suggested by Fredeen (1977)), but may kill or disable on contact. Flannagan et al. (1980) stated that the river downstream was unlikely to be able to support even a fraction of these displaced animals and therefore it must be assumed that most, if not all, of the animals in the catastrophic drift were "ecologically dead". Perhaps the only way the fate of animals induced to drift by toxicants will ever be known, is if species were somehow marked before treatment and then sampled at sites downstream after treatment to determine the % surviving. This would be very difficult if not impossible to conduct on a river the size of the Athabasca.

Flannagan et al.(1979, 1980) did not replicate drift samples, so no level of variability was indicated, and

statistical analysis could not be performed on the data. In addition, drift samplers were all placed in close proximity to the river bank so that variation in numbers due to location across the river was not investigated.

Haufe et al. (1980) concluded that a methoxychlor application at 0.3 mg/litre for 7.5 min in 1976 had a detectable stimulatory effect on the behavioural drift of some organisms in the Athabasca River. The activity attributed to the pesticide was within an order of magnitude of natural variations related to diel periodicity, current-related disturbances, scouring due to flood, and behavioural exodus for emergence. Constant drift tended to occur within the same order of magnitude in pre- and post-treatment periods indicating no major downstream displacement or "sweeping" of fauna occurred. However, Haufe et al. (1980) did not take simultaneous control samples in an untreated portion of the river. Instead, they compared changes in drift density in the 1976 treatment with samples taken in 1977, a year in which the river was not treated. However in 1977, the river was in flood stage, and this disrupts the normal patterns of drift (Waters 1972). Hence, the 1977 data cannot be compared with those from 1976.

Haufe et al. (1980) showed that drift densities of mayflies increased up to 12 h prior to arrival of the pulse, and maximum densities were similar to before and after the pulse-exposure period (Fig. 1). However, drift densities

were plotted logarithmically rather than arithmetically. This served to reduce drastically the amplitude of drift densities during the exposure period. Furthermore, the points plotted represent running three-interval means of the actual data. Thus, each plotted point is the average of three consecutive samples collected hours apart. This type of presentation greatly reduces the apparent amplitude of a short-term peak in a curve, and produces an apparent rise in the curve prior to the actual occurrence of the peak, which explains why drift densities appear to have increased in advance of arrival of the methoxychlor. The raw data of Haufe et al. (1980) should be plotted on an arithmetic scale as was used by Flannagan et al. (1980). Catastrophic drift of most taxa as proposed by Flannagan et al. (1980) may have been present in both studies.

Haufe et al. (1980) also separated drift samples into a living portion and a dead portion immediately after collection. It was implied that all of the organisms classed as alive would remain alive if left drifting in the river. This may not be a valid assumption, and it was not tested in the study. It is interesting that Flannagan et al. (1980) arrived at an opposite conclusion. They suggested that the majority of organisms induced to drift by the insecticide would die. Haufe et al. (1980) observed that of 46 genera sampled in the drift, only nine genera exhibited casualty rates as high as 15-40%. These were

represented in three orders: Trichoptera- Hydropsyche spp. and Cheumatopsyche spp., Ephemeroptera- Rhithrogena spp., and Plecoptera- Isogenus spp. No casualty rates exceeded 5% in the other 37 genera. It was concluded that since none of the casualty rates exceeded 40%, the toxic impact of methoxychlor did not approach the order of natural attrition inherent in the aquatic invertebrate ecosystem.

Non-target species were apparently more sensitive to the methoxychlor than were the target black fly species, according to the method used by Haufe et al. (1980) to determine casualty rates of the organisms sampled in the drift. The maximum casualty rate for black fly larvae was about 10%, whereas the maximum casualty rates of some non-target species was much higher e.g. Baetis and Ephemerella -20%, Isoperla and Rhithrogena -30%, and Cheumatopsyche -40%. Eight of the nine taxa which showed maximum casualty rates (15-40%) were numerous at all stations sampled, and at all times of the year on the Athabasca River (Depner et al. 1980). Thus, although they represented only a small proportion of the total number of taxa in the drift, these taxa possibly represent a very significant component of the biomass in the river.

Depner et al. (1980) sampled 12 stations from 65 km upstream of the treatment site to 240 km downstream over the four-year methoxychlor impact assessment project (1974-1977) on the Athabasca River. For each sample, an area of river

bottom 0.6 m wide x 3 m long was disturbed using a garden cultivator and the resulting aquatic invertebrates were caught in a downstream net. Reductions were observed in some downstream sites after treatment, however, recovery of the non-target populations was rapid and occurred within one month. The numbers of kinds of organisms at all stations increased within each year of the study. The authors concluded that the treatment resulted in an actual increased diversity of organisms over the four years.

Depner et al. (1980) apparently did not use replicate sampling at any site, and used no statistical analysis. Thus, it is not known which differences in the average frequency values recorded are statistically significant. Therefore their conclusions concerning reductions and recovery of invertebrate populations after methoxychlor treatment lack statistical support. The total number of genera found at two untreated sites was compared with the total number at six (1973-74) or eight (1975- 1977) treated sites. This comparison is invalid because more samples from more sites would naturally lead to greater diversity. Depner et al. (1980) should have compared the number of genera collected at the two sites in the untreated section with the number from any two sites in the treated section.

Fredeen (1983) summarized environmental impacts of the increasingly intensive methoxychlor larviciding program on the Saskatchewan River (1977 to 1980). He concluded that

almost all non-target taxa, representing a complete range in feeding habits, activity patterns and life cycles, and all major contributors to biomass, either increased or remained unchanged in abundance during those four years. The fact that significant long-term increases occurred suggested that some factor or factors extrinsic to the larviciding program may have been responsible.

The most frequently used method to monitor invertebrate populations from 1977 to 1980 in the Saskatchewan River involved artificial substrates (polypropylene rope pieces) anchored in mid-river, floating just below the water surface (Fredeen 1983), but this may not be representative of the benthos. No specific untreated control sites were consistently used throughout the study. Only one artifical substrate sample was analyzed each week at each site, so the degree of variation in the sampling technique could not be established. Taxa were identified only to order or family levels, so effects at the genus or species level are masked, and elimination of any one species is not detectable. Since no control sites existed, it is not known whether changes noted as significant can be attributed to the methoxychlor treatment or other causes.

An alternate method used by Fredeen (1983) to monitor invertebrate populations from 1977 and 1978 involved Surber sampling from river margins. Replicate samples from each site were pooled into one sample, and identifications were

made to family or sub-family, except for 1980 when samples were identified to the species level. No control sites were used and the level of natural sample variation was not established. Hence, it was impossible to interpret any gross changes between years in terms of the methoxychlor treatments, and one cannot conclude from this study that " bottom samples showed increases in numbers and taxa of benthic organisms."

Byrtus (1981) reported on two injections of methoxychlor into the Athabasca river. A dosage of 293 ug/litre/7.5 min resulted in an adverse effect on Isoperla spp., Hastaperla spp., Hydropsyche spp., and Cheumatopsyche spp. The Ephemeroptera were not significantly affected by the treatment. There was no significant impact on diversity of benthic organisms at the treated sites relative to the control sites. A second treatment at 299 ug/litre/7.5 min caused an initial decrease of Baetis sp. at the treated sites followed by an increase over control site levels for about seven weeks before the population stabilized. Isogenus sp. (Plecoptera) were adversely affected and populations at the treatment sites did not return to control site levels for about seven weeks. Ephemerella sp., Hydropsyche sp. and Cheumatopsyche sp. were apparently not affected by the treatment. Again, no difference in diversity was observed between control and treatment sites. Byrtus (1981) hypothesized that the initial decrease of

Baetis sp. followed by an increase over control site levels may have resulted due to a lack of predators, e.g. Isogenus sp., which did not recover for seven weeks following treatment. He suggested that high discharge rates during both treatments likely acted as a flushing mechanism limiting the exposure of non-target organisms.

Byrtus (1982) analyzed the impact of a methoxychlor treatment at 299 ug/litre/7.5 min on the invertebrate population of the Athabasca River. The treatment produced adverse effects on invertebrates inhabiting sites immediately downstream of the treatment, whereas sites further downstream were not affected to the same extent. It was suggested that the methoxychlor settled to the bottom within 120 km of the treatment site, because of low discharge. Taxa affected most were members of the Plecoptera, and the only genus not affected was Ephemerella sp. (Ephemeroptera). Recovery at 80 km downstream required one week; however recovery at 240 km took approximately nine weeks.

Byrtus (1982) found that a second injection of 298 ug/litre/7.5 min into the Athabasca River in May 1981 resulted in a limited impact on non-target fauna. He proposed that seasonal emergence of many invertebrates was occurring during this period and these invertebrates were in a terrestrial adult stage during the treatment period. A second treatment of 199 ug/litre/15 min in June 1981

produced serious long-term reductions of many non-target organisms. Baetis sp. required 4 to 7 weeks to recover, Heptagenia sp. 3 to 4 weeks, and Isogenus sp. 7 weeks. Impact on Hydropsyche sp. and Cheumatopsyche sp. was not evident. Most of the non-target invertebrate population was increasing in the river when the second (June) application occurred. It was concluded that proper timing of river treatments could significantly reduce the impact on non-target organisms.

Byrtus (1981,1982) estimated bottom densities of invertebrates using the "kick sample" method. This method has generally been shown to be a qualitative rather than a quantitative sampling technique. The criterion for recovery was based on the population returning to control site levels. However, since there was no analysis to compare the abundance of different genera at the control and treatment sites prior to treatment (no baseline data), it is not known how similar these communities were, and any conclusions based on this criterion are questionable. In addition, because no replicate samples were taken in most of the collections, no level of variability was indicated, therefore statistical analysis could not be performed on the data.

Lehmkuhl (1981) reported that treatment of the Saskatchewan River with methoxychlor (300 ug/litre/15 min) caused significant increases in downstream drift of aquatic

insects, up to distances 70 miles downstream from the treatment. Non-target organisms comprised from 80 to 96% of the drift. Species composing the pesticide-induced drift were not always those that were abundant in benthic samples. The groups of insects most affected in the drift and benthos were the baetid mayflies (herbivore), Isoperla spp. (carnivore) and Isonychia sicca (Walsh) (filter feeder). Groups that were little affected included the carnivorous Odonata, and the herbivorous stonefly P. dorsata.

In this study, replicates of kick samples or "stovepipe samples" used to estimate bottom densities were pooled; no level of variation was given. The same sampling technique was not used for each sampling date because of varying field conditions. The actual numbers therefore, cannot be used quantitatively for comparison of one treatment date with another. Statistical analysis performed on the data (cluster analysis) is also limited because of the use of different sampling techniques. Baseline data, comparing the control and treatment sites before treatment, were not available, making any conclusions regarding methoxychlor impact tenuous.

Lehmkuhl (1982) attempted to determine long-term effects of methoxychlor treatment on the invertebrate community of the Saskatchewan River. Benthos samples (7 replicate stovepipe samples at each of three sites) were collected in May 1982 prior to treatment (300 ug/litre/15 min) and 10

months after treatment. The sites were all riffles consisting of rock or gravel substrates. A comparison of the three sites which had different treatment histories, showed some differences in species dominance in the heavily treated sites, but species lists and populations were not strikingly different. The mayfly, Tricorythodes minutus Traver was rare at the control site (Cecil Ferry upstream, untreated in 1980 and 1981) but was the dominant species at the Gronlid Ferry site (treated since 1976). Stoneflies, mostly Isoperla bilineata (Say) and I. longiseta Banks were abundant at the control site but were present only in small numbers at the treatment sites. Large numbers of small individuals of different taxa were observed in the treated areas, whereas in the control area there were fewer individuals but of a large size (greater total biomass). Stoneflies (Isoperla sp.) appeared to be the main invertebrate predators which fed on small, newly hatched individuals. The absence of these stonefly predators at the treated sites allowed for a large population of smaller sized individuals of various taxa (Lehmkuhl 1982).

It is difficult to interpret the results of major studies conducted on the Athabasca and Saskatchewan Rivers dealing with impact on non-target organisms for the following reasons: 1) the lack of suitable untreated sites to serve as controls with which to compare changes at downstream treated sites; 2) the lack of baseline data and analysis to

show similarity of the control to treated sites; 3) the identification of organisms only to the generic level or higher, which may mask effects at the species level; 4) the lack of a sufficient number of replicate samples to produce confidence in the results obtained from sampling communities where large variation was encountered; 5) the lack of a description of habitat types sampled to ensure that the same communities are being sampled (NRCC 1983). It must be recognized that when the earlier studies began, techniques for sampling large, fast rivers had not been established, and consequently much time had to be spent in developing satisfactory ones. Much of the basic biology of the invertebrates was unknown, and the keys for identification of immature stages were not, and in many cases are still not, available. None of these studies has adequately answered the question of the ability of the impacted invertebrate communities to recolonize and recover following methoxychlor injection.

2.2.3 Secondary effects of pesticide application

Applications of insecticides may potentially affect energy and nutrient flow within stream ecosystems in more far-reaching manners than previously recognized. Macroinvertebrate consumers, primarily insects, are important in regulating rates of detritus processing and availability to downstream communities. Wallace et al.

(1982) treated a small Appalachian forest stream with a 24% emulsifiable concentrate of methoxychlor at a rate of 5.0 mg/litre for 10 continuous h. This was supplemented with a five-h, 5.0 mg/litre, hand-sprayer release onto leaf packs, seeps, and backwater areas. Subsequent 10 mg/litre hand-sprayer releases were conducted at later dates. The treatments caused massive downstream insect drift (1,000 times densities before treatment and of an untreated stream). Drift rates of Plecoptera, Ephemeroptera, Trichoptera, Coleoptera, and Diptera remained significantly higher in the treated than in the reference stream one week following application. Total numbers of insects, shredders, and predators, as well as leaf-breakdown rates and transport of particulate organic matter, were significantly lower in the treated than in the reference stream. No significant differences existed prior to treatment. Although dosages used in this study were far greater than those applied in conventional black fly larviciding programs, the results indicate that the action of pesticides on stream ecosystems extends beyond simple mortality of invertebrates (purely toxicological questions).

2.3 THE TOXICITY AND SUBLETHAL EFFECTS OF METHOXYCHLOR ON CRUSTACEANS

Sanders and Cope (1966) suggested that Daphnia populations may be diminished even at low concentrations of methoxychlor, and this could have adverse effects on fish-food supplies. It was also observed that methoxychlor was 8-18 times more toxic to D. pulex (de Geer) than to Simocephalus serrulatus (Koch).

There were no methoxychlor residues in crayfish sampled 36, 63, and 118 days after a 0.005 mg/litre application of the chemical to a pond (Burdick et al. 1968). It was concluded that aquatic insects and crayfish were able to metabolize methoxychlor.

Daphnia magna Straus concentrated methoxychlor at about the same rate as DDT when exposed to 0.003 mg/litre of both pesticides (Reinbold et al. 1971). However, after transfer to clean water, Daphnia excreted or cleared methoxychlor at a slightly faster rate than DDT. Gammarus (an important fish-food organism) was the most seriously damaged organism in the aquatic system after exposure to methoxychlor (Merna and Eisele 1973). The 96-h TL_{50} for adults of Gammarus pseudolimnaeus Bousfield was 0.75 ug/litre. The least susceptible organism to methoxychlor was the crayfish (Orconectes virilis (Hagen)). Oxygen concentration was very important in determining toxicity to the crayfish. The 96-h TL_{50} at normal dissolved O_2 levels was 7.05 ug/litre whereas

it was only 2.15 ug/litre in exposures having low dissolved O_2 levels. These observations were confirmed by Eisele and Hartung (1976) who found that the amphipod Hyalella azteca (Saussure) was absent for nine months after a stream was dosed continuously at 0.2 ug/litre with methoxychlor for over one year.

Flannagan et al. (1979) found no significant effect on blood calcium and no mortality in caged crayfish, Orconectes virilis (Hagen) after an application of 0.3 mg/litre/15 min methoxychlor into the Athabasca River approximately 200 m upstream from the cages. Whole-body methoxychlor residues increased, but declined to normal levels in about 1 week. Numbers of crustaceans sampled 67 km downstream from the treatment, were drastically reduced. Depner et al. (1980) reported that populations of Amphipoda recovered to pre-treatment population levels, one month after treatment.

Anderson and DeFoe (1980) found the isopod Asellus communis Say to be the most sensitive species tested in a flowing-water test system. Ten % of the isopods died during the first 4 days at 1.3 ug/litre methoxychlor. Following the first 4 days, the number of deaths increased with increased exposure time. The LC_{50} decreased from 1.75 ug/litre at 7 days to 0.42 ug/litre after 28 days.

Generally, crustaceans appear to be very sensitive to methoxychlor with Gammarus sp., H. azteca, and A. communis being the most seriously affected organisms in many studies involving methoxychlor exposures. A noted exception is the crayfish, O. virilis, which appears very tolerant to methoxychlor at dosages used in black fly larviciding operations. Methoxychlor becomes more toxic to crustaceans with increasing exposure time, a trend also observed for many species of aquatic insects.

2.4 THE TOXICITY AND SUBLETHAL EFFECTS OF METHOXYCHLOR ON MOLLUSCS

Wilson (1965) observed that oysters exposed to 0.05 mg/litre methoxychlor in flowing seawater for 10 days concentrated the pesticide to a level of 5,780 times the exposure concentration. Hard clams (Mercenaria mercenaria (Linnaeus)). were unaffected by an exposure of 1,300 ug/litre methoxychlor over a period of 96 h (Eisler and Weinstein 1967). In contrast, Butler (1963) reported that oyster shell growth was decreased by exposure to DDT, and to a lesser extent by methoxychlor. Bedford et al. (1968) found that the mussel, Lampsilis siliquoidea (Barnes) contained 0.07-0.22 mg/kg methoxychlor when exposed to 0.06-0.1 mg/litre methoxychlor for 6-10 weeks in a river. Anodonta grandis Say had a comparable level of methoxychlor (0.07 mg/kg) after 6-10 weeks of exposure. However, Burdick et al. (1968) could find no residues in snails when

methoxychlor was applied to ponds at 0.005 mg/litre. Kapoor et al. (1970) concluded that methoxychlor was stored in snails (Physa) in large quantities and that this organism appeared unable to metabolize it rapidly. When exposed to 0.003 mg/litre of DDT and methoxychor in the lab, snails (Physa) concentrated methoxychlor much more than DDT (18 mg/litre of DDT vs. 38 mg/litre methoxychlor after 6 days). When placed in water free of insecticide, the snails excreted DDT more rapidly than methoxychor.

Butler (1971) examined the differential uptake after a 5-day exposure to methoxychlor (1.0 ug/litre) by the soft clam Mya arenaria Linne and the hard clam, M. mercenaria. Methoxychlor was initially concentrated 1500 times and 470 times by the soft and hard clams, respectively. Upon removal to clean water, residues dropped below detectable levels in 7 days in the soft clam and in 14 days in the hard clam (Gardner and Bailey 1975). Hansen et al. (1972) determined the LC_{50}'s for Physa and Lymnaea to be 5.5 and 8.5 mg/litre, respectively. Estimated pesticide breakdown rates in both Physa and Lymnaea were reported to be much lower than in the mosquito fish, Gambusia. Consequently it was concluded that snails would tend to accumulate methoxychlor instead of metabolizing it to polar compounds. Fredeen (1975) detected no methoxychlor residues in mussels collected in riverbed sand 21-22 km downstream from a 15-min injection of 0.309 mg/litre methoxychlor into the

Saskatchewan River. Sullivan and Atchison (1977) found no methoxychlor residues in snails (Physa) sampled from the Rouge River, Michigan, after a spray program for Dutch elm disease.

No mortalities attributable to the methoxychlor treatment were observed in caged clams (Lampsilis radiata Gmelin) in the Athabasca River (Flannagan et al. 1979). Methoxychlor was concentrated to about 3 times the treatment level (0.3 mg/litre) by L. radiata. Methoxychlor residues returned to trace or undetectable levels within 23 days after treatment.

The snail Physa integra Haldeman did not die at the highest concentration of methoxychlor tested (4.2 ug/litre) during a 28-day exposure period in a flowing-water test system (Anderson and DeFoe 1980). Snail bioconcentration factors for methoxychlor ranged from 5,000 to 8,570.

Methoxychlor appears to be less toxic to molluscs than to other aquatic invertebrates. Molluscs generally concentrate methoxychlor to extremely high levels with little apparent effect. The high concentrations observed in many species of molluscs seem to result from a low ability to metabolize methoxychlor by these organisms.

2.5 THE TOXICITY AND SUBLETHAL EFFECTS OF METHOXYCHLOR ON INSECT EGGS

There have been very few studies to examine the toxicity of methoxychlor to insect eggs. Friesen (1979) exposed eggs of the burrowing mayfly Hexagenia rigida McDunnough to methoxychlor in Petri dishes at 24±2° C. "Early stage" eggs were exposed to concentrations of 0.06 to 0.7 mg/litre for 10.5 days using river water or reconstituted water. In addition, "mid-stage" eggs were exposed to similar concentrations using reconstituted water only. Effects ranged from partial suppression of hatch at the lowest concentration to total suppression at the highest. LC_{50} values were estimated to be 0.12 mg/litre in Red River water and <0.06 mg/litre in reconstituted water. The most sensitive period to methoxychlor occurred in advanced stages of development. It was suggested that the chemical (which acts on the nervous system) had no "target" tissue until the nervous system of the embryo became developed. The estimated LC_{50}'s are lower than maximum levels (0.3 mg/litre to 0.4 mg/litre) used in black fly larviciding operations. Thus, possible adverse effects of methoxychlor on the egg stage cannot be eliminated.

Belluck and Felsot (1981) examined the bioconcentration of methoxychlor by egg masses of the caddisfly, Triaenodes tardus Milne by exposing them for 24, 72, 96, and 120 h to 100 ug/litre of the pesticide. Methoxychlor continued to

accumulate in the egg masses over the 120-h observation period. The bioconcentration factor was approximately 80 times after 120 h, and the mean concentration was about 2,400 ug/litre. It was concluded that passive biological systems such as caddisfly eggs could accumulate significant quantities of pesticide from water, and that the bioconcentration factor was correlated with physicochemical properties of the pesticide such as water solubility.

2.6 RESIDUES AND DEGRADATION OF METHOXYCHLOR IN AQUATIC INSECTS

Research has once again been very limited with regard to residues and degradation of methoxychlor in aquatic insects. Burdick et al. (1968) stated that aquatic insects were apparently able to metabolize methoxychlor by degradation of the methyl-phenyl-ether linkages to form phenolic derivatives which were eliminated. This was supported by the fact that no methoxychlor was detected in dragonfly larvae in a pond 36 days after application. Kapoor et al. (1970) observed that methoxychlor was detoxified in the house fly by multifunction-oxidase activity. It was concluded that methoxychlor was selectively insecticidal because it was much less rapidly degraded by mixed-function oxidase activity in the insect than in the mammal. Kruzynski (1972) determined residues in caddisfly and stonefly larvae exposed to a concentration of 0.075 mg/litre methoxychlor for 15 min. Organisms collected 24 h after the

initial exposure contained concentrations ranging from 1.5 to 3.0 ug/g.

No methoxychlor residues were detected in the larvae of Odonata or Trichoptera collected alive from the river bed either 6-7 days before or 8-9 days after treatment of the Saskatchewan River with methoxychlor at a concentration of 0.309 mg/litre/15 min (Fredeen 1975). However, larvae of these and other families of aquatic insects, including Plecoptera, Ephemeroptera, and Diptera, that were disabled by methoxychlor during the actual passage of injected water, contained an average of 17.5 ug/g methoxychlor.

Flannagan et al. (1979) collected live benthic invertebrates at four stations up to 400 km downstream from an injection of 0.3 mg/litre/15 min methoxychlor into the Athabasca River. Methoxychlor was concentrated to a maximum of 33 times above the treatment level. This concentration factor was very similar among all the free-living taxa sampled, regardless of trophic level, and decreased with distance downstream. The Ephemeroptera appeared to concentrate the insecticide faster than the Trichoptera and Plecoptera because methoxychlor levels in the first post-treatment mayfly samples were an order of magnitude higher than those in the caddis- or stonefies. In all cases, the methoxychlor residues returned to trace or undetectable levels within 23 days after treatment.

Methoxychlor concentrations in stoneflies (P. dorsata) reached a maximum of 1.46 ug/g at a concentration of 4.2 ug/litre in the water after exposure for 28 days in a flowing-water test system (Anderson and DeFoe 1980). The concentration factor for the stonefly ranged from 348 to 1130 with a mean of 573 and decreased with increasing water concentration.

Aquatic insects accumulate methoxychlor to levels above the concentrations used in black fly larviciding operations. Presumably fish or other predators that eat insects disabled or killed by such a treatment would be exposed to relatively high concentrations. This could represent an alternate route of exposure to the pesticide by these organisms with the potential to produce delayed toxic effects as suggested by (Wallace et al. 1973).

2.7 THE INFLUENCE OF FORMULATION ON THE SELECTIVITY OF METHOXYCHLOR

Wallace (1971) compared the effects on invertebrate stream fauna of methoxychlor in oil solution as an emulsifiable concentrate (EC), to formulations of methoxychlor-treated clay particles. Ground applications of 0.075 mg/litre methoxychlor were administered for 15 min. All major orders of immature stream insects were injured after treatment with the methoxychlor EC. The ranges of numbers of three orders caught in post-treatment drift net

samples from three streams were: 675-7,906 Ephemeroptera, 8,271-36,000 Plecoptera, and 928-3,247 Trichoptera. In comparison, ranges of 55-2,511 Ephemeroptera, 275-1,026 Plecoptera, and 55-5,335 Trichoptera were obtained from three streams treated with methoxychlor-impregnated clay particles.

The drift of Ephemeroptera naiads was generally lower in streams treated with methoxychlor-treated clay particles than in streams treated with the EC. Baetid naiads were the most numerous mayflies in the drift nets of the streams treated with methoxychlor-impregnated clay particles.

Fewer Plecoptera naiads were collected after treatment with methoxychlor-treated particulates than after treatment with EC methoxychlor. Of those collected, only Leuctridae naiads were present in high numbers in the drift net samples in the streams treated with particulates whereas Perlodidae and Nemouridae naiads were most abundant in the streams treated with the EC.

The overall numbers of drifting Trichoptera larvae were comparable between the two formulations, however, only the numbers of Philopotamidae larvae increased after application with particulate methoxychlor, whereas several families of caddisflies were numerous in the drift nets following treatment with the EC.

Helson (1971) and Helson and West (1978) treated several streams in the vicinity of Baie Comeau Quebec with methoxychlor-treated celite particles which were metered into streams at a dosage rate of 0.1 mg/litre/15 min. Surber and drift net samples indicated that these treatments were less harmful to many species of non-target invertebrates when compared to similar treatments using an EC formulation of methoxychlor. However, Philopotamidae and Chironomidae larvae in some streams were harmed severely by methoxychlor-treated celite particles. Some members of these groups are filter-feeding organisms. They collect particles of a size range corresponding to the insecticide-treated particles, and thus may have ingested the chemical inadvertently. Organisms such as Baetidae, Heptageniidae, Leuctridae and Nemouridae naiads, Elmidae larvae and adults, and Dytiscidae larvae were also slightly affected (increased drift and lower bottom densities) by the particulate formulation. Most of these taxa, excluding Dytiscidae larvae, are reported to be herbivores or detritus foragers that feed on material of small size. The insecticide-treated particles had a specific gravity heavier than water and some of the material probably settled out on the stream bottom in areas of sedimentation of detritus and silt (Egglishaw 1964). Subsequently, these organisms may have ingested enough insecticide-treated particles to be harmed and swept downstream.

Black fly larvae exposed in the lab to particulate formulations (methoxychlor-impregnated celite particles) and ethanol solutions of methoxychlor at concentrations of 0.075-0.1 mg/litre/15-30 min, concentrated the particulate preparation in much greater amounts (Wallace et al. 1976). The larvae concentrated the ethanol formulation to levels ranging from 82 to 688 ug/kg, and the particulate formulation to 1556-2310 ug/kg. However, accumulations in Trichoptera were greater with the ethanol formulation (1453-1563 ug/kg) than with the particulate one (615-782 ug/kg). This result may be due to adsorption of the ethanol formulation through the large gill surface area of the Trichpotera larvae while the majority of the particulate formulation was screened out.

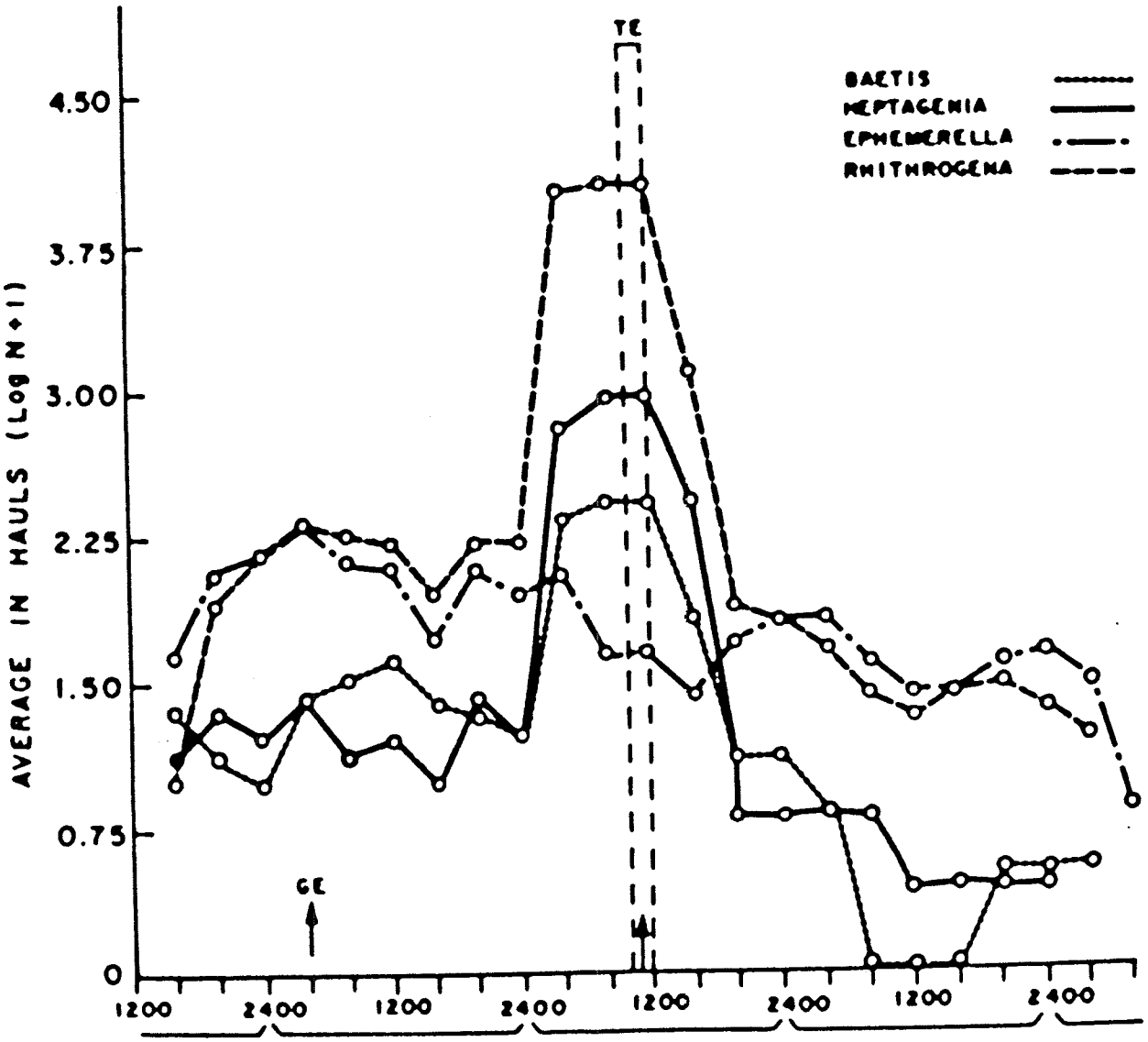
Sebastien and Lockhart (1981) compared the toxicity and availability to aquatic organisms of two formulations of methoxychlor. After 24 h exposure to 0.3 mg/litre EC, stonefly nymphs (P. dorsata) were all moribund, but only 25% were similarly affected when exposed to a particulate formulation (methoxychlor-impregnated celite particles). After exposure for 1 and 12 h to the EC formulation at a concentration of 0.3 mg/litre, stonefly nymphs of the species Acroneuria lycorias (Newman) accumulated about 6-10 times as much methoxychlor as nymphs exposed to the particulate material for the same time periods. P. dorsata nymphs under these conditions accumulated twice as much of the EC formulation as the particulate formulation.

About 98% of Chironomus tentans Fabricius larvae left their burrows and showed a continuous wiggling motion when exposed to the EC formulation at a level of 0.1 mg/litre after 96 h, whereas only 55% of the larvae exposed to the particulate formulation displayed this type of response (Sebastien and Lockhart 1981). There was also 98% mortality in larvae exposed to the EC formulation as compared with 21% mortality with the particulate formulation. All larvae abandoned their burrows after 96 h in the EC exposure and 87% had done so after exposure to the particulate formulation at a level of 0.3 mg/litre. In this experiment, there was 99% mortality in larvae exposed to the EC formulation and 36% in larvae exposed to the particulate formulation. Chironomid larvae exposed to methoxychlor at 0.1 and 0.3 mg/litre, accumulated significantly higher levels from the EC formulation than from the particulate formulation throughout the duration of the experiment (96 h). The two formulations had equal toxicity to black fly larvae, the only filter-feeding animal used, but the particulate formulation was accumulated more readily than the EC. Both formulations induced larvae to detach from the substrate but the EC acted more quickly. At the time of detachment, larvae exposed to the particulate formulation contained an average of 68 times more methoxychlor than larvae exposed to the EC. It was concluded that black fly larvae and other filter feeders should be selectively poisoned by particulates in the size classes most

effectively retained by feeding activities. The effectiveness and selectivity of particulate formulations might be improved by experimenting with parameters such as dosage, particle size, specific gravity, type of carrier particle, or combinations of these.

Theoretically, methoxychlor applied as water insoluble particles would have to be ingested to be toxic and should only selectively poison filter feeders. Animals belonging to other functional feeding groups such as predators and scrapers should not be affected because they would have no way of directly taking up the pesticide. Although the impact on non-target organisms other than filter feeders is reduced by the use of particulate formulations compared to EC formulations, it is not totally eliminated.. Ingestion of methoxychlor-treated particles after settling out by organisms feeding on small particles (e.g. scrapers), may explain the observed toxicity to these organisms. Incomplete adsorption of the pesticide onto the carrier particle leading to direct uptake from the water may account for the impact on other groups including predators. Clearly, particulate formulations are not a panacea for completely eliminating the effects of methoxychlor treatments on non-target organisms.

Figure 1. Methoxychlor addition of Haufe et al. (1980) to Athabasca River. Average catch of Ephemeroptera taxa with time, 18-22 May 1976, from the downstream drift in the thalweg. Data shown as running three-interval means at Pelican Rapids. Toxic exposure to the methoxychlor pulse indicated by TE.


TE
BAETIS
HEPTAGENIA
EPHEMERELLA
RHITHROGENA
AVERAGE IN HAULS (Log N+1)
4.50
3.75
3.00
2.25
1.50
0.75
0
GE
1200
2400
1200
2400
1200
2400
1200
2400

Chapter III

MATERIALS AND METHODS

3.1 STUDY AREA AND SITES

The Souris River enters Manitoba from the United States 6.58 km southwest of Lyleton, Manitoba (101°15'W, 49°00'N). The river follows a northeast path through southwestern Manitoba, passing through or near the towns of Coulter, Melita, Hartney, Souris, Wawanesa and Treesbank before entering the Assiniboine River 4.1 km north of Treesbank (99°36'W, 49°40'N). The Souris is a typical prairie waterway, deriving most of its annual flow from snowmelt and early spring rains. It is characterized by a fairly slow current, following a meandering course, with occasional turbulent (riffle) sections. The water usually contains a high concentration of suspended solids and there is very little emergent vegetation. Temperatures rise dramatically during the summer months, often exceeding 26° C during mid-July. The depth of the Souris River is quite variable, depending on the location and time of year. Depths range anywhere from 30 to 400 cm with an average of approximately 80-100 cm. Flow rates are to some degree controlled by the presence of dams along its course. Dams at Melita, Hartney, Souris and Wawanesa regulate water flow, except in spring

when severe flooding can occur and flood waters cover the dams. In 1976, major flooding occurred, and abnormally high peak flows in May reached 285.0 cubic meters per second (m^3/sec), while the minimum discharge was 0.03 m^3/sec in October. Discharge levels vary greatly in different localities, and from year to year (Environment Canada).

Two riffle areas, designated as the "control" and "treatment" sites, were selected for the study (Fig. 2). The control site was located about 450 m north of the bridge on Provincial Road #348 at Bunclody, Manitoba. The site was a shallow riffle (mean depth 50 cm) that was 34-43 m wide and 65 m long. The substrate consisted of cobble-sized stones and large boulders. The treatment site was located about 450 m south of the bridge (approximately 900 m downstream from the control riffle site). It was also a riffle (mean depth 50 cm) that was 32-45 m wide and 140 m long. The substrate was very similar to the control site. Preliminary sampling indicated that the species of aquatic invertebrates inhabiting both sites were similar. The river area separating the two riffle sites was quite deep (up to 3 m), with a slower more uniform flow. Substrate there consisted of a sandy-clay bottom with a few large boulders.

3.2 EXPERIMENTAL APPLICATION OF METHOXYCHLOR

Methoxychlor used for the experimental application was an emulsifiable concentrate formulation (240 g active ingredient per litre), registration no. 11590 P.C.P. act, manufactured by Chipman Inc., Stoney Creek, Ontario. The injection was made on 15 July 1982 at 1200 h at the head of the treatment riffle site. Four litres of methoxychlor (amt. calculated to give a theoretical dosage of 0.3 mg/litre for 15 min based on the river discharge) were thoroughly mixed with 92 litres of river water that had been filtered through a fine mesh screen into a 200-litre drum. A rotary hand crank pump was used to pump the chemical mixture through a length of garden hose which was divided, after 20 m into two 10-m sections. Each 10-m portion of the hose was submerged below the surface and manually moved back and forth across half the width of the river just upstream of the treatment riffle to ensure complete exposure of the riffle to the chemical. The injection required 15.5 min.

3.3 WATER CHEMISTRY AND DISCHARGE

Water samples for chemical analysis were collected in 1000 ml amber glass jars. Approximately 900 ml of water was sampled from the centre of both the control and treatment riffle sites during each sampling period. Samples were collected by submerging the jar just above the substrate. Immediately after collection, 1 ml of saturated mercuric

chloride solution was added to each jar to prevent microbial degradation. The jars were then placed in an ice cooler for transportation to the laboratory where they were stored at 5° C until analyzed. Samples for water chemistry analysis were collected on 08, 15, 22, 29 July, 17 August and 16 September 1982. Analysis included pH, total suspended solids, suspended carbon and suspended nitrogen. Analyses were performed by the Analytical Chemistry Unit, Water Chemistry Laboratory at the Freshwater Institute, Winnipeg. In addition water temperatures (° C) were recorded at mid day at both sites throughout the study.

Daily discharge measurements (m^3/sec) for 1982 and 1983 were obtained for the Souris River at Souris, Manitoba (about 18 km upstream from the study sites) from Environment Canada. A discharge measurement was made at the site of methoxychlor injection on 14 July 1982 in order to calculate the amount of chemical required to achieve the desired dosage.

3.4 METHOXYCHLOR RESIDUES IN RIVER WATER

Water samples for methoxychlor residue analysis were collected in 1000 ml amber glass jars. Three replicate water samples were taken at both the control and treatment riffle sites at each sample period. Replicate samples were removed from a transect across each riffle site, one from the centre, and the remaining two from the north and south

sides. The sampling procedure involved submerging the jar to obtain a sample of water (900 ml) just above the substrate. Dichloromethane (50 ml) was added immediately to each sample jar to ensure a complete extraction of methoxychlor from the water sample. The jar was shaken repeatedly and then placed on a magnetic stirrer for 5 min to ensure a complete mixing of the solvent with the water sample. Samples were placed in an ice cooler for transportation to the laboratory where they were stored at 5° C until analyzed.

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

In the laboratory the procedures for the extraction, clean-up, and injection of the water samples into a gas chromatograph for residue analysis were as follows: 1. The water sample was poured into a two-litre separation funnel, and the phases allowed to separate. 2. The dichloromethane phase was drained off and passed through a column of sodium sulfate (2.5 cm thick) into a 500 ml flat-bottom flask to

remove any water from the sample. 3. The glass collecting jar was re-extracted with 50 ml of dichloromethane and shaken for one minute. The contents were then poured into the two-litre separation funnel. A further 50 ml of dichloromethane were added to the separation funnel and the entire contents shaken for one minute. The phases were allowed to separate and the dichloromethane phase was again drained off through a sodium sulfate column (2.5 cm thick) into the 500 ml flat-bottom flask. 4. The contents of the flask were transferred to a 250 ml round- bottomed flask. The flat-bottom flask was re-extracted with two additional 10-ml washings of dichloromethane and the contents added to the round-bottomed flask. The dichloromethane in the round-bottomed flask was evaporated just to dryness on a roto-evaporator. Iso-octane (0.2 ml) was added to the flask and the contents re-evaporated just to dryness. 5. Three 1-ml rinsings of hexane:ethyl acetate (1:1) were used to transfer the residue in the flask to a teflon-capped test-tube, which was stored at 5° C. 6. The contents of the test-tube were evaporated just to dryness and 1 ml of hexane:ethyl acetate (95:5) added. This mixture was then pipeted into a 5 ml glass syringe with the plunger removed. The plunger was inserted and the sample was injected onto a silica sep-pak column attached to the bottom of the syringe. The plunger was again removed and 5 ml of hexane:ethyl acetate (95:5) were added and eluted through the silica sep-pak column. The first 2 ml were discarded (contaminants

but no methoxychlor) while the remaining 3 ml were collected in the original test-tube. This cleaned-up sample was evaporated to the appropriate volume (1 ml) and 2 ul were injected, along with a technical methoxychlor standard, into a Tracor Gas Chromatograph for analysis.

3.5 DRIFT STUDIES

Studies concerned with methoxychlor additions to lotic aquatic ecosystems have indicated that benthic invertebrates that are disturbed by the pesticide will leave the substrate and enter the water column. Catastrophic drift, for the purposes of this thesis, is defined as a significant change in the time of day of maximum drift or an increase of more than an order of magnitude in drift densities related to the treatment. Catastrophic drift of organisms has been observed after the addition of methoxychlor to streams and rivers in North America for the purpose of black fly control (Burdick et al. 1968, Wallace et al. 1973, Wallace and Hynes 1975, Flannagan et al. 1979, Lehmkuhl 1981). All of these researchers have measured drift for only a few days prior to, and following treatment, and no sampling was conducted at any later dates. It could be hypothesized that if bottom densities were reduced by the treatment in a well-defined habitat such as a riffle, drift densities leaving the riffle would also be reduced. Recovery of benthic densities would eventually also result in an increase in drift densities to

pre-treatment levels. The drift sampling schedule in the present study was designed to test this hypothesis. Drift samples were collected at regular intervals prior to, and for about a month following methoxychlor injection, and then again the following year. Benthic samples (artificial substrates) were taken in conjunction with the drift samples in an attempt to establish the relationship between benthic density and drift density following a methoxychlor treatment.

Drift is a major source of recolonization for invertebrates inhabiting lotic ecosystems (Williams and Hynes 1976; Townsend and Hildrew 1976). Therefore, drift was measured at a site just upstream of the methoxychlor injection (treatment-inlet site - see below) to determine its importance in the recovery of benthic densities of invertebrates in the riffle immediately downstream. Head capsules were measured on a random sample of 30 larvae of selected species from drift samplers at the treatment-inlet site, and artificial substrates in the treatment riffle, to compare size distributions. Similar species size distributions would indicate if drift was a significant contributing factor to benthic recolonization.

Modified Burton-Flannagan bomb drift samplers (Burton and Flannagan 1976) equipped with 500-um Nitex mesh nets were used to monitor changes in the numbers of drifting aquatic organisms. This sampler has proven to be much more

efficient in the sampling of invertebrate drift than conventional conical drift net samplers (Burton and Flannagan 1976; Flannagan et al. 1979). Modifications involved elimination of stabilizing fins used in deep water and attachment of two iron support rods on either side of the steel cone that could be driven into the river substrate to secure the sampler in position.

Three sets of three drift samplers were positioned along the river. One set located immediately downstream of the control-site riffle, was termed control and sampled the drift leaving the control riffle (Fig. 3). A second set placed just upstream of the treatment site riffle and the point of methoxychlor application was termed treatment-inlet (Fig. 3) and measured the drift entering the treatment riffle (see above). A third set of three drift samplers placed immediately downstream of the treatment-site riffle, was termed treatment-outlet and measured the drift leaving the treatment riffle. The three drift samplers at each site were positioned at mid-depth, evenly spaced across the river.

On each sampling date, drift samples were collected at four-h intervals beginning at 0800 h and continuing for a period of 24 h. This schedule was slightly modified on the treatment date (15 July 1982) at the treatment-outlet site in order to effectively sample large numbers of invertebrates induced to drift by the methoxychlor

treatment. The sampling schedule at the treatment-outlet site on 15 July 1982 was as follows: set at 0800 h, sampled at 1200 h, 1300 h, 1400 h, 1600 h, 2000 h, 2400 h, 0400 h, and 0800 h. Drift samples were collected on the following dates: 07 July 1982 (8 days pre-treatment), 14 July 1982 (1 day pre-treatment), 15 July 1982 (treatment), 21 July 1982 (6 days post-treatment), 29 July 1982 (14 days post-treatment), 17 August 1982 (33 days post-treatment), and 03 August 1983 (384 days post-treatment). All drift samples were immediately preserved in 10% formalin. Rose Bengal was added to each sample to aid sorting efficiency in the laboratory.

Water-velocity measurements were made directly upstream of the opening of each drift sampler using a Gurley model 622 current meter. Readings were recorded at the beginning and end of each 24-h sampling period, and were used to calculate a mean water velocity value for the sampling period. Drift rates (no./4-h period) were standardized to drift densities (no./100 m^3), using the mean water velocity data, to allow comparison between samplers and sites (Elliott 1970). Times of sunrise and sunset were also recorded for each drift sampling date.

Drift samples were sub-sampled in the laboratory using the % weight technique of Sebastien et al. (1986) (Appendix B). Aquatic organisms from subsamples were sorted and identified under 10 times magnification. Three taxa,

Catostomus commersoni Lacepede, Acroneuria lycorias (Newman) and Orconectes virilis (Hagen) were not sub-sampled but were sorted and identified from the entire drift sample due to their low numbers and large size.

3.6 BENTHIC STUDIES (ARTIFICIAL SUBSTRATES)

In river systems where methoxychlor stress produces increased drift of aquatic organisms, large stretches of the river may become depleted of their benthic populations. Benthic recolonization of a depleted section of a river is a commonly used indicator of the ability of a system to recover. The recolonization of benthic aquatic invertebrates in this study was measured by the use of artificial substrates. Very coarse substrate in the experimental riffle sites made quantitative sampling of the natural substrate difficult if not impossible. The artificial substrates were selected to be representative of the type of substrate found in the riffle sites. Although benthic populations colonizing them cannot be truly quantitatively related to natural river bed populations, the artificial substrates were placed in the natural substrate thus providing a representative sample of the surface benthic populations. In monitoring any system to assess impact, it is not sufficient to detect only short-term changes. Therefore, artificial substrates were sampled at regular intervals prior to, and for up to a year following

treatment to obtain a representative indication of recolonization and recovery in the benthos.

Concrete paving stones (Trieste design) made by Genstar Materials Limited, Manitoba Region were used as artificial substrates for benthic invertebrates (Fig. 4). The unique shape of the bricks made them easy to locate in the river when dense growths of filamentous algae covered the natural substrate during the summer months. A 15x10 grid of substrates was placed in the areas of greatest flow in both the control and treatment riffle sites on 8 and 9 June 1982 (Fig. 3). This was four weeks prior to the first sampling date to allow for invertebrate colonization. The bricks were positioned among the natural substrates, approximately one m apart. Ten bricks were removed from each site on each sampling date. Samples were always taken from the row of bricks located furthest downstream on each succeeding date. A large rectangular, long-handled, net (45x20x45 mm) equipped with a 500-um Nitex mesh net was held immediately downstream of the brick to be sampled. The brick was rapidly removed from the river bottom and placed in a 12-litre plastic pail. The net was left in place for approximately 30 sec to catch any invertebrates that had become dislodged from the brick during removal. Any invertebrates found in the net were removed with forceps and placed in the pail with the rest of the sample. Ethyl alcohol (75%) was used to preserve the samples which were

then returned to the laboratory. Sorting was done in white enamel trays, and sorted specimens were placed in vials containing 75% ethyl alcohol for later identification.

Artificial substrates were sampled in 1982 on the following dates: 07 July (8 days pre-treatment), 12 July (3 days pre-treatment), 14 July (1 day pre-treatment), 16 July (1 day post-treatment), 19 July (4 days post-treatment), 23 July (8 days post-treatment), 29 July (14 days post-treatment), 16 August (32 days post-treatment), and 17 September (64 days post-treatment).

Another grid of substrates (15x3) was placed in both riffle sites in 1983 to allow a second year of observation. Substrates were installed on 21 June and sampled on 18 July (368 days post-treatment) and 03 August (384 days post-treatment).

3.7 INSECT EMERGENCE

A reduction in numbers of insects in benthic samples collected after a methoxychlor treatment could result from natural emergence rather than from lethal effects of the pesticide. Reductions in benthic densities at treatment sites in studies involving methoxychlor additions to the Athabasca River were thought to be due in part to natural emergence because of simultaneous reductions at control sites. However, because emergence was not sampled, this

could not be definitely proven. Emergence sampling can also be used to determine what stage the insect is in during the treatment period which may help to explain the effect, or lack of effect, on a particular species. A portion of the species emerging prior to, or during a treatment, would be in the adult terrestrial stage and thus not directly exposed to the pesticide in the river. Recovery could occur in part via egg-laying adults in such cases. In the present study, head capsules were measured on a random sample of 30 larvae of selected species from artificial substrates at both the control and treatment sites on various sampling dates prior to, and following methoxychlor treatment. The resulting size distributions at both sites were compared to determine if recolonization had occurred via egg-laying adults from a previous emergence.

A final reason for sampling insect emergence is that adult specimens can be used to confirm species-level identifications of immatures. Identification of immature stages to the species level is often difficult or impossible, due to the lack of taxonomic keys. A major deficiency of many studies examining the impact of methoxychlor on aquatic invertebrates is that organisms were identified only to the level of genus or higher, thus masking effects at the species level. Interpreting population change requires a knowledge of the ecology of individual species. Whether changes observed are due to

life-history events, environmental heterogeneity and dispersion patterns in the population, artifacts of the sampling protocol, or result directly from the process of concern can only be ascertained by a detailed knowledge of the life history and ecology of the species under study. Identification to the family or generic level is not adequate, because examples can be found in all groups in which species of the same genus or family respond differently to environmental perturbation. Thus studies based on identifications above the species level often have little value in the ecological appraisal of disturbance.

Emergence traps were pyramidal in shape, 1 m^2 at the base, 55 cm high at the apex and fitted with a 500-um Nitex mesh net. A removable glass collecting bottle was fixed over a 5-cm diameter hole at the apex. Polystyrene floats, 13 cm wide and 15 cm deep, were placed around the base of each trap. The trap was secured by a 6-mm nylon cord fixed 3 m upstream to a 1.5-m steel pole. The pole was embedded into the riverbed, allowing the trap to swing with the river current in an approximate 30° arc (Westwood and Brust 1981).

Three emergence traps were placed in both the control and treatment riffles (Fig. 3), and were emptied at various dates before and up to one month following methoxychlor injection. The traps were sampled after a period of 48 h on the following dates: 08 July 1982, 14 July 1982, 17 July 1982, 19 July 1982, 21 July 1982, 28 July 1982, and 18

August 1982. Adult insects were aspirated from the inside of the collection jar, and the inside of the entire trap after slowly raising the trap above the water. Specimens were placed in vials containing 75% ethyl alcohol for later identification in the laboratory.

3.8 STATISTICAL ANALYSES

The effects of methoxychlor on drift densities, numbers on substrates, and numbers emerging, between the control and treatment sites for both individual and total species, were tested using two-way analyses of variance (ANOVA) (Snedecor and Cochran 1981). A total drift density for individual taxa for each 24 h drift sample period was obtained by summing the drift densities (no./100 m^3) observed in each of the six, four-h sampling intervals on each sampling date. Taylor's power law was used to select the proper transformation to remove the dependence of the variance on the mean observed for total drift densities, numbers on substrates, and numbers emerging for individual taxa (Taylor 1961). In all cases a ln(x+1) transformation was indicated. Hydroptila ajax Ross and Cheumatopsyche campyla Ross were the only two species emerging in large enough numbers for statistical analysis on the emergence data.

The two-way ANOVA'S used a split-plot design blocked by sample date. The interaction term was partitioned using a priori multiple comparisons to compare the difference

between the combined control and treatment site means prior to treatment with the difference between the control and treatment site means on each post-treatment sampling date (Snedecor and Cochran 1981). The comparison-wise error rate was adjusted using Sidak's multiplicative inequality index to maintain the experiment-wise error rate at $P<0.05$ (Miller 1978).

The riffle community response to the methoxychlor injection was measured for both the drift and substrates using three diversity indices (species richness, modified Simpson and Shannon-Weaver). Species richness (S) is the "only truly objective measure of diversity" according to Poole (1974, p. 387). The modified Simpson Index (1-Simpson) is an estimate of the probability that two randomly selected individuals will not belong to the same species. It is mostly sensitive to evenness (distribution of individuals among species) and not species richness. The index gives little weight to rare species. The Shannon-Weaver Index (H') combines both species richness and species evenness. The index is most strongly affected by the abundances of the "middle" species in a community, rather than by the common or rare species (Poole 1974). H', 1-Simpson and S diversity indices were calculated for the drift and artificial substrate data. Taylor's power law indicated that the variance was independent of the mean in all cases and no transformation was necessary for

statistical analysis. The same analyses as described above were used to test for differences in drift diversity and diversity on substrates between the control and treatment sites.

In a natural ecosystem such as a river, it is very difficult if not impossible to find two sites in which species abundance or species composition are completely similar. In many studies involving the impact of methoxychlor on non-target organisms in large rivers in Western Canada, the criterion for recovery was based on invertebrate populations returning to control-site levels. However, in practically all cases there was no analysis to compare the abundance of different taxa at the control and treatment sites prior to treatment (no baseline data). Therefore, it was not known how similar these communities were, and any conclusions based on populations returning to control-site levels are questionable. In this study, differences between the control and treatment sites prior to treatment for the various parameters being tested were accommodated by using the comparisons outlined above. The null hypothesis for these comparisons was Ho: the methoxychlor treatment does not affect the observed pre-treatment differences between the control and treatment sites for drift density, numbers on substrates, numbers emerging, or species diversity. This analysis, therefore, gives a much more accurate indication of the effect of

methoxychlor on invertebrates at a treatment site when compared to a control site because it takes into account the difference between the control and treatment sites prior to treatment.

Figure 2. The study area showing location of the control and treatment riffle sites.

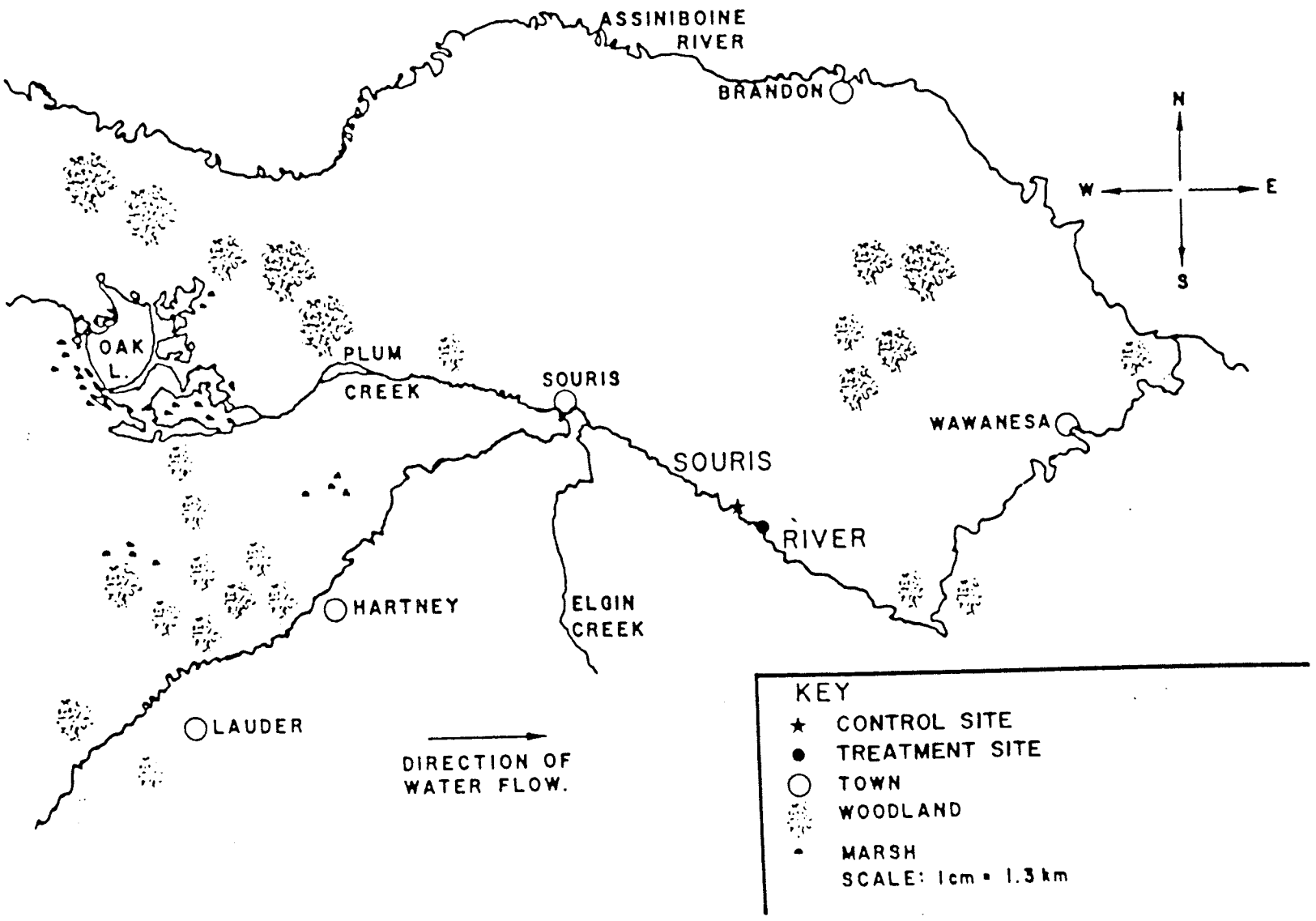

ASSINIBOINE
RIVER
BRANDON
N
W
E
S
OAK
L.
PLUM
CREEK
SOURIS
WAWANESA
SOURIS
RIVER
HARTNEY
ELGIN
CREEK
LAUDER
DIRECTION OF
WATER FLOW.
KEY
CONTROL SITE
TREATMENT SITE
TOWN
WOODLAND
MARSH
SCALE: 1cm = 1.3 km

Figure 3. Diagram of the experimental design used at the study riffle sites showing drift, emergence trap, and artificial substrate samplers. The actual distance from the beginning of the control riffle to the end of the treatment riffle is approximately 1 km.

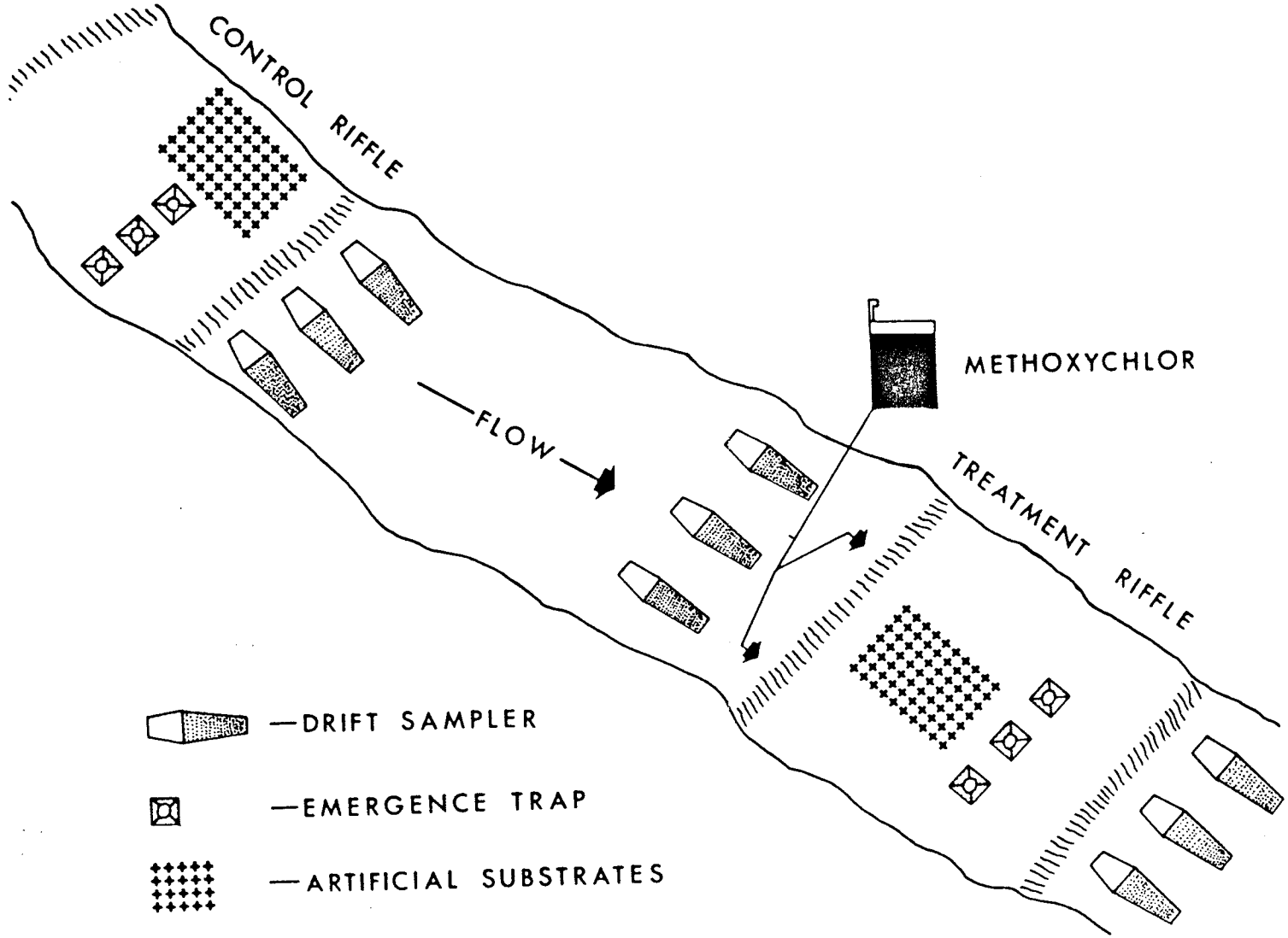

CONTROL RIFFLE
METHOXYCHLOR
FLOW
TREATMENT RIFFLE
—DRIFT SAMPLER
—EMERGENCE TRAP
—ARTIFICIAL SUBSTRATES

Figure 4. Concrete paving stone (Trieste design), made by Genstar Materials Limited, used as an artificial substrate.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Chapter IV

RESULTS

4.1 WATER CHEMISTRY AND DISCHARGE

Water temperatures (recorded at mid-day) reached a maximum of 25.0° C on 15 July (date of methoxychlor treatment) at the control and treatment sampling sites, and declined steadily to 12.5° C on 16 September (Table 1). pH readings fluctuated over the entire sample period but were quite similar between sites on each sampling date (Table 1). Similarly, suspended carbon, suspended nitrogen, and total suspended solid concentrations changed over the sample period, however, observed values were comparable between sites for the majority of sampling dates. Any differences observed for these parameters between sites on certain dates may be due to natural variation because only one sample was collected from each site on each sample date for chemical analysis.

Peak discharges of 78-90 m^3/sec occurred in the spring and declined dramatically to levels below 10 m^3/sec by 01 July in both years (Fig. 5A,B). The actual discharge at the injection site on 15 July 1982 (date of methoxychlor treatment) was 3.32 m^3/sec.

4.2 METHOXYCHLOR RESIDUES IN RIVER WATER

Methoxychlor residues in water following river treatment are listed in Table 2. No detectable methoxychlor residues were observed at the control site at any sampling period before or after methoxychlor injection (Table 2). Water samples taken during the treatment (10 min post-treatment) contained methoxychlor concentrations very close to the desired theoretical concentration of 300 ug/litre in the treatment riffle. Trace levels of methoxychlor were detected up to 12 h post-treatment in the treatment riffle, but no residues were detected by 24 h. A dense growth of filamentous algae probably retarded the passage of methoxychlor through the riffle, and could possibly account for the long duration (12 h) that methoxychlor was detected in the water column. Methoxychlor residues in water samples to which sodium azide was added (10 min and 1 h post-treatment samples) were similar to residues observed in samples collected during the same sampling periods which contained no sodium azide. Thus microbial degradation did not significantly alter the methoxychlor concentrations present in the water samples prior to analysis.

4.3 DRIFT STUDIES

Catastrophic drift

The methoxychlor treatment resulted in an immediate catastrophic drift in all of the observed species of aquatic invertebrates (Table 3). Drift densities at the treatment-outlet site were significantly greater ($p<.0001$) than the control site for all invertebrate taxa during the 24-h treatment period. Moreover, the treatment was not selective for any one functional feeding group. Predators (Acroneuria lycorias (Newman) and Polycentropus cinereus (Hagen)), scrapers (Baetis spp., Caenis tardata McDunnough, Leucrocuta maculipennis (Walsh), Hydroptila ajax Ross, Psychomyia flavida Hagen, and Stenacron interpunctatum (Say)), filter feeders (Cheumatopsyche campyla Ross, Hydropsyche recurvata Banks, and Isonychia sicca Walsh), and collector-gatherers (Ephoron album (Say)) were simultaneously affected. Total drift density and drift densities of the majority of the individual taxa were significantly depressed ($p<.05$) at the treatment-outlet site relative to the control site for all the subsequent sampling dates in 1982 including 17 August (33 days after treatment). Extremely low drift densities and high variability, or absence in the drift, probably prevented the detection of any statistical significant differences ($p<.05$) between the sites on dates other than the treatment date for P. flavida, P. cinereus, A. lycorias, and O. virilis. Drift

densities of only two species (I. sicca and E. album) remained significantly reduced ($p<.05$) at the treatment-outlet site relative to the control on 03 August 1983 (384 days post-treatment). However, low numbers observed in the drift for E. album on the sampling dates after treatment in 1982 and 1983 make any conclusions derived from the use of statistics questionable.

Individual taxa

The response of individual taxa to the methoxychlor treatment was separated into four categories based on propensity to drift under natural conditions (densities drifting into the treatment riffle at the treatment-inlet site) and the length of time required to recover on artificial substrates. The four categories are: 1) species naturally drifting in high numbers and having a very rapid recovery on artificial substrates (less than 10 days, e.g. Baetis spp., C. tardata and L. maculipennis). 2) species naturally drifting in very low numbers, or absent in the drift, and requiring a long period to recover on artificial substrates (longer than 64 days, and possibly up to one year following treatment, e.g. P. flavida and H. recurvata). 3) species naturally drifting in low numbers, or those emerging during the treatment period, and showing either no effect, or a moderately fast recovery on artificial substrates (less than 64 days, e.g. H. ajax and C. campyla). 4) species either absent in the drift or drifting in low numbers under

natural conditions, and not sampled effectively by the artificial substrates (numbers were very low or the species was completely absent). Treatment effects on benthic populations of these species were difficult or impossible to assess (e.g. E. album, I. sicca, S. interpunctatum, P. cinereus, A. lycorias, Hyalella azteca (Saussure), and O. virilis). The drift-density data for representative species from each of the first three categories are presented below. Drift-density data for the remaining invertebrate species and the Chironomidae (which were not identified beyond the family level) are included in Appendix A.

Group 1 species

Group 1 species included taxa that naturally drifted in high numbers and showed a very rapid recovery on artificial substrates (less than 10 days).

All members of the genus Baetis were designated Baetis spp. because the majority of specimens from drift samples were immature or damaged and this precluded species-level identifications. Individual species of the genus Baetis identified from drift and emergence samples included Baetis pygmaeus (Hagen), B. flavistriga McDunnough, B. brunneicolor McDunnough, B. propinquus (Walsh), B. pallidulus McDunnough, and B. intercalaris McDunnough.

Baetis mayflies drifted in high numbers under natural conditions and showed a diel drift periodicity with peaks

corresponding to periods of darkness (Fig. 6). High densities were observed drifting into the treatment riffle at the treatment-inlet site for all sampling dates in 1982. The treatment resulted in an immediate catastrophic drift peak which lasted for about four h and was significantly greater ($p<.0001$) than the control for the 24-h period on 15 July. The number of Baetis spp. drifting during the four-h period following treatment increased 1,914-fold relative to the control site. Total 24-h drift densities were significantly lower ($p<.05$) at the treatment-outlet site compared to the control site for all the post-treatment sampling dates in 1982 (Fig. 6). No significant differences ($p>.05$) were observed for 24-h drift densities between the two sites on 03 August 1983 the following year (Fig. 7).

Group 2 species

Group 2 species included taxa that naturally drifted in very low numbers, or that were absent in the drift, and required a long period to recover on artificial substrates (longer than 64 days, and possibly up to one year following treatment).

The caddisfly Psychomyia flavida rarely drifted under natural conditions (Fig. 8). The methoxychlor treatment on 15 July induced large numbers to leave the substrate and enter the water column. The drift density at the treatment-outlet site increased to a level 12,699 times that

of the control site in the four-h sampling period following injection. Elevated drift densities lasted for a period of about 24 h following treatment at the treatment-outlet site and it was significantly greater ($p<.0001$) than at the control site. Drift densities (24-h) were not significantly different ($p>.05$) between the control and treatment-outlet sites on all the post-treatment sampling dates in 1982 (Fig. 8), or the following summer on 03 August 1983 (Fig. 9). Extremely low drift densities probably prevented the detection of any statistical significant differences ($p<.05$) between the sites on other than the treatment date.

Group 3 species

Group 3 species included taxa which naturally drifted in low numbers, or that were emerging during the treatment period, and showed either no effect, or a moderately fast recovery on artificial substrates (less than 64 days).

The filter-feeding caddisfly *Cheumatopsyche campyla*, drifted in relatively low numbers under natural conditions at all three sites in 1982, but did show a diel drift pattern. Maximum numbers usually occurred during periods of darkness (Fig. 10). The methoxychlor injection caused a pronounced drift which lasted for a period of about 24 h and was significantly greater ($p<.0001$) than the control site. Drift densities increased 10,427-fold relative to the control site in the four-h period following treatment.

Total 24-h drift density was significantly lower ($p<.05$) at the treatment-outlet site compared to the control site on 21 July (6 days post-treatment), but no significant differences ($p>.05$) were found between sites for the remaining sampling dates in 1982, or the following year on 03 August 1983 (Fig. 11).

The caddisfly, Hydroptila ajax, drifted in relatively high densities at the control and treatment-outlet sites but very low densities were observed drifting at the treatment-inlet site (into the treatment riffle). The species showed no definite diel drift periodicity (Fig. 12). The methoxychlor treatment resulted in a catastrophic drift which remained in excess of drift densities at the control site for about eight h and was significantly greater ($p<.0001$) than the control site for the 24-h period following injection. Drift densities increased 104 times relative to the control site in the four h following treatment. Drift density (24-h) was significantly lower ($p<.05$) at the treatment-outlet site compared to the control on 21 July (6 days post-treatment) (Fig. 12). An unexplained increase in drift density occurred at both sites on 29 July which resulted in no significant difference ($p>.05$) for 24-h drift density between the two sites. Drift density (24-h) was again significantly lower ($p<.05$) at the treatment-outlet site relative to the control on 17 August (33 days post-treatment). The sites were not significantly

different ($p>.05$) for 24-h drift density on 03 August 1983 (Fig. 13).

Drift of sucker fry

Catostomus commersoni (Lacepede) fry, drifted under natural conditions in 1982, and exhibited a definite diel drift periodicity with maximum numbers occurring during periods of darkness (Fig. 14). Drift densities (24-h) were not significantly different ($p>.05$) between the control and treatment-outlet sites for any of the sampling dates in 1982 including the treatment date (15 July), or the following year on 03 August 1983 (Fig. 15). The methoxychlor treatment did produce an immediate discernible 3-fold increase in drift density of the fish fry at the treatment-outlet site relative to the control, and the increase lasted for a period of about four h. The absence of fry in the drift on the post-treatment sampling dates in 1982 at the treatment-outlet site relative to pre-treatment levels at that site and post-treatment levels at both the control and treatment-inlet sites, indicates a definite impact of the methoxychlor on fry inhabiting the treatment riffle (Fig. 14).

Community response

Species diversity in the drift at the control and treatment-outlet sites calculated using Species richness (S), modified Simpson (1-Simpson), and Shannon-Weaver (H') diversity indices are summarized in Table 4. The methoxychlor treatment resulted in a significantly higher ($p<.05$) diversity in the drift as measured by all three indices at the treatment-outlet site relative to the control site on the treatment date (15 July). Significant reductions ($p<.05$) in the number of species (species richness) found in the drift were observed at the treatment-outlet site compared to the control site for all the sampling dates after treatment in 1982 including 17 August (33 days post-treatment). Diversity in the drift as measured using Simpson and Shannon-Weaver diversity indices was only significantly reduced ($p<.05$) at the treatment-outlet site relative to the control site until 21 July (6 days post-treatment). Significant differences ($p<.05$) were not observed between the sites for the remaining sampling dates in 1982. Drift diversity measured using all three indices was not significantly different ($p>.05$) between sites the following year on 03 August 1983 (Table 4).

4.4 BENTHIC STUDIES (ARTIFICIAL SUBSTRATES)

Individual taxa

Mean numbers of the 12 taxa colonizing substrates at the control and treatment sites for 1982 and 1983 are summarized in Table 5. Figures showing numbers on substrates for all the individual taxa are included in Appendix A. Artificial substrate data are not presented for E. album because it is a burrowing mayfly, and was never recorded on the substrates. Similarly, juveniles of the crayfish O. virilis and fish fry of C. commersoni were never collected from artificial substrates.

Individual taxa observed on substrates demonstrated different abilities to recolonize following methoxychlor treatment. Some species recolonized very rapidly following treatment. For example, numbers of L. maculipennis and Baetis spp. were not significantly different ($p>.05$) between the control and treatment sites after 4 and 8 days respectively (Table 5). Other taxa remained significantly reduced ($p<.05$) at the treatment site relative to the control site for the majority of sampling dates in 1982 following treatment. For example, numbers of H. recurvata and P. flavida were significantly lower ($p<.05$) at the treatment site compared to the control site 64 days after treatment (last sampling date in 1982) (Table 5). C. campyla showed no significant difference ($p>.05$) between sites 64 days after treatment, however, mean numbers were

still lower at the treatment site, and a large variance in the samples probably prevented the detection of a statistically significant difference. Numbers of some taxa on substrates were unaffected by the treatment, and in fact appeared to be enhanced. H. ajax had significantly higher ($p<.05$) numbers on substrates at the treatment site 1 day following treatment, and numbers remained higher at the treatment site relative to the control site for the majority of sampling dates in 1982 (Table 5). Numbers of C. tardata were higher at the treatment site compared to the control site for all the sampling dates in 1982 and 1983, and were actually significantly greater ($p<.05$) at the treatment site 8 days following treatment (Table 5). Low numbers on substrates probably prevented an accurate indication of the effect of the treatment on benthic populations of C. tardata, however, it appears that they were to a large extent unaffected. Numbers of I. sicca, S. interpunctatum, A. lycorias, P. cinereus, and H. azteca were too low to allow meaningful statistical analysis (Table 5). Any significant differences ($p<.05$) indicated for these species are probably due more to natural variation than an actual treatment effect. H. ajax was the only species to show a significant reduction ($p<.05$) at the treatment site relative to the control site in 1983 (1 year following treatment) (Table 5). This reduction can probably be attributed more to natural variation than to the methoxychlor treatment because numbers of H. ajax on substrates on 18 July 1983 (previous sampling date) were higher at the treatment site.

Total numbers (all taxa) on substrates were significantly reduced ($p<.05$) at the treatment site relative to the control site on consecutive sampling dates up to 8 days following treatment, and not again until 64 days after treatment (Table 5). The significant difference ($p<.05$) observed between sites on 17 September 1982 was due to the contribution of very large numbers of H. recurvata (a mean of 658.9) observed on control substrates. Differences between control and treatment sites are not significant ($p<.05$) when H. recurvata numbers are removed from the calculations.

Community response

Species diversity on substrates at the control and treatment sites calculated using S, 1-Simpson, and H' diversity indices are summarized in Table 6. The methoxychlor treatment resulted in a significantly lower ($p<.05$) diversity on substrates as measured by all three indices at the treatment site relative to the control site 1 day following injection. S was also significantly reduced ($p<.05$) at the treatment site relative to the control site 4 days after treatment, however, significant differences ($p<.05$) in S were not found between sites for the remaining sampling dates in 1982 and 1983. Species diversity on substrates calculated using the 1-Simpson and H' diversity indices was significantly lower ($p<.05$) at the treatment site relative to the control site 14 days following

treatment. Simpson diversity was significantly higher ($p<.05$) at the treatment site 32 days after treatment and again 368 days following treatment. Shannon-Weaver diversity on substrates was also significantly greater ($p<.05$) at the treatment site relative to the control site 368 days following treatment (Table 6).

4.5 DRIFT - BENTHOS RELATIONSHIP

The numbers of many of the taxa and total numbers (excluding H. recurvata) found on substrates had recovered at the treatment site compared to the control site by 14 days after treatment (Table 5). However, drift densities of the majority of taxa and total drift density remained significantly reduced ($p<.05$) at the treatment-outlet site relative to the control site for all the sampling dates in 1982 including 33 days after treatment (Table 3). The patterns of recovery of total invertebrates on artificial substrates and of total drift at the treatment-outlet site are presented in Fig. 16. Total invertebrates on substrates returned to pre-treatment levels about two weeks following methoxychlor injection. The recovery of total invertebrate drift was delayed considerably. Numbers had not returned to pre-treatment levels after about one month, and may have required until the following summer (1 year) to reach pre-treatment levels. The relationship of total drift to total individuals on substrates is further illustrated in

Fig. 17, where total invertebrate drift is graphed against total numbers of invertebrates on substrates for four sampling dates following methoxychlor treatment at the treatment-outlet site. Once totals on substrates had approached a maximum of about 300, which required between 14 and 33 days following treatment, totals in the drift began to increase dramatically.

4.6 INSECT EMERGENCE

Adult emergence was observed for the following species during the sampling schedule in 1982 (08 July-18 August) : P. flavida, P. cinereus, I. sicca, H. recurvata, S. interpunctatum, B. propinquus, B. pallidulus, B. intercalaris, B. flavistriga, H. ajax, C. campyla, Hydropsyche sparna Ross, Hydropsyche bifida Banks, Hydropsyche betteni Ross, and Nixe inconspicua (McDunnough). All but H. ajax and C. campyla were recorded in very low numbers in the emergence traps (usually a mean value of one or less for three replicates).

The caddisfly H. ajax emerged in very large numbers during sampling in 1982. Peak numbers occurred very close to the date of treatment (Fig. 18A). Numbers emerging were significantly lower ($p<.05$) at the treatment site relative to the control on 22 July (7 days post-treatment) and on 28 July (13 days post-treatment). The sites were not significantly different ($p>.05$) on 18 August (34 days post-treatment) (Fig. 18A).

The methoxychlor injection seemed to cause an initial decline in emergence of C. campyla at the treatment site (Fig. 18B), however, no significant differences ($p>.05$) were found between the sites for numbers emerging at any of the post-treatment sampling dates in 1982.

E. album adults were not obtained from emergence traps at any time during the study. However, on 29 July 1982 (14 days post-treatment) a total of 538 adults were recovered from the three drift samplers at the control site, whereas only 14 were collected from the three drift samplers at the treatment-outlet site. This difference is expected, since the methoxychlor treatment had resulted in a catastrophic drift of the nymphs of this species at the treatment-outlet site (Fig. 21, Appendix A). The adult population at the treatment riffle was obviously severely reduced by effects of the injection on immatures. On 03 August 1983, no E. album adults were found in drift nets from either site (the emergence peak was missed). Thus the impact of the treatment on the adult population at the treatment site one year following treatment could not be determined.

Table 1. Water chemistry data for the Control and Treatment study sites on the Souris River, Manitoba, 1982.

Date	Temperature (°C)		pH		Suspended Carbon (ug/1)		Suspended Nitrogen (ug/1)		Total Suspended Solids (mg/1)	
	control	treatment	control	treatment	control	treatment	control	treatment	control	treatment
08 Jul 82	21.0	21.0	7.76	7.90	1770.0	1150.0	289.0	262.0	13.0	12.0
15 Jul 82	25.0	25.0	8.40	8.06	890.0	1280.0	162.0	230.0	9.0	10.0
22 Jul 82	23.0	23.0	7.86	8.16	1400.0	1240.0	244.0	216.0	11.0	10.0
29 Jul 82	22.0	22.0	7.58	7.72	1710.0	2220.0	312.0	290.0	12.0	12.0
17 Aug 82	22.0	22.0	8.24	8.34	1880.0	1790.0	280.0	249.0	15.0	14.0
16 Sep 82	12.5	12.5	8.32	8.32	1230.0	1240.0	134.0	121.0	15.0	14.0

Table 2. Methoxychlor residues (ug/litre) in water samples taken from the north, middle, and south sides of the control and treatment riffle sites at various sampling periods before and following methoxychlor injection (300 ug/litre for 15 min.) into the Souris River, Manitoba, 15 July, 1982.

	Control			Treatment		
Sampling Period	North	Middle	South	North	Middle	South
24 hr. pre-treat	0.00	0.00	0.00	0.00	0.00	0.00
10 min. post-treat	0.00	0.00	0.00	284.44	358.97	223.85
10 min. post-treat*	0.00	0.00	0.00	282.20	306.88	229.74
1 hr. post-treat	0.00	0.00	0.00	2.08	-	2.21
1 hr. post-treat*	0.00	0.00	0.00	2.86	3.08	1.11
3 hr. post-treat	0.00	0.00	0.00	0.26	0.41	0.24
6 hr. post-treat	0.00	0.00	0.00	0.05	0.12	0.03
12 hr. post-treat	0.00	0.00	0.00	0.02	0.06	0.02
24 hr. post-treat	0.00	0.00	0.00	0.00	0.00	0.00
48 hr. post-treat	0.00	0.00	0.00	0.00	0.00	0.00

*Samples to which sodium azide was added immediately following collection.

Table 3. Mean (± S.E.) 24-h drift densities (no./100 m^3) of invertebrates and sucker fry at the control and treatment-outlet sites in the Souris River before and after treatment with methoxychlor

		Pre-treat	15 Jul '82 (Treat)	21 Jul '82 (6 days)	29 Jul '82 (14 days)	17 Aug '82 (33 days)	03 Aug '83 (384 days)
Baetis spp.	C	133.4±45.9	195.6±67.0 **	200.5±18.7 *	427.7±106.7 *	747.0±294.0 *	233.3±45.2
	T	282.5±82.4	13965.6±4023.1	16.6±4.06	144.8±56.2	211.8±66.0	719.9±145.0
Caenis tardata	C	173.3±61.3	308.9±41.7 **	137.1±18.8 *	275.2±118.6 *	997.3±155.2 *	241.0±63.9
	T	663.3±55.2	20230.6±4960.1	24.2±8.2	93.9±35.6	597.0±108.6	383.1±64.4
Leucrocuta maculipennis	C	66.0±29.3	88.2±33.3 **	63.5±16.1 *	133.3±44.3 *	78.4±12.1	43.4±4.6
	T	107.4±41.8	13351.2±3084.5	4.5±2.5	36.4±17.3	32.1±6.8	141.4±35.5
Stenacron interpunctatum	C	6.9±3.1	18.3±7.9 **	2.0±0.7 *	2.9±1.0 *	7.4±2.6 *	44.2±10.3
	T	41.6±27.2	4303.2±1441.4	0.0±0.0	1.6±1.6	3.1±0.5	156.6±50.7
Isonychia sicca	C	29.6±11.6	60.1±21.2 **	26.3±3.3 *	20.9±6.3 *	9.2±3.0 *	10.5±3.3 *
	T	57.2±24.2	11738.8±2875.7	1.0±0.9	0.0±0.0	0.0±0.0	1.5±0.7
Ephoron album	C	10.7±6.8	4.0±4.0 **	0.0±0.0 *	1.2±0.8 *	1.5±0.6 *	0.7±0.7 *
	T	48.1±15.7	2793.3±1080.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Hydroptila ajax	C	177.5±45.3	151.0±66.7 **	24.6±13.3 *	261.3±195.0	38.2±8.1 *	60.0±17.2
	T	391.0±145.0	3124.4±1113.0	1.0±0.9	141.8±71.3	6.9±0.4	108.2±25.8
Cheumatopsyche campyla	C	8.3±5.5	25.1±2.6 **	13.0±3.1 *	10.3±8.6	93.4±25.6	23.6±12.7
	T	15.7±9.9	22419.6±6097.5	0.0±0.0	5.5±2.9	29.8±5.3	56.6±12.7
Hydropsyche recurvata	C	2.5±0.9	3.2±1.9 **	1.6±0.5 *	1.5±1.1 *	18.8±2.2 *	22.7±6.3
	T	13.2±3.5	3594.8±1181.7	0.8±0.8	0.0±0.0	7.8±2.4	60.5±13.7
Psychomyia flavida	C	0.3±0.2	1.0±1.0 **	0.0±0.0	8.4±6.6	3.4±1.8	1.0±0.9
	T	2.75±2.2	12934.8±3372.2	1.5±1.4	1.1±1.1	0.0±0.0	6.5±2.3
Polycentropus cinereus	C	5.7±3.5	6.2±3.0 **	2.1±1.1	6.6±2.3	0.0±0.0	9.4±5.3
	T	0.0±0.0	378.7±137.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Acroneuria lycorias	C	0.1±0.1	0.0±0.0 **	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	T	0.0±0.0	9.4±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Hyalella azteca	C	107.0±25.0	93.0±10.9 **	56.9±7.6 *	54.7±16.3 *	138.3±36.1 *	19.5±3.6
	T	530.5±185.4	7754.0±1895.0	6.2±3.5	16.5±3.9	67.7±15.8	41.7±6.9
Orconectes virilis	C	0.0±0.0	0.8±0.7 **	0.5±0.5	0.0±0.0	0.0±0.0	0.0±0.0
	T	0.0±0.0	39.2±11.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Catostomus commersoni	C	16.2±6.2	12.0±6.9	0.0±0.0	6.1±3.3	9.0±5.6	3.2±1.8
	T	9.9±3.8	4.0±1.1	0.0±0.0	0.2±0.2	0.0±0.0	8.9±7.8
Total taxa	C	737.5±150.5	967.0±131.7 **	528.0±63.3 *	1210.0±465.2 *	2142.0±512.8 *	713.0±15.2
	T	2163.0±278.0	116641.0±30746.3	56.0±17.2	442.0±164.3	956.0±169.5	1685.0±350.0

C - Control site.

T - Treatment-outlet site.

** Means are significantly different ($P<0.0001$).

* Means are significantly different ($P<0.05$).

Table 4. Mean (± S.E.) species diversity in the drift at the control and treatment-outlet sites in the Souris River before and after treatment with methoxychlor

		Pre-treat	15 Jul '82 (Treat)	21 Jul '82 (6 days)	29 Jul '82 (14 days)	17 Aug '82 (33 days)	03 Aug '83 (384 days)
Species richness	C	11.7±0.4	11.7±1.2	10.0±0.0	12.4±0.7	11.7±0.4	11.7±0.4
			*	*	*	*	
	T	11.2±0.3	15.0±0.0	5.0±0.0	6.7±0.4	8.0±0.0	10.7±0.4
Simpson	C	0.80±0.02	0.78±0.01	0.75±0.01	0.72±0.02	0.64±0.02	0.74±0.04
			*	*			
	T	0.77±0.01	0.87±0.002	0.70±0.02	0.73±0.02	0.54±0.05	0.74±0.01
Shannon-Weaver	C	1.82±0.08	1.81±0.06	1.64±0.03	1.56±0.04	1.33±0.04	1.70±0.14
			*	*			
	T	1.70±0.03	2.20±0.01	1.34±0.05	1.44±0.05	1.11±0.09	1.67±0.02

C - Control site.

T - Treatment-outlet site.

* Means are significantly different ($P<0.05$).

Table 5. Mean (± S.E.) number of invertebrates on artificial substrates at the control and treatment sites in the Souris River before and after treatment with methoxychlor

		Pre-treat	16 Jul '82 (1 day)	19 Jul '82 (4 days)	23 Jul '82 (8 days)	29 Jul '82 (14 days)	16 Aug '82 (32 days)	17 Sept '82 (64 days)	18 Jul '83 (368 days)	03 Aug '83 (384 days)
Baetis spp.	C	44.8±4.2	25.3±5.2*	31.2±5.6*	24.3±6.6	39.9±7.3	47.0±9.0	6.2±1.4	12.8±2.4	37.9±4.9
	T	42.0±4.5	0.1±0.1	1.3±0.6	21.4±9.3	43.9±9.6	73.0±16.0	6.0±2.1	18.1±3.0	50.4±9.0
Caenis tardata	C	3.7±3.5	0.7±0.3	0.0±0.0	0.1±0.1*	0.4±0.3	0.0±0.0	0.6±0.3	0.2±0.1	0.0±0.0
	T	6.6±4.4	5.1±2.0	4.0±1.9	9.1±2.8	1.5±0.7	1.0±0.7	1.1±0.4	1.4±0.5	0.4±0.2
Leucrocuta maculipennis	C	7.6±1.1	14.7±4.5*	6.7±3.2	3.5±1.2	9.1±3.3	3.9±1.9	0.7±0.4	1.2±0.5	1.6±0.7
	T	9.8±2.8	0.6±0.3	1.3±0.6	0.8±0.3	3.5±2.1	0.8±0.6	0.6±0.4	6.1±1.7	3.9±1.9
Isonychia sicca	C	3.2±1.4	1.1±0.5	5.8±2.6*	0.3±0.2	1.3±0.5	0.4±0.2	0.0±0.0	0.7±0.3	0.9±0.4
	T	7.4±3.7	0.5±0.2	0.2±0.2	0.3±0.2	0.9±0.5	0.2±0.1	0.2±0.1	1.2±0.4	0.4±0.3
Stenacron interpunctatum	C	0.17±0.03	0.3±0.2	0.1±0.1	0.0±0.0	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.0	0.7±0.3
	T	0.27±0.13	0.0±0.0	0.3±0.3	0.5±0.3	0.6±0.4	0.0±0.0	0.0±0.1	0.5±0.3	1.6±0.7
Acroneuria lycorias	C	0.07±0.07	0.2±0.1	0.0±0.0	0.1±0.1	0.3±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.1
	T	0.04±0.04	0.0±0.0	0.1±0.1	0.0±0.0	0.2±0.1	0.0±0.0	0.1±0.1	0.1±0.1	0.1±0.1
Hydroptila ajax	C	64.6±28.1	5.3±2.3*	2.5±2.0	2.9±1.7	52.1±13.3	22.3±3.4	5.2±1.5	4.5±1.3	168.3±18.8*
	T	100.2±4.4	39.9±5.6	4.1±1.1	31.1±11.4	189.0±77.8	93.8±11.2	3.7±2.2	22.0±6.9	55.7±3.9
Hydropsyche recurvata	C	21.6±3.5	7.4±3.6	21.8±8.4*	13.7±4.8*	20.5±5.1*	371.1±97.0	658.9±145.0*	156.4±26.7	176.1±50.3
	T	9.6±1.6	0.3±0.2	0.6±0.3	0.0±0.0	2.2±1.1	104.2±32.2	131.2±60.8	103.6±30.4	129.5±43.3
Cheumatopsyche campyla	C	8.5±2.0	7.3±1.8	8.7±1.7*	20.7±6.6*	24.6±9.3*	59.7±12.8*	207.4±27.8	16.6±3.6	34.7±8.1
	T	10.0±3.5	2.7±0.7	0.1±0.1	0.3±0.2	2.7±0.9	21.8±5.2	126.0±19.9	23.3±3.5	46.7±10.1
Psychomyia flavida	C	12.2±10.6	89.2±15.7*	134.8±25.5*	40.3±15.2*	4.9±0.8	1.9±1.5	32.6±3.4*	27.3±4.1	4.2±0.9
	T	19.8±14.9	2.2±0.7	1.1±0.6	2.8±1.1	2.8±1.3	2.9±1.1	7.8±3.1	17.1±2.6	9.2±2.1
Polycentropus cinereus	C	0.3±0.06	1.3±0.5*	0.7±0.3	0.4±0.3	0.6±0.3	0.0±0.0	0.0±0.0	0.1±0.1	0.1±0.1
	T	0.44±0.3	0.0±0.0	0.3±0.2	0.1±0.1	0.9±0.3	0.0±0.0	0.0±0.0	0.2±0.1	0.1±0.1
Hyalella azteca	C	13.5±12.7	0.0±0.0	0.0±0.0	0.2±0.1	1.7±0.9	0.4±0.3	25.9±5.9*	0.1±0.1	0.0±0.0
	T	9.2±5.0	1.1±0.5	1.4±0.8	3.4±1.3	2.0±0.9	0.1±0.1	12.5±3.9	0.4±0.2	0.2±0.1
Total taxa	C	180.2±41.2	152.8±17.3*	212.3±23.1*	106.5±16.4*	155.5±25.3	506.7±93.1	937.5±137.1*	219.9±28.7	424.7±61.4
	T	215.3±3.0	52.5±5.6	14.8±2.8	69.9±21.1	250.2±79.8	297.8±48.6	289.3±76.3	194.0±30.7	298.2±57.6

C - Control site.

T - Treatment site.

* Means are significantly different ($P<0.05$).

Table 6. Mean (± S.E.) species diversity on artifical substrates at the control and treatment sites in the Souris River before and after treatment with methoxychlor

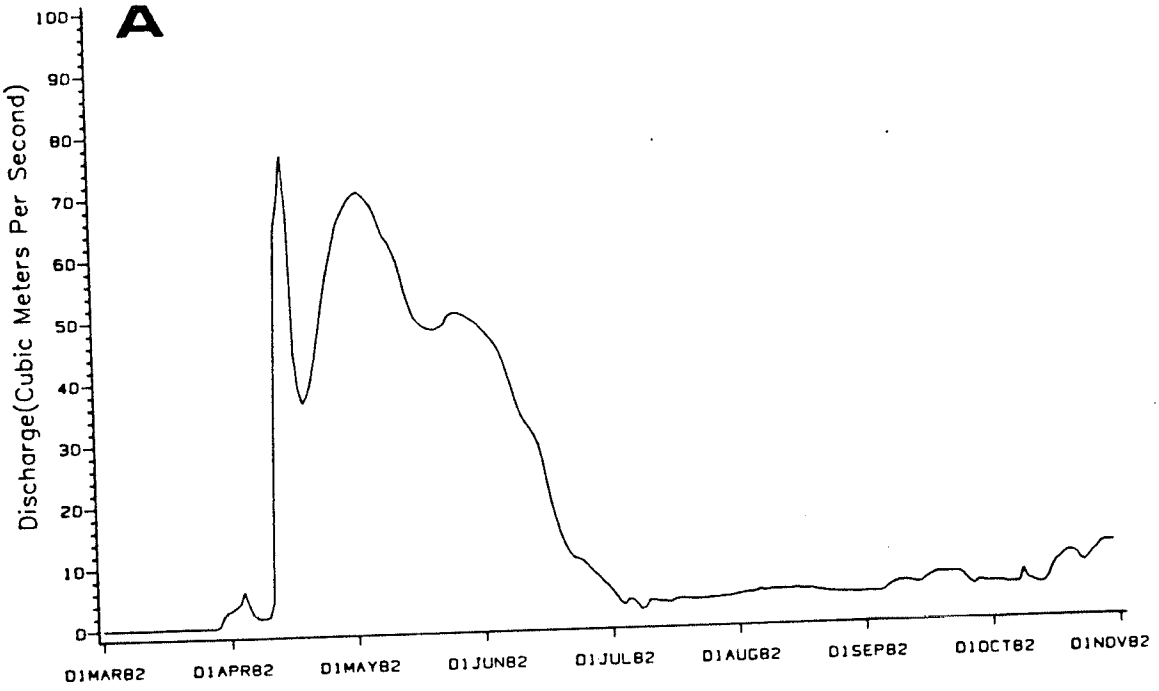
		Pre-treat	16 Jul '82 (1 day)	19 Jul '82 (4 days)	23 Jul '82 (8 days)	29 Jul '82 (14 days)	16 Aug '82 (32 days)	17 Sept '82 (64 days)	18 Jul '83 (368 days)	03 Aug '83 (384 days)
Species richness	C	7.1±0.23	6.7±0.47	6.6±0.27	5.7±0.33	7.7±0.33	5.3±0.42	6.4±0.34	6.1±0.28	6.5±0.54
	T	7.5±0.22	5.1±0.28 *	4.3±0.56 *	5.3±0.56	6.7±0.60	5.4±0.34	6.5±0.37	7.8±0.47	7.1±0.50
Simpson	C	0.69±0.01	0.59±0.04	0.54±0.06	0.58±0.05	0.68±0.02	0.47±0.06	0.46±0.05	0.50±0.05	0.62±0.02
	T	0.66±0.02	0.40±0.04 *	0.66±0.04	0.58±0.07	0.47±0.05 *	0.65±0.02 *	0.54±0.04	0.67±0.05 *	0:71±0.03
Shannon-Weaver	C	1.40±0.03	1.20±0.07	1.09±0.12	1.12±0.09	1.40±0.06	0.94±0.11	0.88±0.08	1.02±0.10	1.19±0.05
	T	1.38±0.05	0.81±0.07 *	1.13±0.11	1.12±0.13	0.91±0.09 *	1.23±0.05	1.03±0.10	1.42±0.10 *	1.45±0.07

C - Control site.

T - Treatment site.

* Means are significantly different ($P<0.05$).

Figure 5. Discharge for the Souris River at Souris, Manitoba (located 18 km upstream from the study sites). A) 1982 B) 1983. (Environment Canada 1984).


A
Discharge(Cubic Meters Per Second)
100
90
80
70
60
50
40
30
20
10
0
01MAR82
01APR82
01MAY82
01JUN82
01JUL82
01AUG82
01SEP82
01OCT82
01NOV82

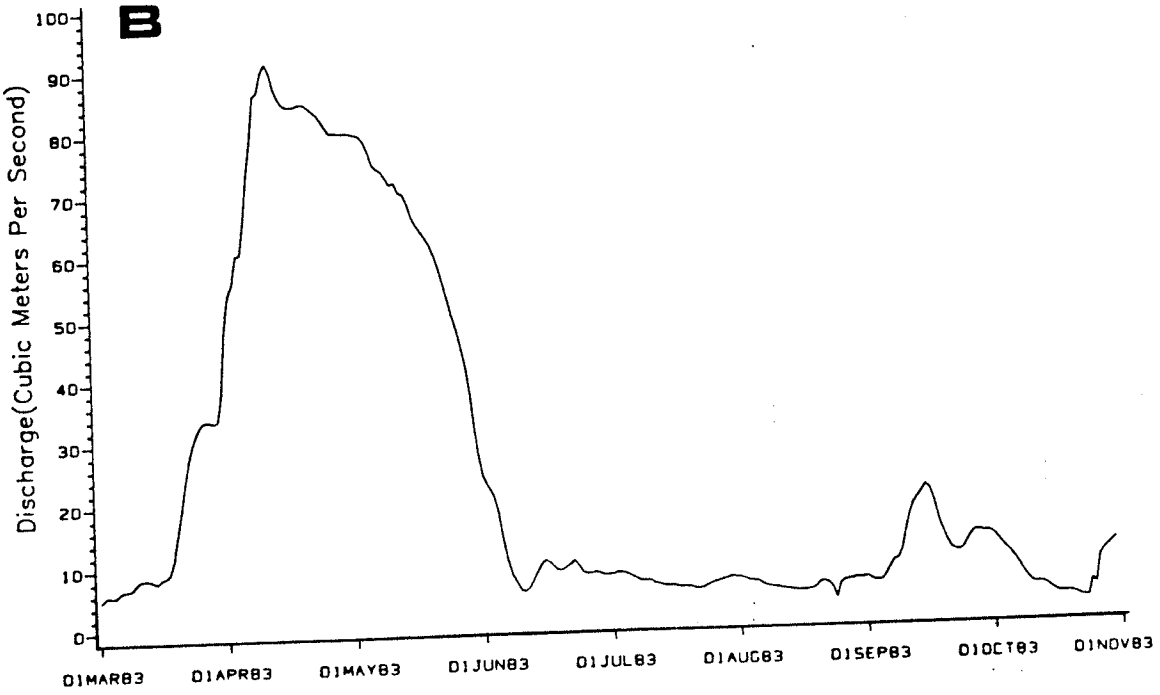

B
Discharge(Cubic Meters Per Second)
100
90
80
70
60
50
40
30
20
10
0
01MAR83
01APR83
01MAY83
01JUN83
01JUL83
01AUG83
01SEP83
01OCT83
01NOV83

Figure 6. Mean (±s.e.) drift density of *Baetis* spp. larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

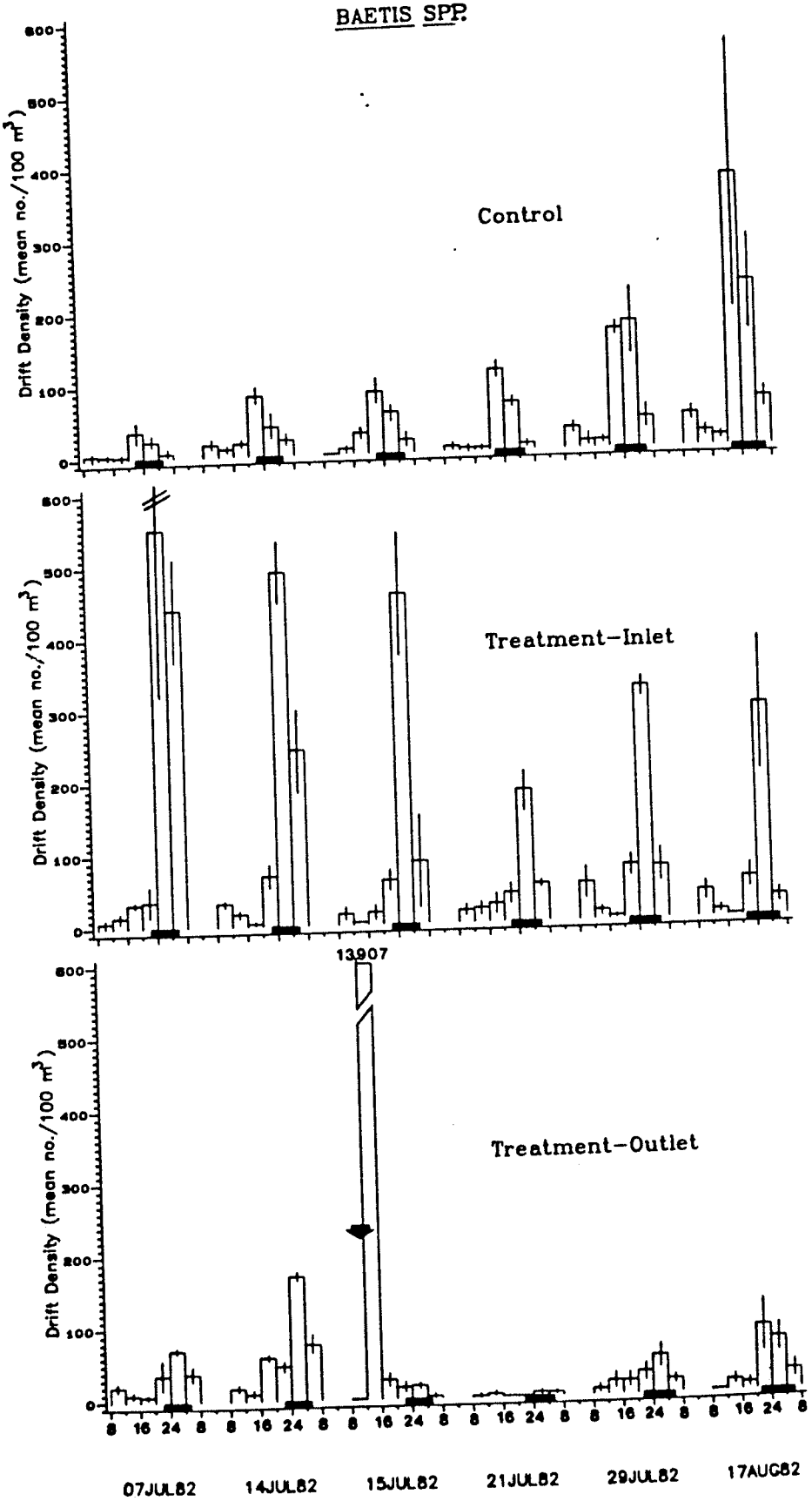

BAETIS SPP.
Drift Density (mean no./100 m³)
Control
Treatment–Inlet
13907
Treatment–Outlet
8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8
07JUL82
14JUL82
15JUL82
21JUL82
29JUL82
17AUG82

Figure 7. Mean (±s.e.) drift density of *Baetis* spp. larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

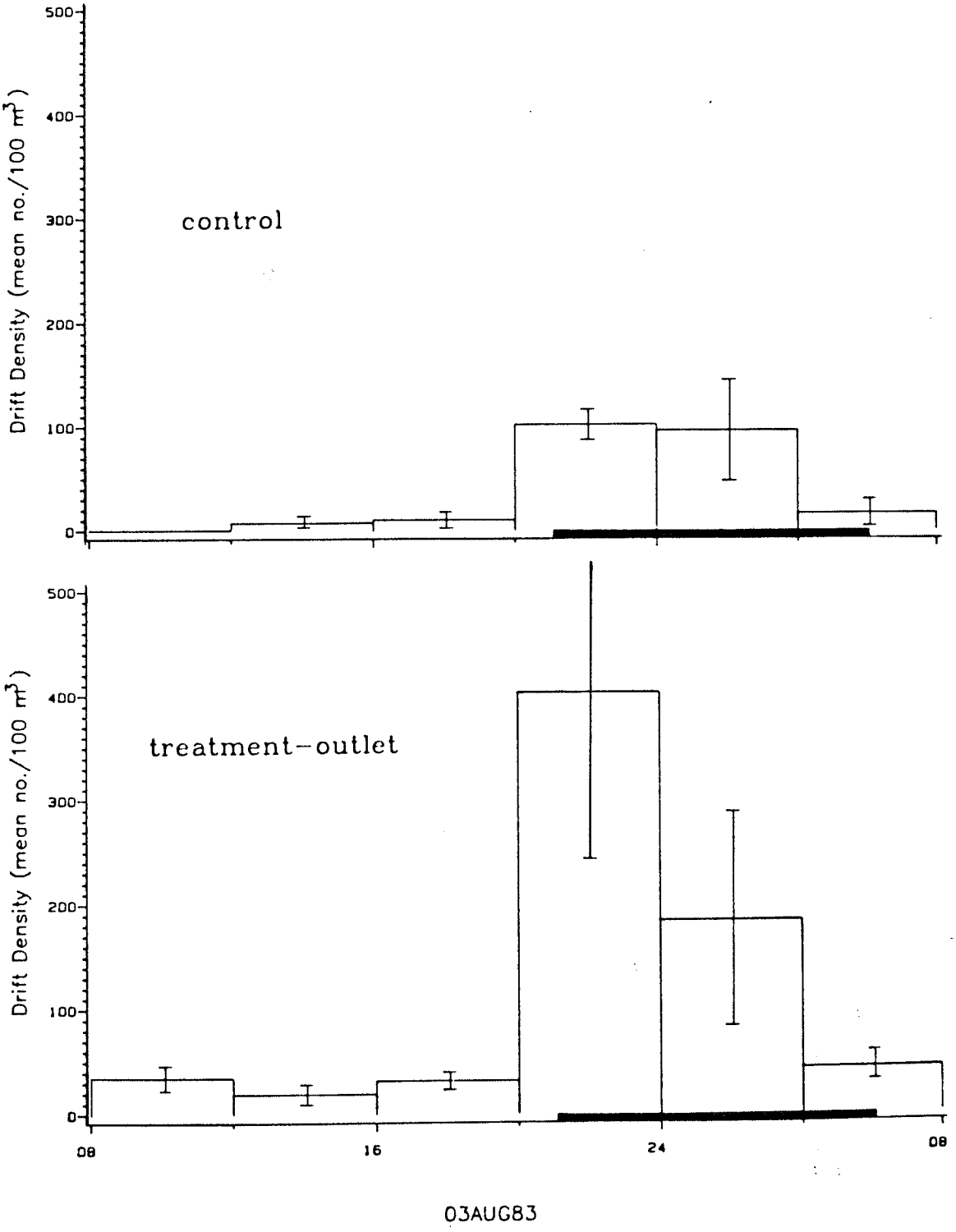

Drift Density (mean no./100 m³)
500
400
300
200
100
0
control
Drift Density (mean no./100 m³)
500
400
300
200
100
0
treatment-outlet
08
16
24
08
03AUG83

Figure 8. Mean (±s.e.) drift density of Psychomyia flavida larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

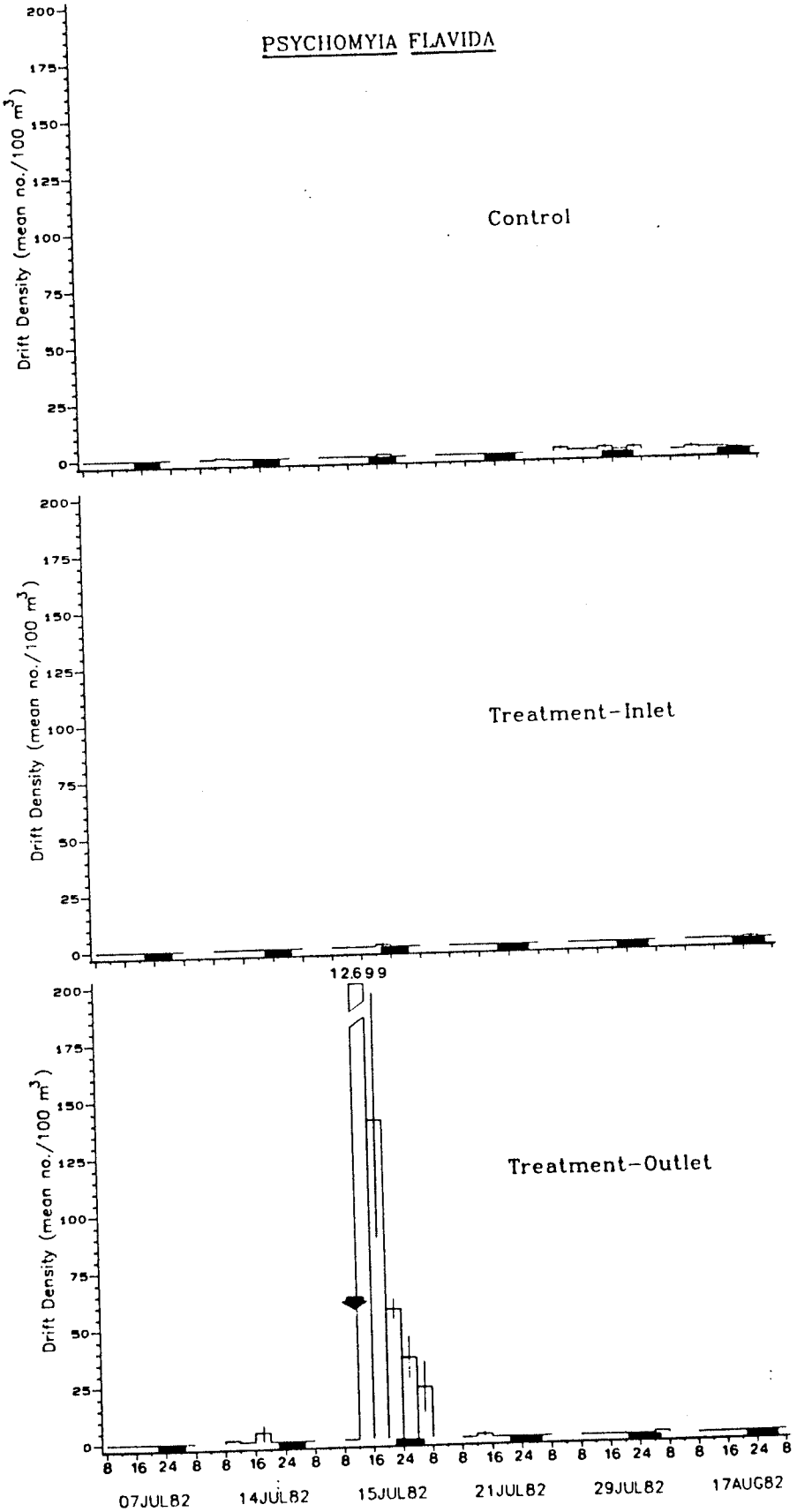

PSYCHOMYIA FLAVIDA
Drift Density (mean no./100 m³)
Control
Treatment-Inlet
12699
Treatment-Outlet
8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8
07JUL82
14JUL82
15JUL82
21JUL82
29JUL82
17AUG82

Figure 9. Mean (±s.e.) drift density of Psychomyia flavida larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

PSYCHOMYIA FLAVIDA

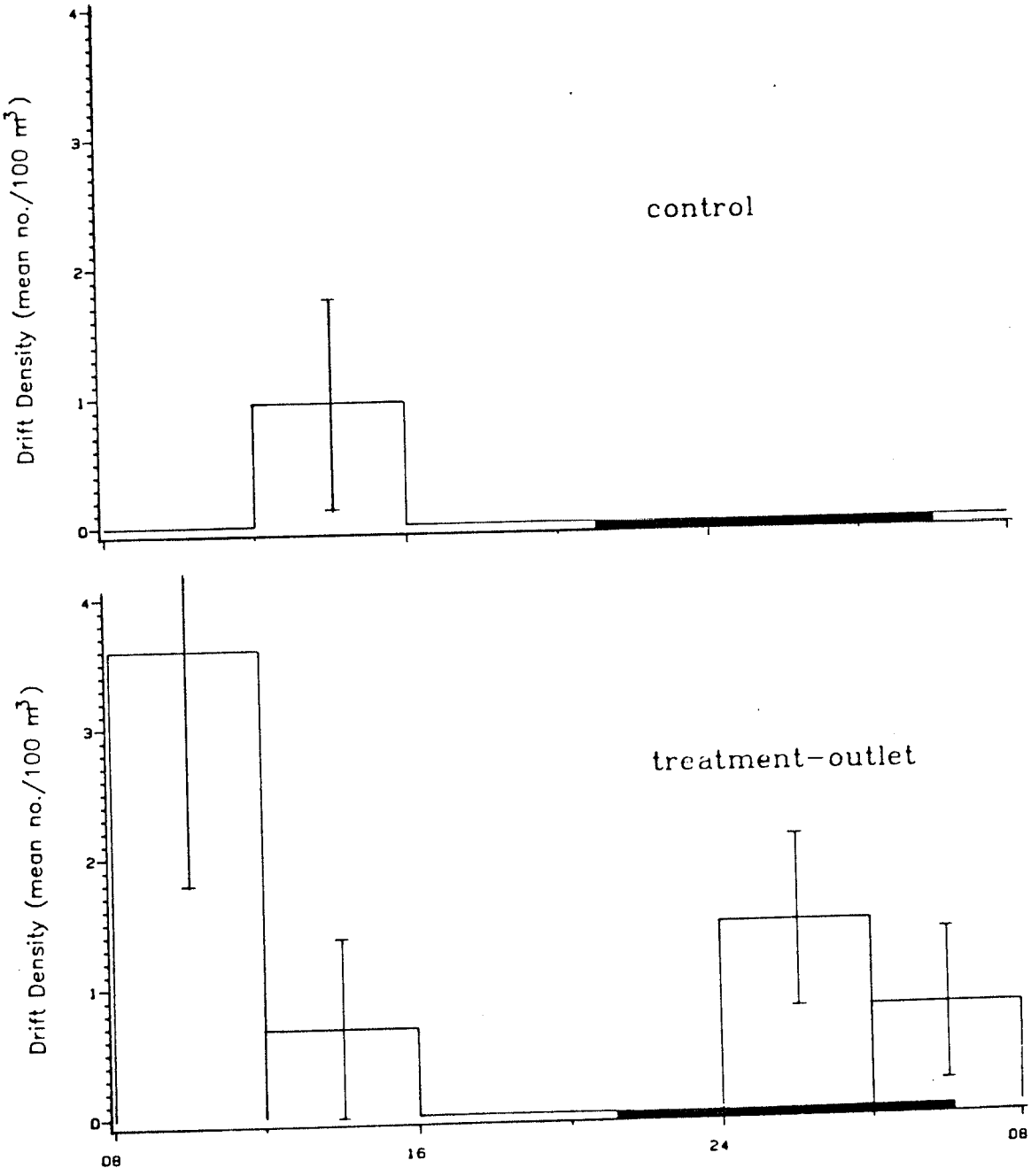


Figure 10. Mean (±s.e.) drift density of Cheumatopsyche campyla larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

CHEUMATOPSYCHE CAMPYLA

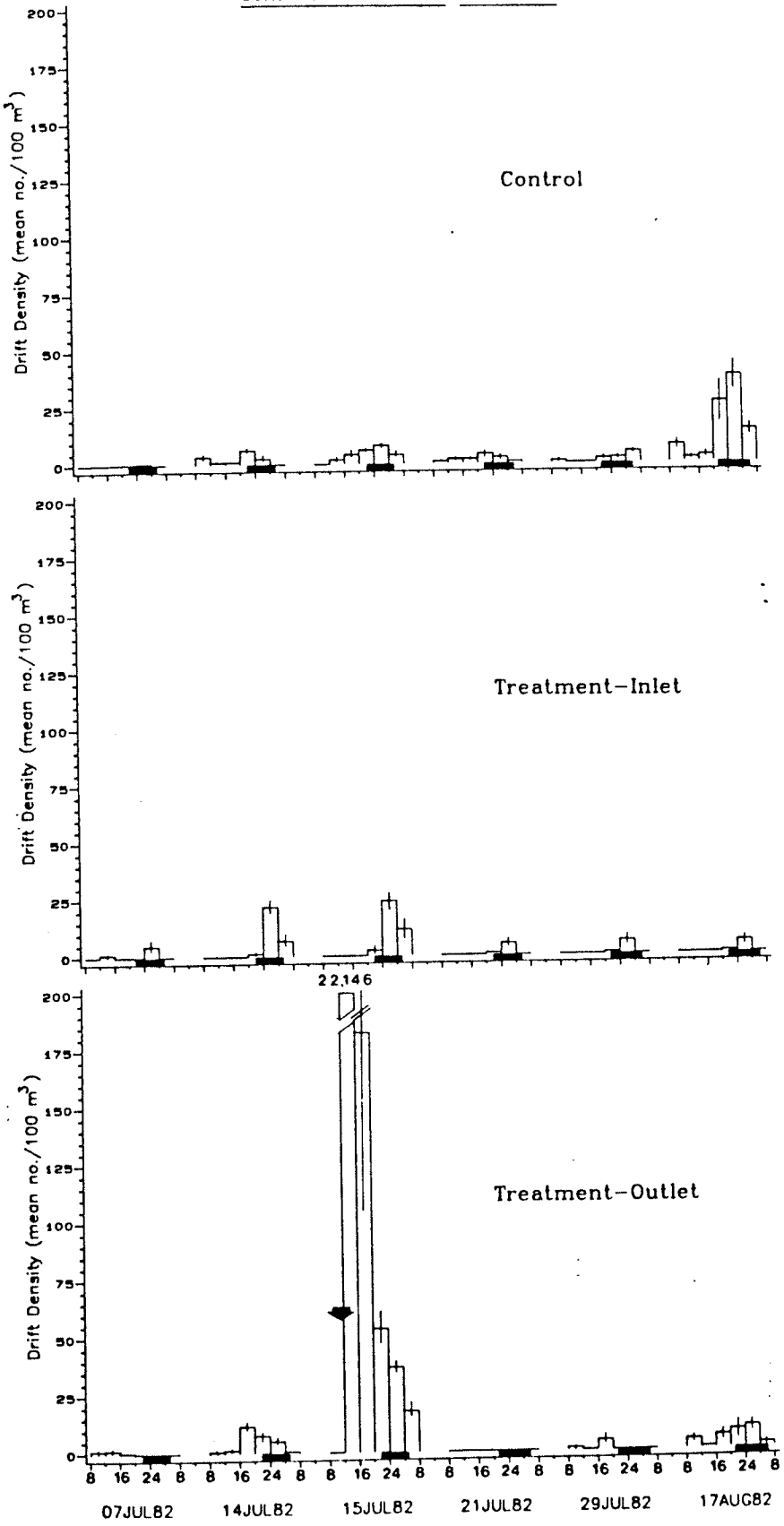


Figure 11. Mean (±s.e.) drift density of Cheumatopsyche campyla larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

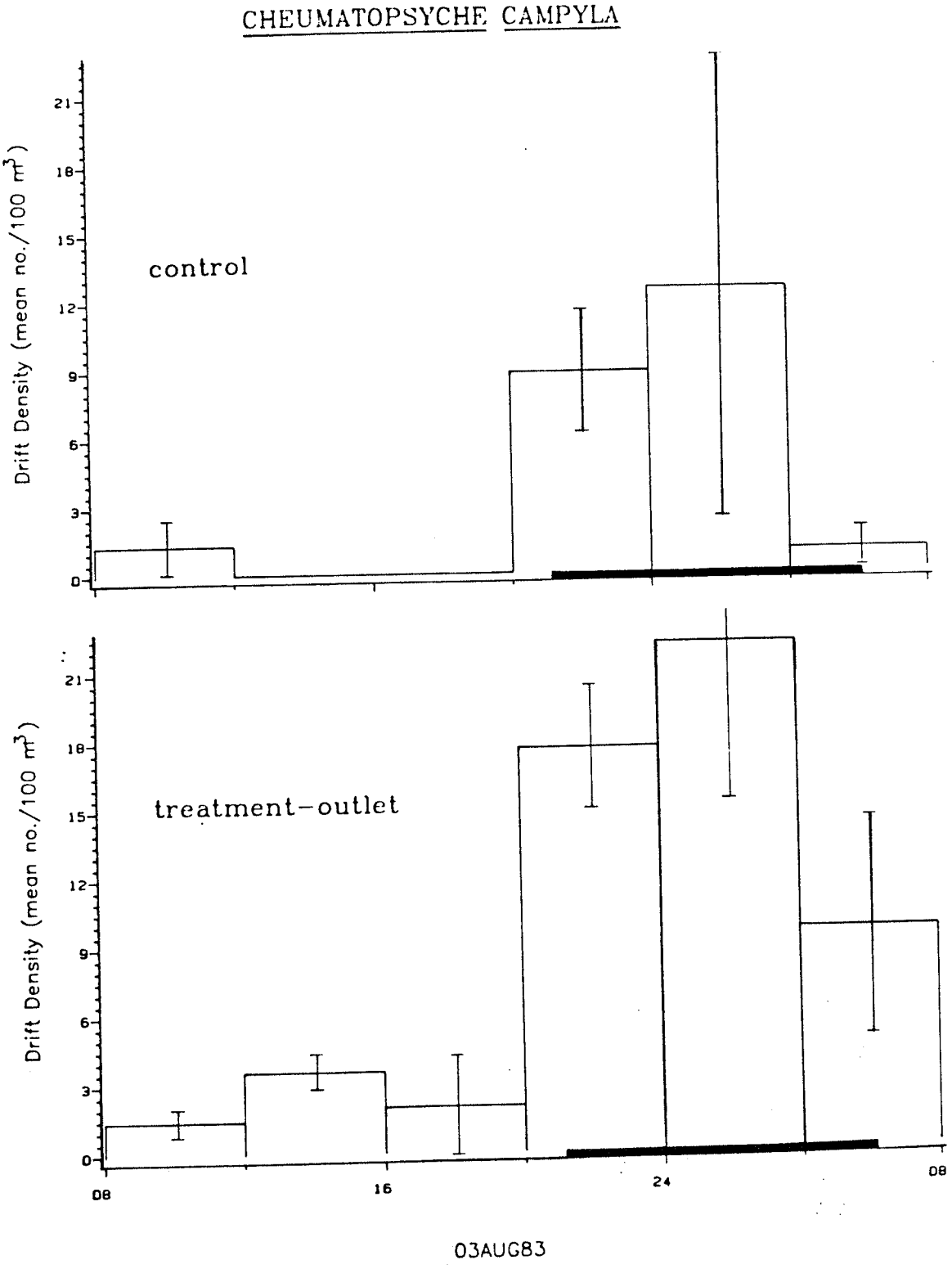

CHEUMATOPSYCHE CAMPYLA
Drift Density (mean no./100 m³)
control
treatment-outlet
0
3
6
9
12
15
18
21
08
16
24
08
03AUG83

Figure 12. Mean (±s.e.) drift density of Hydroptila ajax larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

HYDROPTILA AJAX

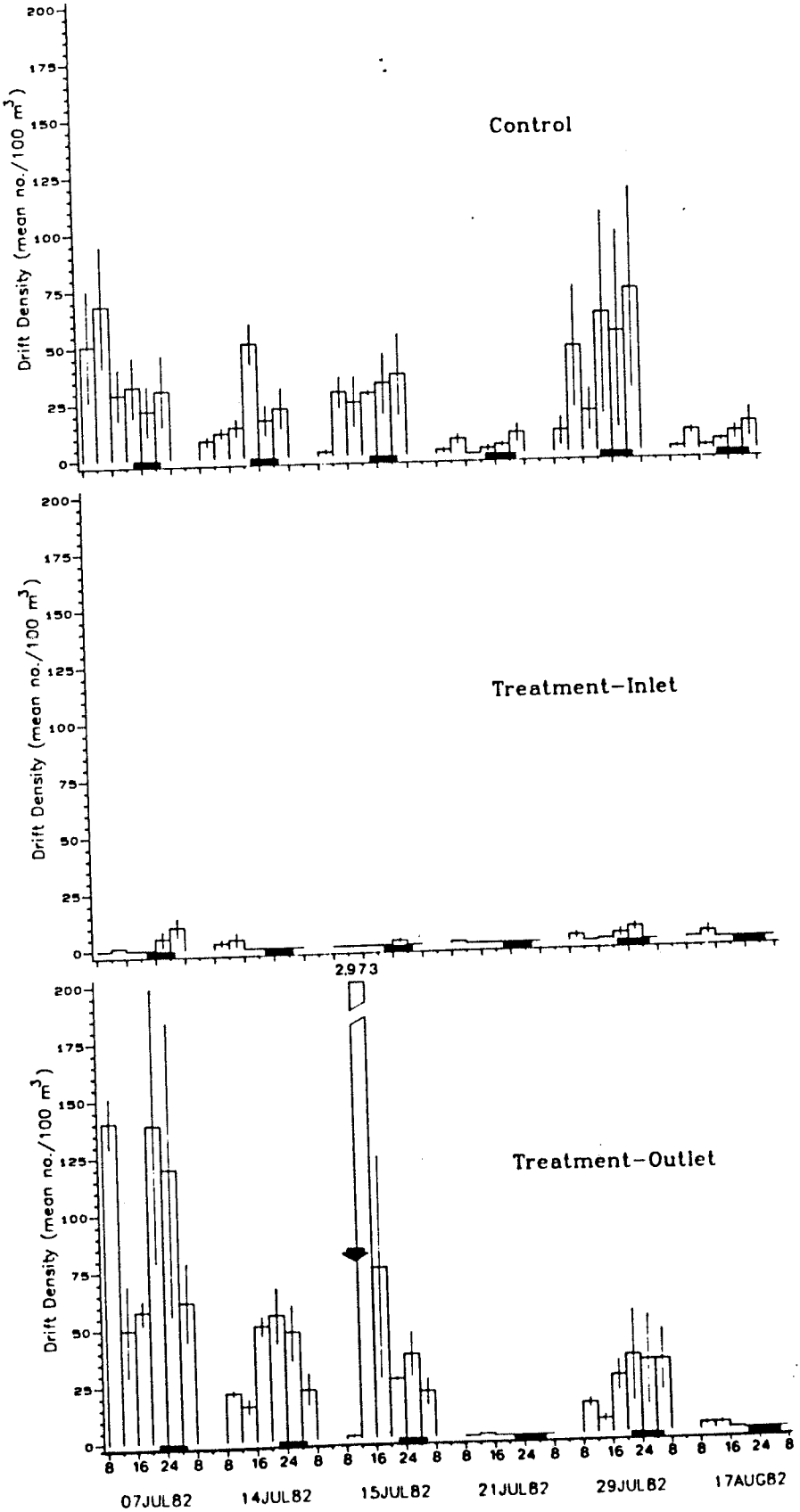


Figure 13. Mean (±s.e.) drift density of Hydroptila ajax larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

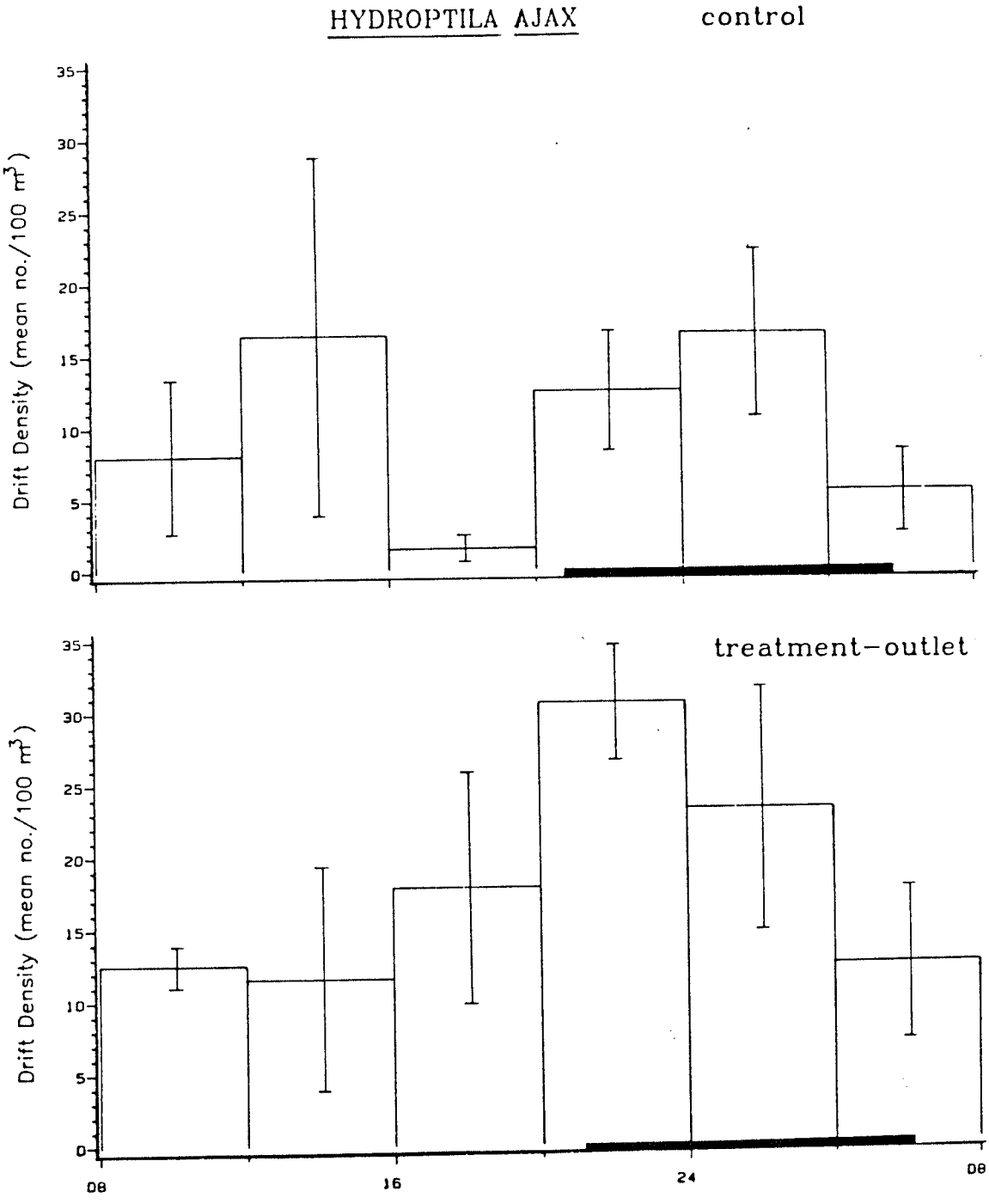

HYDROPTILA AJAX
control
Drift Density (mean no./100 m³)
35
30
25
20
15
10
5
0
treatment-outlet
Drift Density (mean no./100 m³)
35
30
25
20
15
10
5
0
08
16
24
08
03AUG83

Figure 14. Mean (±s.e.) drift density of Catostomus commersoni fry at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

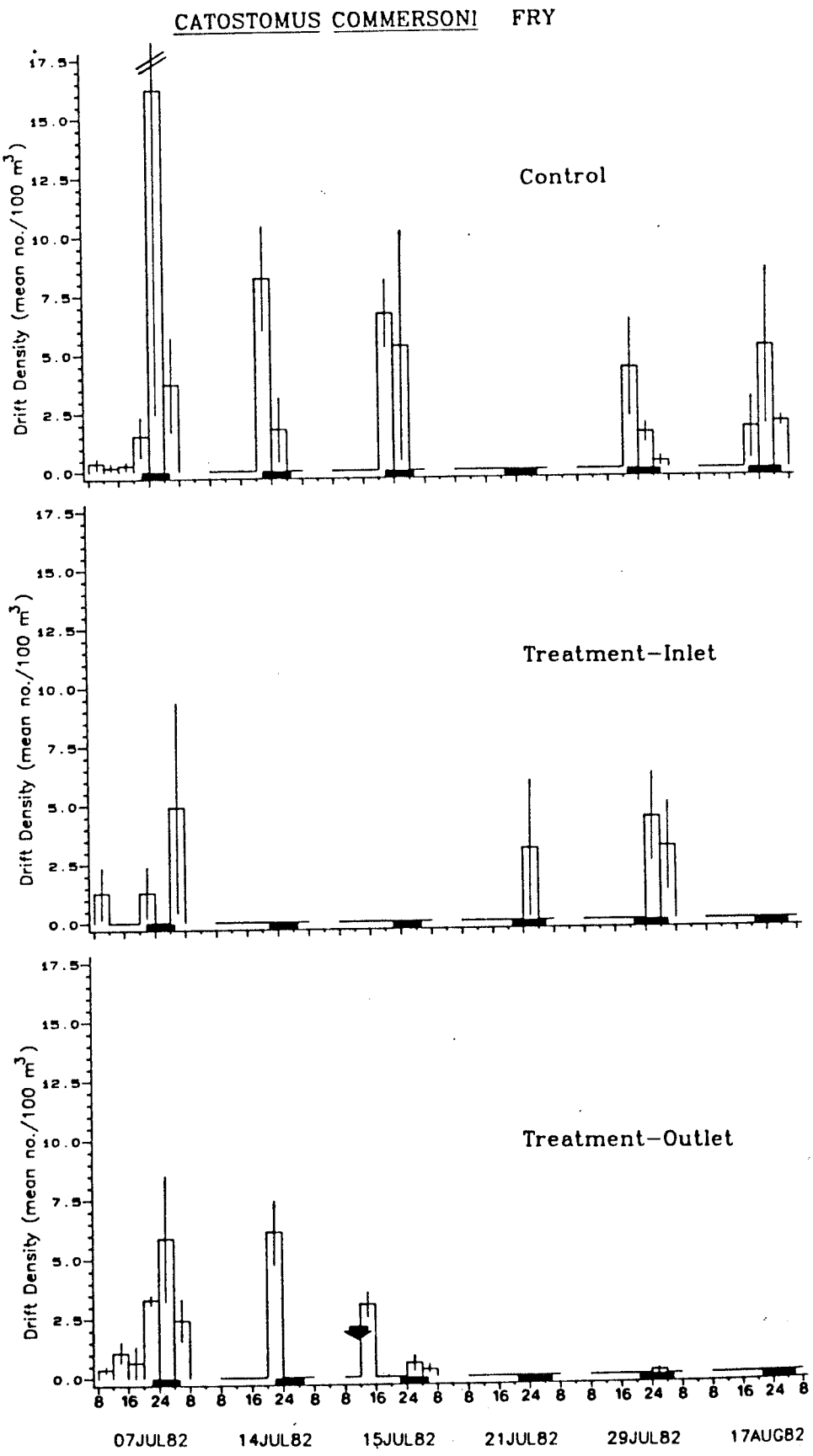

CATOSTOMUS COMMERSONI FRY
Drift Density (mean no./100 m3)
Control
Treatment–Inlet
Treatment–Outlet
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8
07JUL82
14JUL82
15JUL82
21JUL82
29JUL82
17AUG82

Figure 15. Mean (±s.e.) drift density of Catostomus commersoni fry at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

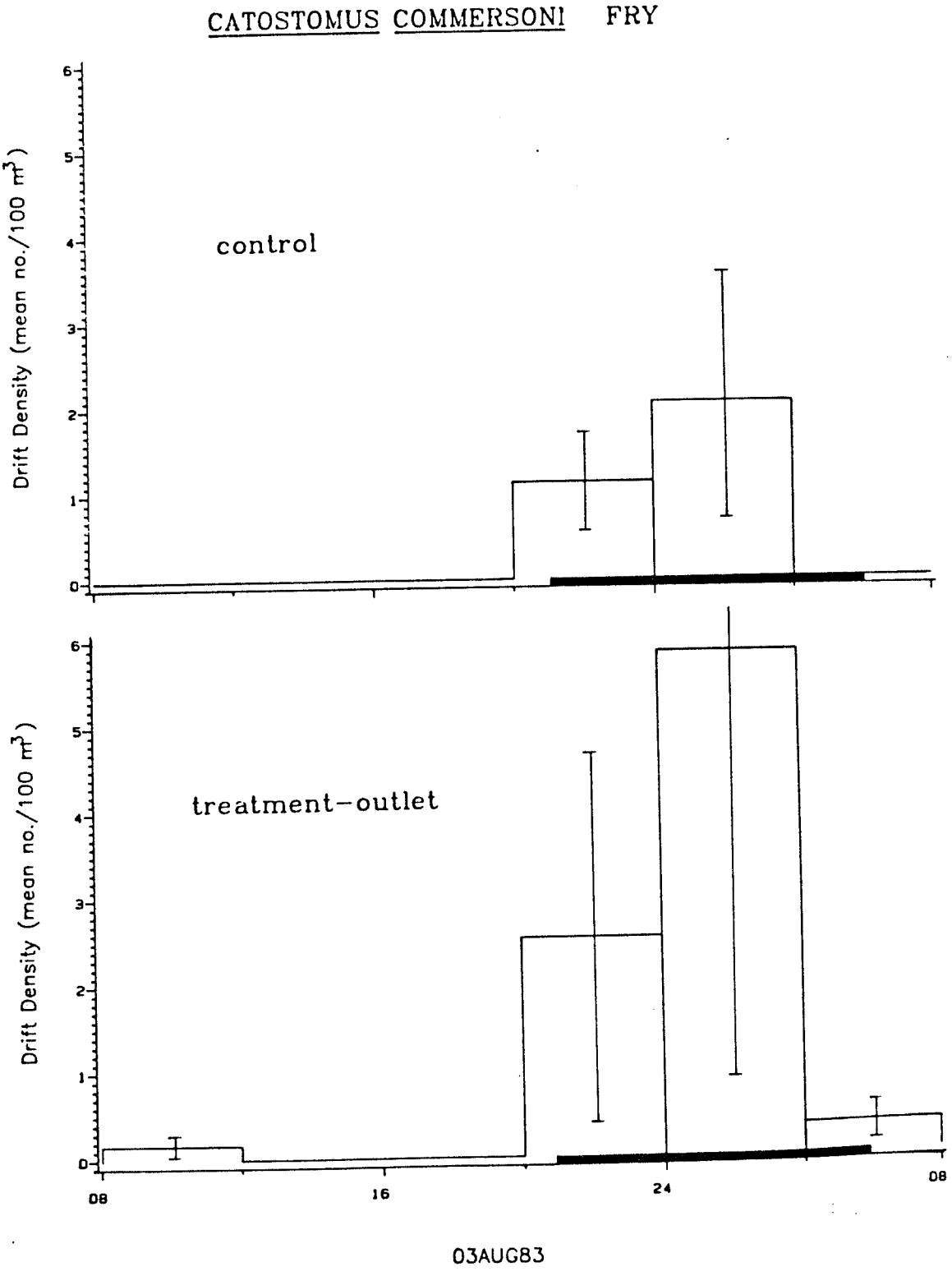
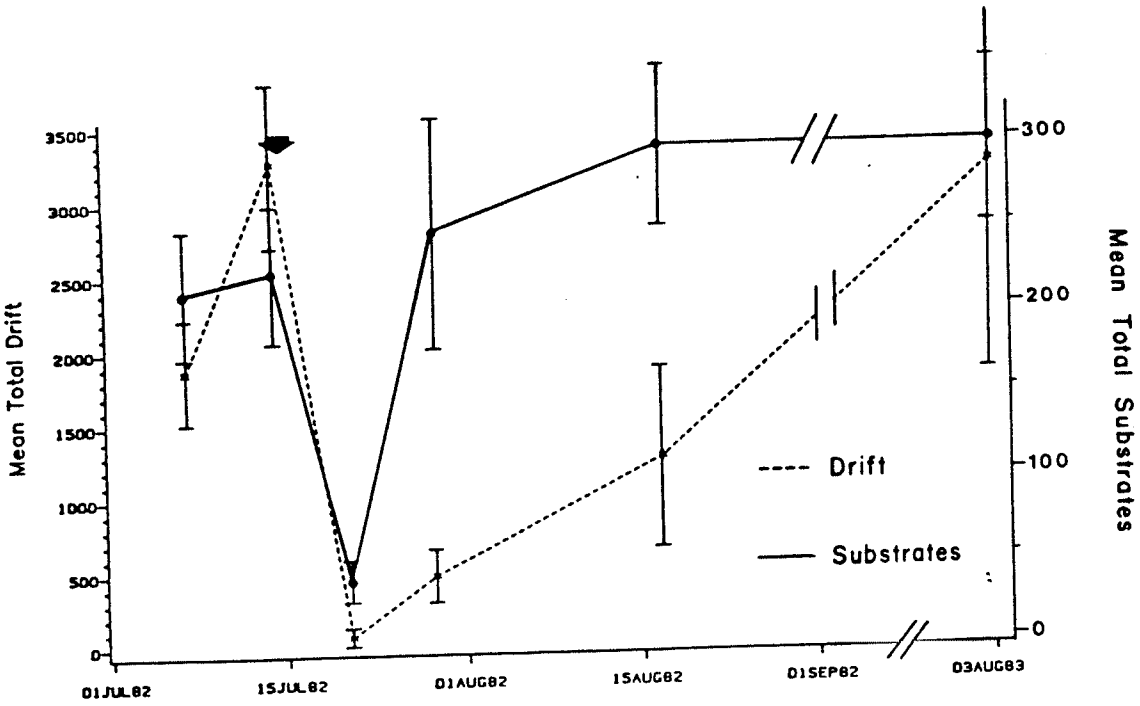

CATOSTOMUS COMMERSONI FRY
control
treatment-outlet
Drift Density (mean no./100 m3)
Drift Density (mean no./100 m3)
0
1
2
3
4
5
6
08
16
24
08
03AUG83

Figure 16. Mean (±s.e.) number of total invertebrates in the drift and on artificial substrates at the treatment-outlet site. Treatment date (15 July 1982) not included. Arrow indicates time of Methoxychlor injection.

Figure 17. Relationship between number of total invertebrates in the drift to totals on artificial substrates at the treatment-outlet site. Numbers on curve indicate days elapsed since Methoxychlor injection.


Mean Total Drift
Mean Total Substrates
3500
3000
2500
2000
1500
1000
500
0
300
200
100
0
Drift
Substrates
01JUL82
15JUL82
01AUG82
15AUG82
01SEP82
03AUG83

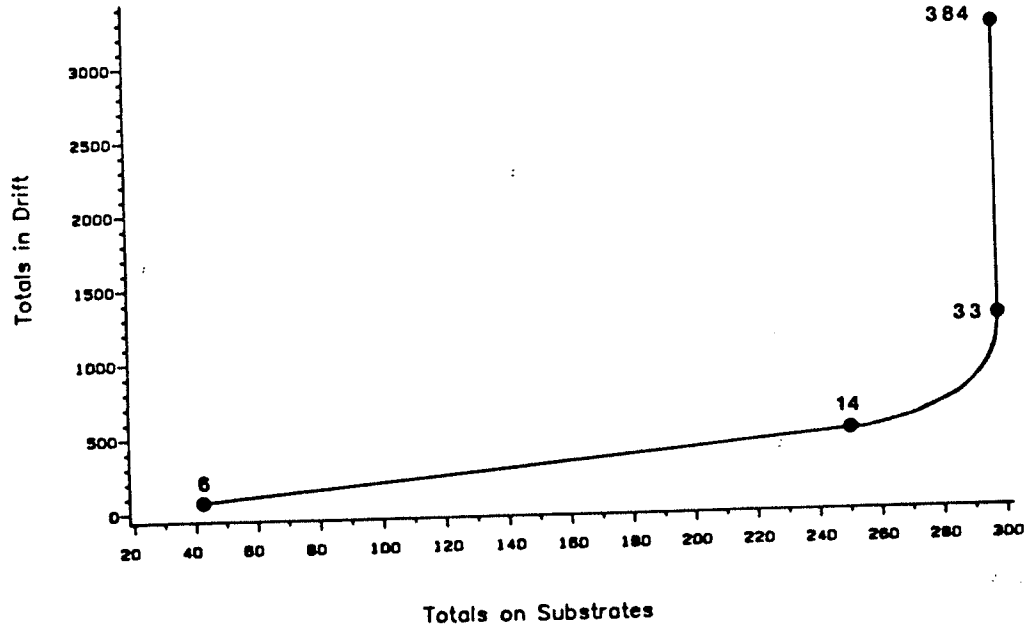
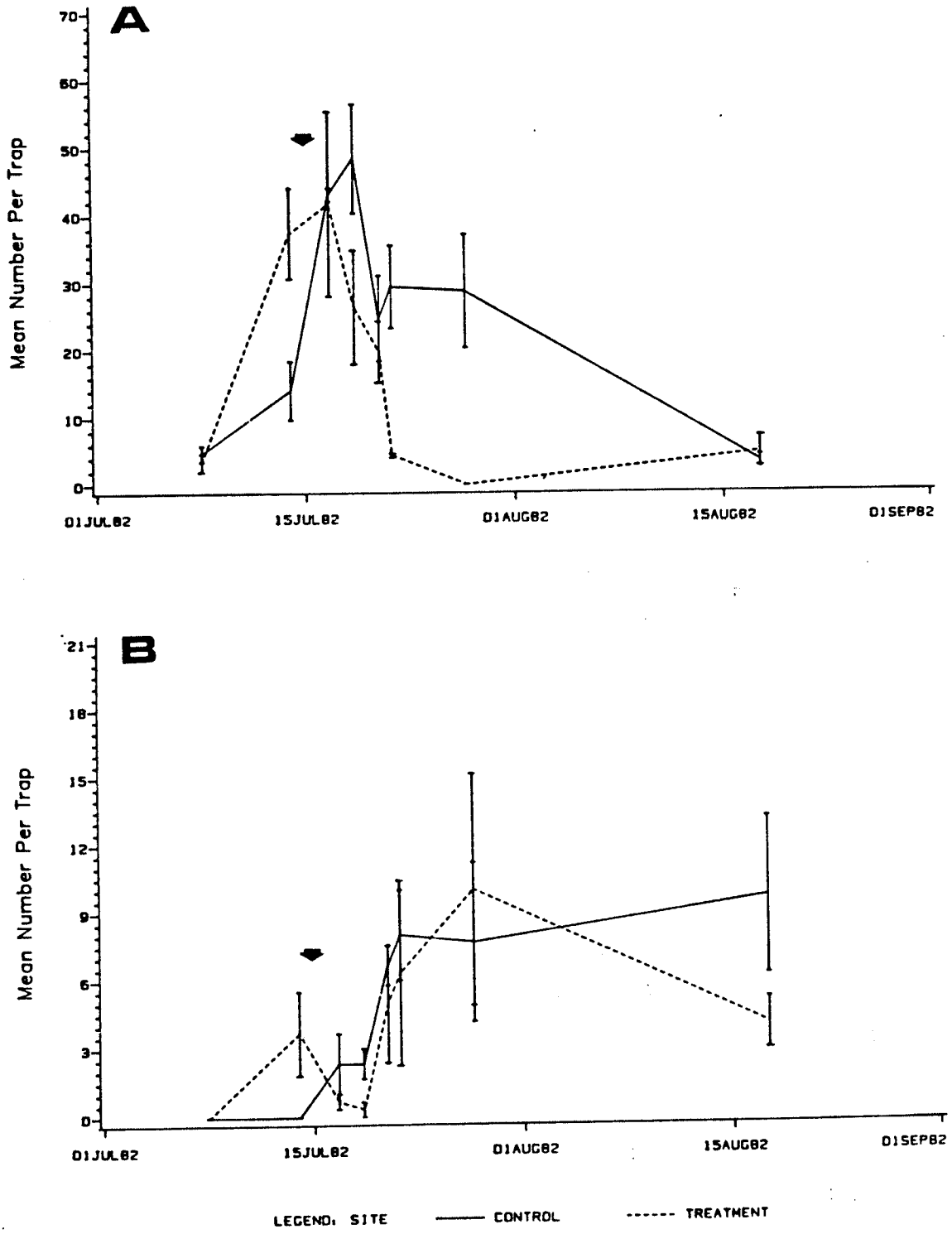

Totals in Drift
3000
2500
2000
1500
1000
500
0
384
33
14
6
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
Totals on Substrates

Figure 18. Mean numbers (±s.e.) emerging at the control and treatment sites. A) Hydroptila ajax B) Cheumatopsyche campyla. Arrow indicates time of Methoxychlor injection.


A
Mean Number Per Trap
70
60
50
40
30
20
10
0
01JUL82
15JUL82
01AUG82
15AUG82
01SEP82
B
Mean Number Per Trap
21
18
15
12
9
6
3
0
01JUL82
15JUL82
01AUG82
15AUG82
01SEP82
LEGEND: SITE
CONTROL
TREATMENT

Chapter V

DISCUSSION

Species responses

Individual taxa responded differently to the pesticide addition and demonstrated varying abilities to recolonize the artificial substrates following treatment. Some recolonized very rapidly (within days), whereas others required a much longer period of time (months) to recover relative to the control site. This great variation in ability to recolonize can probably best be explained by the major ways used by different species for recolonization. Downstream drift may be the most important of these ways. Williams and Hynes (1976) demonstrated that animals recolonizing an area of denuded stream substrate came from four main sources: downstream drift, upstream migration, vertical migration from within the substrate (hyporheic), and egg-laying adults (aerial sources). Drift from upstream was the most important source accounting for 41.4 % of the total number that settled in a denuded area. Townsend and Hildrew (1976) determined that drift from areas upstream was responsible for 82 % of the recolonization observed in their study.

Group 1 species responses

Baetis spp. have a high propensity to drift under natural conditions and this probably represents the main method used by nymphs of this genus to recolonize denuded areas of stream. Numbers of Baetis spp. on substrates at the treatment site were not significantly different ($p>.05$) from the control after only eight days following injection (Table 5). The rapid recovery on the artificial substrates can probably be explained by the very high densities drifting into the treatment riffle at the treatment-inlet site (Fig. 6). The size of Baetis nymphs observed on the substrates at the treatment site a few days after treatment was very similar to that of nymphs observed in the drift at the treatment-inlet site following injection. The mean head capsule widths for a random sample of 30 nymphs from the drift at the treatment-inlet site on 21 July 1982 and on substrates at the treatment site on 23 July 1982 were 0.58 and 0.63 mm respectively. In addition, five of the six species of Baetis collected in this study have bivoltine life cycles (Clifford 1982). (Only B. intercalaris is univoltine). Therefore, because of the high propensity of Baetis spp. to drift and their bivoltine life-cycles, it is doubtful if any of the species of Baetis suffered any long-lasting reductions due to a single methoxychlor treatment.

L. maculipennis also recovered very rapidly on artificial substrates following methoxychlor treatment (Table 5). Similar to Baetis spp. this can be explained by high densities drifting into the treatment riffle at the treatment-inlet site (Fig. 25, Appendix A).

The methoxychlor treatment did not significantly reduce numbers of C. tardata nymphs on artificial substrates at the treatment site. Numbers on substrates were actually significantly higher ($p<.05$) at the treatment site relative to the control on 23 July (8 days post-treatment) (Table 5). Numbers on artificial substrates were not significantly different ($p>.05$) between sites for the remaining post-treatment dates in 1982 and 1983. C. tardata was obviously susceptible to the treatment because of the catastrophic drift observed immediately following injection (Fig. 19, Appendix A). Williams and Hynes (1974) found Caenis sp. to be common in the hyporheos to a depth of 40 cm, with very small instars occurring to a depth of 70 cm. Perhaps the apparent rapid recolonization of the substrates at the treatment site observed after methoxychlor injection occurred as a result of C. tardata nymphs moving up from the hyporheos. Wallace et al. (1973) concluded that fast repopulation of the substrate surface, after application of insecticides may have been due to immigration from hyporheic sources. Recolonization also probably occurred from downstream drift because high densities were found drifting

into the treatment riffle at the treatment-inlet site on all sampling dates in 1982 (Fig. 19, Appendix A). Head capsule measurements of a random sample of 30 nymphs from the drift at the treatment-inlet site on 21 July 1982 showed a similar size distribution to a random sample of 30 nymphs from artificial substrates at the treatment site on 23 July 1982.

Group 2 species responses

Numbers of P. flavida were significantly reduced ($p<.05$) on artificial substrates at the treatment site relative to the control site at all post-treatment sampling dates in 1982 excluding 29 July and 16 August (Table 5) when numbers were also low at the control site. Numbers again increased on substrates at the control site by 17 September (64 days post-treatment) and were significantly greater ($p<.05$) than at the treatment site (Table 5). The increased numbers at the control site on this date resulted from the contribution of a new generation of early instar larvae. Head capsule measurements of a random sample of 30 larvae from the control site on both 19 and 23 July 1982 indicated a uniform larval size distribution. Mean head capsule widths were 0.42 and 0.43 mm respectively. A similar sample taken on 17 September 1982 showed a bimodal size distribution of larvae. Approximately 50% of the sample had a mean head capsule width of 0.58 mm and the remaining 50% had a mean head capsule width of 0.32 mm. An observed emergence of P. flavida from mid-July to mid-August in the Souris River

could also explain the reduced numbers at the control site on 29 July and 16 August 1982 resulting in no significant differences ($p<.05$) between sites. Reduced numbers at the treatment site on the post-treatment sampling dates in 1982 was probably due to both the methoxychlor treatment and an emergence. The long period required for this species to recover on substrates at the treatment site relative to the control site (perhaps until the following summer) can be explained by its absence in the drift under natural conditions. Few were recorded drifting into the treatment riffle at the treatment-inlet site on any of the sampling dates in 1982 (Fig. 8). The major source of colonists seemed to be egg-laying adults. Thus, a much longer period of time was required to achieve recovery. P. flavida is univoltine in the Roseau River, Manitoba (Flannagan 1977). A univoltine species such as P. flavida that has a very low propensity to drift would be very susceptible to long-term reductions by methoxychlor larviciding operations.

The long period of time required for H. recurvata to recolonize the artificial substrates following methoxychlor treatment (Table 5), can also be explained by its very low propensity to drift naturally (Fig. 29, Appendix A).

Group 3 species responses

Numbers of C. campyla on artificial substrates at the treatment site were not significantly reduced ($p<.05$) relative to the control site until four days following

treatment and did not recover until 17 September (64 days post-treatment) (Table 5). The species drifted in relatively low numbers under natural conditions, and few individuals drifted into the treatment riffle at the treatment-inlet site (Fig. 10). C. campyla, however, was emerging throughout July and August at both sites (Fig. 18B). The population at the treatment site on 16 August and 17 September 1982 had shifted to smaller instars than at the control site, although size distribution was comparable between the sites prior to treatment. Head capsule measurements of a random sample of 30 larvae from both the control and treatment sites on 07 July 1982 indicated a similar uniform size distribution. The mean head capsule width was 0.70 and 0.69 mm respectively. The mean head capsule width at the control and treatment sites on both 16 August and 17 September was 0.71 and 0.53 mm respectively. Thus, recolonization of the treatment riffle probably occurred mainly by egg-laying adults since the treatment did not result in a significant decrease ($p<.05$) in emergence at the treatment site relative to the control (Fig. 18B). Minor recruitment also probably came from upstream drift, and the hyporheic because Williams and Hynes (1974) observed that C. campyla occurred to a depth of 20-40 cm in the substrate. C. campyla is univoltine in the Roseau River, Manitoba (Flannagan 1977). Recolonization at the treatment site probably would have required a much longer period of time had emergence not been occurring prior to and during

the period of methoxychlor treatment, due to the low behavioural drift and univoltine life cycle of this species. Emerging species of aquatic invertebrates, that are in the terrestrial stage and thus not exposed to the pesticide in the river, can contribute to recolonization by laying eggs in treated sections of the river, a point raised by Byrtus (1982).

Numbers of H. ajax on substrates at the treatment site were not significantly reduced by the methoxychlor treatment. In fact, numbers were significantly higher ($p<.05$) at the treatment site relative to the control on 16 July (1 day post-treatment) (Table 5). The species was obviously susceptible to the treatment because of the catastrophic drift observed immediately following injection (Fig. 12). The apparent lack of effect of the methoxychlor injection on numbers on substrates at the treatment site was probably due to a combination of mature larvae and pupae occurring on the substrates. These life stages were all counted together. The mature larvae were affected by the treatment resulting in a catastrophic drift whereas the pupae, which were completely encased in silk cases, may not have been directly exposed to the pesticide moving through the treatment riffle. As can be seen in Fig. 18A, H. ajax emerged in high numbers during the 1982 sampling period. Specimens counted on substrates immediately after treatment at the treatment site on 16 July and again on 29 July were

almost all pupae, with few larvae being observed. The species was much more numerous on substrates at the treatment site relative to the control on the pre-treatment sampling dates immediately before injection (Table 5). If only a portion of these numbers (larvae) were affected by the treatment, it would leave the overall impression that the methoxychlor treatment had little effect on total numbers on substrates at the treatment site. Emergence was eventually significantly depressed ($p<.05$) at the treatment site relative to the control site because of the adverse effect of the treatment on larvae (Fig. 18A). Recolonization probably occurred mainly by egg-laying adults from the emergence peak, because very low densities were observed drifting into the treatment riffle (Fig. 12). Again, this demonstrates the ability of a species in the pupal stage, which appears to be less susceptible to the chemical, or one which is emerging and thus not exposed to the pesticide in the river, to act as an important source for recolonization. If the pesticide was applied at a time when H. ajax was not emerging, the impact would probably have been much more severe because of the low propensity of H. ajax to drift under natural conditions.

Responses of other taxa

Ephoron album was adversely affected by the methoxychlor treatment as indicated by the large catastrophic drift observed immediately following treatment (Fig. 21, Appendix

A). E. album is a burrowing mayfly, indicating that the methoxychlor was able to penetrate the burrows formed in the gravel substrate and produce a response by the nymphs. E. album is known to have a univoltine life-cycle (Clifford 1982). Hatching, growth and emergence take place in the summer, and the population overwinters in the egg stage. Emergence occurred in late July and early August in the Souris River in 1982. Giberson (1984) observed females emerging into a swarm of males and mating occurred almost immediately in the Valley River, Manitoba. Females then flew to the water surface, expelled their eggs and died. The entire adult life span was less than four h, and very limited dispersal occurred prior to egg laying. A species such as E. album that drifts in very low numbers under natural conditions, has a univoltine life cycle, and a short-lived adult stage should be very susceptible to long term reduction by repeated methoxychlor treatments. Drift density remained significantly depressed ($p<.05$) at the treatment-outlet site relative to the control on 03 August 1983 (384 days post-treatment) (Table 3). However, low numbers of E. album observed in the drift at both sites on the sampling dates after treatment in 1982 and 1983 make statistics indicating a significant reduction ($p<.05$) in drift densities at the treatment-outlet site questionable. The data do not allow any definite conclusion regarding the impact of methoxychlor on E. album in 1983 (1 year following treatment).

Acroneuria lycorias, a predatory stonefly, also demonstrated a catastrophic drift following methoxychlor injection (Fig. 33, Appendix A), but drift densities did not increase to the same magnitude as the other invertebrate taxa. This was probably for two reasons: 1) it was rare at the study sites, and 2) it may not have been sampled effectively by the drift samplers used. A. lycorias is a strong swimmer and was observed crawling out of a drift net on one occasion, hence its absence in the drift under natural conditions may be misleading. The elevated drift observed following treatment (Fig. 33, Appendix A) does indicate that the species is susceptible to methoxychlor, (see also Sebastien and Lockhart 1981), and the specimens observed in the drift nets were probably moribund and could not escape before the sample was collected. A. lycorias was found on artificial substrates at the treatment site at various post-treatment sampling periods, although numbers were too low to permit any meaningful statistical analysis (Table 5). Some individuals were able to survive the methoxychlor injection as suggested by its presence on substrates at the treatment site only four days after treatment.

Orconectes virilis juveniles were seldom observed in the drift. However, the methoxychlor treatment resulted in a substantial catastrophic drift (Fig. 38, Appendix A). Merna and Eisele (1973) concluded that O. virilis was the least

susceptible organism to methoxychlor of their laboratory test animals. Flannagan et al. (1979) found no significant effect on blood calcium and no mortality in caged O. virilis after an application of 0.3 mg/litre/15 min methoxychlor into the Athabasca River approximately 200 m upstream. Both these researchers used mature specimens in their experiments. Many of the juvenile specimens collected in the catastrophic drift from the Souris River were moribund or dead indicating a high susceptibility to the methoxychlor treatment. O. virilis juveniles may be far more sensitive to methoxychlor than has been previously indicated for mature specimens.

Catostomus commersoni fry showed a definite diel drift periodicity with maximum numbers occurring during periods of darkness (Fig. 14). Clifford (1972) also observed a diel drift during the downstream migration of the fry. It occurred during periods of darkness because the fry lost their orientation to the substrate and entered the water column. Although 24-h drift densities were not significantly different ($p<.05$) between the control and treatment-outlet sites for any of the sampling dates in 1982 or 1983, the treatment did produce an immediate 3-fold increase in drift density of the fry at the treatment-outlet site relative to the control (Fig. 14). Fish mortality was not observed at any time during the experiment. However, the elevated drift indicated an avoidance response to the

chemical by the fish fry, and some were moribund in the pool downstream of the treatment riffle immediately following injection. Flannagan et al. (1979) observed a very large increase in numbers of alevins and fry of C. commersoni in drift nets located 400 km downstream of a methoxychlor injection into the Athabasca River. It was not established if the increase was due to a disturbance or kill of these animals by the methoxychlor, or if it was simply a natural distributional phenomenon.

Larval fish would be more susceptible to acute poisoning than more mature fish. Most of the research conducted on the acute toxicity of methoxychlor to native fish species during black fly larviciding operations (e.g. Lockhart et al. 1977, Lockhart 1980) has been focused on fish specimens in excess of 100 g and has excluded consideration of larval fish. Lockhart (1980) reported that exposure of 3- to 240-g rainbow trout to methoxychlor resulted in a statistically significant inverse relationship between fish size and methoxychlor uptake. Recently, Holdway and Dixon (1984) concluded that methoxychlor was very harmful to juvenile white suckers in laboratory pulse-dosed methoxychlor experiments. Pre-exposure of 1-, 3- and 6-day old fertilized sucker eggs to methoxychlor significantly increased the number of deformed white sucker larvae compared to controls. Two-day-old white sucker larvae from control eggs exposed to 0.1 mg/litre methoxychlor and higher

for two h failed to develop swim bladders. Pre-exposure of 1-day-old white sucker eggs reduced the growth of larvae exposed to 0.1 mg/litre compared to controls. Ten-day-old fed white suckers from control eggs had a 96 h LC_{50} of 0.002 mg/litre methoxychlor. This is a considerably lower concentration than the 0.3 mg/litre level they would be exposed to immediately downstream from the injection site in a river black fly larviciding operation. Spawning activities of white suckers (C. commersoni) could potentially coincide with a methoxychlor larviciding operation, and more research is required to determine the impact of treatments on early life-stages of this species. It has also been suggested that methoxychlor larviciding could result in a detrimental effect on fish populations by reducing food supplies (Flannagan et al.1980, Lockhart 1980, Wallace et al. 1973), which could prove devastating for larval fish.

Community responses

Diversity in the drift, calculated using the modified Simpson Index and the Shannon-Weaver Index, was only significantly reduced ($p<.05$) at the treatment site relative to the control for six days following treatment (Table 4). Total numbers in the drift, however, were significantly reduced ($p<.05$) at the treatment-outlet site relative to the control for all the post-treatment sampling dates in 1982 including 17 August (33 days post-treatment) (Table 3).

Similarly, many individual taxa including Baetis spp., C. tardata, E. album, H. azteca, H. recurvata, H. ajax, I. sicca, and S. interpunctatum showed significant reductions ($p<.05$) in 24-h drift densities at the treatment-outlet site compared to the control for all the post-treatment sampling dates in 1982. Most diversity indices used for environmental impact assessment work on the assumption that the pollution source will reduce the number of intolerant species while favoring the tolerant species. A non-selective pollution source or stress such as methoxychlor which reduces the density of most species without favoring any one taxon will not necessarily result in lower index values. Species richness in the drift was also significantly reduced ($p<.05$) at the treatment site relative to the control for all the post-treatment sampling dates in 1982 (Table 4). However, most of the species that were eliminated from the drift by the methoxychlor injection were very rare in the drift under natural conditions (e.g. E. album, O. virilis, P. cinereus, I. sicca). Both the Simpson and Shannon-Weaver indices are not sensitive for rare species as previously mentioned. The inability of both the Simpson and Shannon-Weaver diversity indices to respond to reduced densities of most species in the drift and the elimination of rare species questions their use for a non-selective stress such as a methoxychlor treatment. Species richness seems to be a more sensitive indicator of biological change induced by the pesticide treatment, an opinion also shared by Green (1979).

Species diversity on substrates calculated using the modified Simpson index and the Shannon-Weaver index fluctuated at the treatment site in the two-week period following injection. Diversity was significantly reduced ($p<.05$) at the treatment site relative to the control site on 16 July (1 day post-treatment), and 29 July (14 days post-treatment) (Table 6). Total numbers on substrates (not including H. recurvata) were significantly lower ($p<.05$) at the treatment site compared to the control site for only 8 days following treatment (Table 5). Only three taxa found on the substrates (H. recurvata, C. campyla, and P. flavida) were significantly reduced ($p<.05$) at the treatment site relative to the control site for most of the post-treatment sampling periods in 1982 (Table 5). Species richness on substrates was significantly reduced ($p<.05$) at the treatment site for just four days following treatment (Table 6). Thus species richness and total abundances required a much longer period of time to recover in the drift than on substrates, indicating that drift was a more sensitive measure of impact due to methoxychlor treatment than numbers on substrates.

Drift-Benthos relationship

The recovery of total invertebrate drift was delayed compared to the rapid recovery of invertebrates on substrates at the treatment-outlet site following methoxychlor injection (Fig. 16). Assuming that the numbers

of invertebrates on artificial substrates are representative of actual numbers in the benthos, there may be a density relationship between drift and benthos. This relationship is further illustrated in Fig. 17 where total invertebrate drift is plotted against total invertebrates on substrates for the post-treatment sampling dates at the treatment-outlet site. If drift of aquatic invertebrates is not related to benthic density, numbers drifting should be uniform regardless of bottom densities, even after recovery. However, if drift is density related, the proportion entering the drift would increase as the total benthic population increased. A density relationship between total numbers in the drift and totals on substrates is indicated in Fig. 17. The increasing bottom component of the total fauna has reached the "carrying capacity" of the artificial substrates at about 33 days post-treatment. Fig. 17 indicates that drift continues to increase up to the last sample date (384 days post-treatment), suggesting recruitment from sources other than the artificial substrates. Benthic invertebrates can occur in quite large numbers down to depths of 1 m or more in the interstices that are formed in the mixture of course sand, gravel, and rocks typically found in the bottom of streams. In running water, this habitat has been termed the hyporheic zone (Orghidan 1959). Williams and Hynes (1974) estimated that only a maximum of 20% (by numbers) of the total fauna occurred in the top 5 cm of substrate in an Ontario stream.

Other researchers report similar observations (e.g. Poole and Stewart 1976; Godbout and Hynes 1982). The artificial substrates used in the present study only gave a quantitative estimate of shallow, surface benthos populations. The increased total drift component observed after 33 days post-treatment probably represented a recruitment from the hyporheic zone. Total drift would eventually level off when the hyporheic habitat had recovered from pesticide treatment. However, an accurate conclusion regarding a drift-benthos relationship cannot be made because: 1) only four samples were taken after treatment; 2) sampling was not continued until total drift had maximized; 3) artificial substrates were used to estimate bottom densities (the hyporheic habitat was not sampled).

Drift resumes being a significant process only after population recovery of the standing crop. Dimond (1967) found that it required one or two seasons after bottom populations had recovered for the drift to regain its normal abundance and composition in streams with different DDT treatment histories. Drift seemingly results from population surpluses, as was suggested by Waters (1966), and provides a means of maintaining balance between populations and resources.

Drift could be used as an effective measure of stream recovery or as an indication of water quality in place of

benthos samples. Larimore (1974) compared the drift of organisms in 100 m^3 of water during the peak period of drifting with the benthos from 1 m^2 of stream bottom in streams having different levels of domestic and industrial pollution. Numbers and weights of both drift and benthos followed similar quantitative relationships indicating the level of water quality. In large, deep, turbid rivers where benthos sampling is all but impossible at mid stream, the use of drift sampling to indicate recovery after an insecticide treatment or the level of water quality merits further investigation. Benthos samples taken in close proximity to the river banks, a common practise of many studies involving the impact of a toxicant on the benthos of large rivers, would seem to be a poor indicator of the impact near the centre of the channel where concentration of the chemical and ecological conditions may be completely different. The major disadvantages of using drift as an indication of water quality are: 1) the habitat from which the species originated is unknown, and 2) not all benthic species drift.

Application of the theory of island biogeography to invertebrate recolonization

The theory of island biogeography can be applied to invertebrate recolonization following black fly larviciding operations in large rivers. MacArthur and Wilson (1963, 1967) suggested that changes in the number of species in a

community over time result from interaction between an extrinsic immigration process that adds species and an intrinsic extinction process in which species are eliminated by ecological processes and chance. Equilibrium (stability) will be achieved when the immigration rate (I) is equal to the extinction rate (E). A number of predictions can be made about immigration, extinction, and the resultant species equilibrium, including: 1) the more distant a habitat patch is from the source of colonization (or the lower its I for any other reason), the fewer the species that will be found there; 2) reduction of the species pool of immigrants will reduce the number of species; 3) the smaller the size of the habitat patch (or the greater its E for any other reason), the fewer the species. Minshall et al. (1983) studied the long-term invertebrate recolonization of a portion of the Teton River, Idaho which was re-flooded after being diverted to facilitate channel restoration. The results were examined in terms of the theory of island biogeography. The rate of colonization or recovery varied directly with distance from the source of colonizers. The number of taxa present, total abundance and Shannon-Weaver diversity generally were inversely correlated with distance downstream. Similarly Gore (1982) observed that the time to faunal recovery (achievement of 85 % similarity of species composition relative to a control site) was approximately doubled with each 200 m increase in distance from the primary source of colonists in a reclaimed section of the Tongue River, Wyoming.

Sites closest to the pesticide injection following a black fly larviciding operation (although exposed to the highest pesticide concentrations) should have the greatest potential to recolonize rapidly because of their proximity to a source of colonizers such as drift and aerial adults from upstream. The time required to recolonize should progressively increase with increasing distance from the treatment site and hence upstream source of colonists. Flannagan et al. (1979) found a significant reduction in standing crop at their two furthest downstream sampling sites one year after a methoxychlor treatment into the Athabasca River, whereas the upstream sampling sites had completely recolonized. The greatest long-term effect on non-target invertebrates was observed at the furthest downstream sample site (400 km) from the treatment site. In streams, the effect of distance will break down eventually since with time the source of colonization will progressively shift downstream and distances from the source of colonists will decrease.

Potential impacts on lotic functional processes

Although the purpose of this study was to examine structural changes in a riffle community produced by a methoxychlor treatment, functional processes such as energy transfer and nutrient cycling may also be adversely affected. Wallace et al. (1982) observed that total numbers of insects, shredders, predators, as well as

leaf-breakdown rates and transport of particulate organic matter were significantly lower in a stream treated with a 24 % emulsifiable concentrate of methoxychlor than in a reference stream. Perhaps the action of pesticides on stream ecosystems extends beyond simple mortality of invertebrates in the treated area and may significantly affect communities in downstream areas due to altered food (energy) supplies.

Many species of aquatic insects play an important role in the processing and cycling of nutrients in lotic ecosystems. Filter-feeding insects have evolved an array of mechanisms that enable different species to consume different particle sizes and types of materials. This reduces export and enhances storage of these particulates, thereby increasing the efficiency of organic input utilization within a stream reach. By increasing the cycling rate of inorganic constituents, such as phosphorus and heavy metals sorbed to the surfaces of these particulates, filter-feeding insects can potentially affect movement of inorganic ions in streams (Wallace and Merritt 1980). Burrowing insects (e.g. chironomids and burrowing mayflies) can influence the release of nutrients from sediments to the overlying water (Gardner et al. 1981). Aquatic insects are also thought to play a significant role in nutrient cycling by influencing community structure and turnover rates of the algal and microbial populations that constitute their food (Merritt

et al.. 1984). The application of a non-selective pesticide can thus potentially affect functional processes such as energy and nutrient flow within lotic ecosystems in more subtle ways than previously recognized, and should be a topic for future research.

Stability of the Souris River riffle community

Stability has been defined as the ability of ecosystems to recover from external disturbances (Webster et al. 1983). An ecosystem is considered stable if its response to a disturbance is small and its return to its original state is relatively rapid. An ecosystem is considered unstable if it is greatly changed by disturbance, returns slowly to its original state, or if it never recovers to the original state (Webster et al. 1983). The riffle community in the Souris River was greatly changed by the methoxychlor injection, however it did return to its original state relatively rapidly. Species richness, species evenness (as measured by the Simpson index), species diversity (as measured by the Shannon-Weaver index), and total abundance in both the drift and on substrates were not significantly different ($p<.05$) between sites one year following treatment. Thus according to the above criteria it can be considered stable. Factors contributing to the high resilience of the Souris River riffle community, and stream ecosystems in general, include: 1) the short life cycles of the organisms in the community, 2) rapid recolonization

mechanisms (e.g. drift, flying adults, recolonization from the hyporheic zone), 3) annual renewal of allochthonous inputs, and, probably most important 4) the continual flushing by the current (Webster and Patten 1979).

Although general recovery of the treated riffle invertebrate community had occurred by the following year, the results of this study should be put into proper perspective when attempting to determine the impact of methoxychlor treatments on entire river invertebrate ecosystems. The treatment riffle site in the present study was located immediately downstream from the methoxychlor injection. Invertebrates inhabiting the riffle were exposed to the pesticide at the target dosage of 0.3 mg/litre/15 min after which residues in the water declined to trace levels for about 12 h and completely disappeared after 24 h. Species located at sites some distance downstream from a methoxychlor injection would be exposed to the pesticide under different conditions. The methoxychlor injection would have been attenuated as it moved downstream and invertebrates would be exposed to the chemical at lower concentrations but for a longer period. Also, the majority of methoxychlor should have adsorbed onto suspended solids in the water. The toxicity of methoxychlor was observed to increase with increasing exposure time for most organisms examined under laboratory conditions (e.g. Anderson and DeFoe 1980; Merna and Eisele 1973; Sanders and Cope 1968).

Taxa colonizing downstream sites that are exposed to the pesticide under chronic conditions may be at a higher risk. Recolonization could also be prolonged because of the further distance from untreated-upstream sources of colonizers (e.g. drift and aerial adults). Multiple methoxychlor injections (as has occurred in some black fly larviciding operations in western Canadian rivers), may be more harmful to benthic invertebrates than a single application such as was investigated in the present study. Finally, potential effects of the treatment on functional processes such as energy transfer and nutrient cycling were not examined in this study.

Chapter VI

CONCLUSIONS

1. The methoxychlor treatment was not selective for any one taxon or functional feeding group of invertebrates monitored. All species observed showed an immediate adverse response to the methoxychlor injection in the form of a pronounced catastrophic drift which lasted from 4 to 24 h.

2. Individual taxa demonstrated varying abilities to recolonize the substrates following treatment. Species that had a high propensity to drift under natural conditions recolonized most rapidly (e.g. *Baetis* spp., *C. tardata*, and *L. maculipennis*).

3. Taxa that required the longest period of time to recolonize following methoxychlor treatment were generally univoltine species that had a very low propensity to drift and a limited ability to disperse as adults (e.g. *P. flavida*).

4. The impact of the methoxychlor treatment on some species was influenced by the phenology of those species. Taxa about to emerge that were in the pupal stage and apparently not affected by the pesticide, or that were in the terrestrial adult stage and thus not exposed to the

pesticide in the river, acted as important sources for recolonization (e.g. H. ajax and C. campyla).

5. The methoxychlor treatment resulted in an immediate 3-fold increase in drift density of white sucker (C. commersoni) fry, and some were observed to be moribund in the pool downstream of the treatment riffle immediately following injection. Juvenile fish are more sensitive to methoxychlor than are adults. Spawning activities of C. commersoni could potentially coincide with a methoxychlor larviciding operation, and more research is required to determine the impact of treatments on early life stages of this species.

6. The methoxychlor treatment resulted in a catastrophic drift of crayfish (O.virilis) juveniles which were moribund or dead. O. virilis juveniles may be far more sensitive to methoxychlor than has been previously indicated for mature individuals.

7. Species diversity in the drift was significantly reduced ($p<.05$) at the treatment site relative to the control for six days following treatment. Species richness and total numbers in the drift were significantly reduced ($p<.05$) at the treatment site relative to the control for at least 33 days following treatment and may have required until the following summer to recover relative to the control site.

8. Species diversity on substrates was significantly reduced ($p<.05$) at the treatment site relative to the control for 14 days following treatment. Species richness and total numbers on substrates were significantly lower ($p<.05$) at the treatment site compared to the control site for 4 and 8 days respectively following injection.

9. Diversity indices such as the Shannon-Weaver and Simpson do not respond to a non-selective pollution source or stress such as a methoxychlor treatment which reduces the density of most species without favoring any one species, and initially only eliminates rare taxa. The use of these indices for studies involving non-selective pollution sources is seriously questioned. Species richness seems to be a better indicator of biological change induced by pesticide treatments.

10. The delayed recovery of total invertebrate drift compared to the rapid recovery of invertebrates on substrates at the treatment-outlet site following methoxychlor injection suggests a density relationship of drift to benthic standing crop. Drift becomes a significant process only after population recovery of the standing crop. It seemingly results from population surpluses and provides a means of maintaining balance between populations and resources. Invertebrate drift seems to be a more sensitive measure of the impact of pesticide treatments on aquatic invertebrate communities than are benthic densities as measured using artificial substrates.

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Appendix A

DRIFT AND ARTIFICIAL SUBSTRATE FIGURES FOR ADDITIONAL AND TOTAL TAXA

Appendix A consists of 1982-83 drift figures for species showing similar responses to the representative species included in the Results section for groups 1 and 2, and all of the group 4 species. Taxa included here are: group 1) C. tardata and L. maculipennis, group 2) H. recurvata, and group 4) E. album, I. sicca, S. interpunctatum, P. cinereus, A. lycorias, H. azteca, and O. virilis. The 1982-83 drift data for the Chironomidae and total taxa are also presented. Figures for all species observed on artificial substrates including Baetis spp., C. tardata, L. maculipennis, S. interpunctatum, H. ajax, P. flavida, I. sicca, H. azteca, H. recurvata, A. lycorias, P. cinereus, C. campyla, and total taxa are also included here.

Figure 19. Mean (±s.e.) drift density of Caenis tardata larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density significantly lower ($p<.05$) at the treatment-outlet site relative to the control site on 21 July, 29 July, and 17 August.

CAENIS TARDATA

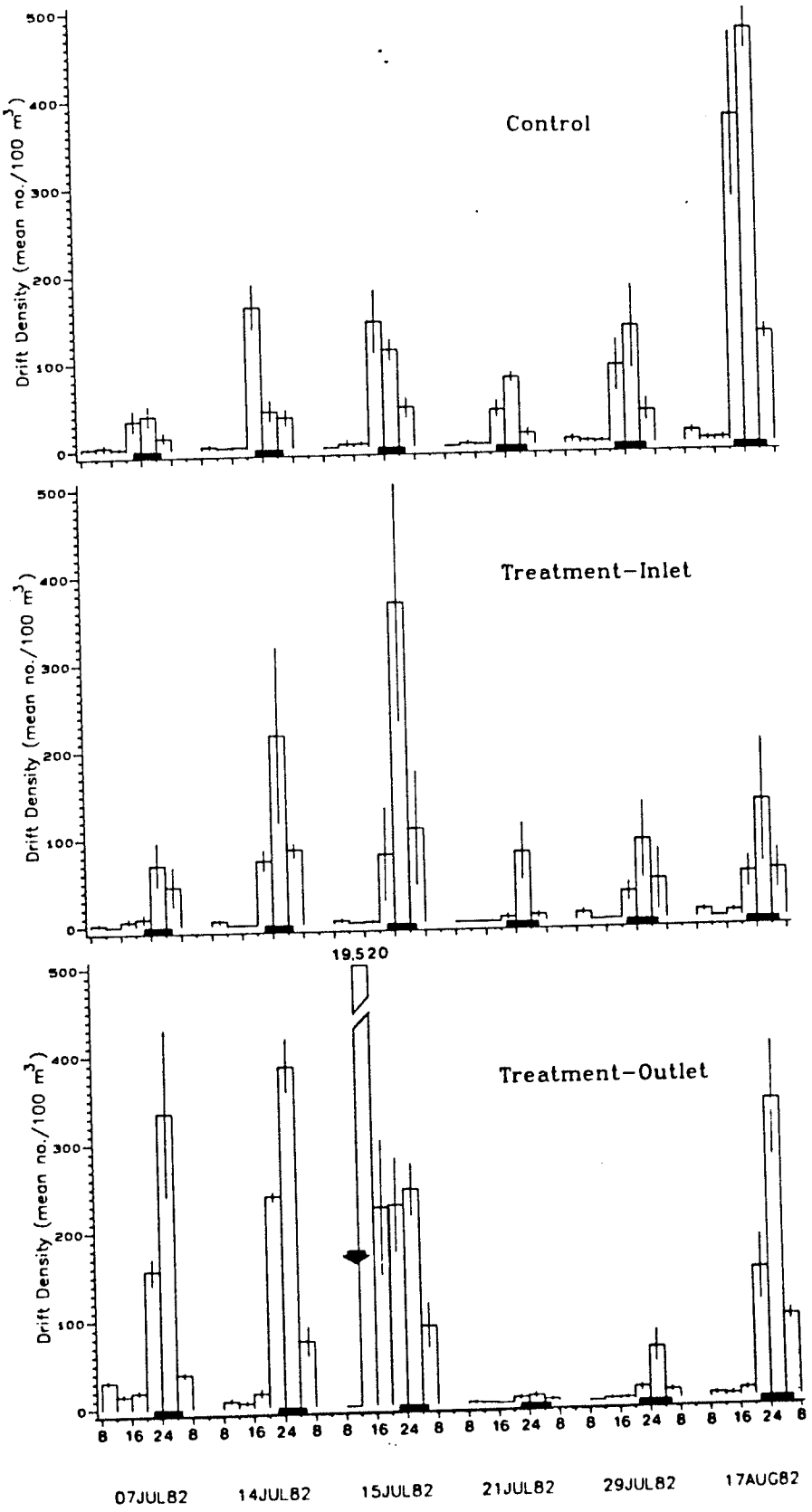


Figure 20. Mean (±s.e.) drift density of Caenis tardata larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites.

CAENIS TARDATA

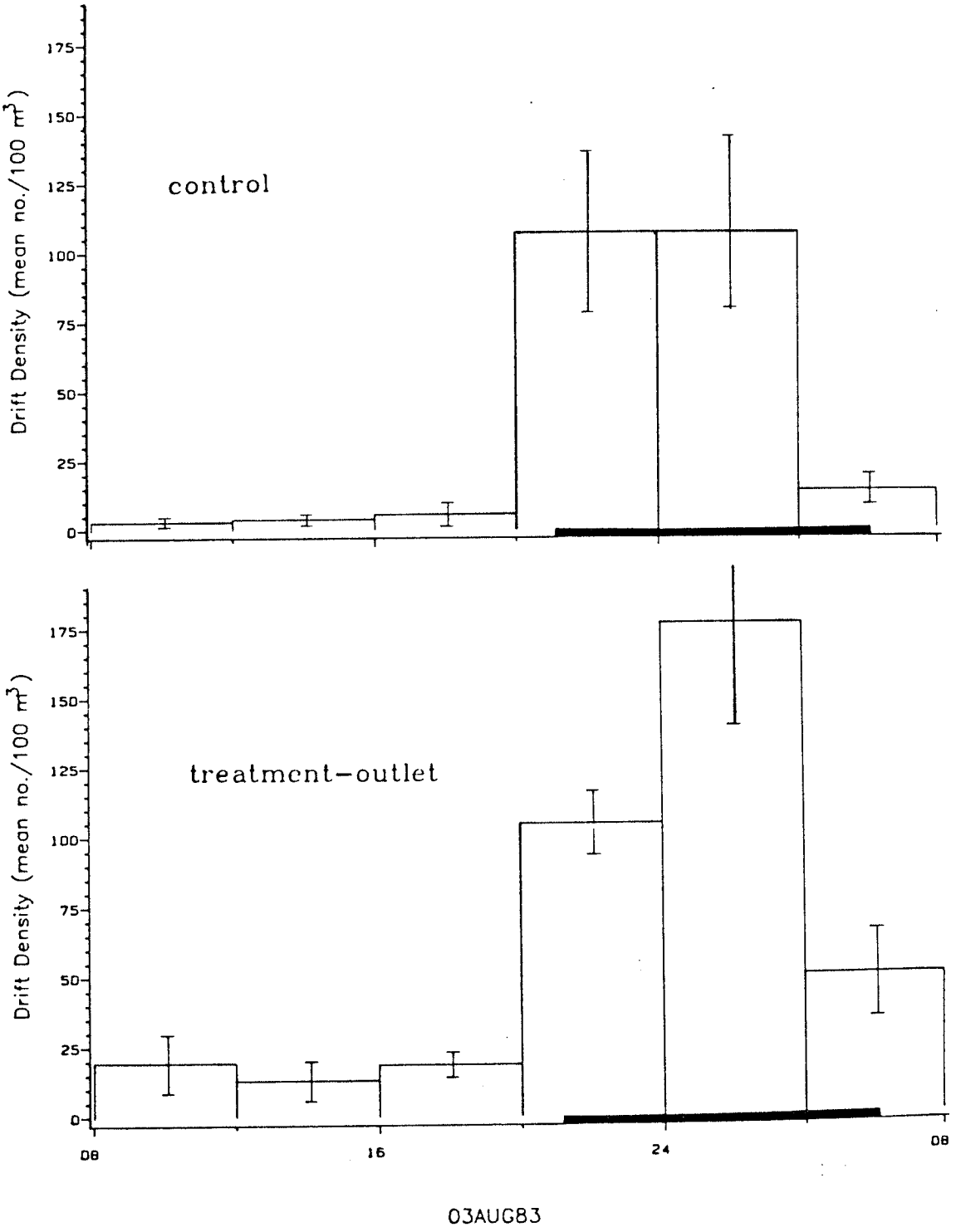


Figure 21. Mean (±s.e.) drift density of Ephoron album larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density significantly lower ($p<.05$) at the treatment-outlet site relative to the control site on 21 July, 29 July, and 17 August.

EPHORON ALBUM

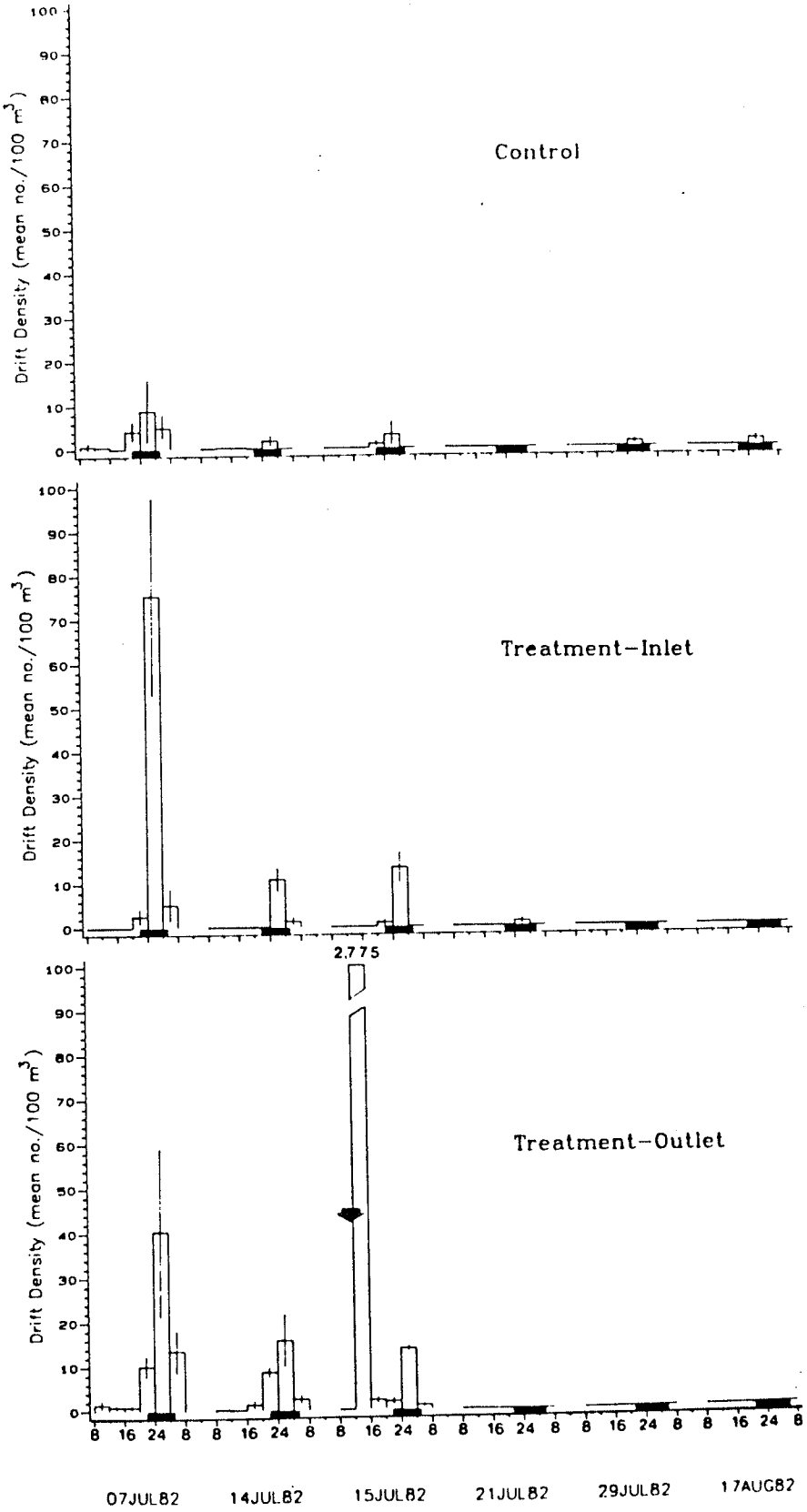


Figure 22. Mean (±s.e.) drift density of Ephoron album larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

Drift density significantly lower ($p<.05$) at the treatment-outlet site relative to the control site.

EPHORON ALBUM

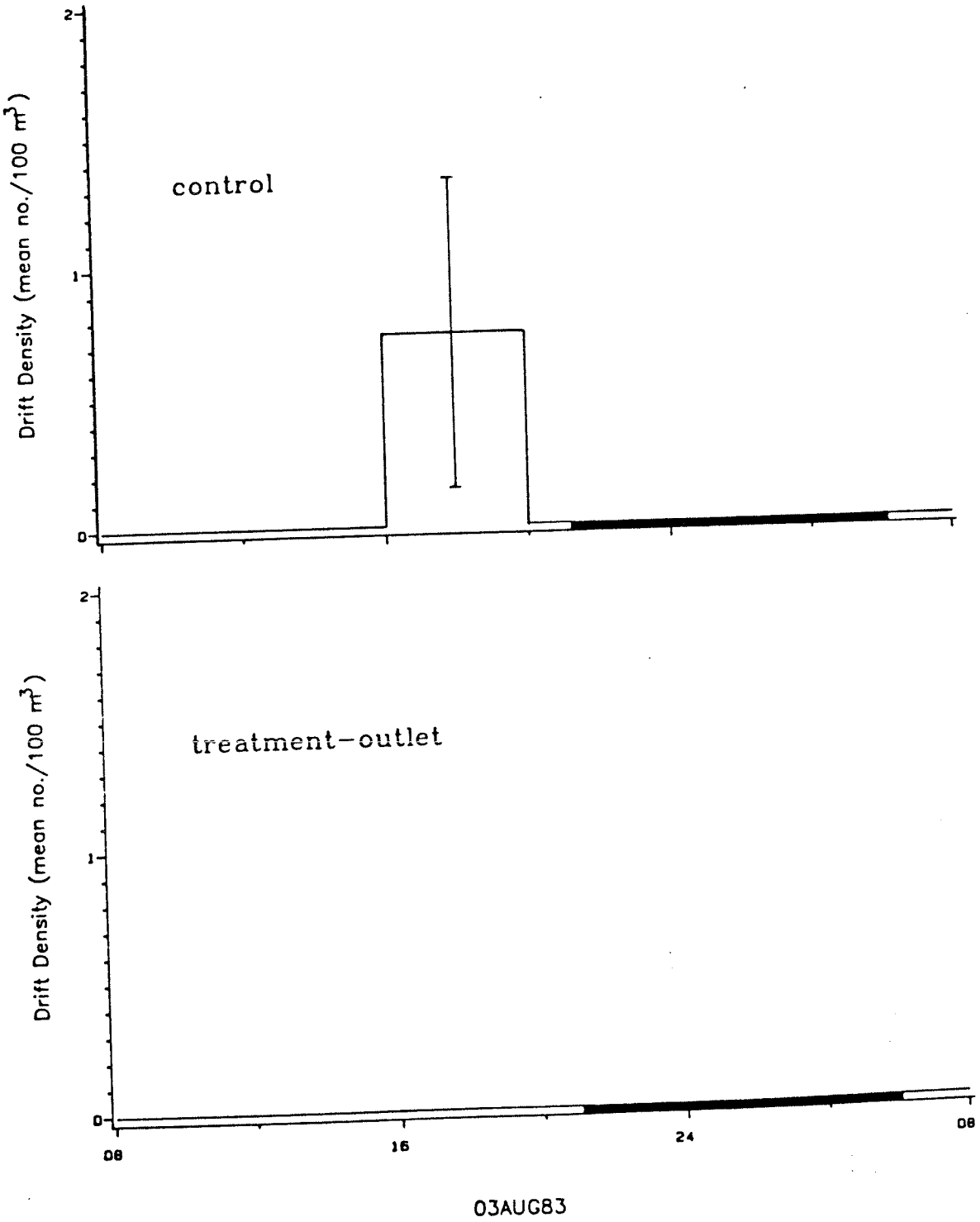


Figure 23. Mean (±s.e.) drift density of Isonychia sicca larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density significantly lower ($p<.05$) at the treatment-outlet site relative to the control site on 21 July, 29 July, and 17 August.

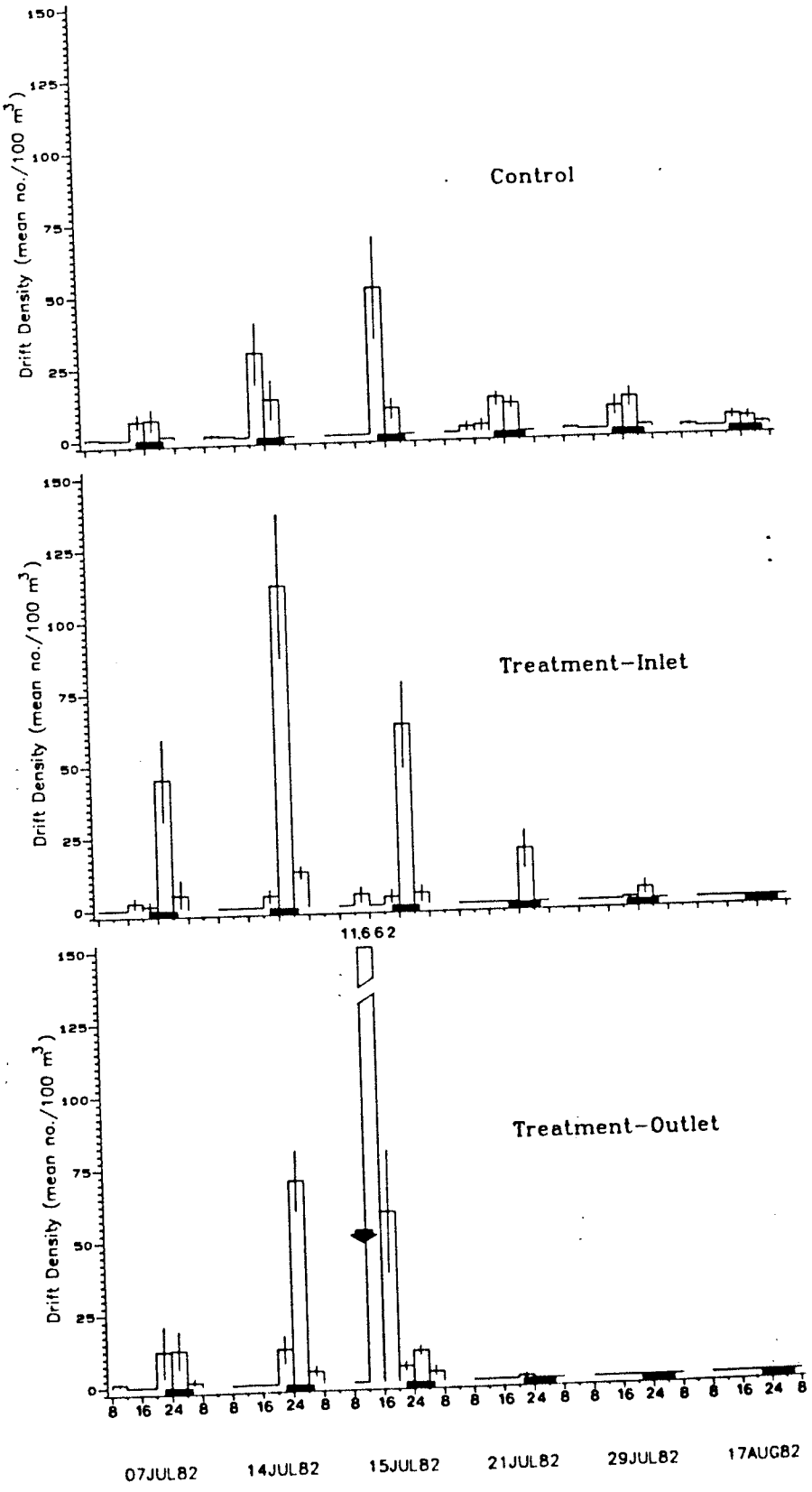

Drift Density (mean no./100 m³)
150
125
100
75
50
25
0
Control
Treatment–Inlet
Treatment–Outlet
11.662
8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8
07JUL82
14JUL82
15JUL82
21JUL82
29JUL82
17AUG82

Figure 24. Mean (±s.e.) drift density of Isonychia sicca larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

Drift density significantly lower ($p<.05$) at the treatment-outlet site relative to the control site.

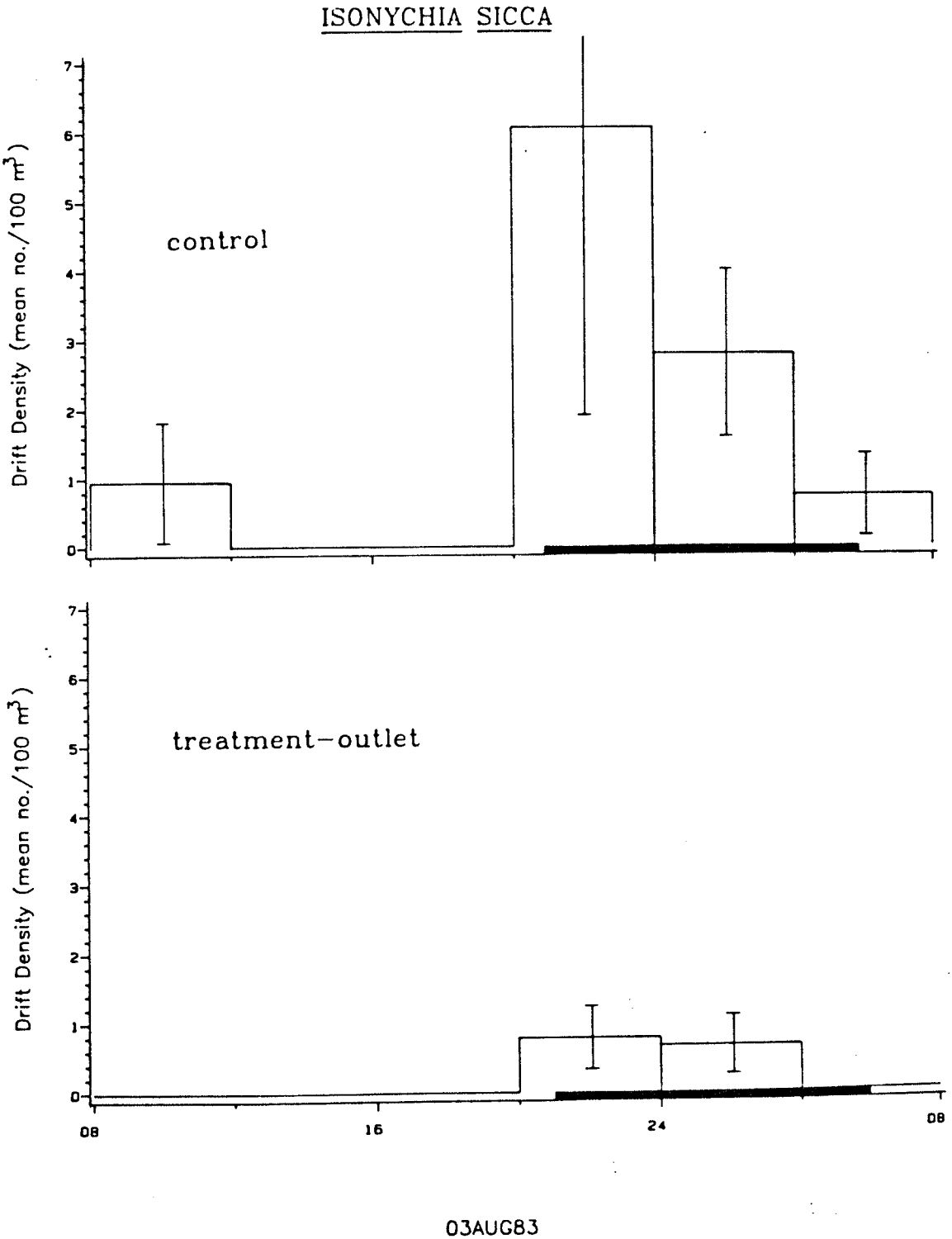

ISONYCHIA SICCA
control
treatment-outlet
Drift Density (mean no./100 m³)
0
1
2
3
4
5
6
7
08
16
24
08
03AUG83

Figure 25. Mean (±s.e.) drift density of Leucrocuta maculipennis larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density significantly lower ($p<.05$) at the treatment-outlet site relative to the control site on 21 July and 29 July.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites on 17 August.

LEUCROCUTA MACULIPENNIS

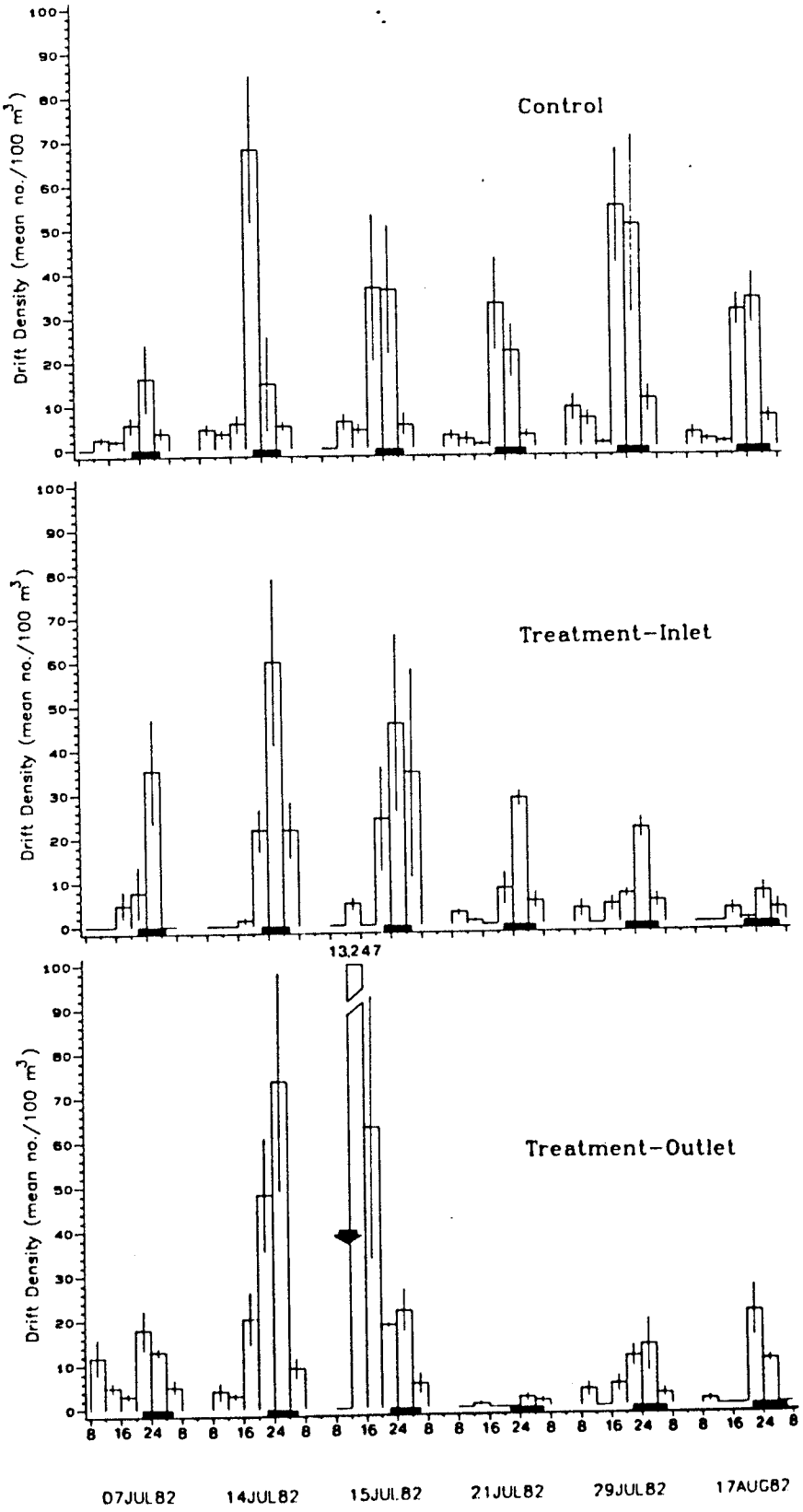


Figure 26. Mean (±s.e.) drift density of Leucrocuta maculipennis larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites.

LEUCROCUTA MACULIPENNIS

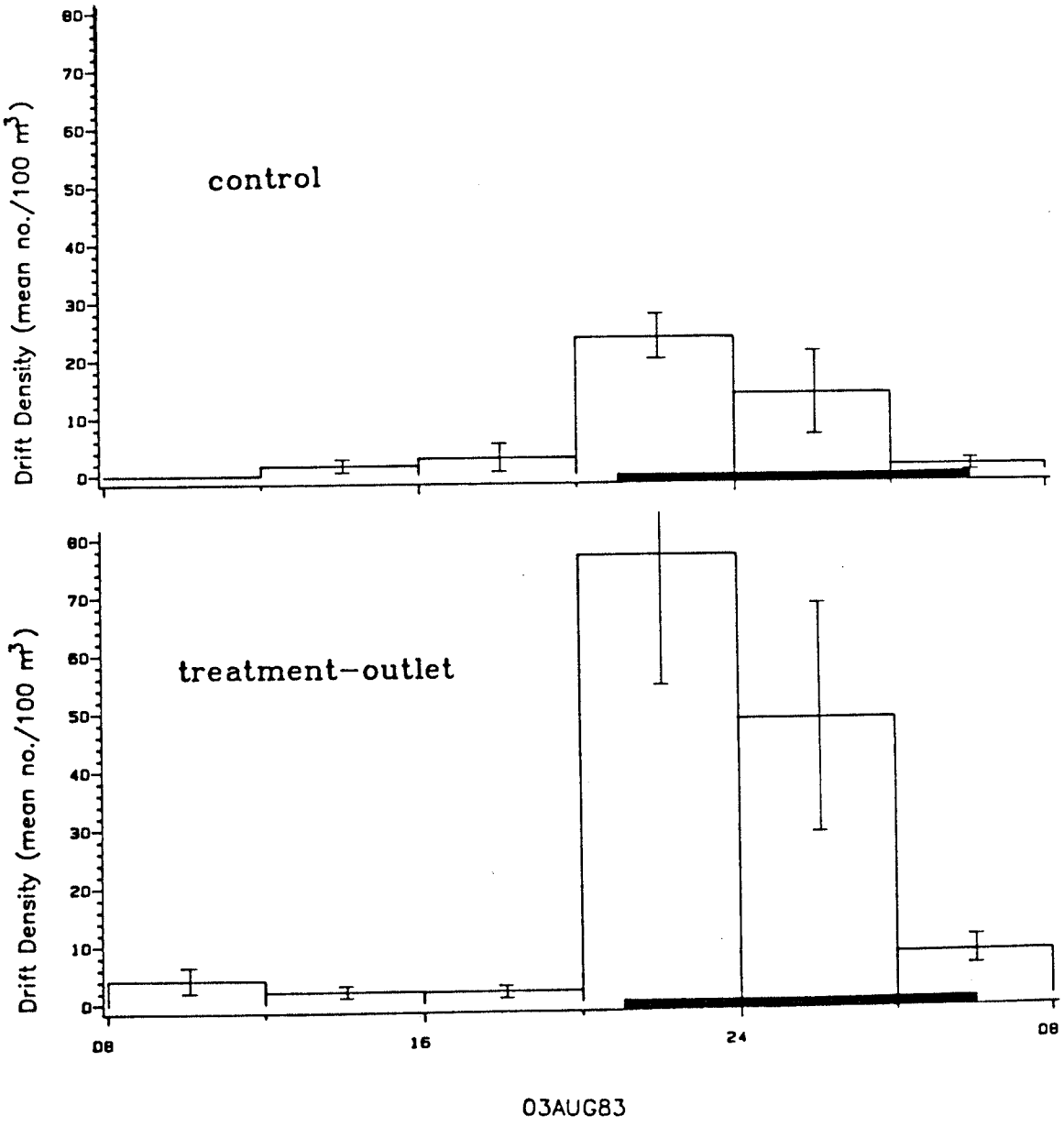


Figure 27. Mean (±s.e.) drift density of Stenacron interpunctatum larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density significantly lower ($p<.05$) at the treatment-outlet site relative to the control site on 21 July, 29 July, and 17 August.

STENACRON INTERPUNCTATUM

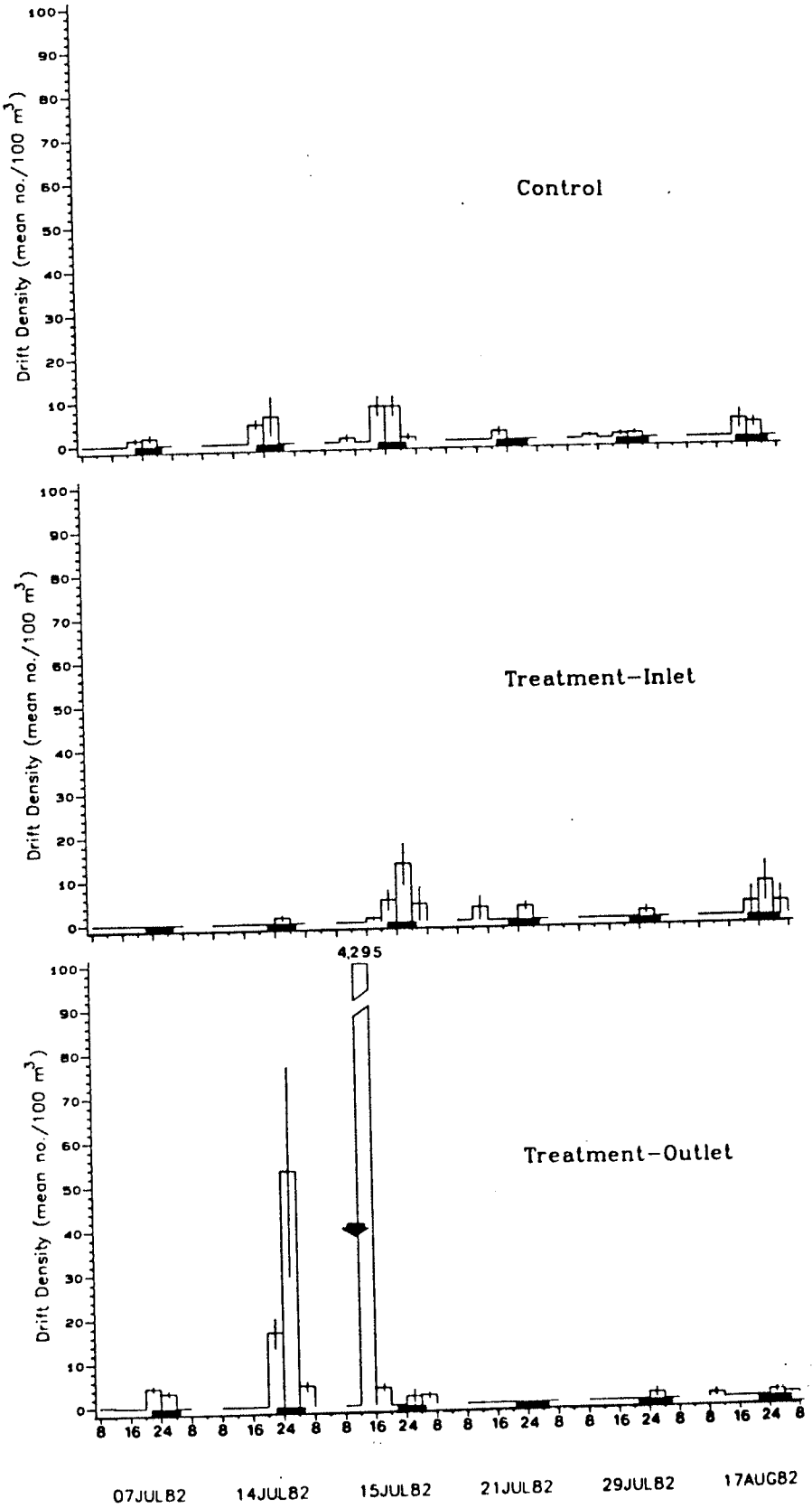
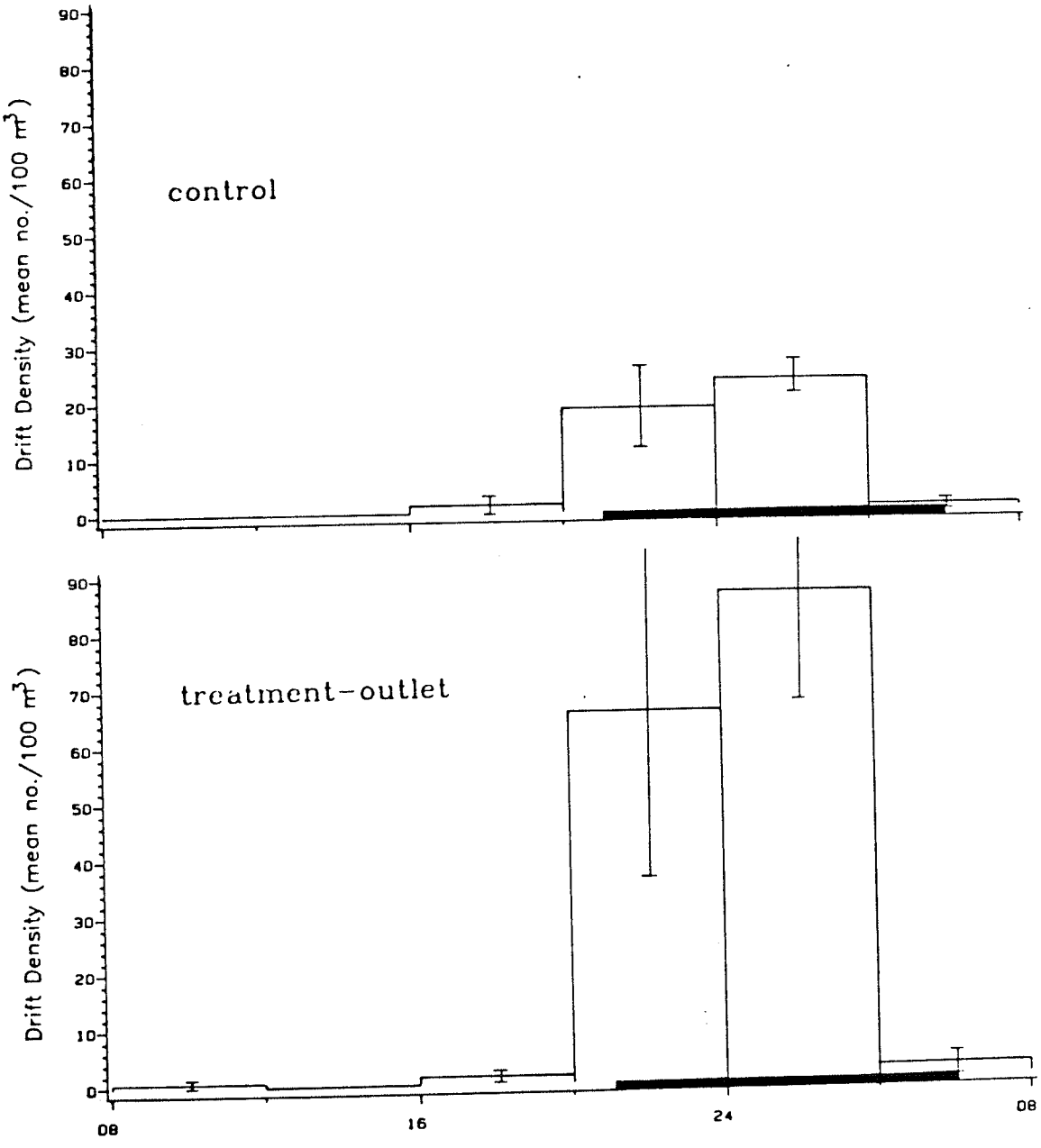


Figure 28. Mean (±s.e.) drift density of Stenacron interpunctatum larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites.

STENACRON INTERPUNCTATUM



03AUG83

Figure 29. Mean (±s.e.) drift density of Hydropsyche recurvata larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density significantly lower ($p<.05$) at the treatment-outlet site relative to the control site on 21 July, 29 July, and 17 August.

HYDROPSYCHE RECURVATA

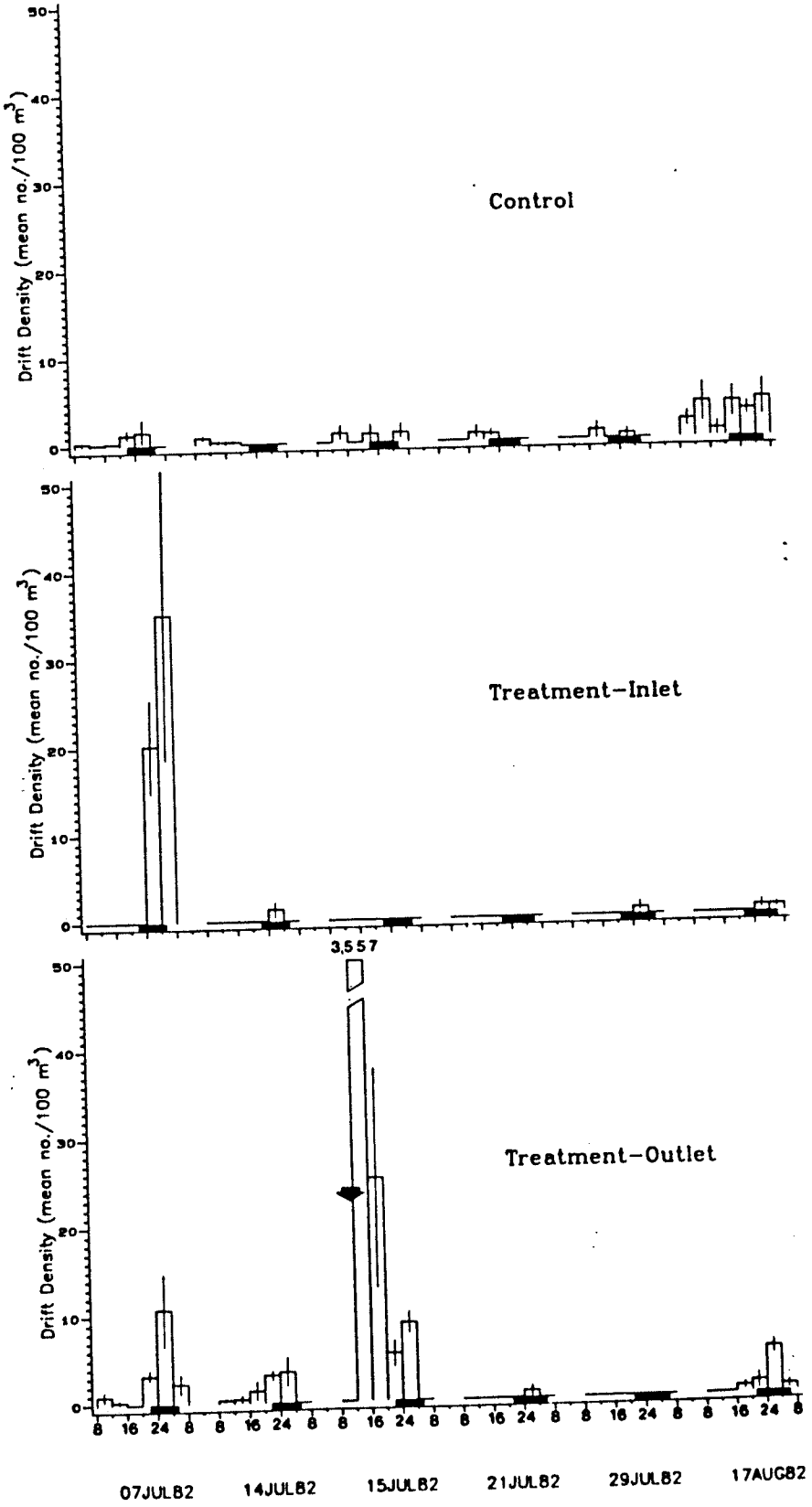


Figure 30. Mean (±s.e.) drift density of Hydropsyche recurvata larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites.

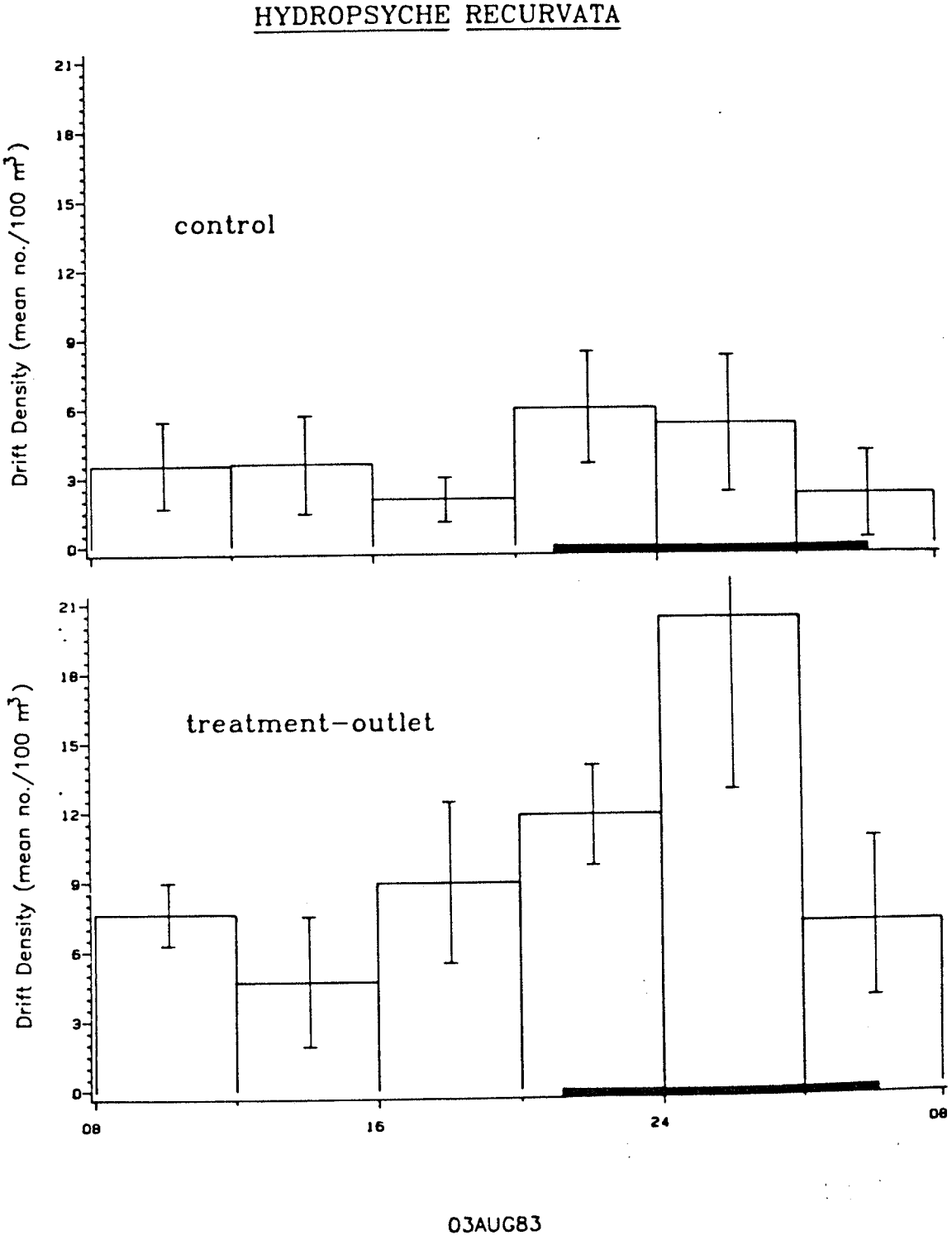

HYDROPSYCHE RECURVATA
Drift Density (mean no./100 m³)
control
treatment-outlet
0
3
6
9
12
15
18
21
08
16
24
08
03AUG83

Figure 31. Mean (±s.e.) drift density of Polycentropus cinereus larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density not significantly different ($p>.05$) between sites on 21 July, 29 July, and 17 August.

POLYCENTROPUS CINEREUS

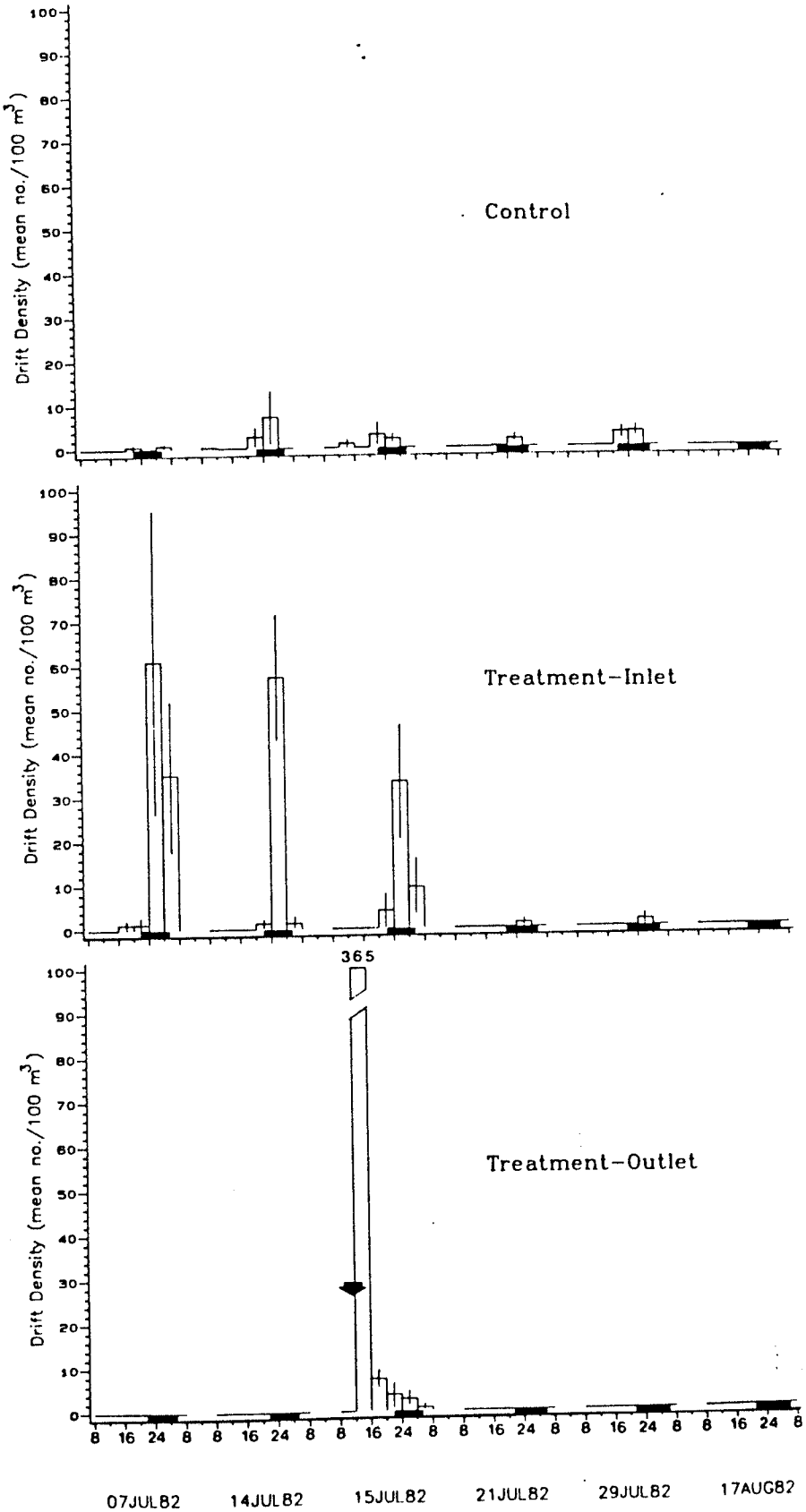


Figure 32. Mean (±s.e.) drift density of Polycentropus cinereus larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites.

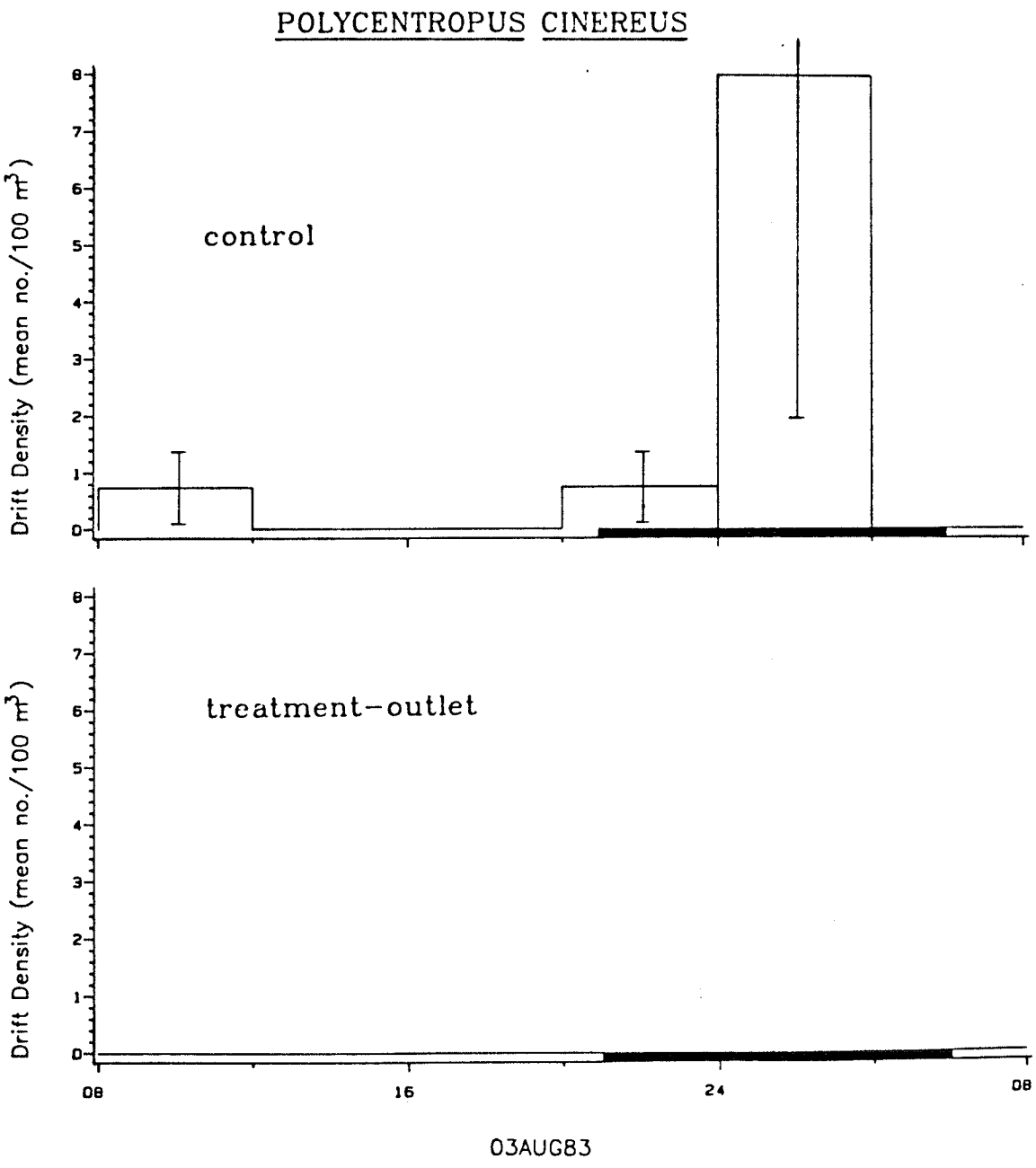

POLYCENTROPUS CINEREUS
control
treatment-outlet
Drift Density (mean no./100 m³)
Drift Density (mean no./100 m³)
0
1
2
3
4
5
6
7
8
08
16
24
08
03AUG83

Figure 33. Mean (±s.e.) drift density of Acroneuria lycorias nymphs at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites on 21 July, 29 July, and 17 August.

ACRONEURIA LYCORIAS

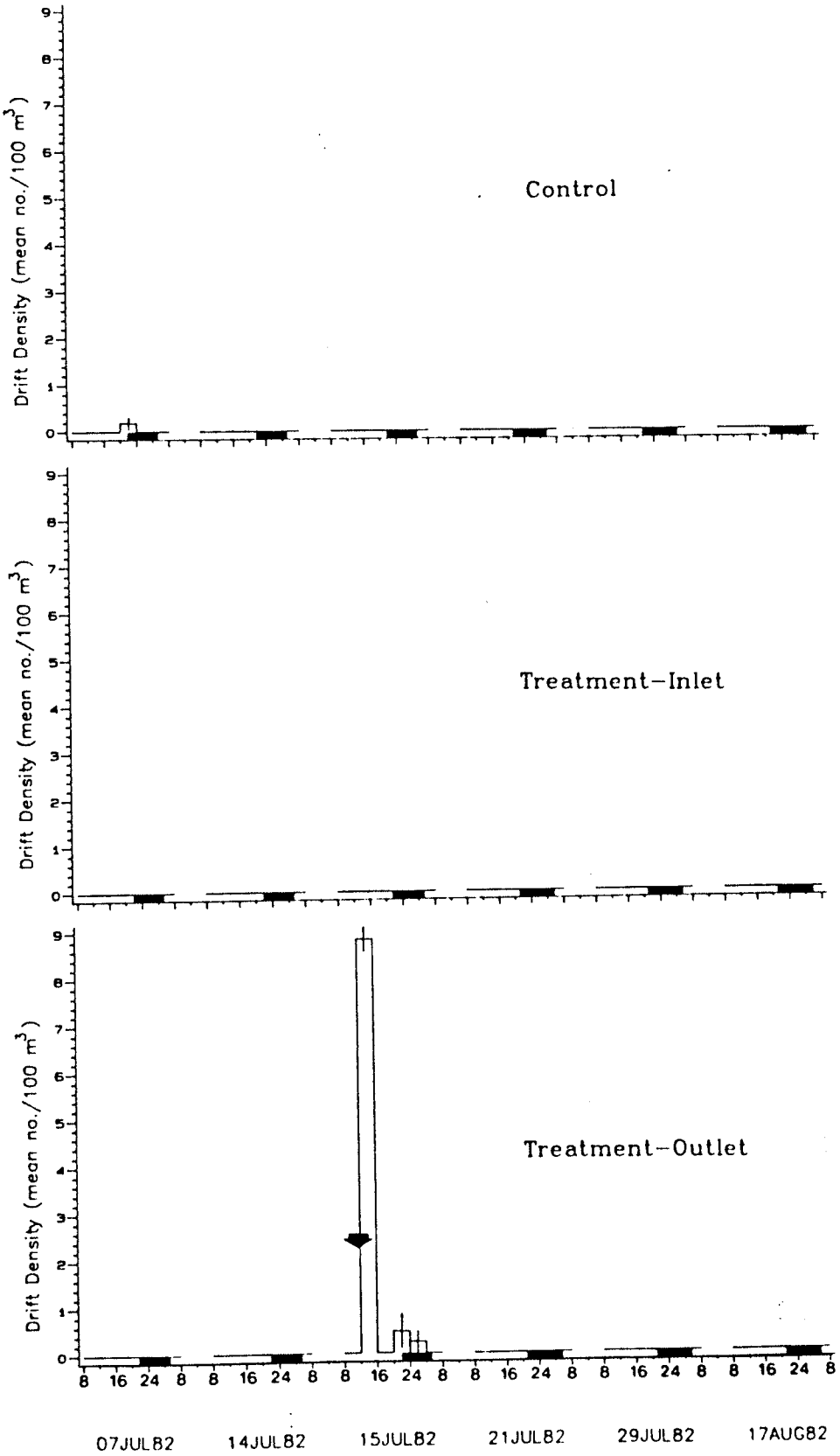


Figure 34. Mean (±s.e.) drift density of Chironomidae larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites on 21 July, and 29 July.

Drift density significantly lower ($p<.05$) at the treatment-outlet site relative to the control site on 17 August.

CHIRONOMIDAE

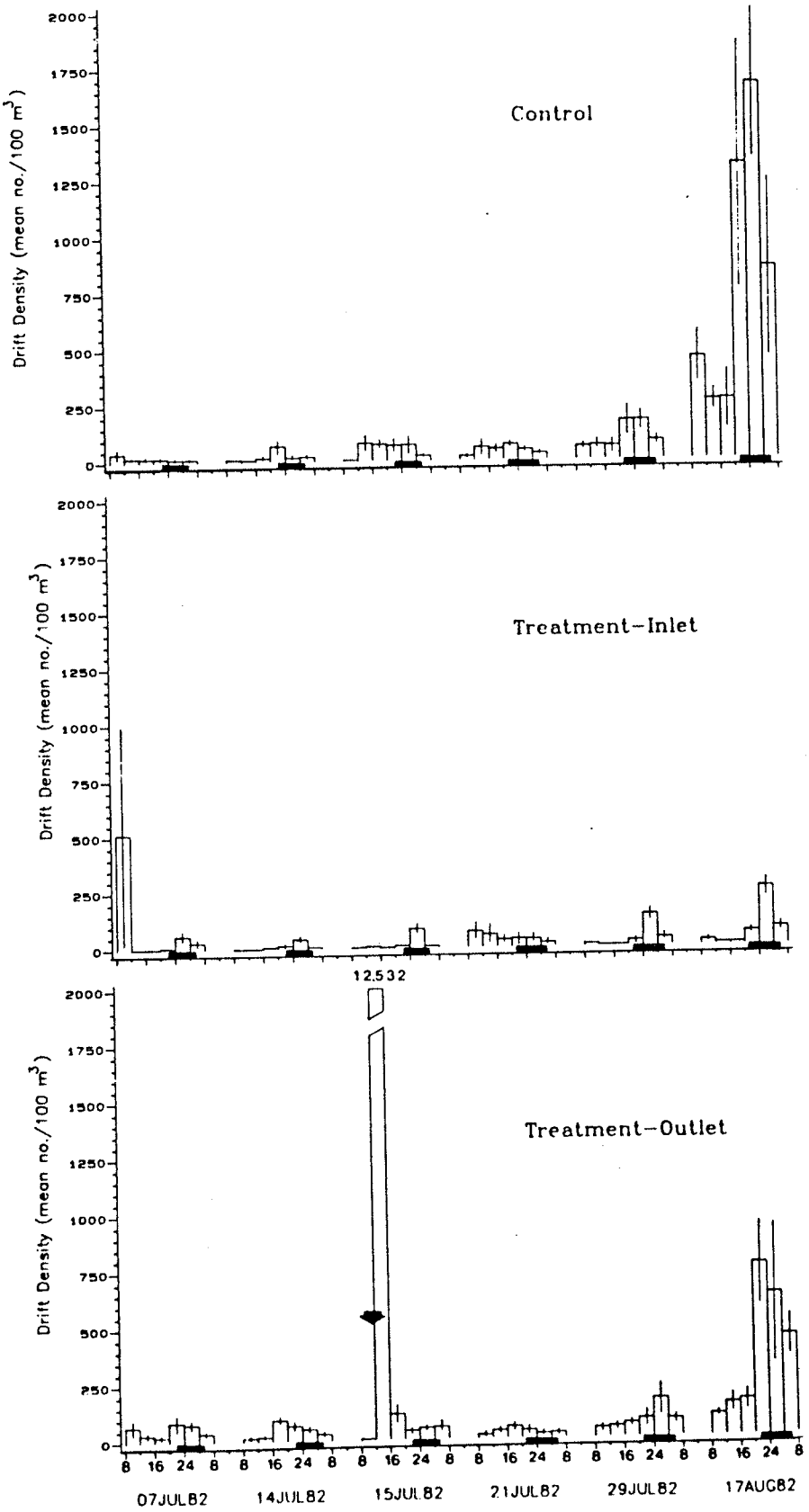


Figure 35. Mean (±s.e.) drift density of Chironomidae larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites.

CHIRONOMIDAE

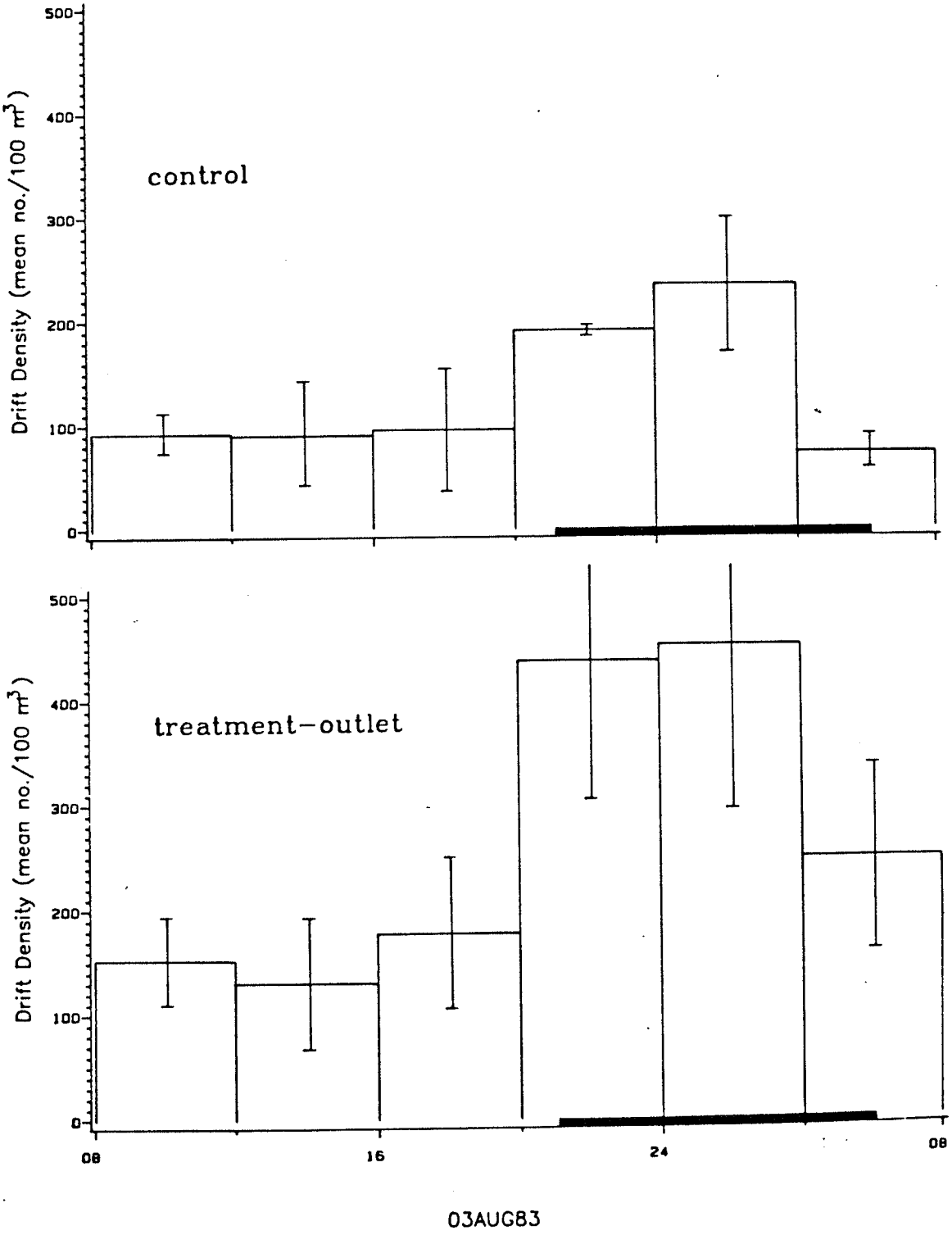


Figure 36. Mean (±s.e.) drift density of Hyalella azteca larvae at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density significantly lower ($p<.05$) at the treatment-outlet site relative to the control site on 21 July, 29 July, and 17 August.

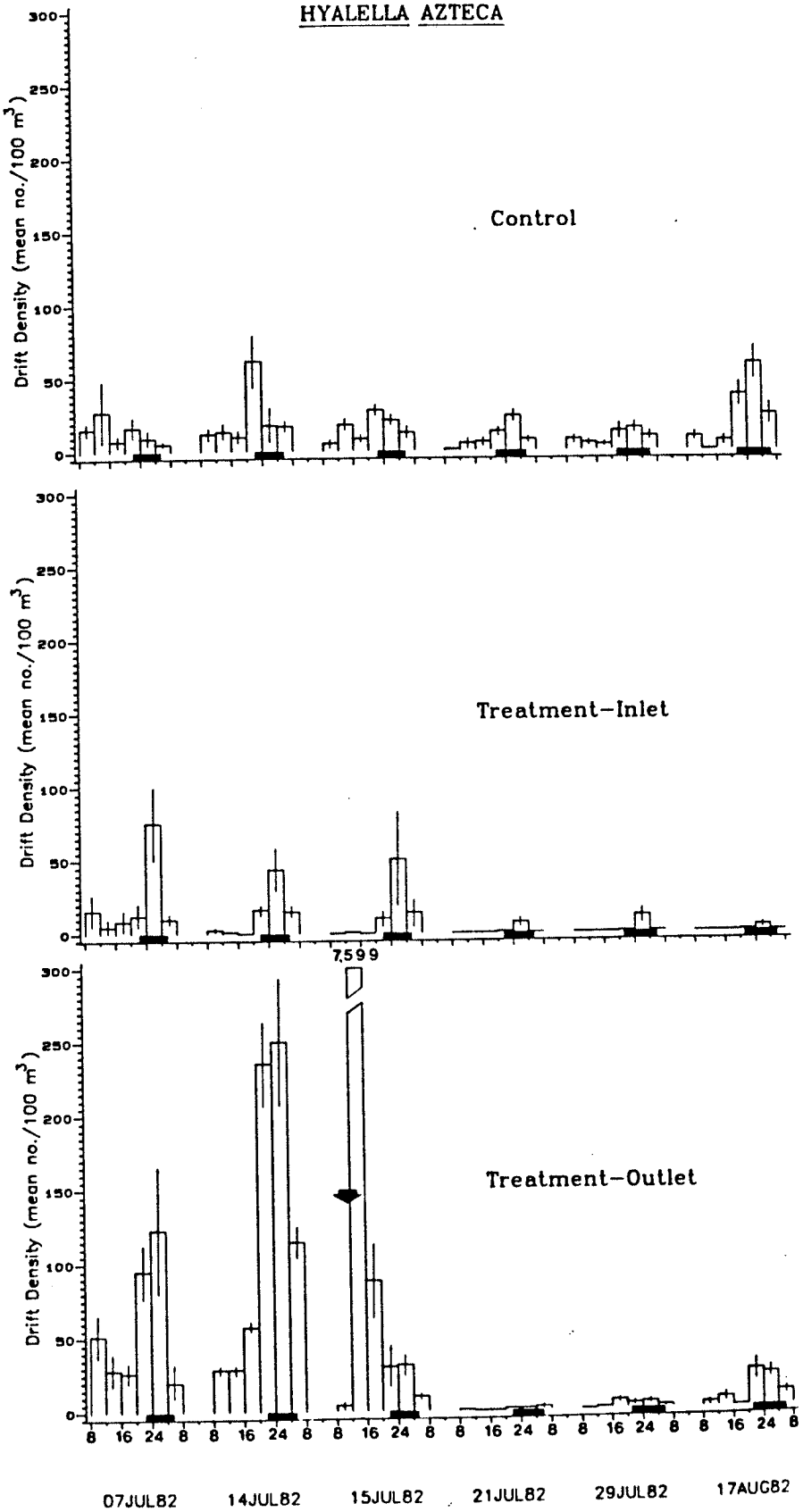

HYALELLA AZTECA
Drift Density (mean no./100 m³)
Control
Treatment–Inlet
Treatment–Outlet
7599
0
50
100
150
200
250
300
8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8 8 16 24 8
07JUL82
14JUL82
15JUL82
21JUL82
29JUL82
17AUG82

Figure 37. Mean (±s.e.) drift density of Hyalella azteca larvae at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites.

HYALELLA AZTECA

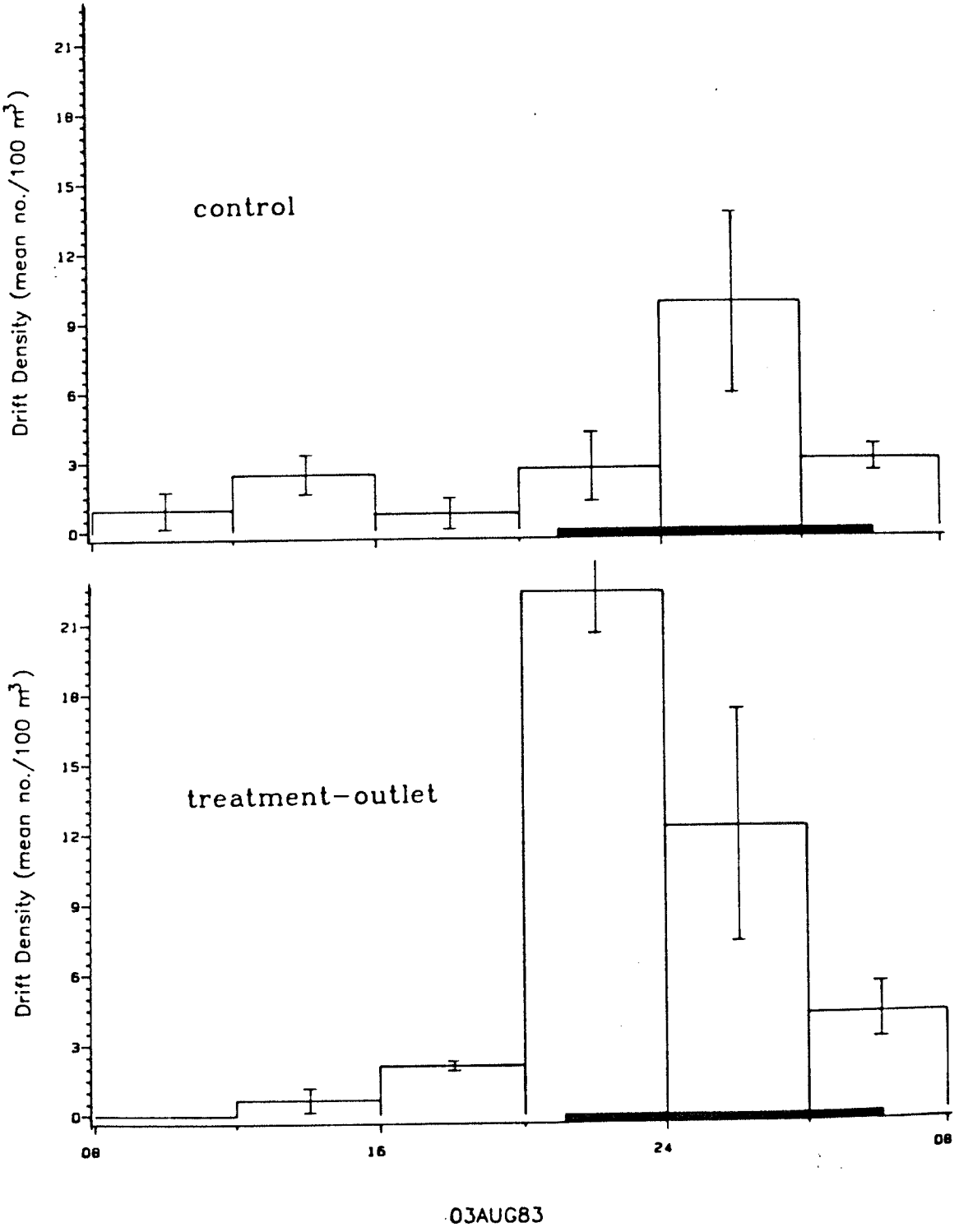


Figure 38. Mean (±s.e.) drift density of Orconectes virilis juveniles at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites on 21 July, 29 July, and 17 August.

ORCONECTES VIRILIS JUVENILES

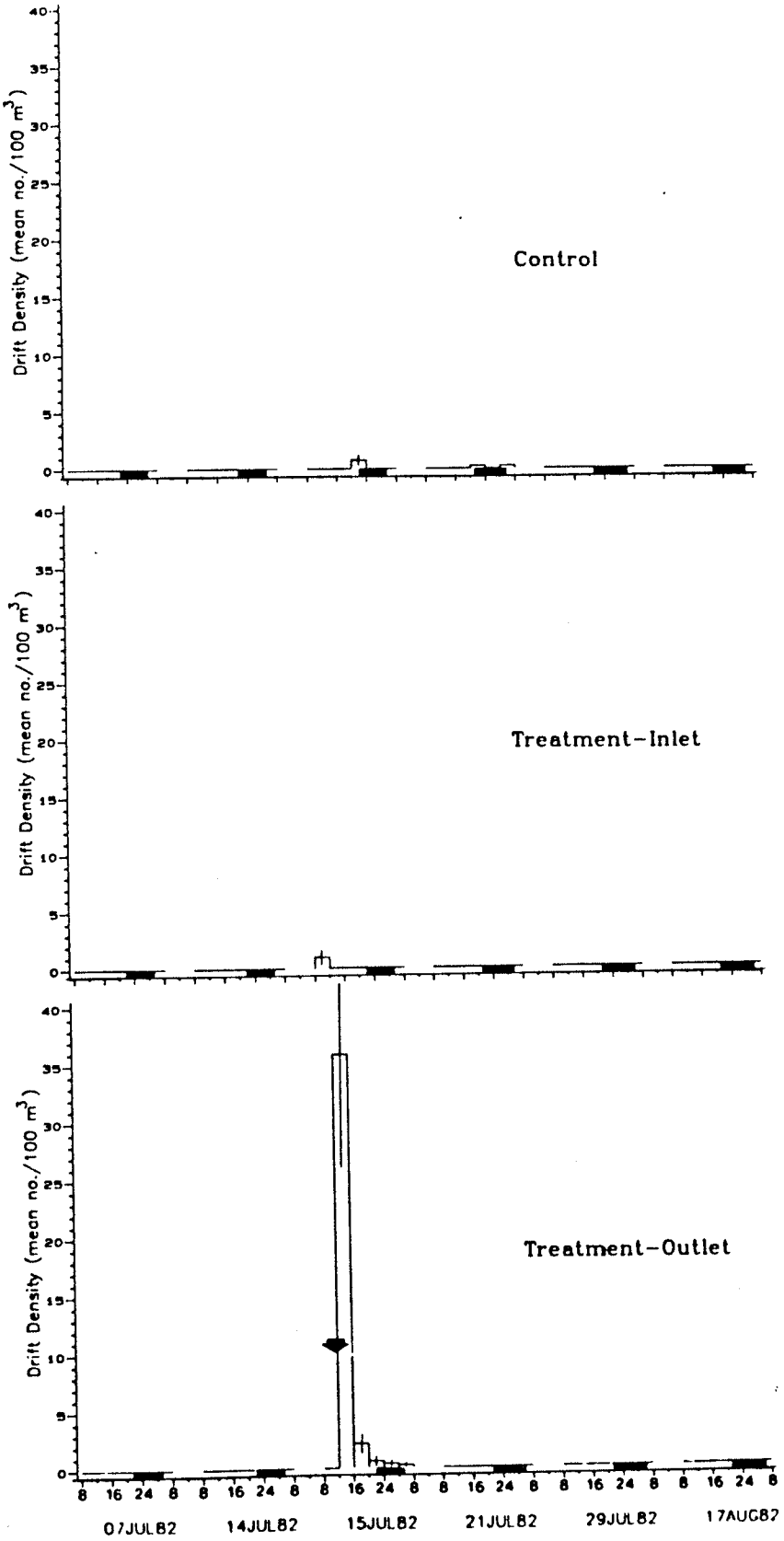


Figure 39. Mean (±s.e.) total drift density at the three sampling sites for 1982. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time. Arrow indicates time of Methoxychlor injection.

Drift density significantly higher ($p<.0001$) at the treatment-outlet site relative to the control site on 15 July.

Drift density significantly lower ($p<.05$) at the treatment-outlet site relative to the control site on 21 July, 29 July, and 17 August.

TOTAL SPECIES DRIFT FOR 1982

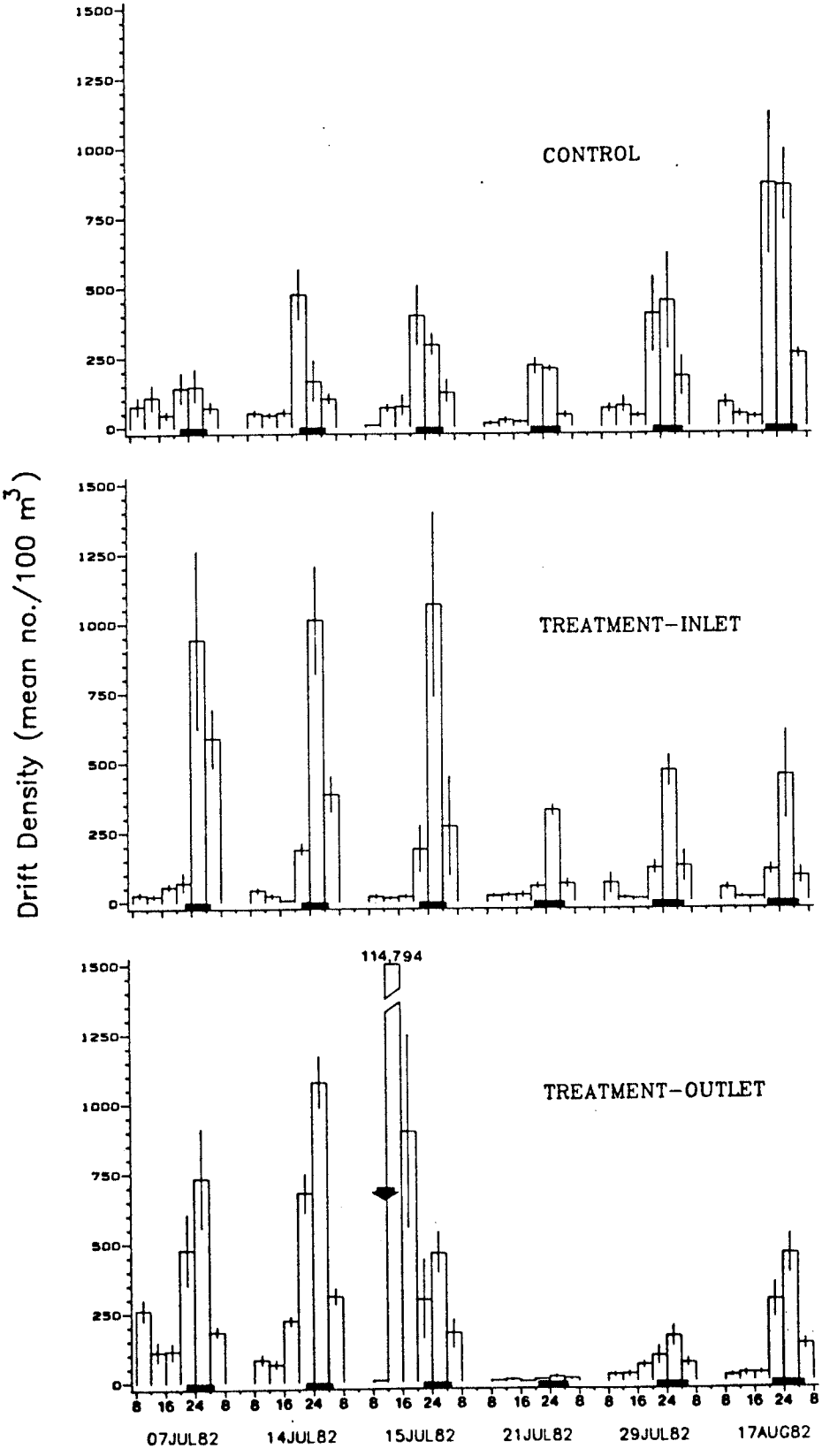


Figure 40. Mean (±s.e.) total drift density at the control and treatment-outlet sampling sites on 03 August 1983. Dark bars represent periods of darkness. Numbers along bottom represent 24-h time.

Drift density not significantly different ($p>.05$) between the control and treatment-outlet sites.

TOTAL SPECIES DRIFT FOR 1983

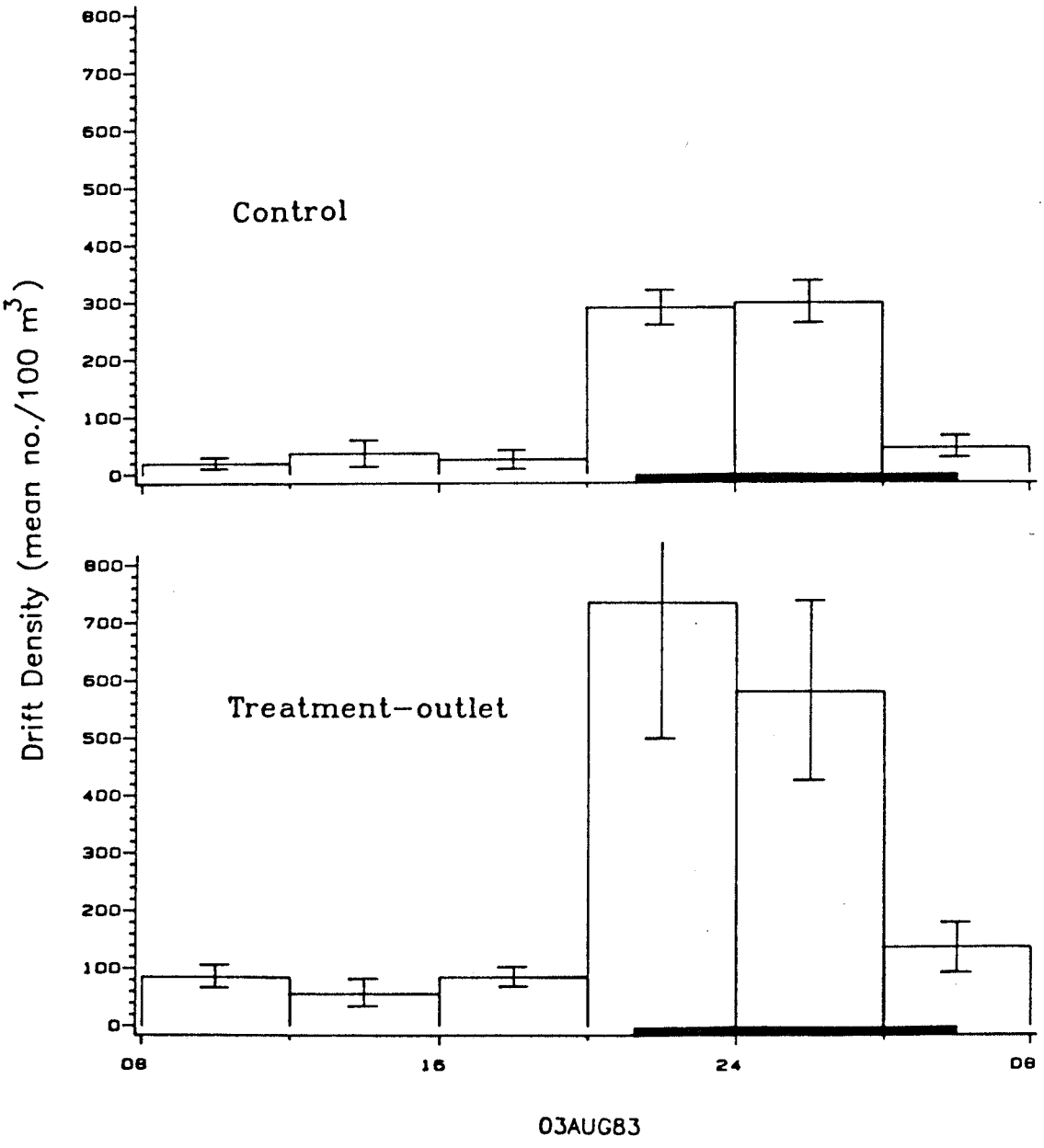


Figure 41. Mean (±s.e.) number of invertebrates on artificial substrates at the control and treatment sites. A) Baetis spp. B) Caenis tardata. Arrow indicates time of Methoxychlor injection. Black dot indicates a significant difference ($p<.05$) between the control and treatment site (based on a comparison of the difference between the combined control and treatment site means prior to treatment with the difference between the control and treatment site means on each post-treatment sampling date).

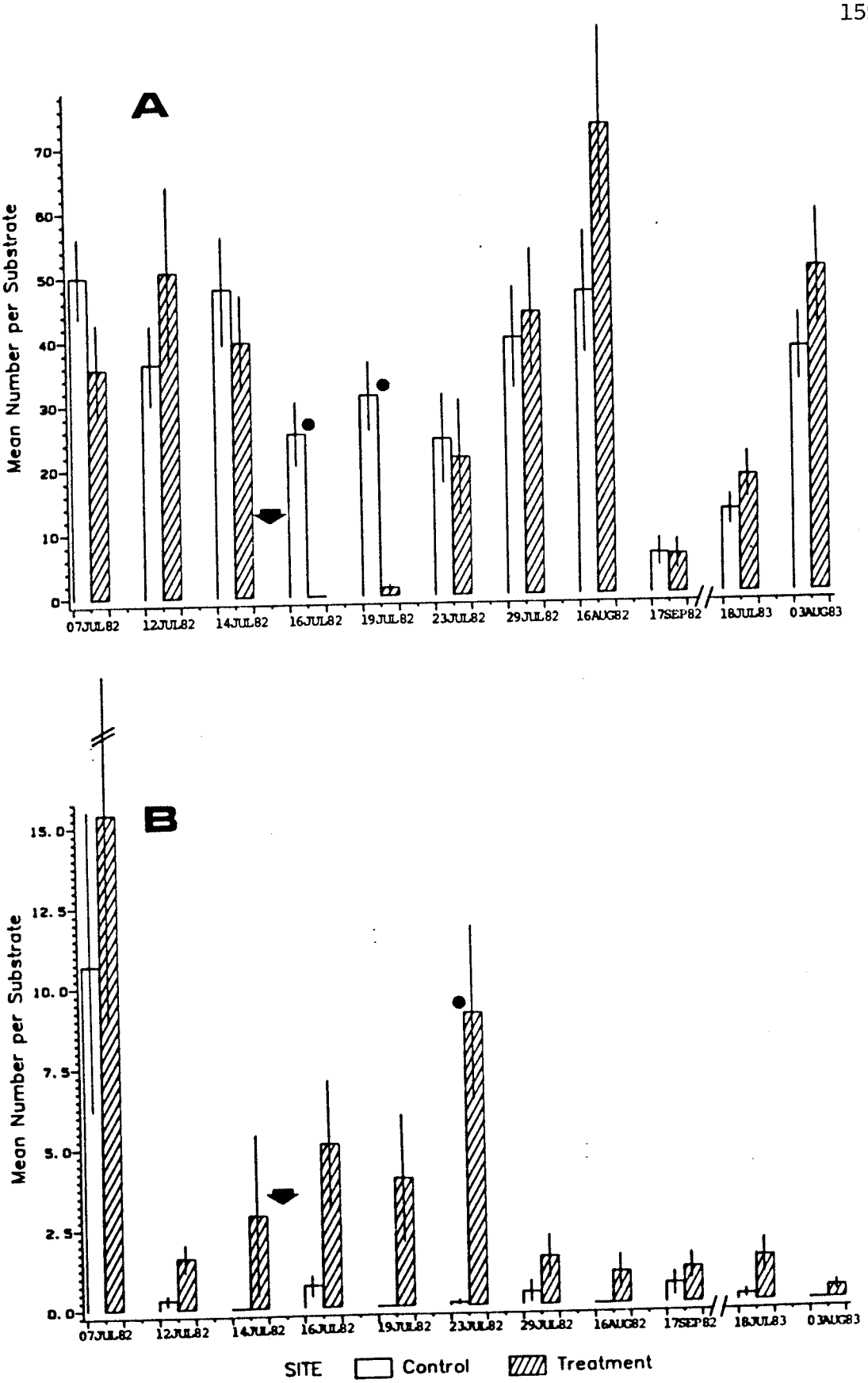

A
Mean Number per Substrate
0
10
20
30
40
50
60
70
07JUL82
12JUL82
14JUL82
16JUL82
19JUL82
23JUL82
29JUL82
16AUG82
17SEP82
18JUL83
03AUG83
B
Mean Number per Substrate
0.0
2.5
5.0
7.5
10.0
12.5
15.0
07JUL82
12JUL82
14JUL82
16JUL82
19JUL82
23JUL82
29JUL82
16AUG82
17SEP82
18JUL83
03AUG83
SITE
Control
Treatment

Figure 42. Mean (±s.e.) number of invertebrates on artificial substrates at the control and treatment sites. A) Leucrocuta maculipennis B) Stenacron interpunctatum. Arrow indicates time of Methoxychlor injection. Black dot indicates a significant difference ($p<.05$) between the control and treatment site (based on a comparison of the difference between the combined control and treatment site means prior to treatment with the difference between the control and treatment site means on each post-treatment sampling date).

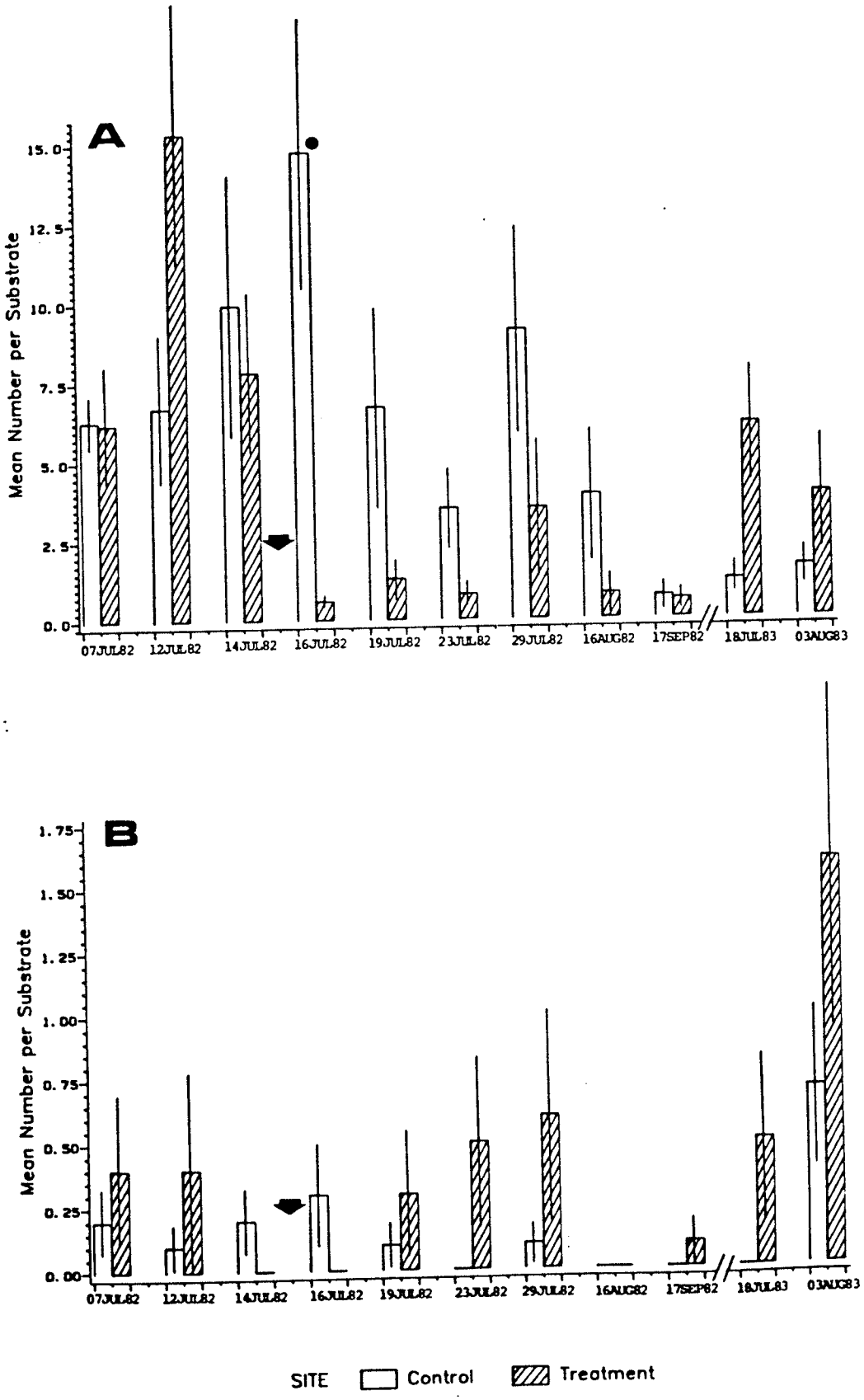

A
Mean Number per Substrate
15.0
12.5
10.0
7.5
5.0
2.5
0.0
07JUL82
12JUL82
14JUL82
16JUL82
19JUL82
23JUL82
29JUL82
16AUG82
17SEP82
18JUL83
03AUG83
B
Mean Number per Substrate
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
07JUL82
12JUL82
14JUL82
16JUL82
19JUL82
23JUL82
29JUL82
16AUG82
17SEP82
18JUL83
03AUG83
SITE
Control
Treatment

Figure 43. Mean (±s.e.) number of invertebrates on artificial substrates at the control and treatment sites. A) Hydroptila ajax B) Psychomyia flavida. Arrow indicates time of Methoxychlor injection. Black dot indicates a significant difference ($p<.05$) between the control and treatment site (based on a comparison of the difference between the combined control and treatment site means prior to treatment with the difference between the control and treatment site means on each post-treatment sampling date).

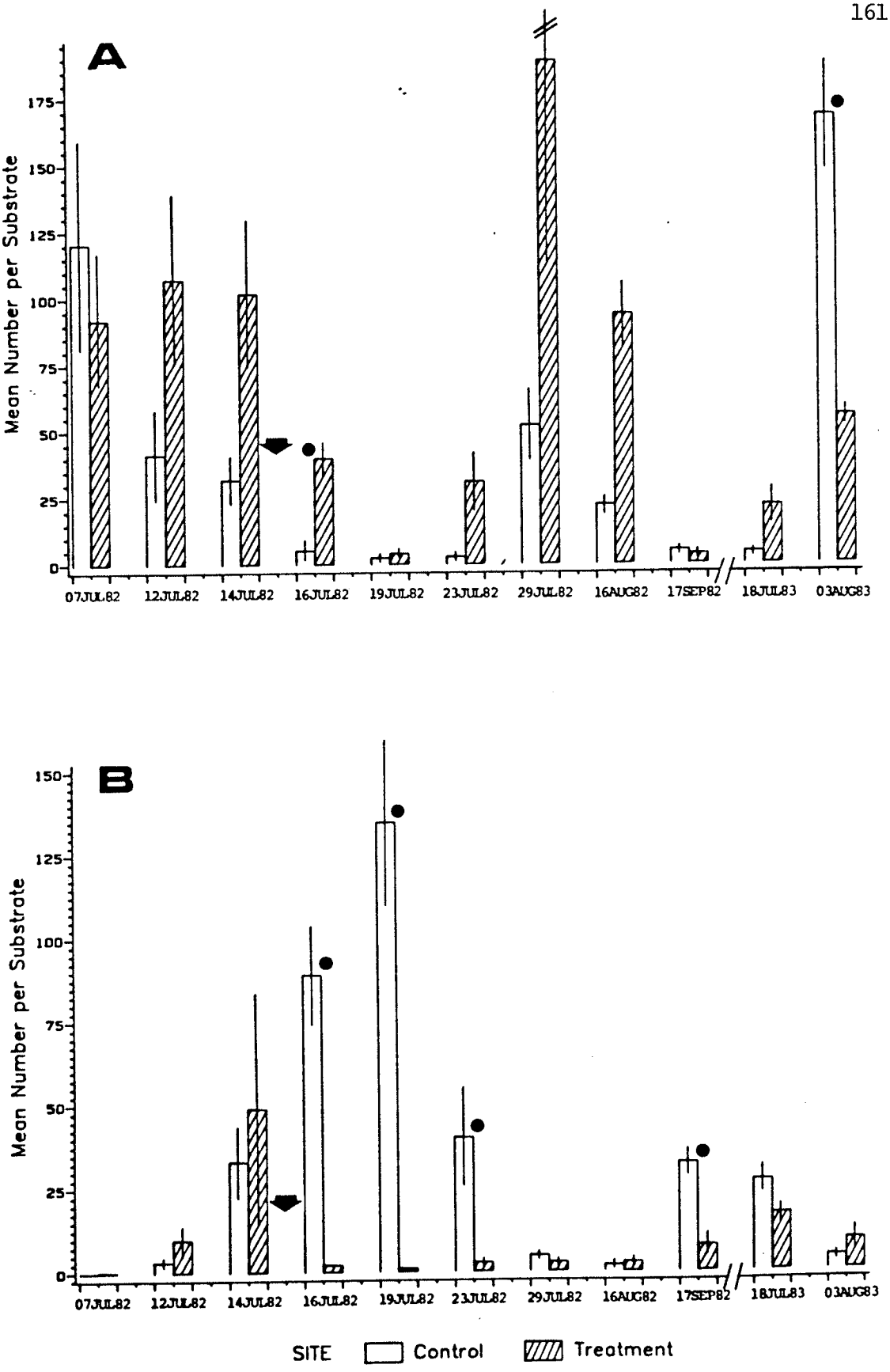

A
Mean Number per Substrate
0
25
50
75
100
125
150
175
07JUL82
12JUL82
14JUL82
16JUL82
19JUL82
23JUL82
29JUL82
16AUG82
17SEP82
18JUL83
03AUG83
B
Mean Number per Substrate
0
25
50
75
100
125
150
07JUL82
12JUL82
14JUL82
16JUL82
19JUL82
23JUL82
29JUL82
16AUG82
17SEP82
18JUL83
03AUG83
SITE
Control
Treatment

Figure 44. Mean (±s.e.) number of invertebrates on artificial substrates at the control and treatment sites. A) Isonychia sicca B) Hyalella azteca. Arrow indicates time of Methoxychlor injection. Black dot indicates a significant difference ($p<.05$) between the control and treatment site (based on a comparison of the difference between the combined control and treatment site means prior to treatment with the difference between the control and treatment site means on each post-treatment sampling date).

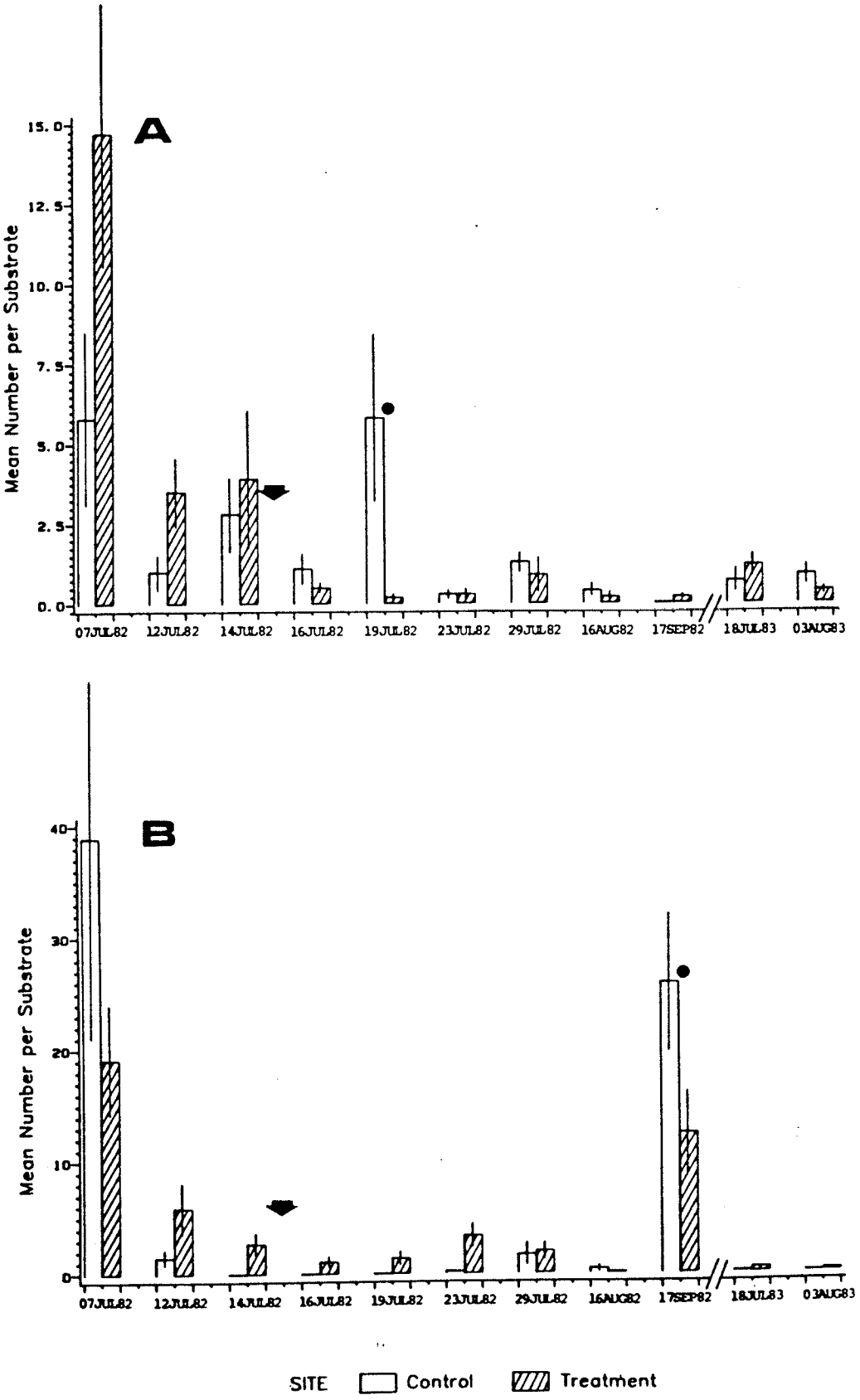

A
B
Mean Number per Substrate
15.0
12.5
10.0
7.5
5.0
2.5
0.0
40
30
20
10
0
07JUL82
12JUL82
14JUL82
16JUL82
19JUL82
23JUL82
29JUL82
16AUG82
17SEP82
18JUL83
03AUG83
SITE
Control
Treatment

Figure 45. Mean (±s.e.) number of Hydropsyche recurvata larvae on artificial substrates at the control and treatment sites. Arrow indicates time of Methoxychlor injection. Black dot indicates a significant difference ($p<.05$) between the control and treatment site (based on a comparison of the difference between the combined control and treatment site means prior to treatment with the difference between the control and treatment site means on each post-treatment sampling date).

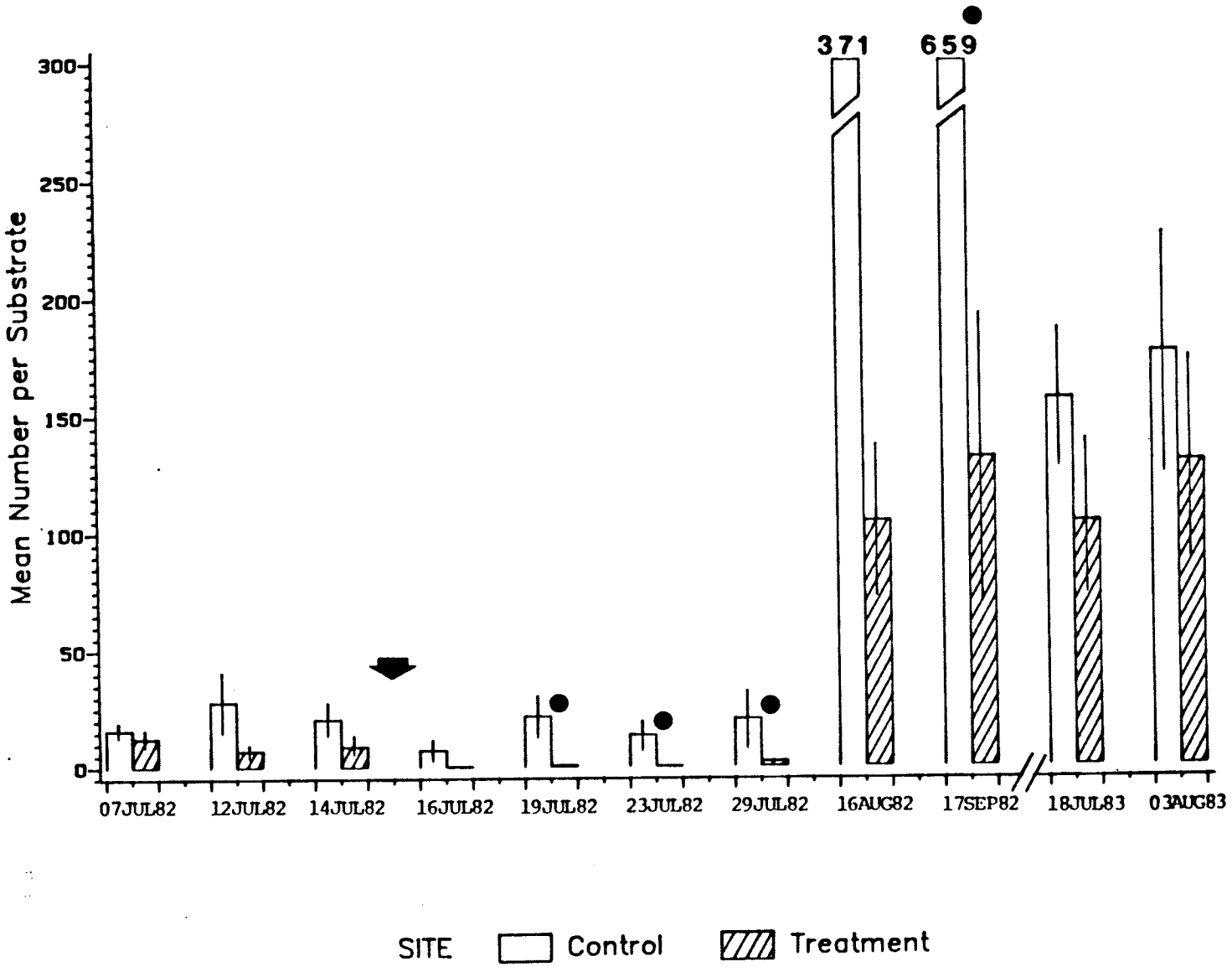

371
659
300
250
200
150
100
50
0
Mean Number per Substrate
07JUL82
12JUL82
14JUL82
16JUL82
19JUL82
23JUL82
29JUL82
16AUG82
17SEP82
18JUL83
03AUG83
SITE
Control
Treatment

Figure 46. Mean (±s.e.) number of invertebrates on artificial substrates at the control and treatment sites. A) Acroneuria lycorias B) Polycentropus cinereus. Arrow indicates time of Methoxychlor injection. Black dot indicates a significant difference ($p<.05$) between the control and treatment site (based on a comparison of the difference between the combined control and treatment site means prior to treatment with the difference between the control and treatment site means on each post-treatment sampling date).

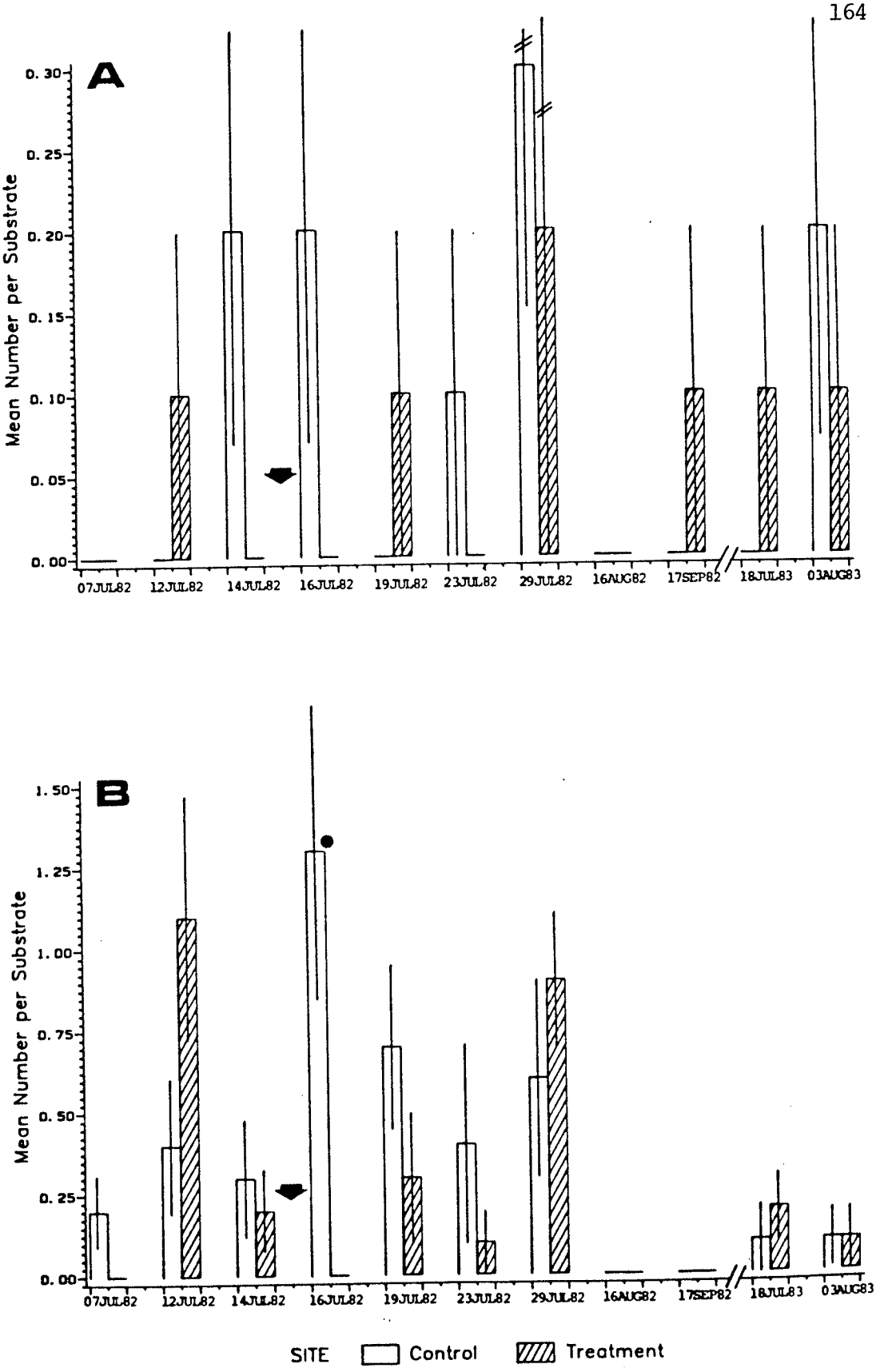
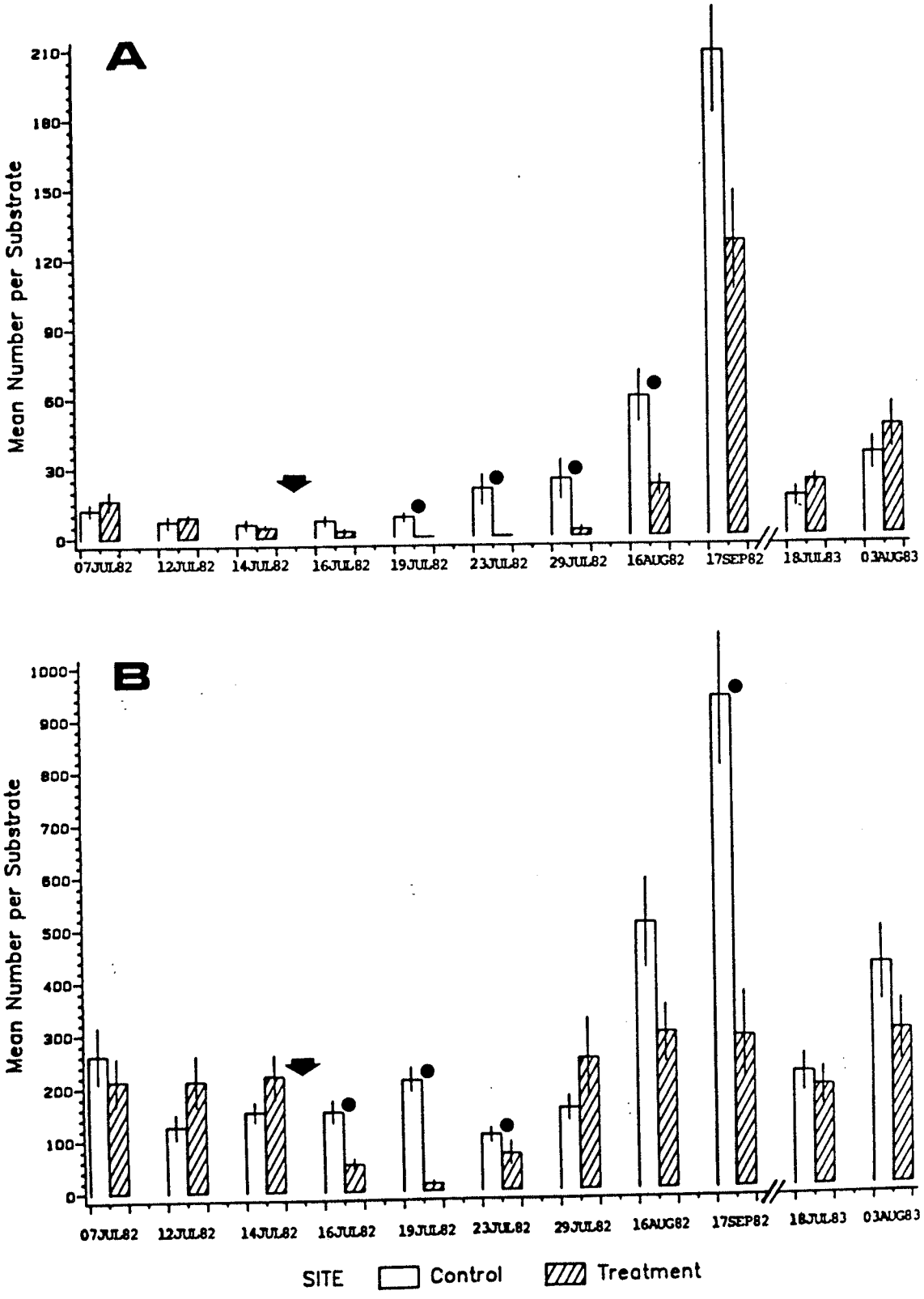

A
B
Mean Number per Substrate
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.00
0.25
0.50
0.75
1.00
1.25
1.50
07JUL82
12JUL82
14JUL82
16JUL82
19JUL82
23JUL82
29JUL82
16AUG82
17SEP82
18JUL83
03AUG83
SITE
Control
Treatment

Figure 47. Mean (±s.e.) number of invertebrates on artificial substrates at the control and treatment sites. A) Cheumatopsyche campyla B) Total numbers. Arrow indicates time of Methoxychlor injection. Black dot indicates a significant difference ($p<.05$) between the control and treatment site (based on a comparison of the difference between the combined control and treatment site means prior to treatment with the difference between the control and treatment site means on each post-treatment sampling date).


A
B
Mean Number per Substrate
0
30
60
90
120
150
180
210
100
200
300
400
500
600
700
800
900
1000
07JUL82
12JUL82
14JUL82
16JUL82
19JUL82
23JUL82
29JUL82
16AUG82
17SEP82
18JUL83
03AUG83
SITE
Control
Treatment

Appendix B

A METHOD FOR SUBSAMPLING UNSORTED BENTHIC MACROINVERTEBRATES BY WEIGHT

A METHOD FOR SUBSAMPLING UNSORTED BENTHIC MACROINVERTEBRATES BY WEIGHT[167]

by

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Keywords: benthos; filamentous algae; dispersion; variability; drift samples; artificial substrates

Abstract

A simple method for subsampling unsorted benthic macroinvertebrates by weight is described for different types of samples obtained from lentic and lotic environments. It is especially useful for samples containing large amounts of filamentous algae that preclude the use of conventional subsampling methods. The method provided random dispersions of individuals in the original samples. Overall variability of the subsamples was low for artificial substrate and catastrophic drift samples. Variability was higher for regular drift samples, which had the lowest numbers of individuals of the three sample types. The method produced considerable savings in time spent sorting. Subsampling approaches for community level studies are discussed.

Introduction

A number of strategies are used to reduce the time and labor required to process samples of benthic macroinvertebrates (Resh et al. 1985). One such strategy is subsampling (e.g. Hickley 1975; Rosenberg 1975). The basis of the many subsampling techniques available is volumetric, whereas weight has been virtually ignored (except see Van Ark and Pretorius 1970 for light-trap catches of insects). It may be necessary to use weight as a basis for subsampling when working with samples containing large amounts of filamentous algae. The macroinvertebrates in such samples usually are entangled, making volumetric subsampling, as well as other processing methods such as elutriation or flotation, difficult to do.

A number of methods for processing samples of macroinvertebrates in algae have been reported. Klattenberg (1975) described a plexiglass box for subsampling Cladophora containing benthic macroinvertebrates, but he provided no data for which estimates of variability could be calculated. The vegetation elutriator of Cross and Minns (1969, p. 316) also was used on "...samples containing a high proportion of filamentous algae", but its efficiency was reduced. They reported "...good results...with a considerable time saving over hand-sorting provided the vegetation was teased apart..." and processed in small (20-g) batches. Once again, no data were presented to substantiate these claims. Pollard and Melancon (1984) developed an efficient field-washing method for recovery of benthic macroinvertebrates from algal mats and watercress, but their method requires live samples. Obviously, in some field situations, samples cannot be processed immediately, so they must be preserved and sorted later.

Preserved samples containing masses of filamentous algae usually have to be handpicked in their entirety, a long and tedious undertaking which taxes the resources of most benthic research programs. The purpose of this paper is to describe a simple yet effective method for subsampling benthic macroinvertebrates by weight before sorting. Although primarily intended for samples containing filamentous algae, the method is also useful for samples that are free of extraneous debris. In addition, the paper considers approaches for dealing with the subsampling of rare species in community level studies.

Methods

Sample types

Tests were conducted on artificial substrate samples that had been colonized along newly flooded shorelines in the Southern Indian Lake reservoir, Manitoba (Resh et al. 1983; Wiens and Rosenberg 1984 and unpublished data), and on drift samples collected from an experimental methoxychlor addition to the Souris River, Manitoba (Sebastien 1986). The artificial substrates were chicken barbeque baskets filled with freshly cut sticks of three species of tree commonly found along the shoreline of Southern Indian Lake (*Alnus rugosa* (DuRoi) Spreng., *Picea mariana* (Mill.) B.S.P., *Salix* spp.). A total of three basket samples was used in this test.

Drift samples of 4-h duration were collected from the Souris River using a Burton-Flannagan bomb sampler (Burton and Flannagan 1976) equipped with a 500-μm mesh net. Two types of drift samples were used here: those taken as part of regular, diurnal monitoring (three sets of three samples each, comprising a total of nine samples), and those

measuring catastrophic drift in response to the methoxychlor addition. Only one catastrophic drift sample was used in this test because of the extremely high numbers of invertebrates that were captured.

The artificial substrate and regular drift samples contained large amounts of filamentous algae, whereas the catastrophic drift sample was relatively free of extraneous debris. Samples used in the tests were selected arbitrarily from all those collected in the course of the two studies.

Sample treatment

Individual artificial substrate and regular drift samples were thoroughly mixed in a 1- or 2-l beaker, to ensure a random distribution of the invertebrates, by stirring with a glass rod. Samples were then poured onto a pre-weighed sieve (U.S. Standard no. 70 mesh, 212 µm, ID 20.3 cm) and allowed to stand for 15-20 min until excessive preservative had drained off. The moist sample was stirred again while on the sieve, and was then weighed on an electronic pan balance to the nearest 0.1 g. Four approximately equal amounts by weight were removed at random. Thus, each portion comprised ≈25% by weight of the total sample. Total weights of artificial substrate samples varied from 8-41 g, whereas those of regular drift samples varied from 18-99 g.

The invertebrates in each quarter were sorted, identified, and enumerated. Organisms in the artificial substrate samples were identified as Chironomidae and other invertebrates, whereas organisms in the regular drift samples were identified to species whenever possible.

The catastrophic drift sample was treated in the same manner as the artificial substrate and regular drift samples. However, because of the

high number of invertebrates involved, 10 subsamples, each only ≈1% by weight of the total sample, were removed. Thus, a total of ≈10% by weight of the original sample was subsampled. These subsamples as well as the remainder of the sample were sorted, and organisms were identified to species whenever possible.

Statistical treatment

Dispersion - In order to estimate the total number of organisms in each sample from subsample counts, it was first necessary to establish that the organisms were dispersed randomly in the original sample. The index of dispersion (I), which is based on the variance to mean ratio of the subsample and tests for conformity to a Poisson series (Elliott 1977; Wrona et al. 1982), was calculated for all samples. This index approximates a χ^2 distribution, allowing the following relationship to be used (Elliott 1977):

$$I(n-1) \approx \chi^2_{df=(n-1)} = \frac{s^2(n-1)}{\bar{x}} \quad \text{when } n < 31$$

where n = number of subsampling units taken

s^2 = sample variance

$\bar{x}$ = sample mean

df = degrees of freedom

Agreement to a Poisson series was checked by examining whether the calculated χ^2 approximation value occurred between the 0.975 and 0.025 probability levels of the χ^2 distribution (Elliott 1977; Snedecor and Cochran 1980).

Variability - Variability of the subsamples was measured by the coefficient of variation (Elliott 1977):

$$CV = s\left(\frac{100}{\bar{x}}\right)$$

where CV = coefficient of variation

s = sample standard deviation

$\bar{x}$ = mean number in the sample

Results and Discussion

Dispersion

χ^2 values calculated for numbers of benthic macroinvertebrates in subsamples of the three artificial substrate samples used, indicated that Chironomidae and total numbers of invertebrates were randomly dispersed in the samples prior to subsampling (Table 1). Chironomidae were emphasized because they comprised >80% of total numbers in these samples, and were the taxon of greatest interest in the Southern Indian Lake study.

Chironomidae were also abundant in the drift samples (regular: ≈25-70% of total numbers; catastrophic: 11.5% of total numbers), but the Ephemeroptera, Trichoptera, and Amphipoda were emphasized because they were the taxa of interest in the Souris River methoxychlor-addition study. χ^2 values calculated for one of the three sets of regular drift samples are shown in Table 2. In only two instances (Hyalella azteca (Saussure), replicate 3; Others, replicate 1) were the dispersions significantly different from random ($p \leq 0.05$). In fact, the dispersions were uniform in these two instances. Most of the dispersions from the other two sets of samples also were random, and a summary of the results from all three sets (9 replicates) is shown in Table 3. Most species

yielded high percentages of random counts. However, Caenis tardata McDunnough was a conspicuous exception to the general trend (Table 3), their dispersions in the original samples being contagious in four out of the nine replicates.

Subsamples of species in the single catastrophic drift sample consistently agreed with a Poisson series, thus confirming effective subsampling (Table 4). Note, however, that for Isonychia sicca Walsh χ^2 = 18.9, a value very close to the upper limit of χ^2 values.

Unlike in some of the regular drift samples, C. tardata in the catastrophic drift sample appeared to be randomly dispersed in the original sample. Nymphs of this species may have a body shape or some morphological feature that predisposes them to entanglement in the filamentous algae that pervaded the regular drift samples. This may have prevented a random dispersion when some of these samples were mixed prior to subsampling.

Variability

Relative variability of subsamples from the artificial substrates was only ≈3% (Tables 1, 5). However, CV's were usually higher and more variable for the regular drift subsamples due to the relatively low numbers in these samples. CV's for one of the three sets of regular drift samples are shown in Table 2, and values for all of the sets are summarized in Table 5. Although 13 of 24 CV's for the set of regular drift samples shown in Table 2 were $\leq$30%, eight of the values exceeded 50%. Most of the CV's for subsamples in the catastrophic drift sample were $\leq$15% (Tables 4, 5), indicating a relatively low variability. The highest CV in this sample was recorded for Polycentropus cinereus

(Hagen) (37.9%), a taxon that was present in the lowest numbers in each subsample. Although variability was highest in samples with low numbers of invertebrates, values of χ^2 indicated that dispersions usually were not significanty different from random in both large and small samples (see above).

Subsampling approaches

A central problem of subsampling in community oriented studies is the strategy that should be used in dealing with rare species. Basically, there are two approaches that can be taken to deal with this problem, depending on the objective of the study (Wrona et al. 1982). The first approach would be to adapt the numbers counted to the desired accuracy. Once randomness has been demonstrated, the accuracy of the estimated total count for a given taxon depends on the total number of individuals counted rather than the number of subsampling units taken (e.g. at least 100 individuals would be required to yield an accuracy of ±20% at 95% confidence limits) (Elliott 1977; Wrona et al. 1982). Therefore, only enough subsamples would be sorted to give sufficient numbers of an abundant taxon to achieve a desired accuracy, and thereafter these taxa would be ignored. In contrast, rare taxa would be counted in all subsamples and additional subsamples would be taken if the error terms for these rare taxa were still unacceptable. This approach would be used when accurate estimates of population density are required.

The second approach would be to standardize the number of subsamples taken, and to accept the substantial error associated with subsampling rare species. In a community level study such as one

monitoring the effects of pesticide application on non-target organisms, continued counting of rare taxa defeats the original purpose of the subsampling, which is the saving of time and effort. Additionally, accuracy for rare taxa such as P. cinereus in the catastrophic drift sample (Table 4) can remain low even if substantial extra effort is expended in counting higher numbers. For some taxa present in samples with low numbers (e.g. regular drift samples), the error will be high even if all the individuals are counted.

Conclusion

Based on our results, and the time required to sort and identify each subsample (see below), only one subsample (25% by weight) was analyzed for artificial substrate and regular drift samples. Numbers of each taxon were multiplied by four to obtain an estimate of total numbers in each sample. This relatively large subsample size was chosen because it yielded relatively high numbers of individual taxa and lower variabilities than for a smaller subsample (cf. Van Ark 1975; Madoni 1984). Also, there was less probability of missing rare species with a larger subsample. These considerations were especially important for the regular drift samples which had low total numbers of organisms.

For the catastrophic drift samples, only one subsample (1% by weight) was sorted and identified because of the high numbers of invertebrates in each sample, and numbers of each taxon were multiplied by 100 to yield an estimate of the total number in the entire sample. P. cinereus was the only taxon that had numbers too low in one subsample to obtain an accuracy of at least ±50% (i.e. a count of 16 at 95% confidence limits - see Hickley 1975; Elliott 1977; Wrona et al. 1982) (Table 4).

The time saved by using the subsampling method was proportional to the size of the subsample. Thus, whereas the entire artificial substrate, regular drift, and catastrophic drift samples required approximately 40 h, 16 h, and 200 h respectively to sort, the subsamples only required approximately 10 h, 4 h, and 2 h respectively to sort.

Van Ark and Pretorius (1970) used mass as a basis for subsampling light-trap catches of insects, and Van Ark (1975) showed that mass was more reliable than volume. Although we have not compared the weight-based subsampling method presented here with one based on volume (samples with filamentous algae would preclude this comparison), the method presented appears to be a simple and reliable way to subsample collections of benthic macroinvertebrates, especially those containing filamentous algae.

Acknowledgments

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Table 1. Subsample counts for Chironomidae and total invertebrates in artificial substrate samples from Southern Indian Lake, Manitoba. Each subsample is 25% of the total weight of the sample. $I(n-1) = \chi^2_{3df, \alpha=0.05} = 0.22\text{-}9.35$, i.e. if χ^2 value lies between 0.22-9.35, agreement with a Poisson series is accepted at the 95% probability level. CV = coefficient of variation (see text for definition of terms).

Species of tree used in artificial substrate	Subsample A	B	C	D	Calculated value of χ^2	CV (%)
A: Alder (*Alnus rugosa* (DuRoi) Spreng.)						
Numbers of Chironomidae	766	731	731	785	2.87	3.6
Total numbers of invertebrates	917	878	897	924	1.43	2.3
B: Spruce (*Picea mariana* (Mill.) B.S.P.)						
Numbers of Chironomidae	983	997	942	1010	2.65	3.0
Total numbers of invertebrates	1161	1156	1121	1197	2.50	2.7
C: Willow (*Salix* spp.)						
Numbers of Chironomidae	924	930	919	861	3.38	3.5
Total numbers of invertebrates	1023	1014	1025	962	2.63	3.0

Table 2. Subsample counts for benthic macroinvertebrate species in regular drift samples from the Souris River, Manitoba. Each subsample is 25% of the total weight of the sample. $I(n-1) \simeq \chi^2_{3df,\alpha=0.05} = 0.22\text{-}9.35$, i.e. if value of χ^2 lies between 0.22-9.35, agreement with a Poisson series is accepted at the 95% probability level. CV = coefficient of variation (see text for definition of terms).

Taxon	Replicate No. 1 Subsample counts						Replicate No. 2 Subsample counts						Replicate No. 3 Subsample counts					
	A	B	C	D	χ^2	CV (%)	A	B	C	D	χ^2	CV (%)	A	B	C	D	χ^2	CV (%)
Hyalella azteca (Saussure)	2	6	0	1	9.23	116.9	8	4	5	4	2.05	36.1	6	6	5	6	0.13	8.7
Isonychia sicca Walsh	3	2	2	3	0.41	23.1	1	9	5	4	6.90	69.6	2	6	2	9	7.33	71.7
Caenis tardata McDunnough	11	9	10	5	2.37	30.1	11	11	12	6	2.20	27.1	27	22	24	17	2.36	18.7
Leucrocuta maculipennis (Walsh)	6	4	9	6	2.04	33.0	7	7	9	6	0.65	17.4	23	17	16	12	3.65	26.7
Baetis spp.	46	47	27	38	6.51	23.4	48	36	31	39	3.97	18.6	35	42	39	30	2.22	14.2
Cheumatopsyche campyla Ross	2	4	2	0	4.01	81.7	2	1	3	0	3.34	86.1	1	0	2	1	2.01	81.7
Others[1]	1	1	1	1	0	0	6	4	0	1	8.27	100.1	1	2	1	3	1.57	54.7
Total	71	73	51	54	6.21	18.2	83	72	65	60	4.26	14.2	95	95	89	78	2.17	9.0

[1] Includes: Ephoron album (Say), Psychomyia flavida Hagen, Stenacron interpunctatum (Say), Hydroptila ajax Ross, Hydropsyche recurvata Banks, and Polycentropus cinereus (Hagen).

Table 3. Number of times subsample counts were outside the value of $I(n-1) = \chi^2_{3df,\alpha=0.05} = 0.22\text{-}9.35$ for regular drift samples from the Souris River, Manitoba. The maximum number of times = 9 unless otherwise indicated.

Taxon	No. of times table values were outside of χ^2	% of counts random
Hyalella azteca	1	88.9
Isonychia sicca	0[a]	100.0
Caenis tardata	4	55.6
Leucrocuta maculipennis	0	100.0
Baetis spp.	1	88.9
Cheumatopsyche campyla	0[b]	100.0
Others[c]	1	88.9
Total	1	88.9

[a] 8 replicates used.

[b] 7 replicates used.

[c] Includes six species (see Table 2, footnote).

Table 4. Subsample counts for species occurring in a catastrophic drift sample from the Souris River, Manitoba. Each subsample is 1% of the total weight of the sample. I = index of dispersion ($I(n-1) = \chi^2_{9df, \alpha=0.05}$ = 2.7-19.0, i.e. if value of χ^2 lies between 2.7-19.0, agreement with a Poisson series is accepted at the 95% probability level). CV = coefficient of variation (see text for definition of terms).

Species	Subsample 1	2	3	4	5	6	7	8	9	10	Σx	χ^2	CV (%)	Total no. in entire sample
Hydropsyche recurvata	34	41	39	40	44	41	43	28	46	43	399	6.3	13.3	4462
Cheumatopsyche campyla	232	243	251	248	274	227	281	266	266	288	2576	14.8	8.0	28458
Polycentropus cinereus	2	2	5	2	5	4	2	3	4	4	33	4.3	37.9	418
Psychomyia flavida	121	108	116	98	126	102	131	125	128	134	1189	11.8	10.5	12689
Hydroptila ajax	23	18	19	21	24	25	23	19	24	29	225	4.5	14.9	1920
Isonychia sicca	97	91	113	98	127	126	131	133	121	127	1164	18.9	13.4	9230
Caenis tardata	107	101	123	118	126	131	116	128	124	130	1204	7.4	8.3	11210
Leucrocuta maculipennis	105	103	113	113	127	125	127	130	141	138	1222	12.7	10.7	14126
Stenacron interpunctatum	34	41	39	40	43	32	46	45	52	44	416	7.4	14.0	3215
Ephoron album	27	35	37	32	30	28	24	31	26	28	298	5.0	13.6	2854
Baetis spp.	142	152	155	136	163	147	160	149	172	167	1543	7.5	7.4	18270
Hyalella azteca	94	90	92	94	93	72	99	95	109	119	957	14.1	12.8	9011

Table 5. Summary of frequencies of occurrence of coefficients of variation (CV) for subsamples of artificial substrates (AS), regular drift samples (RDS), and catastrophic drift samples (CDS).

Sample type	% range					
	0-10	10-20	20-30	30-40	40-50	>50
AS (n=6)	6	0	0	0	0	0
RDS (n=24)	3	6	4	3	0	8
CDS (n=12)	3	8	0	1	0	0

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Our file / Notre référence

10 June 1988

Dr. Robert J. Sebastien
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Dear Bob:

This letter is to grant permission to include the manuscript entitled "A method for subsampling unsorted benthic macroinvertebrates by weight", for which we are co-authors with yourself, in your Ph.D. thesis (pages 167-184).

David M. Rosenberg

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/dl

cc: H. Agar

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