

Long-term effects of the mosquito control agents *Bti* (*Bacillus thuringiensis israelensis*) and methoprene on non-target macroinvertebrates in wetlands in Wright County, Minnesota (1997-1998)

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INTRODUCTION

This study was designed to determine the effects of the mosquito control agents *Bti* (*Bacillus thuringiensis* var. *israelensis*) and methoprene on non-target aquatic macroinvertebrates in 25 wetlands in Wright County, Minnesota after 7 to 8 years of continuous treatment application. The project began in 1988 when the Metropolitan Mosquito Control District (MMCD) and the Natural Resources Research Institute (NRRI) at the University of Minnesota-Duluth designed a study to determine the effects of *Bti* and methoprene on aquatic invertebrate communities and wetland birds. Background information was collected from 1988 to 1990. Treatment applications on 27 wetlands began in 1991 and continued through 1998. NRRI monitored the wetlands from 1991 through 1993, and found significant reductions in the density and biomass of many macroinvertebrate taxa during the first three years of treatment (NRRI 1995). No monitoring of the macroinvertebrate community occurred from 1994-1996. In 1997 MMCD contracted scientists at the University of Wisconsin-Superior's Lake Superior Research Institute to begin a follow-up study to determine if long-term application of the insecticides had led to substantial changes in community structure.

The current study was conducted in a manner similar to the previous study so that the results could be compared. In the initial study benthic macroinvertebrates were sampled quantitatively with core samplers from 25 X 50 m rectangular plots within each wetland. This core sampling program was repeated in both 1997 and 1998. In addition, in 1998 UW-Superior scientists used artificial substrates to collect invertebrates from a much larger region of each wetland to determine whether the invertebrate community found in the standard plots was

representative of the entire wetland.

During our 1997 study, fish were found in several of the wetlands. This year minnow traps and fyke nets were used to qualitatively sample the wetlands to determine the relative abundance and species composition of fish at each site that might be affecting the composition of the invertebrate community. Members of the UW-Superior sampling team also assisted in a multi-agency sampling program to assess the abundance and health of the frogs and toads present in the wetlands.

METHODS

Field Work

Site Selection This study was designed to follow procedures and methods used for the benthic macroinvertebrate portion of the previous multi-year study on the wetlands in Wright County, Minnesota (NRRI 1995). Each year five sampling periods, spaced approximately three weeks apart, were chosen based on the planned treatment schedules of *Bti* and methoprene: one date for pretreatment and four post-treatment dates. In 1997 collection dates were May 2 to 4, May 27 to 29, June 11-13, June 26-27, and July 16-17. The sampling dates in 1998 were April 24-26, May 19-20, June 9-12, June 30 to July 3, and July 19-22.

In order to ensure comparability of study results, attempts were made to utilize the same wetlands and sampling locations that were employed in the 1989 to 1993 study. In 1997 and 1998 we were able to visit 25 of the original 27 wetlands including sites 3-7, 9-14, and 51 in Corinna township, and sites 17-27, and 29-30 in Victor township. A 50-meter baseline transect was established for each wetland that matched as closely as possible the location of the baseline

previously used for that wetland. The baseline was located near the perimeter of each wetland usually partially overlapped the area where the collection buckets for spraying were located.

Sample plots measured 50 m x 25 m.

Core samples A set of 16 core samples were pooled to constitute a single macroinvertebrate sample for each wetland on each sampling date. Two cores were taken from each of four stations located on two separate transect lines that ran perpendicular to the baseline in each wetland. Prior to each sampling date, random even numbers were drawn to establish the location of the two transect lines. One line was located between 0 and 25 m on the baseline, and the other transect was located between 25 and 50 m. Four additional numbers between 2 and 24 were then drawn to determine the location of the sampling stations along each of the transects. On each date the location of two additional transect lines and four additional stations were selected in case any of the originally selected sites were unsuitable for sampling (i.e., the water was too deep [>60 cm in depth], the site had an unstable bottom, or was so dry that no water seeped up around the sampler's foot after standing in an area for a minute). The same transect lines and stations were used for each wetland during a single sampling period and then new locations were selected for the next trip.

Four or five researchers comprised the core sampling team on each date. In order to assure quality control in the field, no more than two new team members were added on any date. The group leader was responsible for recording data and ensuring that all sampling protocols were followed. Upon arrival at each wetland, a measuring tape was used to locate the starting point of each transect along the baseline. Two team members would then proceed out along each transect using 2 m sticks to measure out the distance to their first randomly selected sampling

site. At each site water depth, vegetation cover, and sediment consistency were recorded.

To collect the cores, one member of each group would place a 5 cm diameter hand corer (Wildco model 2420-H45) down in an undisturbed location adjacent to the transect line and, using the meter stick as a guide, push the corer approximately 10 to 15 cm into the sediment. The corer was twisted clockwise to cut through the vegetation, and then rocked gently from side to side. A rubber stopper was inserted firmly into the top of the core tube to create a partial vacuum and retain the sample as the corer was removed from the sediment. (We found that the stopper created a better seal than the flapper originally supplied with the corer). The second team member, wearing rubber trapper's gloves, would place one hand near the bottom of the core tube and cover the bottom of the tube as their partner rapidly extracted it from the bottom. The corer was then placed over a sieve bucket (250- μ m mesh) and the cork was removed, freeing the sample into the bucket. A thin metal rod with a flat plate attached was used to scrape any remaining sediment out of the corer prior to collection of additional samples. A second core was then collected a few inches away from the site of the first. After collecting two cores, the sampling team would move to the next site on their transect and repeat the process. Prior to moving to the next wetland, all sampling equipment was cleaned to prevent cross contamination of the sites.

The standard sampling procedure occasionally had to be modified in order to obtain representative samples and retain the core material. If the sampling site contained very loose unconsolidated bottom sediments, the corer was pushed in to a greater depth; up to 30 cm. When working in areas with deep water (>60 cm) and thick floating mats of vegetation, the corer was pushed into the vegetation mat as far as possible without punching through the bottom of the

mat. In these cases samples consisted of a portion of the mat, rather than actual bottom sediments. In relatively dry areas with clay substrates, sampling depth was reduced to the top 6 to 10 cm of sediment. If material fell out of the corer before it could be emptied into the sieve bucket, the rest of that core was discarded and a new complete core was collected.

The 16 individual core samples from each wetland, representing a total surface area of 314.16 cm², were consolidated and placed in labeled 5 quart buckets with enough water to cover the sediment, sealed, and then transported to a local hotel where they were sieved. All samples were sieved in a 250- μ m mesh sieve bucket before the end of the day on which they were collected. A small portion of the sample was placed in the sieve bucket which was suspended in a 5 gallon bucket of water. The bucket was agitated while clean water from a hose was sprayed over the sample until most of the fine sediment was removed. The sieved material was rinsed into in a clean, labeled 5 quart pail and the process was repeated until the entire sample had been cleaned. Samples were preserved with 10% formalin and stored until processed in the lab.

Artificial Substrates Artificial substrates are an effective means of sampling organisms that colonize objects within the water column, such as aquatic vegetation. Liber, Schmude, and Rau (1998) found that chironomid larvae readily colonized 10-cm diameter masonite disks in a wetland in Minnesota. Flies in the family Ceratopogonidae, caddisflies in the family Polycentropodidae, oligochaete worms in the family Naididae, and amphipods (*Hyalella azteca*) were also found on their disks. The use of artificial substrates reduces the variability that might occur when sampling sites contain a variety of vegetation types and substrate textures. In this study, artificial substrate samplers were employed to sample a wider area of each wetland than was possible with core samples and ultimately to reduce sample variability.

Twenty-four 10 cm diameter, 0.5 cm thick masonite disks were randomly placed in each wetland except sites 12 and 27 which did not have suitable water levels for any disks, and sites 9 and 24 which only had suitable locations for 16 disks. Eight of the disks were placed within the standard 50 X 25 m plots used for the core studies and the remaining 8 to 16 substrates were spread throughout the remainder of the wetland. The region of each wetland that would be sampled with artificial substrates was determined in conjunction with staff from MMCD in order to ensure that all samples would be taken from treated areas.

The random locations selected for placement of the eight disks within the standard plots were 14-14, 14-18, 18-8, 32-8, 32-20, 34-10, 40-2, and 44-14, where the first number refers to the distance (m) along the baseline, and the second number is the distance into the wetland perpendicular to the baseline. If any of these locations was inappropriate for sampling (water depths <25 cm or >100 cm) alternate locations were selected from the following randomly selected set of sites; 42-6, 32-14, 40-16, 34-24, 14-2, and 40-12.

The remaining 16 disks were placed outside of the main plot. If the main plot was centrally located in the wetland, 8 desks were placed on each side of the main plot. If the main plot was located at one edge of the wetland, all disks were placed on one side of the plot. A random walk technique was used to determine placement locations for the disks. A set of compass headings and distances (ranging from 5 to 16 m) were randomly selected prior to reaching the wetlands. Researchers would start from one end of the baseline or from a readily recognized landmark on shore, and follow the first compass heading along the perimeter of the wetland for the required distance. An artificial substrate sampler would be placed at this site which would then be the starting position for the next stage of the random walk. New compass

headings would be added to (or subtracted from) the previous reading to keep the next stage of the random walk within the wetland. When deep water or unstable mats were encountered, the researchers would continue to walk the remaining distance along the edge of the deep site.

The masonite disks were slid onto wooden dowels and secured with rubber bands. The dowels were then inserted into the substrate and adjusted so that the top of the dowels were visible above the water's surface and the disks were suspended 8 to 10 cm above bottom. When working in areas with floating mats, a hole was made in the mat and the dowel was attached to a set of floats with the disk located below the mat. Vegetation near the site of each artificial substrate was sprayed with fluorescent orange or red spray paint to aid in locating the samplers on later trips.

The first set of artificial substrates was deployed between April 24 and 26, 1998. Twenty-four disks were used at each of the other sites except site 5 where only 16 disks were deployed because of frozen ground, site 9 where only 16 disks were used due to the small size of the wetland, and site 24 where only 16 disks were used because of the unstable nature of the floating mat. On May 19 an additional 8 disks were placed at site 5. Disks were checked after three weeks and their depths were adjusted as necessary to prevent dessication.

The research design called for allowing 6 weeks for the colonization of the substrates. The first set of disks were retrieved on June 9-12, 1998. A second set of disks were set out at this time and were retrieved on July 19-22, 1998. During retrieval, vegetation was moved away from the support rod, the rod was raised to the surface and a labeled ziplock plastic bag was placed over the disk as it was removed from the water. The rubber band was removed from the rod and the band and disk were immediately placed in the bag which was then sealed. At the

end of each day the disks were preserved in 95% ethanol and then stored until processed in the lab.

Collection of Other Aquatic Organisms During the 1987 surveys, fish and frogs were observed in several of the wetlands. This year, we attempted to determine the species composition and obtain estimates of the relative abundance (catch/effort) of these organisms.

Minnow traps and small mesh fyke nets were used to collect fish in wetlands that contained a minimum of 30 cm of water. Sites 12, 21, 23, and 27 were not sampled due to their tendency to dry out in 1987. All other wetlands received 5 minnow traps. In addition, small mesh fyke nets (0.5 m diameter) were placed at sites 7, 13, 14, 18, 20, 25, 29, 30, and 51. In order to maximize potential to collect fish, the nets and traps were placed both in portions of the main study plot and in adjacent regions of the wetland that contained water of sufficient depth (25 cm for minnow traps and 50 cm for fyke nets). The traps and nets were baited with a combination of pelleted floating pond fish food, dry cat chow, and dry dog chow. Fish samples were collected from June 30 to July 2, and from July 19-22, 1998. Traps and nets were deployed at each site for a 48 hour period, and were checked each morning and late afternoon/evening. All fish were identified and enumerated. A subsample of the fish were preserved in 10% formalin until returned to the lab where they were weighed and measured (total length).

During the core sampling trips we noted the wetlands in which we observed or heard frogs. We also noted the presence of frogs and tadpoles in minnow traps. This information was used to select a subset of the wetlands to survey for frogs. Eighteen of the 25 sites (six from each treatment) were identified for further study. On August 17-20, 1998 UW-Superior researchers joined scientists from the MMCD, the University of Illinois, and the University of Minnesota-

Duluth. The research team spent a standard number of person-hours in each wetland using long-handled nets in an attempt to capture all frogs that were observed. The frogs were identified, enumerated and checked for deformities. A subsample of the catch was preserved and returned to the University of Illinois for further analysis.

Laboratory Work

Splitting core samples Formalin was decanted under a hood in the lab and the material from each core sample was placed in a 250-um sieve rinsed thoroughly in water. Samples from the first three collection dates in 1998 were split into quarters using a large, modified Folsom splitter that was created (and used extensively) by personnel at the Lake Superior Research Institute (O' Halloran et al. 1996, Schmude et al. in press). Samples were initially split in half, one-half was randomly chosen and split again into quarters. After splitting, one of the quarters was randomly chosen for processing. On the last two sampling dates, one of the quarter samples was split an additional time to provide a sample size of one-eighth of the original sample.

Samples with large amounts of material were split in two stages. The first part was split in half and stored in separate buckets (A and B). The next part was split in half (C and D), with parts A and D, and B and C subsequently combined to form two equal halves. One half was randomly chosen to be split into quarters, and after splitting, one quarter was randomly chosen for processing. Material not immediately chosen for further splitting or processing was preserved in formalin and kept until splitting and picking quality assurance checks had been completed. All subsamples chosen for analysis were stained with a few grains of powdered rose bengal and stored in water in a refrigerator until they could be processed; samples were processed in a timely fashion and no sample remained unprocessed in this condition for more than five

days.

An attempt was made to randomly choose samples from two sites from each collection date for a Quality Control (QC) check on the splitting technique; a total of 10 of the 125 samples were processed for QC (8%) in 1998. For the splitting QC check one quartered subsample from each of the two halved subsamples was randomly chosen, and both were processed. The splitting efficiency averaged 5.25% (Table 1) so no changes were made in our techniques.

Picking core samples Each sample selected for processing was fractionated into three different sizes of material for easier and faster completion: large material that remained in a 4000- Φ m mesh sieve, medium material that ranged from 450-4000 Φ m, and fine material that was caught on a 250- Φ m mesh sieve. The large material was examined by eye, while the medium and fine material was viewed through a Bausch and Lomb or Leitz dissecting microscope at 7-12 X magnification. A light table and overhead light was used for illumination.

Large material to be examined by eye was placed in a 51 x 44 x 7 cm white, plastic photo-processing tray (Ted Pella, Inc., Cat. #26554). The tray had two raised ridges in the shape of an "X," and water was added so that the ridges were not quite submerged. The ridges dissected the tray into four compartments that could more easily be examined. Overhead lamps and lighted magnifiers were used for illumination and examination of the sample. Medium and fine material to be viewed through the microscope was placed in a 14-cm diameter gridded petri dish. A small amount of material (1-2 teaspoonfuls) was evenly distributed in shallow water and the grids were systematically examined. All organisms except Nematoda and zooplankton were picked and placed in a labeled vial with 70% ethanol. Extremely small middle segments of oligochaetes were not picked from the debris.

Approximately 10% of the samples were chosen for a QC check on picking efficiency and were repicked by a second person. If the picking error exceeded 20%, all samples picked by that person were reexamined. If more than one person was involved in picking the original sample, then the last 10 samples picked by the team were picked a second time. QC checks were done more frequently when new staff members were being trained. New staff members were involved in picking samples collected in April and May. Table 2 shows that errors were made initially, so all of the April and many of the May samples were repicked by a second person. Picking efficiency averaged 10.4% for the rest of the May samples and the June and early July samples. We noticed an increased number of errors in the samples collected July 19-22 (25% average error), possibly due to the lower density of organisms and the greater amount of vegetative matter. All 25 samples from this date were picked a second time.

Artificial Substrates Prior to analysis, the artificial substrates that were collected from each wetland on each date were divided into three groups for processing. Group 1 consisted of 5 of the 8 disks from the main plot in each wetland. Groups 2 and 3 each consisted of 5 of the 16 total disks that had been deployed outside of the main disks. Random numbers were drawn to determine which disks would be placed in each group. If a selected disk had been lost or placed in an area of the wetland that had dried up, another disk was selected as a replacement.

Organisms were gently scraped from all surfaces of each disk and were collected in a 250 um mesh sieve. The material from each set of 5 disks was pooled in the sieve, washed well and then rinsed into a labeled sample jar and preserved with 70% ethanol.

All benthic macroinvertebrates were picked from the disk samples using a microscope as described in the previous section. After identification, densities (#/m²) were determined by use

of the formula $D = \text{Number found} / A * 10,000$, where A = the surface area of the disks. During the exposure, the disks had swollen to a diameter of 10.2 cm and a thickness of 0.5 cm, so $A = 179 \text{ cm}^2$.

Identifications Identification of insects, crustaceans, and gastropods were to the generic level; oligochaetes, leeches, and clams were identified as such. Pupae and specimens that were very immature, damaged, or taxonomically difficult were identified at higher taxonomic levels. Chironomid larvae and some beetle larvae were mounted on slides in Hoyer's mounting medium to facilitate identification. Taxonomic references included Hilsenhoff (1995), Merritt and Cummins (1996), Thorp and Covich (1991) and Wiederholm (1983).

Measurements and biomass of benthos from cores In 1998 lengths of chironomids, clams and gastropods were determined using an ocular micrometer while viewing through a dissecting microscope. Methods for measurements of insects followed those of Smock (1980); snails were measured for greatest height and clams for greatest width. No correction factor was used for possible shrinkage due to preservative. Additional variables included damaged specimens, which may or may not have been included in measurements depending on the degree of damage, and the effect of telescoping of body parts, measurements of which were not standardized. Equations for biomass calculations were obtained from Smock (1980) and Hershey (1990), and generally follow the equations used in the previous study (NRRI 1995). The formula used for chironomids in the groups Orthocladiinae and Chironominae is $\text{dry weight} = \exp(-5.279 + 2.32 * \ln(\text{length}))$ while dry weights of Tanypodinae were calculated from the equation $\text{weight} = \exp(-5.573 + 2.41 * \ln(\text{length}))$. Biomass of molluscs was determined from the relationship $\text{dry weight} = 1000 * \exp(0.189 * \ln(\text{length}) - 0.289)$ Length measurements are in mm

and dry weights are calculated as mg.

Dry weight biomass of oligochaetes was determined directly in 1998. All oligochaetes from an individual sample were pooled and rinsed with distilled water prior to being placed in tared weighing pans. The animals were dried at 60 C for 48 hours and were cooled for at least 1 hours in a desiccator prior to weighing (McCauley 1984).

Statistical analysis The statistical design of the study was described in NRRI (1995) and consisted of a two-way (block, treatment) analysis of variance (ANOVA - Model C) followed by a test for multiple pairwise comparisons that compared effects from the two treatments (*Bti* and methoprene) with control responses on each individual sampling date. For the core samples repeated measures tests using a time series covariance model were also used to determine if sampling dates had a significant effect on the results for 1997 and 1998. Finally a repeated measures test using an unstructured covariance model was used to examine differences between data from the core samples for the two years. For the artificial substrates, an initial ANOVA was run to determine whether there were significant differences in abundance from samples collected inside the main plot and from other areas of the wetland. In the event of no differences, the samples were pooled and analyzed as described above.

The number of replicates in each treatment group varied slightly due to missing data, but generally included 9 control sites, 9 *Bti* sites, and 7 methoprene sites. The nine blocks included in the model were those established by NRRI for the original study. Endpoints included density and biomass estimates (for core samples) for macroinvertebrate groups at various taxonomic levels. To accomplish meaningful analyses, groups were included only if the total number of organisms of each taxon met a minimum level (100 organisms was used by Hershey 1994).

Effects were considered significant at $p \leq 0.05$ for all statistical tests.

Taxonomic and functional feeding groups that were examined in the statistical analyses, if abundance warranted, included

Total Macroinvertebrates

Total Non-Insects

Total Annelida

Total Mollusca

Bivalvia

Gastropoda

Total Insects

Total Coleoptera

Hydrophilidae

Dytiscidae

Scirtidae

Other Coleoptera

Total Diptera

Non-chironomid Diptera

Ceratopogonidae

Stratiomyidae

Odontomyia

Other Stratiomyidae

Tipulidae

Culicidae

Other Non-Chironomid Diptera

Chironomidae

Tanypodinae

Orthoclaadiinae

Chironominae

Paratendipes

Polypedilum

All Other Chironomini

Tanytarsini

Nematocera

Brachycera

Remaining Insects

Total Insect Predators

Dipteran Predators

Predatory Chironomids

Non-Chironomid Dipteran Predators

Total Non-Dipteran Predators

Prior to analysis, density data were transformed using $\ln(x+Y)$ animals/m² where Y is the density (#/m²) that would be represented by one organism. The addition of at least one organism to each pooled sample ensured that analyses were performed on the minimum number of organisms that could be detected in a sample. When core samples were split in quarters, one organism was equivalent to 127/m²; when one eighth of a core sample was analyzed one organism represented 255/ m². In 1997 some samples contained less than 16 cores and in this case one organism represented 56/m². For consistency, we let Y = 56 for density transformations for core samples for both years. For the artificial substrate samplers, one organism per pooled sample was equivalent to 11/ m².

Biomass data were transformed using natural log with the addition of a constant as listed below for each taxon or functional group. The constant used was lowest biomass (or slightly less than the lowest biomass) for each taxonomic group that was present at any sampling site. Values used in 1998 included : Total Macroinvertebrates 0.00, Total Non-insects 22.0, Bivalvia and Gastropoda 1800, Total Mollusca 4000, Total Annelida 7.00, Total Insects 1.00, Total Diptera 1.00, Tanytarsini 0.06, all other groups 0.5.

In this report we will use the term *average* density (or biomass) to represent arithmetic mean values for treatment groups and the term *mean* density (or biomass) to represent the back-transformed least squares estimates of mean density (or biomass).

RESULTS

Site Characteristics

Water depths within the main sampling plot ranged from seepage (<5 cm) to 103 cm in 1998 (Table 3). Four of the sites (9, 10, 12, and 27) generally had water depths less than 20 cm, seven sites averaged 20 to 40 cm of water (6, 7, 11, 18, 19, 21, and 51), and six sites were over 40 cm deep (13, 14, 23, 25, 26, and 30). Site 17 showed a great deal of variation in water levels, varying from 51 cm at one location in April, to 12 cm at the same spot in June. The regions where we had placed many of the artificial substrates in Site 17 dried up completely in June, resulting in a loss of samples (Table 4). The second set of disks that were deployed in this wetland in June were moved to deeper water, while the disks in the other wetlands were moved down their support rods until they rested just above the sediment. Sites 3, 4, 5, 20, 22, 24, and 29 contained dense floating mats of vegetation and water depths were often recorded above the mat. At these sites the majority of the core samples were taken from the mat itself and not from the bottom. Floating mats were also observed at sites 12, 17, 18, and 26. Zones outside of the main plots where we placed the artificial substrates were generally similar in characteristics to the main plots (Table 5). The bottom sediment at several of the sites, both within and outside the main plots, was still frozen on the first sampling date in April.

Cattails were the dominant type of vegetation in all of the wetlands and exhibited considerable changes in density during the course of the sampling season as the dead material present in April was replaced by live growth by May and June. A few of the sites contained grasses and sedges in addition to cattails (Tables 3-5). Some of the sampling plots had small

regions of open water. The main sampling plot in site 14 was located along the edge of a lake and had very limited vegetation.

Non-invertebrate fauna

Last year utilization of bottle traps revealed that 13 wetlands (sites 5, 6, 7, 10, 11, 13, 14, 17, 22, 24, 25, 26, and 29) contained fish. Most of the fish that were collected were mud minnows (*Umbra limi*), sticklebacks (*Culaea inconstans*), and minnows (unknown species). One northern pike (*Esox lucius*) was collected at site 22, while site 25 contained a number of very large carp.

This year 13 of the 21 wetlands that we sampled with minnow traps and fyke nets were found to contain fish (Tables 6 a and b). Six of the sites were control sites (5, 13, 14, 17, 26 and 51), five were treated with *Bti* (7, 11, 20, 24 and 30) and two (25 and 29) were treated with methoprene. Mud minnows were the most abundant and commonly found species, occurring at 10 of the sites. Stickleback, *Pimephales* minnows, and bluegill sunfish (*Lepomis macrochirus*) were each found in 3 wetlands. A large number of bullheads (*Ictalurus melas*) were found at sites 17 and 26. Other species that were found included yellow perch (*Perca flavescens*), two species of darters (*Etheostoma nigrum*, and *E. flabellare*), smallmouth bass (*Micropterus salmoides*), a northern pike (*Esox lucius*) and a tadpole madtom (*Noturus gyrinus*). The highest densities of fish were found at sites 11, 14, 17, 26 and 30, while the two methoprene sites (25 and 29) had low catch rates per effort. Most of the fish collected in the minnow traps and fyke nets were between 50 and 75 mm in total length. The fyke nets generally captured the same species that were found in the minnow traps.

Four species of frogs were found in the 18 wetlands that were surveyed in mid-August,

1998 (Table 7.) While *Rana pipiens* was present at every site samples, it was most abundant at site 23. Green tree frogs (*Hyla cimerea*) were only collected from site 4 where they were quite abundant. Two green frogs (*R. clamitans*) were found at site 5 and a wood frog (*R. sylvatica*) was captured from site 3. The average density of frogs from control sites (5, 14, 18, 19, 26, and 51) *Bti* sites(4, 6, 11, 23, 24, and 30) and methoprene sites (3, 9, 10, 25, 27, and 29) were remarkably similar (27 to 29 frogs per standard effort). Scientists from the University of Illinois are tabulating the data on the health of the frogs and will present their results in a separate report.

Benthic Macroinvertebrate Fauna from core samples

In 1997 the core samples collected at least 106 genera of aquatic insects, including 36 chironomid genera. In 1998 we found at least 102 genera of insects with 41 chironomid genera (Table 8). Isopoda, Amphipoda, Bivalvia, at least 9 genera of Gastropoda, Hirudinoidea, and Oligochaeta were present in both years. Appendix table 2 lists the density for each taxon at each site during 1998 while Appendix Table 4 contains biomass values for the taxa.

Total Macroinvertebrates In 1997 the total density of macroinvertebrates collected in core samples was generally similar in all treatments, with annual mean densities ranging from 14,888 to 15,763 organisms/m² (Table 9). Oligochaetes, chironomids, bivalves, and stratiomyids were the dominant groups in core samples in 1997 comprising 45%, 19%, 12% and 5% respectively, of the total density (Figure 1). Total density in 1998 was slightly higher than in 1997 with annual mean densities of 15,753 to 17,778 organisms/m². Chironomids now dominated the invertebrate community (42% of total). Oligochaetes, bivalves, and gastropods were also common representing 13%, 11% and 6% of the total density. Total biomass values were a bit more variable, with annual mean biomass ranging from 74 to 105 g/m² in 1997 and

from 64 to 167 g/m² in 1998 (Table 10). Bivalves accounted for the majority of the biomass in 1997 (Fig. 2 a) while bivalves and gastropods were both important in 1998.

Seasonal changes in total invertebrate density were observed in 1997. In early May control sites had significantly fewer total macroinvertebrates (15,808 organisms/m²) than *Bti*- (22,372 organisms/m²) and methoprene-treated (23,586 organisms/m²) sites. This trend was also reflected in biomass, where controls averaged 125,600 mg/m², and treatments averaged 173,900 mg/m² (*Bti*) and 206,400 mg/m² (Fig.3). These differences were not statistically significant (Tables 9, 10, Appendix Tables 3, 6).

In 1998 there were no significant differences in total macroinvertebrate densities between the control and treatment sites on any sampling dates (Appendix Table 1), or over the entire sampling season (Table 9, Appendix Table 3). Total biomass in control sites (seasonal mean 64 g) was generally lower than that of the *Bti*- (149 g) and Methoprene-treated sites (167 g) (Figure 3, Table 10, Appendix Table 6). In early July the differences were significant (Appendix Table 5).

Non-insects The differences in total macroinvertebrate abundance and biomass that were observed in 1997 were due to lower densities of Annelida and Bivalvia in control sites (Figs. 5, 7). Average densities became more similar during the study, although control sites continued to contain a lower density and biomass of bivalves than the treated sites (Fig. 5). These differences were not statistically significant over the entire sampling period (Tables 9, 10, Appendix Table 3). In 1998, the density of annelids and bivalves was again lower in the control sites than in the treatment sites. The control sites contained significantly fewer bivalves than the *Bti* sites on July 1, 1998 (Fig. 5, Appendix 1). Biomass of both annelids and bivalves were significantly reduced

in the control sites in early July (Fig. 5, 7, Appendix Table 5).

No significant differences in the density or biomass of gastropods could be attributed to treatment levels during either 1997 or 1998 (Table 10, Appendix Tables 1, 5). However, a significant increase in both the density and biomass of gastropods was noticed between 1997 and 1998 (Fig. 2 a, Appendix Tables 3, 6)

Total Insects There was a slightly lower density of total insects in core samples for the methoprene sites than at control sites during all of the post-treatment dates in 1997 (Fig. 7 b), and on May 19, 1999. These differences were not statistically significant due to high variability among sites (Table 9, Appendix Tables 1, 3). Total insect biomass was not reduced by either of the treatments in 1998 (Appendix Table 5).

Diptera - Chironomidae The family Chironomidae was the most abundant family of dipterans found in both 1997 and 1998. Average annual densities increased from 913 to 1900 organisms/m² in 1997 to 4581 to 6974 organisms/m² in 1998 (Table 9, Fig. 8). Biomass also increased between the two years (Table 10, Fig. 2 b).

The subfamily Chironominae dominated this family, with highest densities in the tribe Chironomini. The Chironomini showed a significant decrease in density and biomass at *Bti*-treated sites during the last sampling date in 1997 (Fig. 9). The major taxa in this group were *Paratendipes*, *Polypedilum*, and *Chironomus*, and all showed lower densities in *Bti* sites versus control sites on this date (Figs. 10, 11). In 1998 the biomass of the Chironomini in the *Bti* sites remained significantly lower than the controls during May (Appendix Table 5) and over the course of the entire year (Table 10, Appendix Table 6). Significant differences in density were noted for the “other Chironomini” in April of 1998 (Appendix table 1).

At the methoprene sites, the density of *Chironomus* was significantly lower than controls on July 16, 1997 which likely accounted for the significant decrease in the density and biomass of the test group "all other Chironomini." on that date and for the entire sampling season (Table 9, Appendix Tables 3, 6). The biomass of the "other Chironomini" was significantly reduced again in June 1998 (Appendix Table 5). Overall, the biomass of this group was lower than the controls for both years (Appendix Table 6).

Bti also appeared to have a significant effect on members of the second tribe of the Chironominae, the Tanytarsini (Fig 12). Density was significantly decreased in both 1997 and 1998 (Table 9, Appendix Tables 1, 3) while biomass showed significant decreases in 1998 and over the two years combined (Table 10, Appendix Tables 5, 6). Density and biomass were much lower than the controls in April and May, 1998.

The biomass and density of chironomids in the subfamily Orthocladiinae were generally lower at control sites than in treatment sites during both 1997 and 1998 (Fig. 13). These differences were significant in April, 1998. (Appendix Table 1). No significant treatment effects were noticed on the Tanypodinae, the predatory group of chironomids (Fig. 14, Appendix Tables 1, 3).

Other Diptera Members of the families Stratiomyiidae, Ceratopogonidae, and Tipulidae were present at many of the sampling sites in both 1997 and 1998 (Appendix tables 2 and 4). No significant treatment effects were observed on density or biomass of these groups on any date (Figs 15, 16, Appendix tables 1 and 5). Density and biomass of dipterans was also examined when they were grouped into their suborders Nematocera and Brachycera. While they appeared to be a slightly lower density of Nematocerans in the methoprene sites in 1998 (Fig 17), these

differences were not significant.

Coleoptera The Coleoptera were the major non-dipteran order of insects found in the core samples. There were no significant differences in abundance for the treatments on any date in 1997 (fig 18) however, density of the family Dytiscidae was reduced at the *Bti* sites on two dates in 1998 (Appendix Tables 1, 3).

Predators Average density of insect predators in core samples ranged from 426 to 973/m² in 1997 and from 1214 to 2554/m² in 1998 (Figure 21, Appendix Tables 1, 3), with the majority of them being Diptera (Chironomidae: Tanyptodinae). No significant treatment effects were observed for this group of organisms. Non-dipteran predators were much less abundant (59 to 373/m²), and on only one date (June 26, 1997) was a significant reduction in density observed for methoprene sites (Fig.20); this difference was not evident in biomass.

Non-predators In 1998 significant decreases in the density and biomass of non-predatory dipterans, and especially non-predatory chironomids were observed in the *Bti* sites (Fig 19, Appendix tables 3,5), mainly due to the decreases that were observed in the Chironominae.

Macroinvertebrate Fauna from Artificial Substrates

The density of macroinvertebrates collected on artificial substrate samplers was quite variable, some disks contained no organisms, while others contained over 400 invertebrates per disk (4400/m²) (Appendix Table 7). An analysis of variance was conducted to determine if there were any statistical differences in the density of invertebrates on substrates placed within the main plot (group 1) and those placed outside the main plot (groups 2 and 3). Two models were used, one that included the blocks that were originally assigned to the wetlands, and one that omitted the blocks. The results from both tests showed that densities for the three groups of

substrates were similar (Appendix Tables 8 and 9). The three groups were combined for further analyses to determine whether the *Bti* or methoprene treatments affected densities. Because some of the sampling sites had dried up during the colonization period, the analysis of variance models were adjusted to account for an unbalanced design.

The first set of artificial substrate samplers was collected in early June, after six weeks of exposure. At that time, mean density of macroinvertebrates ranged from 363 to 478 organisms/m² (Table 11). Chironomids were the dominant taxa present (>50%), followed by annelids (Figure 21). While annelids were not present on as many substrates as the chironomids, very high densities of small leeches (up to 3667/m²) were found on a few of the substrates (Appendix table 7). The mean density of macroinvertebrates on substrates that been exposed for six weeks between June and July was much lower, ranging from 84 to 130 organisms/m². The slower rate of colonization by chironomids accounted for most of this decrease in abundance (Table 11).

The use of artificial substrate samplers revealed few significant differences in macroinvertebrate density due to treatments. The density of gastropods at the control sites was lower than at either of the treatment sites in both June and July (Table 11, Appendix Tables 10, 11). A lower density of predatory chironomids in the subfamily Tanypodinae was also noted at the control sites in July.

DISCUSSION

In this study, the effects of *Bti* and methoprene on benthic macroinvertebrates were assessed primarily from data on density and biomass that were obtained from core samples. A great deal of variation existed in the data collected from different wetlands, even from those assigned to the same treatment groups. We attempted to find factors, besides treatment level, that might account for some of this variation. In 1998 we observed that in some of the wetlands a majority of the core samples were actually being taken from the vegetative mat and not from the bottom sediments. We also determined that fish were present in many of the wetlands and were concerned that the effects of predation might be altering invertebrate density. Two new blocks (mat / no mat, and fish / no fish) were added to our analysis of variance models. Due to the uneven distribution of these factors among treatment levels, the new blocks did not greatly reduce variation and thus were not used in the final analyses.

Artificial substrate samplers were used this year to examine whether data obtained from a standard 25 x 50 m plot was representative of an entire wetland. In a previous study (Liber et. al. 1998), similar substrates that were placed in a wetland setting were colonized by over 1000 chironomids/m² when the disks were suspended 10 cm below the water's surface for 40 days. Disks that were placed near the bottom for 48 days were not colonized as readily (< 500 chironomids/m² in June). Our disks were initially placed within 15 cm of the surface, but due to declining water levels during the treatment period, many of the disks were moved close to the bottom prior to retrieval. Mean density of chironomids on our substrates ranged from 183 to 261/m² in the various treatment groups in June. Density was greatly decreased in July with only

15-31 chironomids/m². Liber et. al (1998) found significant reductions in chironomid abundance on artificial substrates when they were exposed to *Bti* at levels 10 times greater than the rate recommended by the Minneapolis-St Paul Metropolitan Mosquito Control District for early summer mosquito control. They noticed the greatest reductions in the Chironomini and Tanytarsini. In our study utilizing artificial substrates, we found no significant reductions in the abundance of chironomids or any other taxa of macroinvertebrates in wetlands receiving the standard application of *Bti* or methoprene. Data from the main sampling plot in each wetland was similar to that from other regions of the wetlands.

This study found that the mean density of macroinvertebrates in core samples was similar between control wetlands and those treated with *Bti* and methoprene, ranging from 14,888 to 15,763 organisms/m² in 1997 and from 15,753 to 17,778 /m² in 1998. Oligochaetes and Chironomids were the dominant organisms in 1997 (45% and 19% respectively of density). Their relative rankings were reversed in 1998 when chironomids increased to 42% and oligochaetes declined to 13% of total density. The only significant treatment effects observed during this study were the reduction in density and biomass of Chironomids in the subfamily Chironominae which contains mainly non-predators. Members of the Chironomini were affected at both the *Bti* and methoprene sites, while some of the Tanytarsini were impacted by exposure to *Bti*.

In a previous study (1991-1993) of these same wetlands, the mean density of macroinvertebrates in core samples averaged 1236 organisms/m² in methoprene treated sites, 1349/m² at *Bti* sites and 3123/m² at the control sites (NRRI 1995). Chironomids were the dominant taxa found, comprising approximately 60% of the invertebrate community in all three

years. Significant treatment effects were observed for many taxa, including total macroinvertebrates, total Insecta, total predators, total Diptera, Chironomidae, Ceratopogonidae, Culicidae, Tipulidae, Stratiomyidae, Nematocera, Hydrophilidae and Dytiscidae larvae. Reductions in abundance and biomass occurred soon after treatment with *Bti* and methoprene, and persisted throughout the sampling period.

The differences in total density and treatment effect observed between this study and the previous study (NRRI 1995) may be due to several factors. The earlier study commenced after several years of drought which may have impacted population density and stressed some species, increasing their susceptibility to the control agents. In 1997 and 1998, water levels were generally higher and promoted the growth of thick mats of vegetation. The vegetation may have provided additional substrate and food for benthic organisms while limiting the insect control compounds from reaching the substrate.

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