AN ASSESSMENT OF NON-TARGET EFFECTS OF THE MOSQUITO LARVICIDES, BTI AND METHOPRENE, IN METROPOLITAN AREA WETLANDS

A REPORT FROM

THE SCIENTIFIC PEER REVIEW PANEL

TO

THE METROPOLITAN MOSQUITO CONTROL DISTRICT

January, 1996

Scientific Peer Review Panel
Of the Metropolitan Mosquito Control District

Richard Anderson, PhD
Scientific Chair
USEPA Research Laboratory
6201 Congdon Boulevard
Duluth, Minnesota 55803

Judith Helgen, PhD
Fresh Water Ecology

Stuart Hurlbert, PhD
Department of Biology
San Diego State University

Roger Moon, PhD
Department of Entomology
University of Minnesota

Robert Naiman, PhD
Center for Streamside Studies
University of Washington

William Schmid, PhD
Department of Ecology,
Evolution, and Behavior
University of Minnesota

Kenneth Simmons, PhD
Entomology

Keith Solomon, PhD
Director
Centre for Toxicology
University of Guelph

Harrison Tordoff, PhD
Professor Emeritus
Department of Ecology,
Evolution, and Behavior
University of Minnesota

Michael Zicus, PhD
Zoology

John Genereux
192 Charles Avenue
St. Paul, Mn 55103
Administrative Chair

January 23, 1996

Joseph Sanzone, Director
Metropolitan Mosquito Control District
2099 University Avenue West
St. Paul, Minnesota 55104-3431

Dear Joe:

The SPRP was formed in 1986 as an independent research group, funded by the MMCD, with an aim to describe long-term consequences of the MMCD mosquito larvicide program. This letter accompanies a report that summarizes the research we promoted and the results of that research. It also marks the end of the SPRP.

During our operation we sponsored both laboratory and outdoor studies. The laboratory studies filled specific gaps in our information on the toxicity of Bti and methoprene. The field studies were designed to measure effects on nontarget invertebrate and bird populations when the larvicides were applied in their usual manner.

Our results and concerns are summarized in the Report's Executive Summary and detailed in the report. I would like to highlight some in this letter. The laboratory studies showed that chironomids may be as sensitive as mosquitoes and that some zooplankton may be affected at low concentrations (Daphnia) while others may not (Diaptomus). The chironomid results were supported in the field studies. Some chironomid species and other primitive flies were affected while no changes in the measured zooplankton populations were found. Interpretation of the field results is never as simple as those found in laboratory studies and we, as you can guess, struggled to produce the joint interpretation found in the report.

I would also like to highlight the SPRP concern about the potential for long-term effects and the need to study them more intently. The major study we sponsored included three application years and the wetlands have had an additional two years of applications. That means that the wetlands have been treated for five years. There is no experimental area that is as well studied or has the potential for assessing long-term larvicide effects.

We ardently recommend that the invertebrate populations in those wetlands be sampled this spring (1996) and that applications be continued so that any environmental effects questions can be answered in more detail and with greater certainty.

However, the SPRP is aware that there is legislation that stops further research by the MMCD and your funding is reduced. It is truly sad that such an opportunity may pass and that answers will not be available for a questioning public.

In closing, the SPRP would like to thank the MMCD for both their "hands-off" approach during our operation as a research panel and the cooperation shown by all the staff when our requests were made. The high professional level made our jobs smoother. You and all your staff are applauded by us.

Sincerely yours,

Richard L. Anderson

Richard L. Anderson
Scientific Chair
Scientific Peer Review Panel

Table of Contents

Executive Summary 1

Section I: Introduction 3

Section II: Literature Review and New Laboratory Studies 5

A. Characteristics of Bacillus thuringiensis israelensis 5

B. Characteristics of Methoprene 8

Section III: Initial SPRP-Sponsored Field Studies 12

Wright County Historical Studies 12

North Metropolitan Area Bird Study 13

Lake Maria Study 14

Mallard Duckling Study 15

Section IV: Wright County Long Term Experiment (WCLTE) 16

Methods 16

Results 19

Bti-Treated Wetlands 19

Methoprene Treated Wetlands 27

Summary and Discussion of WCLTE 31

Section V: Ecological Consequences of Bti and Methoprene use in the Metropolitan Area 33

Appendix I. Operational Use of Mosquito Larvicides by MMCD 37

Appendix II. An Overview of Wetlands Biology 40

Appendix III. An Overview of Selected Aspects of Duck Breeding Biology 43

Appendix IV: References 51

Appendix V. Glossary 58

Index To Figures

Figure 1: Average densities of selected groups of zooplankton collected in the Wright County Long Term Experiment wetlands 20

Figure 2: Species richness of benthic invertebrates in core samples collected in the Wright County Long Term Experiment wetlands 22

Figure 3: Densities of benthic invertebrates in core samples collected in the Wright County Long Term Experiment wetlands 23

Figure 4: Biomass of benthic invertebrates in core samples collected in the Wright County Long Term Experiment wetlands 26

Figure 5: Abundance of nineteen different species of birds in the Wright County Long Term Experiment 28

Index to Tables

Table 1: Summary of 1991 sampling and treatment events and outcomes in WCLTE 17

Table 2: Summary of 1992 sampling and treatment events and outcomes in WCLTE 18

Table 3: Summary of 1993 sampling and treatment events and outcomes in WCLTE 18

Table 4: Mean number of individuals per square meter (density) of selected benthic invertebrates in experimental wetlands, Wright Co., MN 24

Table 5: Mean biomass (mg/m^2) of selected benthic invertebrates 25

EXECUTIVE SUMMARY

The Scientific Peer Review Panel (SPRP) of the Metropolitan Mosquito Control District (MMCD) first met in 1986 to answer the question: What are the long term ecological consequences of the larvicide program? The SPRP began its work with a review of existing biological and toxicological information on the larvicides methoprene and *Bacillus thuringiensis israelensis* (Bti) used by the MMCD.This assisted the preparation of the Supplemental Environmental Impact Statement of the MMCD in 1987.

Following this review, the SPRP developed a research plan to assess the biological effects of these larvicides . The panel recognized that wetland birds and amphibians were primary concerns of the public, but the SPRP also understood that any non-target effects of these larvicides would most likely be upon other invertebrates, especially aquatic insects, whose larval development occurs in wetlands. The SPRP further realized that studies relevant to non-target organisms would require assessment of both direct and indirect effects. Direct effects would be through acute or chronic toxicity; e.g., death or impaired reproduction. Indirect effects could result from changes in the food web; e.g., food supply, competition or predation. A significant reduction of aquatic invertebrates (as a direct effect of larvicide treatments) could be the basis for indirect effects on species that feed upon them; e.g., waterfowl, other wetland-associated birds, amphibians, and predatory invertebrates like dragonflies.

These considerations led to a research plan with both laboratory studies and multiple year field studies. The laboratory studies were needed to fill specific gaps in the toxicity information for wetland zooplankton, insects and amphibians. The field studies were commissioned to describe and define effects in natural wetland communities when methoprene and Bti were applied in their usual manner for mosquito control.

The laboratory studies with Bti showed that *Paratanytarsus* chironomids were affected at concentrations near or below those that kill mosquitoes, although a longer exposure time was required. Feeding studies on crayfish and amphibians using Bti-killed mosquitoes showed no effects. Laboratory tests sponsored by the SPRP using methoprene found the most sensitive animal species was the cladoceran *Daphnia* where reproduction decreased at concentrations of methoprene around 5 to 10 µg/l, about 5 to 10 times higher than the methoprene concentration expected in water during operational use. The copepod *Diaptomus* and the amphipod *Hyalella* were much less sensitive than *Daphnia.* Methoprene did not affect amphibian growth or survival, even when the exposure concentration exceeded 1,300 µg/l for 100 days.

The SPRP sponsored four outdoor studies and helped support, in part, a duckling study sponsored by the Legislative Commission on Minnesota Resources. The studies sponsored entirely by the SPRP included:

- The Wright County Long Term Experiment (WCLTE), where treatments were assigned and applied for three consecutive years in 27 wetlands in western Wright County;
- A divided wetland experiment where reference and treated sectors were established by dividing several wetlands; and
- Two historical studies which compared previously treated and untreated wetlands.

A five point summary of these studies is that:

1. Densities of some of the aquatic insects, particularly chironomids and other benthic flies, were reduced by applications of both methoprene and Bti in the WCLTE.
2. Zooplankton were not affected by applications of either larvicide in the WCLTE.
3. Reproduction by Red-winged Blackbirds nesting in the wetlands was not affected by applications of either larvicide in the WCLTE.
4. Densities of 18 other bird species were not affected by application of either larvicides in the WCLTE.
5. No effects of the larvicides on aquatic insects, on zooplankton, or on birds were seen in the two historical studies or in the divided pond study.

The SPRP remains concerned about possible effects of the applications of control materials on food resources. The significant effects associated with Bti and methoprene applications in the WCLTE were to reduce populations and biomass of chironomids and several other benthic fly taxa. No food web effects on foraging bird populations were found. However, many components of the wetland community food web were not examined, in particular predominant predators such as dragonflies, beetles, water bugs, damselflies, and the invertebrates associated with the wetland aquatic vegetation. In addition, bird species that are more strictly dependent on wetland foods (including ducks) were not examined closely. Measurements of some zooplankton parameters, as well as measurements of densities of breeding birds, were also relatively imprecise.

The WCLTE provided an optimal but imperfect model to begin study of the MMCD larvicide program in the environment. Despite the study's strengths, it is still unknown whether or not similar results would be seen if the larvicides, particularly Bti, were applied in other sites at the rates and frequencies similar to those used by the MMCD in most years. We believe there is overlap between the types of wetlands studied in the WCLTE and those treated by the MMCD. However, there is uncertainty as to exactly what types of wetlands are treated and how appropriate the WCLTE sample was for inference to the MMCD program. Further, the three-year WCLTE was too short to fully investigate all potential chronic effects. However, the WCLTE was designed to include continued treatment beyond three years to make follow-up sampling of benthic invertebrates and zooplankton possible. Continued treatment and sampling is extremely important.

The MMCD has supported the most comprehensive assessment of the effects of a mosquito larviciding program anywhere to date. This research has provided a partial basis for evaluating the non-target effects of the larvicides now being used. Questions concerning non-target effects remain unanswered and new ones likely will arise in the future, should mosquito treatment programs continue. Social responsibility dictates that a mosquito control district with a mandate to provide protection from arthropod and tick-transmitted diseases and relief from mosquito and black fly annoyance pursue control in ways that minimize ecological consequences.

The demand for mosquito control in the Metropolitan Area is likely to continue at least in the near future. Thus, the MMCD will need to pursue its mission in the face of ecological uncertainties. This task will be difficult, but not impossible. An adaptive management approach, whereby ecological risks and social benefits are continually reassessed by the Technical Advisory Board with inputs from affected citizens and continued research, will ensure the MMCD's mandate is achieved in a responsible way.

SECTION I. INTRODUCTION

The Metropolitan Mosquito Control District (MMCD) is a joint powers authority established to suppress mosquitoes that transmit diseases and to reduce mosquito and black fly populations so that outdoor activities of people are not disrupted. The District now emphasizes control of mosquito larvae with applications of several formulations of two larvicides: an insect growth regulator, methoprene, and a bacterial spore, *Bacillus thuringiensis israelensis* (Bti), with its associated toxin. General operation of the MMCD was described in an EIS (MMCD, 1977) and a supplement (MMCD, 1985), and in the agency's Self-Assessment of Performance (MMCD, 1994a).

In 1981, the MMCD established a Technical Advisory Board (TAB) which meets annually to discuss the MMCD operational decisions. In 1985, this board recommended that the MMCD investigate the effects of mosquito control practices on organisms other than mosquitoes; i.e., non-target effects. The MMCD formed a Scientific Peer Review Panel (SPRP) in 1985 in response to a recommendation of the TAB and a petition to the EQB by concerned citizens (Anonymous, 1985a, a petition from National Audubon Society, et al., to EQB August 1, 1985; and Anonymous, 1985b, a response to petition by MMCD, October 18, 1985). This group of scientists was charged with developing and conducting, through contracts, a research program with the goal of gaining a better understanding of the ecological consequences of the District's larvicide program.

The SPRP first met in 1985 to consider what might occur when the larvicides methoprene or Bti are applied on a routine basis in Metropolitan Area wetlands. The problem had two levels: 1) to define potential impacts on non-target organisms of the two pesticides and 2) to define what measures were needed to research these effects on non-target species.

The panel recognized that wetland birds and amphibians were primary concerns of the public. The SPRP also understood that any non-target effects of these larvicides would most likely be upon other invertebrates, especially aquatic insects, whose larval development occurs in wetlands. The SPRP also realized that studies relevant to non-target organisms would require assessment of both direct and indirect effects. Direct effects would be through acute or chronic toxicity; e.g., death or impaired reproduction. Indirect effects could result from changes in the food web; e.g., food supply, competition or predation. A significant reduction of aquatic invertebrates (as a direct effect of chemical treatments) could be the basis for indirect effects on species that feed upon them; e.g., waterfowl, other wetland-associated birds, amphibians, and predatory invertebrates like dragonflies.

The literature review and special concerns over certain non-target species suggested that a number of studies would be required. The literature indicated that direct toxic effects of the larvicides were mostly limited to targeted insects and closely related species (Mian and Mulla, 1982, Mulla et al., 1979). The review also found specific gaps in toxicity information for wetland zooplankton, insects and amphibians. In addition, laboratory studies of toxicity were done on limited numbers of species, some of which were not native to Minnesota wetlands. And also, the laboratory tests were usually of a short-term (acute) nature.

The SPRP recognized the limitations of laboratory toxicity tests and decided that studies of the larvicides on natural Minnesota wetlands were necessary for several reasons. First, the literature lacked information about the chronic effects of repeated applications of mosquito larvicides. In addition, by studying these effects in natural wetlands, a broader spectrum of invertebrate and vertebrate non-target species could be examined, and indirect effects through food-web interactions could be evaluated.

The SPRP defined two critical areas that required more information:

1. Laboratory toxicity tests, acute and chronic, of direct effects of Bti and methoprene on specific wetland invertebrates and amphibians.

2. Direct and indirect effects of both larvicides on naturally-occurring animals through long-term field studies with repeated treatments.

In Sections II-IV, this report reviews pertinent literature and summarizes new laboratory and field studies that were commissioned by the SPRP. Section V interprets results and relates them to previous research and to the MMCD control programs. Some unresolved questions about the results are discussed and recommendations to the District for future studies complete the main body of our report.

Background information on the District's operations and wetlands biology and ecology is provided in separate appendixes. Appendix I describes the Bti and methoprene programs used by the MMCD in the seven-county metro area wetlands in recent years. Appendix II gives a general overview of the biology and trophic relations among the different kinds of animals that would be exposed to the larvicides in the treated wetlands. Appendix III reviews selected aspects of waterfowl feeding ecology. References used in the text and appendixes and a glossary are also included as Appendixes IV and V.

SECTION II. LITERATURE REVIEW and NEW LABORATORY STUDIES

A. Characteristics of *Bacillus thuringiensis israelensis*

Bacillus thuringiensis (Bt) is a gram positive, rod-shaped bacterium that can produce a spore. During spore formation, a paraspore inclusion is produced. This inclusion, commonly called the crystal or delta-endotoxin, is a protein that becomes toxic after activation in an insect's gut. There are many forms of Bt (Martin and Travers, 1989) and the crystal produced by each form is usually only toxic to a few related insect species. Five forms are registered by the Environmental Protection Agency for the control of pests in beehives, gardens, orchards and forests. The form used by the MMCD is *Bacillus thuringiensis*, Serotype H-14 which is also called *Bacillus thuringiensis israelensis* (Bti). This bacterium was first isolated in 1976 from a stagnant pool in the Negev Desert in Israel (Margalit and Dean, 1985). It is registered by the Environmental Protection Agency for control of mosquitoes and black flies.

Exposure Route and Mechanism of Bti Toxicity

Bti, to be toxic, must be ingested and the protein crystal must be activated by unique conditions found in the gut of susceptible insects. If the pH is less than 9.0, the crystal is not activated and is either digested or passed through the system. If the gut pH is highly alkaline, exceeding 9.0, then the crystal toxin is changed. The crystal proteins are activated by natural gut proteases and the activated proteins bind to specific receptors on the midgut surface (Ravoahangimalala et. al. 1993). After binding, pores develop in the gut cell membranes and the cells break apart, essentially dissolving the gut wall. In sensitive animals, if enough toxin is ingested, the effect occurs within a few hours. For larval mosquitoes, death is usually within 24 hours.

The conditions for toxin activation are ingestion and a highly alkaline gut pH. In most insects, the midgut pH range is 6.0 to 8.0 except in moths, butterflies and caddis flies, where a range of pH 8.0 to 10.0 is usual (Chapman, 1982). In mosquitoes, the anterior midgut pH exceeds 10 and remains above pH 8 until the posterior third of the midgut (Dadd, 1975).The pH in the chironomid gut has not been reported. Since the toxin crystals must be ingested, animals that filter water or ingest particles would be exposed more readily than predators.

Literature on Bti

The effects of Bti on mosquitoes are well established and will not be reviewed here. Margalit and Dean (1985) summarized many reports on the effects to mosquitoes and concluded that Bti is toxic to 72 mosquito species. The same report concluded that Bti is also toxic to almost all the filter-feeding black flies tested, 22 species.

Chironomids, insects closely related to mosquitoes, can be affected but reports often show they are less sensitive than mosquitoes. Ali et al. (1981) exposed *Glyptotendipes paripes*, *Chironomus crassicaudatus*, *C. decorus* and *Tanytarsus* sp. to several Bti formulations and found, after 48 hours, the LC90 values were 13 to 75 times higher than 24 hour LC90 values for the mosquitoes *Aedes aegypti* and *Culex quinquefasciatus*. (In acute toxicity tests, the LC90 is the concentration of toxicant that is lethal to 90% of the individuals in a test in a defined period of time.) They also reported that, for chironomids, formulation, instar and the access to food can change the LC values. Changes in formulation alone could produce a two to nine fold change in the LC values for a species. Access to food produced higher LC values (i.e., made the insect larvae less susceptible to Bti). The increases in LC values were usually not greater than a doubling of the LC value for unfed larvae. First instar chironomids were more sensitive than later instars.

Kondo et al (1995) also studied the comparative susceptibility of several chironomid species to Bti. Their study exposed the last instar of six species from five genera--*Chironomus, Dicrotendipes, Glyptotendipes, Paratanytarsus* and *Stictochironomus*--to a log series of concentrations over five days. They showed that the difference in susceptibility ranged from 10 to 1,000 times among the five genera. The *Chironomus* and *Paratanytarsus* species were the most sensitive. *Dicrotendipes* and *Glyptotendipes* were about 10 times less sensitive and the least sensitive were *Stictochironomus*. Since all the species have similar feeding behaviors and feed on similar food, the results suggest some factor other than feeding behavior affects sensitivity.

Other laboratory studies have shown that Bti does not directly affect *Daphnia* (Vaishnav and Anderson, 1995), *Pteronarcys* (Plecoptera: stonefly) (Flum, 1988), *Gammarus* (Amphipoda) (Brazner and Anderson, 1986), Fathead Minnows (*Pimephales promelas*) (Snarski, 1990) or Brook Trout (*Salvelinus fontinalis*) (Fortin et al., 1986).

Effects in SPRP Commissioned Laboratory Studies

Research sponsored by the SPRP tested the effects of exposing mosquitoes and other aquatic animals to Bti (Brooke et al, 1988). To verify the Bti activity, fed and unfed mosquitoes were exposed to a range of concentrations for 24 hours. The EC_{50} for fed *Aedes aegypti* larvae was 2,600 Bti spores (measured as CFU or Colony Forming Units)/ml of water. (The EC_{50} is the concentration of toxicant that causes an effect on 50% of the organisms in a test.) For unfed larvae, the EC_{50} was 750 CFU/ml. These 24-hour EC_{50} values are significantly ($P<0.05$) different. Similar tests with another insect, a chironomid *Paratanytarsus* yielded a 96 hour EC_{50} of 160 CFU/ml. A direct comparison of these values for the mosquito and chironomid is not possible since the exposure time was greater for the chironomid (96 hour) than for the mosquito (24 hour).

When mosquito larvae are killed by Bti, their bodies fall to the bottom of the water column and may be consumed by other animals. The potential for exposure through ingestion of dead mosquitoes was measured in a 28-day crayfish feeding experiment (Brooke, 1988). Each crayfish ingested an average of 353 Bti-killed larvae during the test. This is equal to a dose of about 920,000 Bti spores per crayfish. There were no significant ($P<0.05$) differences in death or growth between exposed and control crayfish.

Another SPRP sponsored experiment (LeClair et. al., 1988) was a complex feeding program with three species of amphibians, the Leopard Frog (*Rana pipiens*), American Toad (*Bufo americanus*) and the Spring Peeper (*Pseudacris crucifer*). Tadpoles ate between 6-25 Bti-killed mosquito larvae per week in crowded and uncrowded conditions throughout their development. Tadpole growth, time to metamorphosis and survival were not significantly different in these tadpoles as compared with control tadpoles fed Bti-free mosquito larvae.

In summary, the SPRP sponsored Bti studies exposed a chironomid species, through ingestion of Bti spores and toxin, and conducted a secondary exposure to crayfish and amphibians through ingestion of Bti killed mosquitoes. The chironomids experienced high mortality at low Bti concentrations. These laboratory results pointed to the need to monitor chironomid populations in the long-term field experiment. The laboratory ingestion studies showed no effects on crayfish and amphibians. The prospect of effects through consumption of dead mosquitoes is unlikely, so amphibian and crayfish populations were not assessed in the field experiment.

Literature on Field Studies of the Effect of Bti on Invertebrates

Many studies have shown that Bti is lethal to mosquitoes. There are also reports that Bti can be lethal to populations of chironomids in natural systems. Chironomids are common and can become very abundant in shallow natural and man-made aquatic systems, the same habitats that often produce mosquitoes. In most cases, chironomids are considered beneficial insects. They are prey for other aquatic and terrestrial animals and transform organic material in the sediments. However, if chironomids become very abundant near people, they are considered to be a nuisance; they have caused medical problems in other areas of the country. Consequently, investigators have studied chironomids both as a pest and as a beneficial insect. Pesticides for control of midges have also been studied. Some of the studies have used Bti and the results provide background for this summary. Reviews of the effects of Bti on chironomids and other invertebrates were published by Mulla et al. (1979) and Ali (1991a).

Rodcharoen et al (1991) were interested in reducing chironomid populations. They evaluated a Bti technical powder, a liquid Bti concentrate and two formulations of Bti based on a corn-cob grit formulation in experimental mesocosms and lakes in southern California. The two grits contained 200 and 400 ITU/mg, respectively (MMCD's formulation has 200 ITU/mg). For mesocosm studies, the less potent formulation was applied at 22.4 or 44.8 kg/hectare (20 and 40 lb/acre) and the more potent formulation was applied at 11.2 or 22.5 kg/ha (10 and 20 lb/a). The results were variable. Some chironomid species were affected and some not. In the mesocosms, the lower potency (200 ITU/mg) formulation reduced populations of *Chironomus* sp and *Dicrotendipes* sp. by 85 and 77% respectively, when applied at highest application rate. The more potent formulation (400 ITU/mg) produced the same results at the low application rates. Densities of two other common genera (*Procladius* and *Paratanytarsus*) were not significantly reduced in the same test.

In the lake tests, the lowest application rates of the 200 ITU formulation (applied at 13.5 kg/ha) produced a 22% reduction in *Chironomus decorus* while the higher rates (at 28 and 56 kg/ha) produced 83 and 96% reductions after two weeks. Reduced *Chironomus* populations continued for four more weeks after the higher application rates. The differential susceptibility found in the small area studies was also found in the lake study. While *Chironomus decorus* populations were reduced, other species, *Procladius bellus* and *Tanypus grodhausi* were unaffected, even at the highest application rate (56 kg/ha). In another lake experiment, the 400 ITU/mg formulation was applied at 11.2 and 19.1 kg/ha and those applications produced reductions of 32 and 47% in *Chironomus decorus* for about two weeks. Three weeks after treatment, the population levels were higher in the treated than in the untreated areas. It was concluded that Bti, applied at 5 to 10 times the rates recommended for mosquito control, could reduce densities of some chironomids, but efficacy was neither widespread nor consistent.

Charbonneau et al. (1994) described laboratory and field studies at the Minnesota Valley National Wildlife Refuge in Bloomington, Minnesota. In the field experiments, Vectobac G, the Bti formulation used by the MMCD, was applied to enclosures constructed in Type 4 and Type 5 wetlands used as fish rearing ponds. The experiments were conducted over two years. Each year, a set of enclosures was constructed. In 1989, Bti was applied three times at the recommended application rate (RAR) of 5 lbs/acre. In 1990, both the RAR and 5X RAR were tested (25 lbs/acre). Field tests showed that the applications were sufficient to eliminate mosquitoes. Chironomid taxa were sampled using sediment cores and emergence traps. No reduction in these taxa could be associated with the treatments.

Laboratory tests conducted with sediment and water taken from the enclosures determined if chironomids and mosquitoes were affected under more controlled conditions. Those tests showed that the natural samples were toxic to mosquitoes and that chironomid mortality occurred at the recommended and at the 5X RAR. The chironomid mortality was variable in these laboratory tests, ranging from 3 to 100% at the RAR and 23 to 100% at 5X RAR. The chironomids were a mixture of genera common in Minnesota.

To investigate the lack of effect in the field experiments, Charbonneau et al.(1994) also conducted a more extensive series of experiments with *Chironomus riparius* to examine factors that may have affected the field results. The authors noted that the lack of field effect could be attributed to interactions of water temperature, water depth, surface area covered by macrophytes, and larva instar. The formulation was more toxic at higher water temperatures while deep water produced less mortality. A surface covered with macrophytes did not allow the Bti to sink to the bottom where the samples were taken, lowering it toxic effects in the benthic zone. In addition, second and third instar chironomid larvae are, generally, more sensitive than fourth instar larvae.

B. Characteristics of Methoprene

Methoprene is a synthetic juvenile hormone compound, a form of insect growth regulator that disrupts the normal development of some insects. The research leading to the development of methoprene is described by Henrick (1982). Insect growth regulators are insecticides that were developed after it was found that the addition of small amounts of an insect's hormone at a critical time in the insect's development produced changes in growth, development or behavior. (Appendix I). This finding stimulated research that described the structure of the natural hormones and further research to synthesize similar compounds that might control insect pest populations by interfering with the process of development.

Exposure Route and Mechanism of Methoprene Toxicity

During normal development of holometabolous insects such as mosquitoes and chironomids, metamorphosis proceeds from egg to larva to pupa to adult. Each stage has a distinctly different integument, and transitions in form are under hormonal control. In the larval stage, concentration of natural juvenile hormone in the insect's blood is relatively high. During the last larval instar, before pupation, the level usually decreases. This decrease, coupled with a release of a molting hormone, initiates formation of the future pupa's integument (rather than another larval integument). When artificial methoprene is absorbed through the integument or ingested by a larva, the internal juvenile hormone concentration is increased artificially, thereby disrupting the formation of the new pupal integument. In short, most exposed insects eventually die during their pupal stage and hence don't emerge as adults. Larvae are not affected directly by treatment. The window of susceptibility is thus quite short.

Animals such as insects and related Crustacea, whose metamorphosis is regulated by a juvenile hormone and a molting hormone, could be sensitive to methoprene during their development. In contrast, other animals lacking natural juvenile hormone should not be sensitive. Effects on insects, especially Diptera, are well known, and were reviewed by Mian and Mulla (1982). Wright (1976) concluded that methoprene is not toxic to swine, sheep, hamsters, rats, dogs, rabbits, guinea pigs, and cattle. Also, teratological studies in swine, sheep, hamsters, rats, and rabbits showed no observed effects.

Literature on Methoprene

Methoprene is a synthetic chemical and is not naturally found in waters or sediment. Methoprene added to water or sediment decays rapidly and does not bioaccumulate. Schaefer and Dupras (1973) reported that persistence is related to temperature. Higher temperatures increase the rate of breakdown of methoprene. Wright (1976) noted that methoprene applied to soils, plants, insects or other animals is rapidly degraded and metabolized. Dunham and Miller (1978) summarized analytical methods and reported that methoprene breaks down rapidly in the environment through metabolism by microorganisms and by photodegradation. Madder and Lockhart (1980) characterized the dissipation of methoprene from shallow sod-lined pools treated at 0.056 kg actual ingredient (a.i.)/ha. Methoprene disappeared rapidly from the water. Degradation was thought to be by photoisomerism and microorganism metabolism. The authors suggested that methoprene should not persist in water.

Because methoprene decays rapidly in nature, slow-release formulations have been developed to extend the duration of exposure for weeks to months after the material is applied in wetlands for control of mosquito larvae. The MMCD now applies 150-day briquets, 30-day pellets and a small amount of a liquid formulation (MMCD, 1994b). When the briquets and pellets are actually wet, they slowly and continually release methoprene into the water.

When the SPRP project began, it was thought that recovering and measuring methoprene in treated water would be difficult. Nonetheless, it was desirable to be able to measure or at least verify actual concentrations of methoprene in wetlands as a part of our assessment. Accordingly, SPRP supported studies to characterize methoprene concentrations in treated wetlands, and the results were reported in Hershey et al.(1990) and Niemi et al.(1992).

The investigators confirmed that measuring methoprene concentrations in wetlands is extremely difficult. In part of the Lake Maria Study (Hershey et al., 1990), methoprene-like co-extracted chemicals were found in samples where no methoprene was expected. These "false positives" could be attributed to sample contamination in the laboratory or field collection, to methoprene actually present in the sample, or to a compound of similar chemical structure in the sample. The last possibility was proposed by Sjogren et al. (1986), Dunham and Miller (1978) and Knuth (1989). In the pre-treatment phase of the Wright County Long Term Experiment (Niemi et al., 1992), investigators measured methoprene concentrations and verified that "false positives" were indeed false and that there was no methoprene in the sites before the test. The detection capability developed by the investigators was 0.4 to 1.3 μg/l of methoprene.

While SPRP studies were in progress, another report (Knuth, 1989) described techniques of capillary gas-liquid chromatography and could detect methoprene in treated, natural waters. Knuth found 0.39 to 8.8 μg/l with a detection limit of 0.2 μg/l. This investigator noted that measuring methoprene from field samples is complicated by the large number of co-extracted chemicals, high sample variability and the biological complexity of the collection site.

The data from these papers and reports showed that methoprene concentrations in treated sites are near or below the analytical detection limit. In this circumstance, analyses are costly (compared to samples that have higher methoprene concentrations) and can be unreliable. Because of the high cost and possible unreliability, the SPRP decided to monitor the application by collecting material during the application, rather than do water chemistry analyses.

Effects in SPRP-Commissioned Laboratory Studies

Zooplankton are filter-feeding herbivores and occur in freshwater wetlands that receive mosquito control treatment. The SPRP sponsored laboratory tests with specific zooplankton species to assess their susceptibility. The cladoceran *Daphnia pulex* was tested as a representative of this group (Brooke et al., 1988). Two acute tests and one life cycle test were conducted with S-methoprene, the active agent in the formulated mosquito control chemical Altosid®. For the two tests, the EC_{50} values with 95% confidence limits were 78 (55- 110) µg/l and 71 (67-75)µg/l. The life cycle exposure of *D. pulex*, started with <24-hour old neonates and continued for 21 days. During the test, survival and reproduction were measured. The chronic value is developed as the geometric mean of the highest concentration that had no adverse effect and the lowest concentration that produced a significant effect on either survival or growth. For this test, the effective concentration was based on a reduction in young production and was estimated to be 5 µg/l methoprene, that is effects on *Daphnia* growth or reproduction would be expected at 5 or more µg/l

Fortin (1988) conducted tests with two zooplankton species, the cladoceran *Daphnia pulex* and a copepod (*Diaptomus* sp.). He also studied effects on the amphipod *Hyalella azteca.* Tests were conducted with solutions prepared from the briquet formulation used by the MMCD and with solutions of S-methoprene. For the acute *Daphnia* tests, fed and unfed animals were used and LC_{50}s, the lethal concentration to kill 50% of a sample, were determined and reported for dilutions of a saturated stock solution of methoprene. After 72 hours of exposure, the LC_{50}s ranged from about 35% to 58% of the saturated stock solution. In the chronic exposure, survival was affected at 100 µg/l. Reproduction, measured by the number of young per brood, was reduced at a concentration of 10 µg/l. The adult *Diaptomus* tests conducted with S-methoprene produced LC_{50}s that ranged between about 400 to 700 µg/l. The *Hyalella* tests were conducted with the formulated agent and the LC_{50}s ranged between 10% and about 24% of the stock solution.

Brooke (1988) also studied effects of methoprene on developing frogs. In an acute test, 7-day old larvae of the Leopard Frog were exposed for four days. No deaths occurred at any concentration, although larvae in the highest concentration (1,310 µg/l) were moving slower and less frequently than in the lower concentrations. A frog embryo-tadpole test was also conducted. This experiment started with 48 to 72 hour embryos and continued for over 100 days. Survival and growth were measured at regular intervals during and at the end of the exposure. Body mass was reduced, development of hind appendages was delayed and time to complete metamorphosis was increased in frogs exposed at 720 µg/l, the lowest concentration that had an effect.

These SPRP-sponsored laboratory tests provided data on toxicity of methoprene to selected non-target animals. The results suggested directions for field experiments. The most sensitive animal species tested was the cladoceran, *Daphnia,* whose survival was affected at about 74 µg/l in the acute experiment. Decreases in reproduction occurred after chronic exposure at concentrations around 5 to 10 µg/l. The MMCD formulation is designed to provide sustained concentrations of 0.5 to 3 µg/l, so it could be predicted that chronically exposed *Daphnia* and similar zooplankton populations could be affected. In contrast, the copepod *Diaptomus* and the amphipod *Hyalella* were much less sensitive. Because chronic tests using these animals were not conducted, effects of chronic exposure would need to be examined in the field.

Literature on Field Studies of the Effect of Methoprene on Invertebrates

Many studies have shown that methoprene is lethal to mosquitoes. Methoprene has also been studied as a possible control material for chironomids when they reach nuisance populations. A review of chironomid control materials, including methoprene is in Ali (1991a).

One study used a material similar to that used by the MMCD (Ali, 1991b). Methoprene was applied in 3 solid formulations, (Altosid® Pellet, Altosid® XR Briquet and a granule) and a liquid formulation and effects on chironomid populations were measured. The pellets, similar to those used by the MMCD, were applied at 5.6 kg/ha. The pellets gave initial and prolonged control of specific groups of chironomids; the Tanytarsini (64-99% mortality for 7 weeks), Chironomini (79-94% for 5 weeks) and total chironomids (64-98% for 7 weeks). The conclusion was that Altosid® pellets are lethal to midges.

Norland and Mulla (1975) reported on the effects of repeated treatments of a methoprene emulsifiable concentrate formulation on invertebrates in freshwater experimental ponds. The applications were on a 5 day schedule for about 13 weeks. Each application was set to deliver 0.1 ppm (=100 µg/l) of methoprene to the ponds. The authors described laboratory bioassays and population changes in the ponds for a mayfly, *Callibaetis pacificus*. The laboratory bioassays exposed early life stages and found that, at 100 µg/l, a cumulative mortality of 90% occurred within 5 days. In the pond bioassays, older nymphs were used. Of the older nymphs, 60% exposed at four hours after the application died and 50% of those placed in the pond four days after the application died. The sensitivity of the young stages, reported in the laboratory exposures, was reflected in lower numbers of small mayflies in the early field experiment samples. Near the end of the study, the mayfly populations were similar and on several dates the treated pond population exceeded the control pond mayfly population.

Norland and Mulla (1975) also examined prey and predator relationships by measuring the biomass and species abundance in treated and control ponds. The prey group included mosquitoes, chironomids, mayflies and ostracods. Population size and biomass were measured. For chironomids, abundance in the treated ponds was generally lower than in control ponds. On 7 of the 17 sample dates, the abundance was 1.5 to 2.0 fold lower. However, biomass, as a percent of the total prey biomass, was similar between the control and treated ponds. Ostracods were abundant throughout the test in both the control and treated ponds. Ostracod biomass remained relatively constant throughout the experiment but their contribution to the total measured biomass varied from 22 to 71% due to seasonal changes in other invertebrate populations. The predator population was small, consisting of several species of dragonflies (Odonata) and aquatic beetles (Coleoptera). No effects were detected in the dragonfly population, but the abundance of *Laccophilus*, an aquatic beetle, was less in the treated ponds. An earlier report by Miura and Takahashi (1973) found no *Laccophilus* mortality in a pre- and 4 day post-treatment measurement.

SECTION III: INITIAL SPRP-SPONSORED FIELD STUDIES

The literature review and new laboratory tests indicated that multi-year larviciding could have chronic effects on some wetland invertebrates and other organisms higher in the food web. If true, then the most powerful way to evaluate such effects would be through an extensive, long-term experiment in local wetlands. Toward this end, the SPRP commissioned a multi-year study that began in 1988 in western Wright County (WCLTE), involving wetlands that had never before been treated. After a pre-treatment study, investigators would apply larvicides to chosen wetlands in subsequent years and sample resident populations to assess possible responses. Using this design, differences between the average treated and reference wetland could be attributed with known probability of error to treatments rather than to other uncontrolled factors. Results are summarized in the Section IV.

As the long-term study was beginning, the SPRP supported four additional field studies using two different designs in an effort to seek more immediate evidence for possible effects. One design was a "historical survey." If MMCD's larvicides were having effects, and one compared a set of wetlands with a history of larvicide treatment and a reference set that had never been treated, then differences between the treated and untreated sets might be evident.

The SPRP commissioned two studies using the historical design, here named the "Wright County Historical Survey," and the "North Metropolitan Area Bird Survey." The Wright County Survey (Niemi et al., 1990b) looked for effects on nesting Red-winged Blackbirds (*Agelaius phoeniceus*), on zooplankton and on benthic invertebrates. The North Metropolitan Area Survey (DeJong and Rusterholz, 1989) censused breeding birds. Both studies involved wetlands that had already been treated for 2 or more consecutive years with Bti, with methoprene, or with no larvicide.

A second approach was a "divided pond" design. Small wetlands that had never been treated were partitioned into 2 or 3 water-tight divisions that were as equal as possible. Larvicides and placebos were applied separately to the divisions. Populations of chosen animals in the treated divisions were then compared with those in untreated divisions of the same wetlands.

This divided pond design was used in two field experiments. The "Lake Maria Study" (Hershey et al., 1990) evaluated effects of Bti and methoprene on invertebrates in eastern Wright County. The "Mallard Duckling Study" (Cooper et al., 1989) evaluated effects of methoprene on duckling growth and foraging, and on their food resources in Sherburne County. Results of these two surveys and two experiments are summarized below.

Wright County Historical Survey

This study (Niemi et al., 1990b) examined possible effects of Bti and methoprene on birds and invertebrates, by comparing populations in historically treated wetlands inside MMCD's treatment boundary with non-treated wetlands beyond the boundary in the western half of the county. There were 10 wetlands that had been treated with Bti, 10 that had been treated with methoprene, and 30 that had never been treated. Field work in the 50 wetlands was completed in 1988.

Investigators examined reproduction and growth of Red-winged Blackbirds in plots on all 50 wetlands. No differences between treated and untreated sites were detected in average clutch sizes, egg sizes, nestling growth rates, fledgling masses or fledgling ages. The reproductive success of Red-winged Blackbirds was highly variable among sites, but appeared to be lower in sites where Marsh Wrens (*Cistothorus palustris*) and Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*) were present.

In an accompanying thesis study, Boley (1992) compared the reproductive success of male Red-winged Blackbirds in 8 methoprene treated sites (chosen from the 10 studied by Niemi et al., 1990c) with males in 8 previously untreated sites (chosen from the 30 studied by Niemi et al., 1990a). Males were banded in each site in 1988 and 1989, and were then counted when they returned to the same sites in 1989 and 1990. Male territory size, harem size, and survival probabilities of eggs, fledglings and nests in 1989 and 1990 were also studied. Boley found that return rates in methoprene treated sites (72%) were significantly lower (α=0.05) from untreated sites (77%) in 1989, and also in 1990 (54% vs. 82%, respectively), suggesting that methoprene affected the reproductive success of the males through a food web effect. However, measures of territory size, harem size, and egg and nest survival probabilities revealed no patterns that were consistent with a food-web effect of treatment.

Many of the 50 study sites were also sampled to characterize invertebrates in emergent vegetation zones. Zooplankton were collected using funnel-traps that rely on the tendency of these animals to swim upward at night. Bottom dwelling aquatic insects and other invertebrates were sampled with benthic cores. There was a tendency for densities of some zooplankton to be greater in treated sites, and some species were absent from treated sites. However, overall densities and species richness of zooplankton were statistically independent of treatment history (α= 0.05). Similarly, populations of aquatic insects and other benthic invertebrates were not significantly different among the treated and non-treated wetlands.

The Wright County Historical Survey indicated that Red-winged Blackbird and invertebrate populations were not statistically different in wetlands inside and outside MMCD's treatment boundary (α = 0.05). However, the rigor of this test was limited by four circumstances: (1) The treated sites had not been treated for very many consecutive years. (2) The number of treatments per year in preceding years was relatively low. (3) Sampling for invertebrates was complicated by low water levels in the study year, to the extent that dryness prevented sampling in some of the sites. (4) Densities of many of the invertebrates were so low, perhaps because of drought, that effects of treatment would be difficult to distinguish statistically from natural variation.

Despite these shortcomings, the study served to develop field techniques. It also provided the first comprehensive sets of data on the species composition and densities of invertebrates in metropolitan area wetlands.

North Metropolitan Area Bird Survey

This survey (DeJong and Rusterholz, 1989, and amendments) censused terrestrial breeding birds associated with historically treated and non-treated wetlands in Ramsey, Anoka and Washington Counties. Twenty-three sites with Bti histories and 11 sites with methoprene histories were paired with untreated sites on the basis of their area, shape, vegetation and water regimes. Sites were selected using a double blind approach. Bird populations were censused two times, using the variable circular plot technique, between mid-May and early July 1988. In addition, nests of Tree Swallows (*Tachycineta bicolor*) in wooden nest boxes were monitored in seven matched pairs of sites during 1988-1991 to estimate occupancy rates, clutch size, egg success, nestling growth rates, and fledgling success. The authors did not distinguish between Bti and methoprene treatments in their analyses.

Data were sufficient to compare numbers of 26 different species. This survey indicated that densities of breeding birds of most species were not significantly different between treated and reference wetlands in the north Metropolitan Area. Densities of Yellow-headed Blackbirds were significantly lower on the treated wetlands and their densities were negatively correlated with number of years of previous treatment. Abundances of the remaining species were independent of treatment history. Growth of Tree Swallow nestlings was retarded slightly in treated wetlands during 1988, with nestlings from treated wetlands fledging about 2 days later, but at approximately the same mass as those in non-treated wetlands. Differences in fledging age were not detected in 1990 or 1991.

The strength of this survey was that it was a double blind study. Shortcomings were that many of the species censused are ones that are weakly dependent on wetland resources, that effects on Tree Swallow fledgling growth were variable year to year, that the investigators did not distinguish between sites treated with different larvicides, and that the small number of study sites limited the power of the study to detect small effects of treatment.

Lake Maria Study

The goal of this experiment was to examine short-term effects of Bti and methoprene on aquatic invertebrates in vernal wetlands (Hershey et al., 1990). Field work began in 1988, when six small Type 1 and 2 wetlands in Lake Maria State Park were sampled to characterize their zooplankton and benthic invertebrate communities. In October, three were chosen and trisected radially. Divisions were created with curtains of polyolefin material that were anchored to the pond bottom, supported by a cord to a center post, and positioned such that sectors were as equal as possible.

Larvicides were applied in spring, 1989. One sector of each pond was chosen at random and treated with active Bti granules; the remaining two sectors received placebo granules. Similarly, a second sector received active methoprene briquets and the other sectors received placebos. The third sector of each wetland received placebo granules and briquets. The experiment was a double-blind design; neither the MMCD personnel who applied the larvicides nor the investigators collecting and analyzing the samples knew which sectors received active larvicides. Granules were applied at a rate of 7 lbs/acre (7.8 kg/ha) one week after invertebrates were first seen in the water, and then reapplied at 2-week intervals for a total of 6 applications. Methoprene was applied once as 150-day briquets in April. The zooplankton populations were sampled with funnel traps, and aquatic insects and other benthic invertebrates were sampled with benthic cores.

The results of the experiment were that neither Bti nor methoprene caused statistically significant changes in densities of zooplankton, of insects, or of other benthic invertebrates.

The strengths of the experiment were that it was a double blind, randomized design; that average species diversities of zooplankton and insects were high; that densities of zooplankton were sufficient to provide a reliable test of each larvicide; and that dosages of the two larvicides were high enough to cause effects. Shortcomings of the experiment were that densities of aquatic insects and other benthic invertebrates were too low to provide a rigorous test of either material. In addition, funnel traps and benthic coring techniques, which are valuable in collecting many kinds of organisms, were inadequate to sample fairy shrimp, clam shrimp, and other invertebrates, such as predatory beetles, that swim actively in the water column and which may be unique to vernal wetlands. Finally, chronic effects might not become evident within a single treatment year.

Mallard Duckling Study

Effects of methoprene on the growth and behavior of Mallard ducklings (*Anas platyrhynchos*) were investigated with human-imprinted ducklings in three Type 4 wetlands in Sherburne National Wildlife Refuge (Cooper et al., 1989). This study was funded by the Minnesota Legislative Commission on Minnesota Resources; the SPRP provided funding for collecting and analyzing invertebrate populations. The wetlands had high densities of chironomid larvae, they had no prior mosquito treatments, and they were isolated from sources of agricultural runoff. The ponds were bisected using double plastic barriers, and integrity was tested with a sodium assay. Treatments were paired and assigned randomly to wetland halves, and active or placebo briquets were applied by MMCD personnel using a double blind approach. Water levels in the wetlands receded rapidly during the study and stranded some of the briquets, so extra briquets were applied below water level at two additional times.

Broods of 10 ducklings per wetland half were used during two trials. In the first, growth was measured during a 5-day interval that began after the first briquets were applied. In the second trial, growth and behavior were observed for 31 days. The extra briquets were applied during the second trial. To measure availability of invertebrate food resources, benthic cores were collected just before the first trial began and again after the second trial ended. Floating traps were used each week of the second trial to sample insects as they emerged from the water surface.

Results from the two trials were inconclusive. In the first trial, ducklings from the methoprene treated wetland halves weighed less after 5 days of foraging. In the second trial, ducklings from the treated and reference halves weighed the same after 31 days. Preliminary analysis of the behavioral data suggested broods on treated wetland halves might have traveled farther during the second trial, but no differences in duckling time budgets were evident. Benthic cores and emergence traps indicated the methoprene did not cause significant differences in the density of benthic larvae or the density of emerging adults.

The strengths of the experiment were that it was a double blind, randomized design; and that use of imprinted ducklings is a powerful way to assess treatment effects on duckling food resources. Limitations of the results were that the findings were variable. Treatment effects on duckling growth rates seen in the first 5-day broods were not evident in the second 31-day broods. Effects on invertebrates were unlikely to materialize by the time the first broods were in the wetlands, and benthic cores and emergence traps failed to demonstrate that treatment changed the abundance of invertebrates during the second trial. Unfortunately, declining water levels may have interfered with efficacy, and no independent measures were available to demonstrate that methoprene concentrations were biologically active in the treated wetland halves during either trial. Finally, as water levels declined, the paired halves of some of the wetlands became noticeably dissimilar. Thus, no conclusions about effects of methoprene on growing ducklings can be drawn from this experiment.

SECTION IV: WRIGHT COUNTY LONG TERM EXPERIMENT

The Wright County Long Term Experiment (WCLTE) was the most comprehensive study that the SPRP undertook to address questions about chronic ecological effects of the MMCD's larvicide control of mosquito populations. The research program included two experimental treatments (Bti and methoprene) versus reference wetlands as well as pre-treatment measurements in Wright County wetlands. The experiment included three years of treatment, after two years of pre-treatment investigation. This study of chronic effects is our most realistic evaluation of the MMCD's programs of repetitive annual treatments in Type 3-4 wetlands.

Results of this study were reported by Niemi et al (1990c, 1992, 1994). As of this writing, the SPRP has received the second draft of the final report, and is awaiting the final report. Major findings are summarized below.

Methods

In 1987, the WCLTE started at 28 sites in western Wright County, an area that had never been treated with any form of mosquito control agent. Drought conditions in 1988 limited the sampling of benthic invertebrates and zooplankton populations. Red-winged Blackbird reproduction and bird communities were censused in all sites. Because drought continued into the 1989 field season, all of the wetlands could not be sampled for insects and zooplankton. In 1990, all sites had standing water through May so that sampling for benthic invertebrates, zooplankton and wetland birds was completed.

In the spring of 1991, 27 sites were assigned carefully into 9 groups of 3 similar sites. One site in each group was scheduled to be left untreated (reference site), the second was to be treated with methoprene, and the third was to receive Bti. Access to one of the methoprene sites was denied in 1992 . The final experimental wetlands consisted of: 9 reference + 8 methoprene + 9 Bti-treated sites.

Treatments were monitored with bucket samplers placed in each wetland, to measure the amounts of the two materials that were actually applied to the treated wetlands, and to make sure no inadvertent treatments to untreated sites were made . Dates and rates of applications in the treated sites in 1991 through 1993 are listed in Tables 1–3.

Sampling for mosquitoes was done to assure active larvicides were used. Separate techniques were used for the two larvicides; they are described below under the Bti and methoprene sections.

Populations of zooplankton were sampled in plots of all wetland sites (Tables 1–3), using sets of ten funnel traps placed in each of the treated and reference wetlands at 3–4 week intervals during spring and summer of each year. The zooplankton swim upward at night into the funnel and are trapped in a collection bottle. The numbers of individuals of each taxon caught over a 24-hour period was used as a measure of abundance to test for treatment effects. Body size and reproduction were also examined by measuring specimen length and by counting eggs in the brood pouches of the females.

Populations of benthic invertebrates, which included aquatic insects, snails, and fingernail clams were sampled. On each visit to each site, two hollow tubes (5.0 cm diameter) were pushed into the pond bottom and the enclosed mud and water were collected as a core sample. Eight samples were taken from each wetland. These eight pairs of cores were pooled from each wetland on each sampling date and then sub-sampled. The resulting material represented 118 square centimeters of substrate area.

The invertebrates were extracted from the pooled cores, sorted, identified and counted. The density of animals was converted to the number of specimens per square meter of wetland bottom surface area. Body lengths were measured and converted to estimates of dry weight for biomass comparisons.

In the laboratory, the quality of procedures was assured by reexamining at least 15% of samples to make sure the identifications were complete and accurate.

Tests of treatment effects were similar for both Bti and methoprene. The results from the treated sites were compared with those from reference sites using an ANOVA in 3 ways: date by date within each year, on a yearly basis across dates within each year, and averaged over the three treatment years.

Breeding birds were included in this experiment as potential indicators of food-chain shifts. They were censused in the Wright County wetlands to see if the densities of any of the species were affected by the treatments. Bird censuses were done for 10 minutes/point at one to three listening points per wetland. Each 10-minute count was followed by a 2-minute playback of songs of Soras (*Porzana carolina*) and Virginia Rails (*Rallus limicola*). Thirty-eight points were sampled on two different dates in late May and early June. The number of species detected and number of individuals of each species was normalized to the wetland with the smallest area (site 13 = 2.0 ha).

Reproductive biology and behavior of both Red-winged and Yellow-headed Blackbirds were examined to further assess indirect treatment effects. Nests were monitored in each wetland: average clutch size and egg volume were measured, sex ratios and growth rates of nestlings were recorded, and hatch rates and egg-nestling survival rates were estimated by the Mayfield method (Mayfield, 1961). Some data on foraging behavior were also collected.

Table 1. Summary of 1991 sampling and treatment events and outcomes in WCLTE.

Date (1991)	Samples taken†	Application made	Bti (lbs/acre)††	Methoprene (lbs /acre)††	Chironomids affected††† Bti	Methoprene
May 6	Yes				No	No
20	Yes				Yes	No
21		Yes	9.0	5.5		
31		Yes	6.3			
June 11		Yes	No data	4.2		
13	Yes				No	No
21		Yes	10.3			
July 2		Yes	8.1	1.1		
14	Yes				No	No
17		Yes	8.8			
24		Yes		2.9		
29	Yes				No	No
Aug 21		Yes		4.7		
Sept 10		Yes		4.5		

†Of both zooplankton and benthic organisms.

†† Average dose applied on that date to the 8 or 9 wetlands assigned to receive that treatment.

††† Outcome of ANOVA for test of hypothesis that mean number of chironomid larvae per benthic core was the same in treated and untreated reference wetlands.

Table 2. Summary of 1992 sampling and treatment events and outcomes in WCLTE.

Date (1992)		Samples taken[†]	Application made	Bti (lbs/acre)[††]	Methoprene (lbs /acre)[††]	Chironomids affected[†††] Bti	Chironomids affected[†††] Methoprene
April	23		Yes	7.7[††††]	5.0		
May	6	Yes				No	No
	8		Yes		3.9		
	13		Yes	5.9[††††]			
	25	Yes				No	Yes
	30		Yes	6.5			
June	1		Yes		3.3		
	8	Yes				Yes	Yes
	19		Yes		4.7		
	20		Yes	12.2			
	26	Yes				Yes	Yes
July	9		Yes	17.6	6.2		
	17		Yes	18.6			
	18	Yes				Yes	Yes
	30		Yes		5.0		

[†] Of both zooplankton and benthic organisms.

[††] Average dose applied on that date to the 8 or 9 wetlands assigned to receive that treatment.

[†††] Outcome of ANOVA for test of hypothesis that mean number of chironomid larvae per benthic core was the same in treated and untreated reference wetlands.

[††††] These granules were from an ineffective batch of product.

Table 3. Summary of 1993 sampling and treatment events and outcomes in WCLTE.

Date (1993)		Samples taken[†]	Application made	Bti (lbs/acre)[††]	Methoprene (lbs /acre)[††]	Chironomids affected[†††] Bti	Chironomids affected[†††] Methoprene
April	30		Yes	11.5	13.2		
May	2	Yes				No	No
	14		Yes	17.0			
	21	Yes	Yes	10.0	5.9	Yes	Yes
June	9	Yes				Yes	Yes
	11		Yes	13.3	8.2		
	23		Yes	14.2			
	25	Yes				Yes	Yes
July	2		Yes		4.4		
	15	Yes				Yes	Yes
	20		Yes	14.9	5.9		
Aug	9		Yes		5.1		

[†] Of both zooplankton and benthic organisms

[††] Average dose applied on that date to the 8 or 9 wetlands assigned to receive that treatment.

[†††] Outcome of ANOVA for test of hypothesis that mean number of chironomid larvae per benthic core was the same in treated and untreated reference wetlands

Results

Results of the Wright County Long Term Experiment were obtained for three treatment years, 1991–1993. The data for zooplankton, benthic invertebrates and birds are arranged separately for each of the two larvicide treatments.

Bti-Treated Wetlands

The nine wetlands assigned for Bti treatment were treated six times in each of the three years (Tables 1–3). Average doses in 1991, 1992 and 1993 were 8.5, 10.0 and 13.2 lbs per acre, respectively.

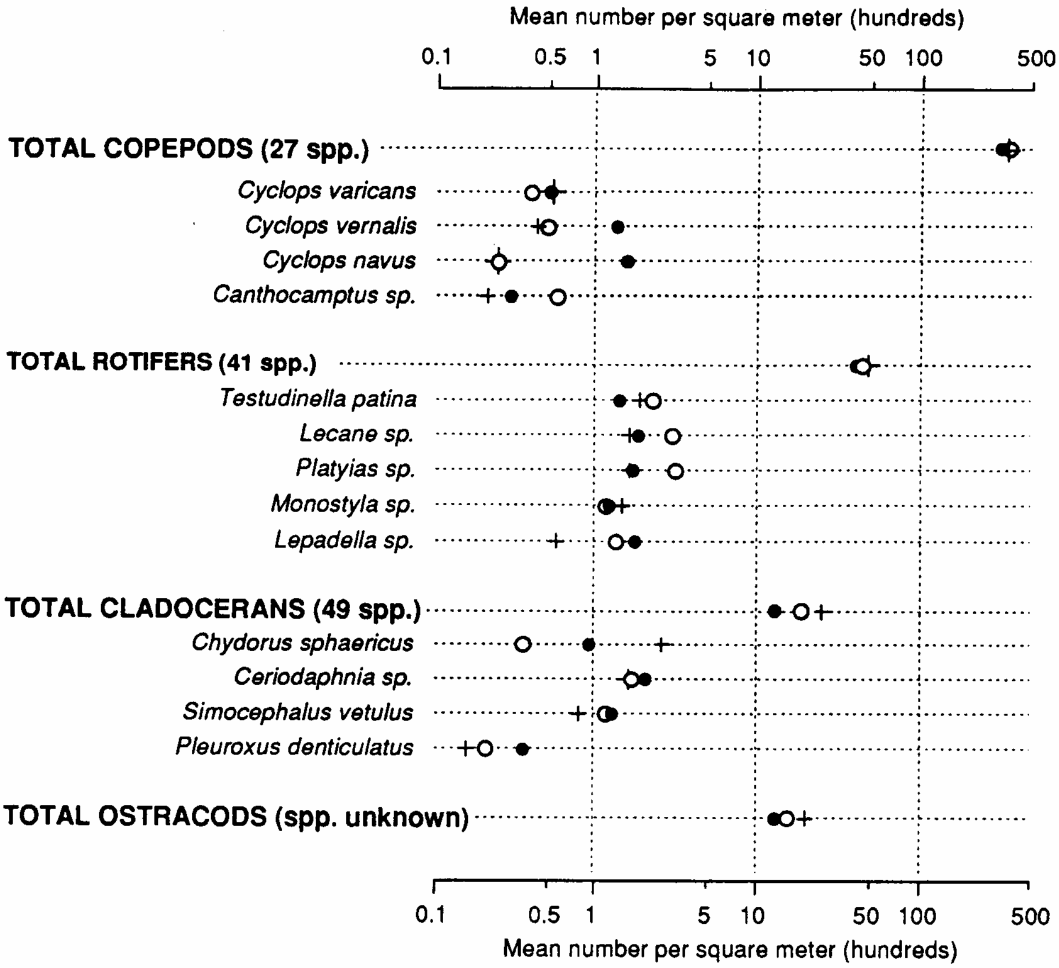
To test for the presence of active Bti in the sites, ten dip counts of mosquito larvae were taken in each Bti and reference wetland. In 1991, the presence of Bti activity was determined by comparing counts of larvae of reference sites with those of Bti-treated sites. In 1992 and 1993, dip samples were compared just before and 24 hours after treatment, and the difference was used to calculate per cent mortality. Bti treatment was generally effective in 1991, with treated sites averaging about 90% fewer larvae than reference sites. In 1993, Bti treatment killed about 80% of the mosquito larvae present before treatment. However, Bti activity was essentially zero for the first three treatments in April and May of 1992 when a unreliable batch of Bti was used. A new batch was used in the last three treatments in June and July, which killed about 70% of the mosquito larvae.

The results of the zooplankton sampling indicated the Bti treatments did not affect the species richness of zooplankton during the three years of treatment. About 180 different taxa were found in the benthic samples from the Wright County wetlands. An average of 15.5 different species of copepods, rotifers and cladocerans were detected in the treated wetlands compared to 15.8 species in the reference sites. Species richness in the two experimental groups were not significantly different. More detailed analyses of data by year, and by date within year, were consistent with the same conclusion . In this experiment, there was an 80% chance of detecting at least a 10% decrease or a 11% increase in the number of zooplankton species in the three treatment years ($\alpha = 0.05$).

Similarly, Bti produced no clear changes in zooplankton density, size or reproduction during the three years of treatment. Densities averaged about 64,500 individuals per square meter in the reference wetlands and about 60,700 in the Bti-treated wetlands (Figure 1). The difference was small enough to be attributed to sampling error. The same conclusion was reached when densities of component taxa were examined, and also when compared on year-by-year and on date-by-date bases. Analyses of data on body size and clutch size similarly showed no consistent effects of Bti on zooplankton populations. This experiment had an 80% chance of detecting at least a 45% decrease or a 82% increase in the density of zooplankton, a 10% change in specimen size, and a 30-43% change in egg production in the three treatment years ($\alpha = 0.05$).

The most abundant and diverse groups of benthic invertebrates in these Wright County wetlands were aquatic insects. These insects consisted mainly of flies (Order Diptera) and secondarily of beetles (Coleoptera). The most common flies were midges (Family Chironomidae), a group closely related to mosquitoes (Culicidae). In addition to the aquatic insects, other benthic invertebrates included snails, clams and worms. A grand total of 23,500 specimens of about 180 taxa were found in the benthic samples from treated and reference wetlands. Most taxa were identified to genus, but some were identified only to family or to order. The number of benthic invertebrate taxa per sample (richness) in the

Figure 1. Average densities of selected groups of zooplankton collected in the Wright County Long Term Experiment wetlands.



Note: Densities are the numbers of individuals in a given sample, expressed as the three year mean number per meter squared, plotted on a log scale.

Key: o Bti treated wetlands (nine)

• Methoprene treated sites (nine in 1991; eight in 1992 and 1993)
+ Untreated reference sites (nine)

reference wetlands declined from nine per sample in 1989 to six per sample in 1990 (Figure 2); numbers of snails, clams and rove beetles declined as water levels recovered from previous drought levels.

Treatment with Bti in 1991-93 had a measurable effect on species richness of some of the benthic invertebrates (Figure 2). Mean richness in the reference sites doubled from 6 to 12 in 1992 and 1993, and this increase was due to increased numbers of Dipteran (aquatic fly) taxa, mainly chironomids. In contrast, richness remained at about six taxa per sample in the Bti-treated wetlands, and failed to increase in 1992 and 1993. By 1993, the treated wetlands had only 37% as many detectable taxa as were evident in the reference sites. This difference was due primarily to increases during 1992 and 1993 of richness in the reference sites; treatments appeared to inhibit the development of richness of Dipteran (aquatic fly) species during the second and third years of treatment.

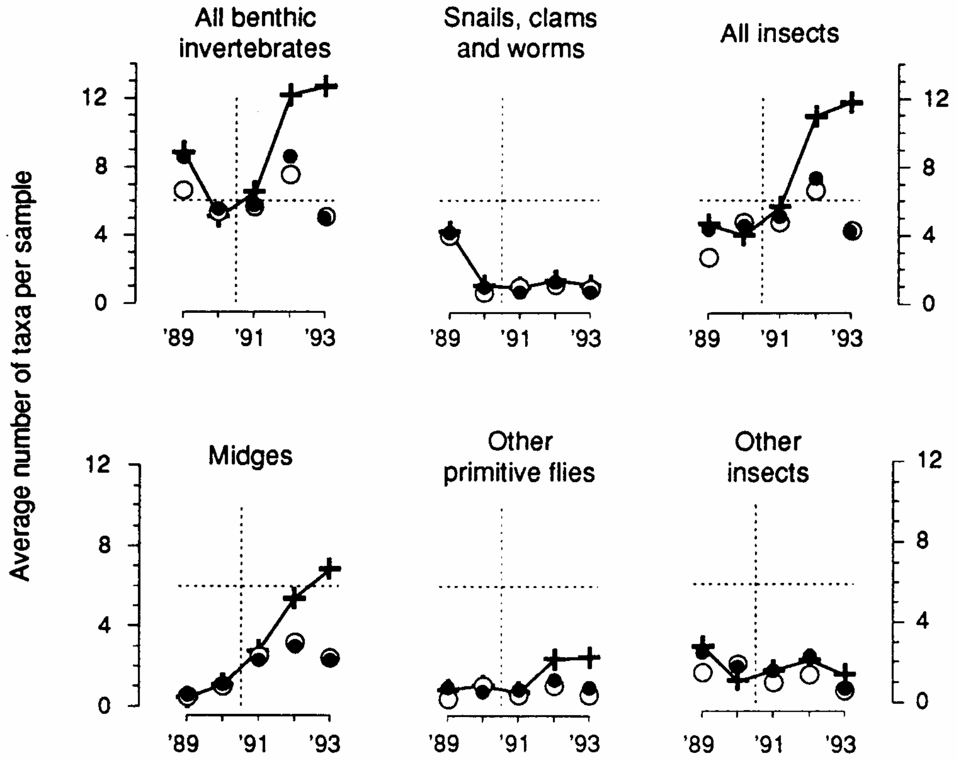
Densities of benthic invertebrates were equal in all wetlands from 1989 through 1991 (Figure 3, Table 4). There was an 88% decline from 1989 to 1990 that occurred mainly in populations of snails, clams and worms.

Bti had no significant effect on density of any of the benthic invertebrates during the first year of treatment. However, treatment effects became apparent during the second and third application years. In reference sites in 1992, the average density of benthic chironomids was 2,700 per square meter and reached a high of 9,500 in early June. Chironomid density in Bti-treated sites averaged 927 and reached a high of 2,200 in late June, or a 66% reduction in relation to reference sites. In 1993, chironomid density in the same reference sites averaged 2,500, with a high of 3,800 in early June. The mean was about 400 in the Bti-treated wetlands, with a high of 1,000 in early May, or an 88% reduction in comparison with the reference sites. Reductions were also seen in populations of other flies such as crane flies (Tipulidae), biting midges (Ceratopogonidae), and soldier flies (Stratiomyidae).

Reductions in biomass of benthic invertebrates due to Bti treatment were similar to the patterns in density (Figure 4, Table 5). The biomass of chironomids and other primitive flies in Bti sites was significantly lower in treated than in reference sites near the end of 1992 and the last four sampling dates in 1993. Seasonal patterns of density and biomass in each treatment year showed that most insect taxa had similar levels at the start of each season, but were significantly different by mid May and into July. By 1993, the third year of treatments, insect biomass in Bti sites was just 17% of the insect biomass that was present in the reference sites (Table 5). The biomass of midges was 13% of the reference sites' biomass.

In summary, Bti had no statistically significant effect on benthic invertebrates in the first year of applications, but differences in density and biomass of insects—especially among the benthic midges and other primitive flies—became significant during the late spring and summer of the second and third treatment years. Chironomid density in treated areas in the third year of treatment was only 16% of reference sites; biomass only 13%.

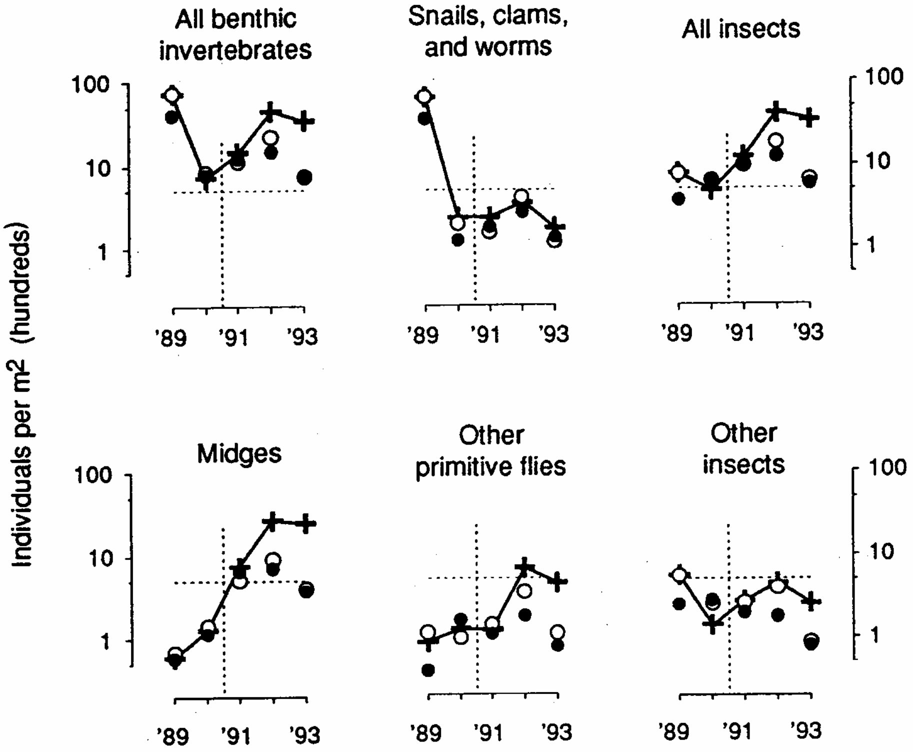
Figure 2. Species richness of benthic invertebrates in core samples collected in the Wright County Long Term Experiment, 1989-93.



Note: Species richness is the number of different kinds of invertebrates in a given wetland. Collections were made on 2-3 week intervals in the spring and summer of 1989-93. Points represent means of wetlands studied.

Key: o Bti treated wetlands (nine)
• Methoprene treated sites (nine in 1991; eight in 1992 and 1993)
+ Untreated reference sites (nine)

Figure 3. Densities of benthic invertebrates in core samples collected in the Wright County Long Term Experiment, 1989-93.



Note: Densities are the numbers of individuals in a given sample, expressed as hundreds per square meter, plotted on a log scale. Collections were made on 2-3 week intervals in the spring and summer of 1989-93. Points represent means of wetlands studied.

Key: o Bti treated wetlands (nine)

- • Methoprene treated sites (nine in 1989-1991; eight in 1992 and 1993)
- + Untreated reference sites (nine)

Table 4. Mean number of individuals per square meter (density) of selected benthic invertebrates in experimental wetlands, Wright Co., MN, 1991-93. (Numbers in parentheses represent the corresponding mean expressed as a percentage of reference sites' mean in the same year.)

	Aquatic midges			Crane flies		
Year	Reference	Bti	Methoprene	Reference	Bti	Methoprene
1991	756	527 (70%)	645 (85%)	15	14 (93%)	11 (73%)
1992	2,713	927 (34%)	703 (26%)	134	39 (29%)	34 (25%)
1993	2,477	398 (16%)	368 (15%)	127	34 (27%)	17 (13%)

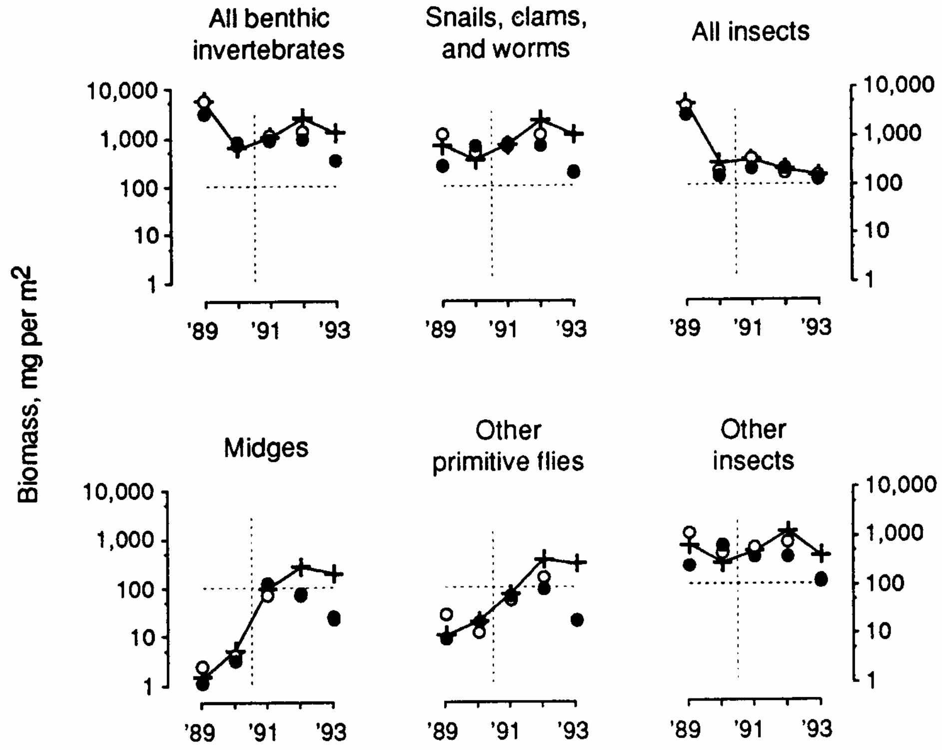
	Other Insects			Other Benthic Invertebrates		
Year	Reference	Bti	Methoprene	Reference	Bti	Methoprene
1991	447	423 (95%)	376 (84%)	238	157 (66%)	181 (76%)
1992	1,271	817 (64%)	491 (39%)	357	419 (117%)	275 (77%)
1993	765	210 (27%)	163 (21%)	176	120 (68%)	136 (77%)

Table 5. Mean biomass (mg/m^2) of selected benthic invertebrates, 1991-93. (Numbers in parentheses represent the corresponding mean expressed as a percentage of reference sites' mean in the same year.)

	Aquatic midges			**Crane flies**		
Year	Reference	Bti	Methoprene	Reference	Bti	Methoprene
1991	98	72	124	0.5	0.4	0.3
		(74%)	(127%)		(80%)	(60%)
1992	273	73	76	13.0	1.1	2.1
		(27%)	(28%)		(8%)	(16%)
1993	195	25	22	17.9	0.7	0.5
		(13%)	(11%)		(4%)	(3%)

	Other Insects			**Other Benthic Invertebrates**		
Year	Reference	Bti	Methoprene	Reference	Bti	Methoprene
1991	603	692	523	325	354	216
		(115%)	(87%)		(109%)	(66%)
1992	1,991	1,059	609	213	178	219
		(53%)	(31%)		(84%)	(103%)
1993	876	164	180	156	156	130
		(19%)	(21%)		(100%)	(83%)

Figure 4. Biomass of benthic invertebrates in core samples collected in the Wright County Long Term Experiment, 1989-93.



Note: Biomass is the weight of the specimens collected, expressed as milligrams per square meter, on a log scale. Collections were made on 2-3 week intervals in the spring and summer of 1989-93. Points represent means of wetlands studied.

Key: o Bti treated wetlands (nine)

• Methoprene treated sites (nine in 1989-1991; eight in 1992 and 1993)

+ Untreated reference sites (nine)

No significant changes in breeding bird population densities were associated with Bti treatment in the Wright County wetlands. There were 19 species common enough for analysis (Figure 5). When counts of each species in each of the three treatment years were considered separately, Virginia Rails were found to be significantly less abundant in Bti treated sites (0.05 birds per hectare) than in reference sites (0.36 birds per hectare) in 1991, but not in the other two treatment years. No treatment effects were apparent in any of the years with any of the other species. This experiment had an 80% chance of detecting at least a 35% decrease or a 54% increase in the density of male or female Red-winged Blackbirds in the three treatment years ($\alpha = 0.05$). Decreases of at least 55 to 70% or increases of 220 to 330% could have been detected in the less abundant species such as Virginia Rails.

In the case of Red-winged Blackbirds, detailed studies of components of reproductive success provided no evidence that Red-winged Blackbird reproduction was affected by Bti applications. In the average treated and reference wetland, clutch size was three to four eggs, and one fledgling left the nest at about 11 days of age. Daily survival probabilities for nests during egg laying, incubation and fledging ranged from 0.96 to 1.00. A small number of tests for differences in reproductive parameters showed statistically significant effects of Bti. However, these effects were inconsistent in direction. In some cases reproductive parameters were reduced in wetlands treated with Bti, whereas in other cases they were increased in the treated sites. High annual variability was noted for almost all measures before and after treatments. In this experiment, there was an 80% chance of detecting a decrease or an increase of 5% in egg volume, clutch size and age at fledging ($\alpha= 0.05$). Minimum increases or decreases in the estimated daily survival rate that could be detected ranged from 5-15%.

An effort was made to measure proximate food-web effects of treatment on foraging behavior of nesting Red-winged Blackbirds. There was little evidence that treatments affected either the number of times food was delivered by parents to their nestlings (four to five times per 30 minute observation interval), or the proportion of time that females foraged in the wetlands (60%–63% of time) vs. in surrounding uplands. The only comparison of statistical significance was for rates of nestling feeding during one of the three treatment years. In this experiment, there was an 80% chance of detecting a 5% decrease or increase in number of foraging trips to nests ($\alpha= 0.05$). Minimum detectable differences in proportion of time spent foraging in the wetlands were -30% to +45%.

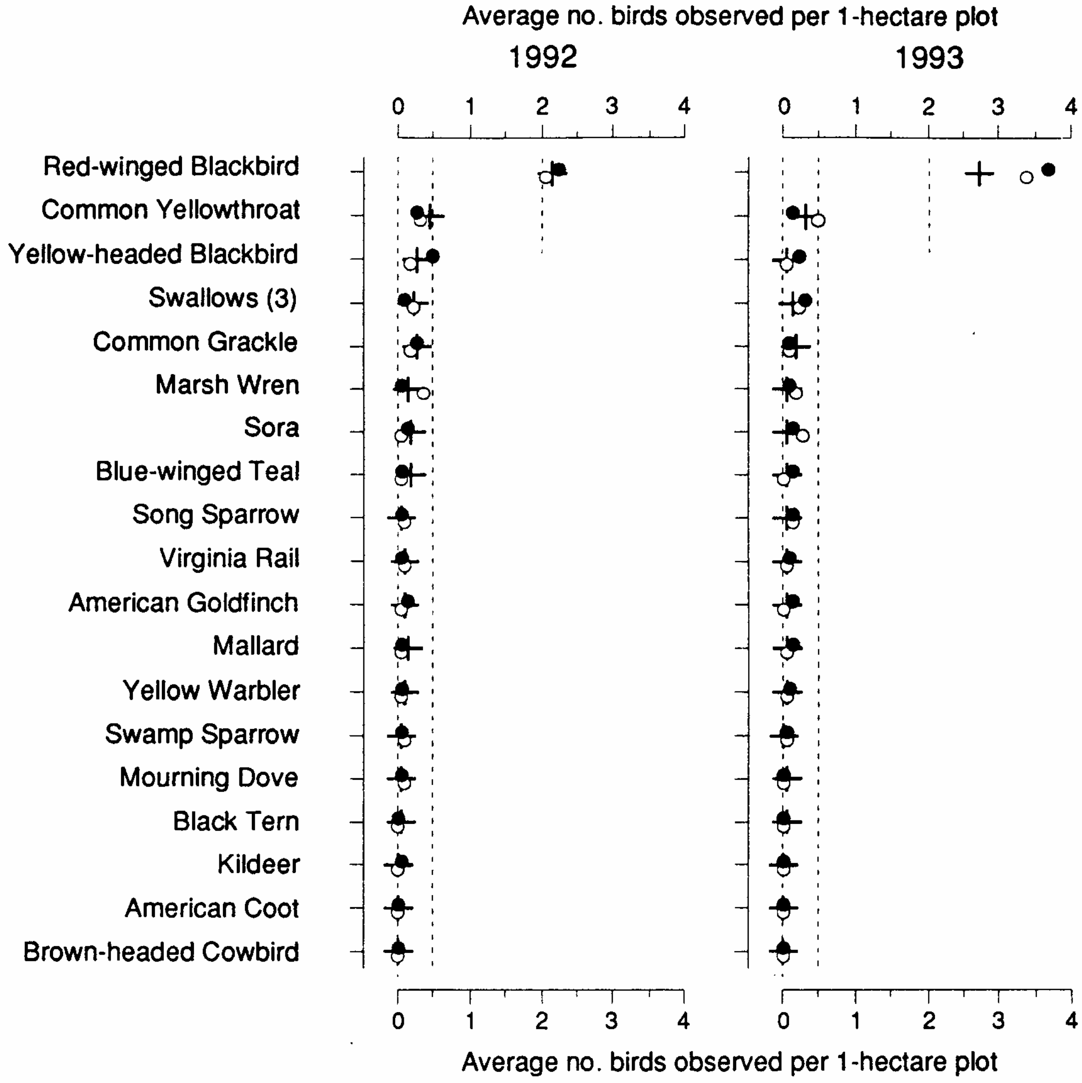
Methoprene Treated Wetlands

The methoprene treatments in 1991 through 1993 are summarized in Tables 1–3. Six applications were made in each of the three years. Because Bti and methoprene have such different biological actions, different tests were required to make sure methoprene was active in the water. Tests were performed with mosquito pupae collected from and maintained in water samples taken from reference and methoprene-treated sites. The differences in emergence success of mosquito pupae (pupa to adult) between reference and treatment wetlands were used to estimate presence of active methoprene in 1992 and in 1993.

In 1992, 72% of the pupae from reference sites and 17% of pupae from treated sites successfully emerged. In 1993, 70% of reference pupae and 10% of treatment pupae emerged successfully. These results represented a 76% reduction in 1992 and an 86% reduction in 1993, and indicate that methoprene was present in treated sites and not in reference sites.

The effects of methoprene treatment on zooplankton populations were similar to the effects of Bti treatments. A grand total of 120 different zooplankton taxa were identified during the

Figure 5. Abundance of nineteen different species of birds in the Wright County Long Term Experiment.



Note: Data was gathered in 1992 and 1993. Species are ranked by deceasing overall abundance. Points represent means of wetlands studied.

Key: O Bti treated wetlands (nine)

- • Methoprene treated sites (nine in 1989-1991; eight in 1992 and 1993)
- \+ Untreated reference sites (nine)

The effects of methoprene treatment on zooplankton populations were similar to the effects of Bti treatments. A grand total of 120 different zooplankton taxa were identified during the experiment, but many occurred sporadically and were too scarce to be analyzed. For those that could be analyzed, no consistent differences in the species composition of treated and reference wetlands could be found. Over the three treatment years, the average sample from reference wetlands contained 15.8 different kinds of zooplankton, whereas the average sample from methoprene-treated wetlands contained 14.4 species. The difference of 1.4 species was too small to be attributed statistically to treatment. The detailed analyses by year and by date within year were consistent with the general conclusion that methoprene did not affect zooplankton species diversity. In this experiment, there was an 80% chance of detecting at least a 10% decrease or a 11% increase in the number of zooplankton species in the three treatment years ($\alpha = 0.05$).

Consistent or persistent changes in zooplankton density, size or reproduction were not detected. Zooplankton densities during the three treatment years were about 64,600 per square meter in the average reference wetland, and about 52,600 in the average treated wetland (Figure 1). The difference was too small to be attributed statistically to methoprene treatments. With two exceptions, the same conclusion was reached when individual taxa were examined, and when densities were examined on a both yearly and date-by-date basis. The exceptions were two copepod species, *Cyclops vernalis* and *C. navus*, whose densities averaged three to six times greater in sites treated with methoprene. *Cyclops vernalis* was more abundant in the methoprene-treated sites on about a fourth of the sampling dates. Body size and clutch size also appeared to be unaffected by methoprene. This experiment had an 80% chance of detecting at least a 45% decrease or a 82% increase in the density of zooplankton, a 10% change in specimen size, and a 30-43% change in egg production in the three treatment years ($\alpha = 0.05$).

Some of the benthic invertebrates were affected by the methoprene treatments. The number of taxa in reference sites before treatment declined from nine to six per sample from 1989 through 1990 (Figure 2), mainly due to losses of snails, clams, worms and rove beetles as water levels increased. After this decline, overall richness doubled to 12 taxa per sample in 1992 and 1993 in the reference wetlands. This increase occurred in numbers of flies, including midges (Chironomidae) and other primitive flies . In contrast, richness remained at 5–8 taxa per sample in the methoprene-treated wetlands.

Changes in the number of taxa detected were an expression of reduced densities of benthic invertebrates (Figure 3, Table 4). During the two years before treatment, and the first year of treatment (1989-90 and 1991 respectively), the densities of the different benthic invertebrates were nearly equal in treated and reference wetlands. An 88% decline from 1989 to 1990 was mainly in populations of snails, clams and worms; whereas the 62% increase from 1990 to 1991 consisted mainly of midges.

During the first year of treatment, methoprene had no statistically measurable effect on density of benthic invertebrates. However, significant reductions of benthic insects were apparent during the second and third years of treatment.

Following the drought recovery, densities of benthic chironomids increased significantly in the reference sites to an average of 2,700, with a high of 9,500 in early June of 1992. In contrast, densities in the methoprene-treated wetlands averaged 700, with a high of about 1,000 in early June. In 1993, the average chironomid density in reference sites was 2,500, with a high of 3,800 in early June. By contrast, they averaged 360 in methoprene sites, with a high of 836 in early May. Methoprene applications also reduced populations of crane flies (Tipulidae), biting midges (Ceratopogonidae) and soldier flies (Stratiomyidae) over the period of the study, compared to untreated sites.

Biomass of several insect taxa was also significantly different and lower in the treated sites on many of the sampling dates (Figure 4, Table 5) compared to untreated sites. Biomass in treated and reference sites was not significantly different during 1991 or on the first sampling dates in 1992 and 1993. It should be noted that the Bti granules which were applied on the first two dates in 1992 were from a defective batch of material.

However, chironomid biomass in treated sites was significantly lower on the last three sampling dates in 1992 and on the last four dates (of five) in 1993 (Tables 2 and 3). Chironomid biomass in treated sites was about one-third of what was in the reference sites in 1992 and about one-ninth in 1993. Reductions in biomass of some of the other benthic insects were also apparent.

Censuses of breeding bird populations indicated bird densities were not significantly affected by the methoprene treatments (Figure 5). For the 19 species tested over three years, there were four species that differed in at least one year. Of these, only the Virginia Rails and Soras rely on aquatic invertebrates for food. In contrast, the Marsh Wren, which also feeds on wetland invertebrates, showed a marked population increase during 1992 in the methoprene-treated sites. This experiment had an 80% chance of detecting at least a 35% decrease or a 54% increase in the density of male or female Red-winged Blackbirds in the three treatment years ($\alpha = 0.05$). Decreases of at least 55 to 70% or increases of 220 to 330% could have been detected in the less abundant species such as Virginia Rails.

Detailed study of Red-winged Blackbirds provided no convincing evidence that their reproduction was affected by methoprene. A small number of tests showed statistically significant differences between birds in treated and reference wetlands. However, the effects were inconsistent in direction. Some components of reproduction increased in treated sites, whereas others decreased in the treated sites. High annual variability was noted for almost all measures before and during treatments. Because so many different statistical tests were conducted, the significant comparisons may have occurred by chance. In this experiment, there was an 80% chance of detecting a decrease or an increase of 5% in egg volume, clutch size and age at fledging ($\alpha= 0.05$). Minimum increases or decreases in the estimated daily mortality rate that could be detected ranged from 5 to 15%.

Studies of Red-winged Blackbird foraging behavior produced little evidence that methoprene affected this species' foraging behavior. Over the three treatment years, the average parent delivered food to its nestlings about 4–5 times per 30 minute period, and this feeding rate was the same in the average treated and reference wetland. These parents spent 60–63% of their foraging time on the wetlands, and the rest was spent foraging in upland areas. On a year-by-year basis, the only comparison of statistical significance was for rates of nestling feeding in 1993, when female parents returned to their nests more frequently in the reference wetlands than in the treated wetlands. However, because so many statistical tests were conducted, this difference may be the result of chance. In this experiment, there was an 80% chance of detecting a 5% decrease or increase in number of foraging trips to nests ($\alpha= 0.05$). Minimum detectable differences in proportion of time spent foraging in the wetlands were -30 to +45%.

Summary and Discussion of WCLTE

Summary

The WCLTE was an extensive experiment designed and conducted to examine mosquito control larvicides effects on non-target animals. The experiment was a before and after design with 1988 to 1990 used as pre-treatment years and 1991 to 1993 as treatment years. Many wetlands were examined and twenty-seven were randomly selected and randomly placed within one of three groups: reference sites receiving no treatment, sites receiving Bti treatment and sites receiving methoprene treatment. Zooplankton, aquatic insects and breeding birds were sampled within each site. Site monitoring assured that Bti and methoprene were applied to treatment sites and not to the reference sites.

No significant effects on zooplankton or breeding bird populations could be associated with Bti and methoprene treatments. In addition, Red-winged Blackbird reproduction was not significantly affected by larviciding treatments.

Significant effects on some aquatic insects were associated with both Bti and methoprene treatments. Although aquatic insects were unaffected in 1991, the first year of treatment, both numbers and biomass were greatly reduced in the 1992 and 1993 treatment years by both Bti and methoprene. Differences were largely due to a reduction in larval chironomids, which made up about 60% of the numbers of all the insects sampled. Both larvicides reduced aquatic insect densities by a range of 57-83%. Biomass was reduced 50-83%. The 1992 reductions occurred by early June and continued through the last sample date July 18. The chironomid populations in treated and untreated wetlands were about the same on the first sample date in 1993, but became increasingly different thereafter. Non-insect invertebrate populations that were sampled were not affected by the treatments.

Discussion

This study was one of the most extensive long-term field studies on the effects on mosquito larvicides. The preliminary work in 1988-1990 developed protocols and analyzed the species in the entire set of wetlands. This knowledge provided a scientific basis for the assignment of the wetlands to treatment and reference groups. In this study, many variables were controlled by the assignment of wetlands, by the control and on-site verification of the treatments, and by the use of consistent methods.

The finding that aquatic insect populations represented in the core samples were reduced by the larvicides indicates those species are physiologically susceptible to both of the larvicides. Furthermore, the results indicate that environmental concentrations of the larvicides produced by the experimental doses and application frequencies were sufficient to affect population growth of the susceptible species in the treated wetlands. The concurrent lack of measurable effect on zooplankton and bird populations indicates that neither of these two groups appear to be susceptible to direct effects. Populations in both groups appear to be independent of changes in densities and biomass of the affected benthic insects.

The wetlands studied in the WCLTE were not randomly chosen from those likely to be treated by the MMCD. Their selection was a compromise between the general types that the MMCD could treat, wetlands that would likely stay wet into at least late July, and wetlands that had good Red-winged Blackbird habitat.

The WCLTE did not measure everything in the wetlands. The benthic core sampling method is appropriate for slow moving or sediment-dwelling animals, but is not an adequate sampler for mobile invertebrates such as fairy shrimp and many predators, such as water beetles, that swim rapidly. Also, the sampler did not measure invertebrate, including chironomid, populations living on aquatic vegetation. Whether the invertebrates living on aquatic vegetation were exposed or would respond like the benthic ones measured in benthic cores is unknown and currently under study.

Bti application rates were not the same as used in the MMCD program. In the MMCD program, Bti is applied once or twice to most sites, at a dose of 5 to 8 pounds per acre, and occasionally up to 10 pounds per acre. In the WCLTE, Bti was applied five to six times each year at a field-measured rate averaging 12 pounds per acre, as measured in the collection buckets. Finally, applications were made later in 1992 and 1993 than is recommended for mosquito control.

Methoprene as applied by the MMCD is usually a single application of a 150-day briquet. This product is designed to release methoprene over 150 days if it is continually in water. In the WCLTE, there were five to six applications of a 20-day release granule formulation to simulate a 150-day briquet.

Chironomid and related primitive fly populations were reduced. However, two findings remain difficult to explain. One is the absence of effects in the first year in treated sites, and the second is the magnitude of the effects seen in the second and third years of treatment. One suggestion that may explain the lack of apparent first year affect is that the recolonization and recovery from the preceding drought years may have meant that invertebrates in all wetlands took a year to recover to normal levels.

The fact that Bti and methoprene caused such similar responses in invertebrate populations was also unexpected. Bti and methoprene have very different modes of action, yet the population reductions were very similar in absolute numbers (Figure 3 and 4).

It should be noted, from a statistical standpoint, that large effects are easier to measure, especially when the species being studied are abundant and variability is low (e.g., with the chironomids). Conclusions about more subtle effects in highly variable populations are more difficult to detect. This is especially true if the populations are hard to sample because of their low abundance or because they are hard to observe (e.g. Virginia Rails). An adequate sampling protocol is frequently not known a priori, or is not possible with available budgets even if known.

SECTION V: ECOLOGICAL CONSEQUENCES OF BTI AND METHOPRENE USE IN THE MMCD TREATMENT AREA

The ecological consequences of the MMCD's larviciding program could be expressed as either direct toxicity to or indirect food web effects on organisms exposed to the larvicides.

It is clear that neither Bti nor methoprene is directly toxic to most animals in the Metropolitan landscape. There is no evidence that Bti and methoprene at field application rates are toxic to birds, mammals, or amphibians, based on literature and SPRP-sponsored studies. However, both Bti and methoprene reduced chironomids and other primitive flies substantially at the doses used in the WCLTE.

Possible indirect effects are much more difficult to detect because of the complex nature of wetland food webs and the fact that many organisms at higher trophic levels were not sampled by the methods used in the WCLTE, or were difficult to analyze due to their low abundance in the benthic cores. Nevertheless, many measurements included in the WCLTE could have detected indirect food web effects had they been sufficiently large. No significant effects were seen.

Many components of the wetland community food web were not examined for effects, particularly on predominant predators such as dragonflies, beetles, water bugs, and damselflies. Measurements of some zooplankton parameters, of populations of predatory invertebrates which utilize other invertebrates as food, and of the density of breeding birds associated with the WCLTE wetlands, were relatively imprecise. For many of them, changes of 50% or more would have had to occur in order to be detected.

The laboratory studies and the WCLTE together represent the most comprehensive assessment of non-target effects yet conducted on any pesticide. The lack of precision for some variables or selection of study end points should in no way be a criticism of the research that was done. Rather, it points to the difficulty of conducting studies involving measurements of possible food web changes, given the inherent variability from site to site and season to season in natural wetlands.

Of special concern to the SPRP is the potential effect of the MMCD larvicide program on wetland-inhabiting birds. Clearly, Red-winged Blackbird reproduction was unaffected by either larvicide. From the start of the project, the SPRP recognized that Red-winged Blackbirds were abundant enough that meaningful measurements and adequate samples could be obtained. However, they were also recognized as a compromise species because Red-winged Blackbirds are not strictly dependent on wetlands for food. Nonetheless, we believed that they were sufficiently dependent on wetlands as a food source that their reproductive performance might have been sensitive to major changes in abundance of invertebrate foods. Unfortunately, results seen for Red-winged Blackbirds cannot be extended to less numerous bird species that are more dependent on wetlands for food.

Knowledge about foods eaten and nutrient needs during reproduction is probably greatest for waterfowl. Pathways exist within the food web by which nesting ducks and ducklings might be affected by the larvicides used by the MMCD. Food web effects could be experienced by nesting hens early in the year in ephemeral wetlands. Effects could also be experienced by ducklings during brood rearing later in the year in more permanent wetlands. This does not mean that any effects are necessarily being realized, but caution and additional investigations may be prudent.

The MMCD treats large areas of productive wetland types or wetland zones used by nesting hens (See Appendices I and II) in the seven-county Metropolitan Area. Furthermore, a relatively large acreage of some types of Metro wetlands receive at least some treatment. However, the exact extent to which wetlands with temporary and seasonal water regimes or to which the temporarily flooded zones of more permanent wetlands are treated cannot be determined. The wetland classification system used by the MMCD is inappropriate for the type of assessment needed. Certainly, some of the wetlands or wetland zones that are treated may have little value for nesting female ducks or broods. Nonetheless, the wetland types or zones that are most important as a food source to egg-laying females (see Appendix III) appear to be widely treated. Obviously, a key unknown in this assessment is the extent to which the wetland types or zones important to egg-laying and incubating ducks in the Metro area landscape are actually treated by the MMCD. Extensive surveys, on-site assessment of treatment acreage, and a change in MMCD record keeping to the wetland classification scheme used in the National Wetland Inventory could help determine the degree of overlap.

MMCD treatment of wetlands with waterfowl value does not mean necessarily that effects on ducks would be realized. Chironomid larvae are food that is frequently consumed by nesting ducks (see Appendix III), and chironomid reductions in the WCLTE occurred at a time when nutrient resource demands by egg-laying females of all ducks is approaching its maximum. Indeed, most egg-laying and incubation related nutrient needs in the duck population occur at a time of year when chironomid reductions were seen in the WCLTE. Whether or not a Bti or methoprene dose-response similar to that seen in the WCLTE occurs with actual operational MMCD treatments has not been measured. Again, caution and additional investigation may be prudent.

Bti was applied in the WCLTE at a rate greater than it is usually used operationally, and applications occurred over a longer time. Possibly, chironomid reductions would be less severe and their recovery would occur earlier in the summer under MMCD operational dosing; however, the nature of the Bti dose-response is unknown. Currently, the SPRP is administering a study investigating Bti and chironomid dose-responses in mesocosms in natural wetlands which should be informative.

Applying methoprene to temporary and seasonal wetlands with 150-day briquets used by the MMCD may produce a methoprene dose in the wetlands that is similar to that experienced in the WCLTE, where methoprene delivery was via 20-day granules. Use of briquets is designed to assure methoprene is being released into the wetland as long as the briquets are wet. Thus, the potential to reduce chironomids to the extent seen in the WCLTE seems greatest where 150-day briquets are used in the current MMCD program. A study commissioned by the SPRP demonstrated the difficulty of measuring the concentrations of field-applied methoprene in natural wetland water with current technology.

Even if many wetlands important to nesting ducks were treated for mosquito control, and MMCD treatments reduced chironomids in these wetlands to the extent seen in WCLTE, these two conditions together would not mean necessarily that nesting ducks would be affected. Waterfowl studies have shown that hens forage intensively while nesting during virtually all of the time they have available to them. It is doubtful that ducks could forage with the same efficiency on other invertebrates if chironomids were removed from wetlands in their home range. However, this is debatable. The extent to which hens can switch foods efficiently and the full impact of a change in diet are unknown. If hens are unable to switch to other protein sources efficiently, a reduction in their reproductive effort could be realized. This reduction, if realized, would likely be expressed primarily as fewer nesting attempts being made per female and as reduced clutch size. It is possible that egg size would be reduced as well. The relationships between reproductive success when chironomids have been removed from hen diets should be studied experimentally.

Effects of larvicides on ducklings are also possible, but to evaluate these possibilities will require answers to a parallel set of questions:

1. How much of the duck brood habitat in the Metropolitan Area landscape is treated for mosquito control?;

2. Is dosing in these wetlands high enough to reduce chironomid availability for young ducklings?; and

3. Can ducklings switch efficiently to other foods?

Effects on local duck populations would occur only if overlap in brood-rearing marshes and MMCD treatments were large, if larvicide doses were sufficient to reduce foods needed by young ducklings substantially, and if ducklings could not efficiently use alternate foods.

We have focused primarily on the possibility of landscape level effects because these could affect the overall Metropolitan Area duck population the most. Local level effects are also possible if dosing in certain neighborhood wetlands was sufficient to cause reductions in important duck foods and ducks were unable to use alternatives in the wetlands efficiently. In this case, ducks in the Metro Area might redistribute themselves with no measurable overall effect on the Twin Cities population level. This situation might also be studied experimentally. Existing duck densities and factors influencing them would make carefully designed studies essential as redistributions would probably need to be examined through behavioral changes.

Suggestions for Further Research

This has been the most comprehensive assessment of the effects of a mosquito larviciding program anywhere to date. Scientific research has provided a partial basis for evaluating the non-target effects of the mosquito control materials. Nonetheless, many questions remain unanswered and, no doubt, new ones will arise in the future.

Two key follow-up studies could be done to continue addressing unanswered questions:

1. The WCLTE sites have been treated since the study ended. The first study would be to collect benthic samples in early May and June 1996 in the WCLTE sites. Analysis of the samples would assess the effects of these continued treatments on benthic organisms.

2. At the same time, it would be appropriate to place and retrieve artificial substrates and activity traps in WCLTE sites to see if benthic effects are mirrored in vegetation layers.

At the very least, treatments should be continued in the WCLTE sites in 1996, to permit re-sampling in 1997 or subsequent years. The long-term nature of the experiment is extremely valuable and needs to be maintained.

The WCLTE provided an optimal but imperfect model to begin study of the MMCD larvicide program in the environment. Despite the study's strengths, it is still unknown whether or not similar results would be seen if the larvicides, particularly Bti, were applied in other sites at the rates and frequencies similar to those used by the MMCD in most years. We believe there is overlap between the types of wetlands studied in the WCLTE and those treated by the MMCD. However, there is uncertainty as to exactly what types of wetlands are treated and how appropriate the WCLTE sample was for inference to the MMCD program. Further, the three-year WCLTE was too short to fully investigate all potential chronic effects. However, the WCLTE was designed to include continued treatment beyond three years to make follow-up sampling of benthic invertebrates and zooplankton possible. Continued treatment and sampling is extremely important.

The MMCD has supported the most comprehensive assessment of the effects of a mosquito larviciding program anywhere to date. This research has provided a partial basis for evaluating the non-target effects of the larvicides now being used. Questions concerning non-target effects remain unanswered and new ones likely will arise in the future, should mosquito treatment programs continue. Social responsibility dictates that a mosquito control district with a mandate to provide protection from anthropod and tick-transmitted diseases and relief from mosquito and black fly annoyance pursue control in ways that minimize ecological consequences.

The demand for mosquito control in the Metropolitan Area is likely to continue at least in the near future. Thus, the MMCD will need to pursue its mission in the face of ecological uncertainties. This task will be difficult, but not impossible. An adaptive management approach, whereby ecological risks and social benefits are continually reassessed the Technical Advisory Board with inputs from affected citizens and continued research, will ensure the MMCD's mandate is achieved in a responsible way.

APPENDIX I. Operational Use of Mosquito Larvicides by MMCD

In the landscape, wetlands range in wetness from seasonally saturated hydric soil to permanent ponds. MMCD follows a modified Circular 39 (Shaw and Fredine, 1956) classification system. Wetlands classed as Type 1.1 (open grassy field), 1.2 (woodland pool in floodplain forest) and 1.3 (woodland pool) are habitats where the common spring Aedes are found. Wetlands classed as Types 2.1 (canary grass meadow), 2.2 (sedge meadow) and 2.3 (lesser known vegetation associated with temporary water) are mosquito breeding sites, as are the Type 3 'inland shallow fresh marshes' that typically hold 6-24" of water, but may be completely dry in a dry year. These sites are termed seasonally flooded wetlands.

MMCD considers a breeding site for mosquitoes as sites that hold rainfall for a week or more. These can be marsh edges, short grass ditches, woodland pools, tire ruts and occasionally hoof prints. For the MMCD, the reed canary grass and sedge meadows (Type 2) sites are the most prolific mosquito breeding sites. Mosquitoes will also breed in more permanent sites such as cattail marshes and ponds.

At the optimal temperature of 25 deg C, *Aedes vexans* reaches the pupal stage in about 7 days, but lower water temperatures extend the time from egg to pupa. At 10, 15 and 20 degrees centigrade development takes 46 days, 22 days or 10 days, respectively (Trpis and Shemanchuk, 1970).

Mosquitoes of the genus *Aedes* are called the 'floodwater' mosquito because the eggs are laid in areas experiencing seasonal or temporary flooding, such as ditches, floodplains and temporary and seasonally flooded depressional wetlands (Horsfall, 1963). The eggs of *Aedes* are adapted to a transient habitat, and typically oviposition takes place in the receding moist margins of the wet areas and depressions that include cattail wetlands, grassy swales and temporary woodland pools. The eggs of the mosquitoes have varying degrees of latency before they hatch, but renewed flooding triggers the hatch by reducing the oxygen levels around the eggs. In temporary pools, reflooding often takes place in spring after snow melt and spring rains and hatches are triggered.

Receding moist edges can occur in any type of wetland as water levels fluctuate. A small seasonally flooded or temporary wetland may recede completely and appear dry in summer. A Type 4 semipermanent wetland will, in most years, retain the open water while the water at the vegetated fringe edge recedes or rises. This fringe area and the cattails are the primary areas for mosquito larval growth in seasonal and semipermanent wetlands, in more temporary or seasonal wetlands the entire wetland can contain mosquito larvae.

The MMCD program emphasizes control of mosquito larvae. Sites are mapped, sampled and prioritized according to mosquito productivity. The sites producing the most mosquitoes are treated with either *Bacillus thuringiensis* var *israelensis* (Bti) or an insect growth regulator called methoprene or Altosid®. Both products are available in several formulations. In usual use, sites less than 3 acres are ground-treated with Altosid 150-day briquets. This formulation provides season-long control. In larger sites, a granule form of Altosid is applied by helicopter. These air sites are usually 3 acres or more, may also be treated with Bti formulated on corn cob granules.

The treatment threshold is 2 larvae per dip in the interior, more populated zones of the MMCD. The threshold for treatment in zones further out varies, depending on:

- The total acres of breeding mosquitoes; and
- The amount of time and material remaining after the inner zones have been treated.

The two larvicides have been described in reports of the MMCD Technical Advisory Board:

> *Bacillus thuringiensis* var *israelensis* serotype H-14 (Bti) is a naturally occurring soil microorganism that was discovered in Israel in 1976. Bti is a bacterium which has a highly specific mode of action against a narrow host spectrum. This selective activity is limited to the order Diptera (flies), specifically mosquito, midge, and black fly larvae. Since its discovery, the organism has shown rapid larvicidal activity against both mosquitoes and black flies. MMCD chose to use Bti because of its extreme selectivity. It contrast to the variety of Bt used in gardens and forests against moth pests (*Bacillus thuringiensis* var. *kurstaki*), Bti has no activity against lepidoptera (moths and butterflies).
>
> In 1975 methoprene was registered as the first biochemical (now termed biorational) agent for mosquito control. Methoprene is a synthetic insect growth regulator (IGR) which mimics insects natural juvenile growth hormone and disrupts normal development. IGR's act on highly species-specific hormonal systems controlling metamorphosis and molting.
>
> Methoprene is a true analog of a mosquito's own juvenile hormone. The immature larvae have an absolute requirement for these hormones to progress through the usual larval stages. During the 3rd and 4th larval instar stages, juvenile hormone levels normally drop to a very low level or zero, which is necessary for the larvae to complete development into an adult mosquito. Methoprene maintains the juvenile hormone concentration, in water, at a higher than normal level. This juvenile hormone is ingested and absorbed through the larvae's cuticle. In the presence of this higher hormonal level in the latter instar stages, the insect does not develop the physical features necessary for adult emergence. Mortality of the insect usually occurs during the pupal stage. (Hayes and Laws, 1991; Zoecon, 1993). (MMCD TAB 1994b)

MMCD treats the majority of the documented mosquito breeding sites in seasonally flooded wetlands, nearly half of the type 4 sites and about a third of the type 5 sites. They also treat breeding sites in Type 4, or semi-permanent wetlands. These have an open water area surrounded by a band of vegetation that consists of sedges, canary grass and other wetland plants in the shallow area and cattails towards the open water. Depth is 6" to 3 ft. Breeding sites in some Type 5 or more permanent open water wetlands and in other types of wetlands are also treated.

MMCD data for methoprene and Bti use for 1993 are shown in Tables 1-3. Methoprene and Bti use are tabulated separately first, then together. A small percentage of the treated wetlands were treated with both insecticides.

Table 1. MMCD Methoprene Use in 1993.

Wetland Type	Total Acres	Number of Sites	% Sites Treated	% Site Acres Treated
1	8,193	13,369	68	17
2	37,318	25,155	72	14
3	40,066	9,953	71	12
4	24,718	8,187	40	11
5	39,157	2,056	4	<1

Table 2. MMCD Bti Use in 1993.

Wetland Type	Total Acres	Number of Sites	% Sites Treated	% Site Acres Treated
1	8,193	13,369	15	16
2	37,318	25,155	7	24
3	40,066	9,953	9	33
4	24,718	8,187	5	26
5	39,157	2,056	29	41

Table 3. Combined Bti and Methoprene Use in 1993.

Wetland Type	Total Acres	Number of Sites	% Sites Treated	% Site Acres Treated
1	8,193	13,369	81	29
2	37,318	25,155	78	36
3	40,066	9,953	75	44
4	24,718	8,187	44	35
5	39,157	2,056	31	42

Source: MMCD records

A detailed presentation of the extent of treatment by the MMCD is not possible in this report. This is due to a database that is based on breeding sites, not actual wetland basins, and difficulty in estimating how much treatment actually occurs at each site.

Treating a site does not necessarily mean the entire wetland is treated. It appears that in terms of acreage of wetlands of each type in the District, roughly over a third of the acreage receives one or other larvicide treatment at least once each year, while 75% or more of Type 1 to Type 3 sites are treated at least once each year. This seems inconsistent with reports from MMCD field personnel, whose operational practice is to treat the complete area of Type 1, Type 2, and most Type 3 sites.

The extent to which metropolitan area wetlands are treated is difficult to fully assess, because of the way wetland classification terminology has been used in MMCD record keeping. The MMCD has used what has become an outdated and inadequate wetland classification system (i.e., Shaw and Fredine, 1956). This system classifies wetlands based on the nature of the entire basin. The District sometimes treats only a specific zone in the wetland basin. These records are confusing because more than one "mosquito breeding site" can be identified in some wetland basins. Mosquito breeding sites are most analogous to specific "wetland polygons" (Cowardin et al., 1974) used in the National Wetland Inventory to delineate zones within wetlands based on their physical, chemical, and biological characteristics.

APPENDIX II. An Overview of Wetlands Biology

The habitats where mosquito eggs and larvae are found are also habitats for certain invertebrates which similarly depend on a detrital and algal food base also available to the mosquitoes. When the site is flooded, the plant litter present begins to decompose into detritus, and algae grow on surfaces of plants, litter and sediments.

Depressions that are seasonally flooded for short periods in the year are characterized by very high densities of aquatic invertebrates and low diversity (Wiggins, Mackay and Smith, 1980). The advantage of the seasonal wetlands for invertebrate growth is attributed to the organic food released upon flooding and the absence of fish and other predators. Invertebrates in these sites grow rapidly, as do the mosquitoes. Some species, such as the fairy shrimp have evolved life history strategies that confine them to seasonal wetlands (Daborn, 1976; Wiggins et al, 1980). Seasonal wetlands can have greater numbers of aquatic beetles than semipermanent wetlands (Kantrud et al, 1989). These top predators are capable of flying from more permanent wetlands to the seasonal ones, and some of the taxa can reproduce in seasonal wetlands (Wiggins et al, 1980).

In many of the Type 1 temporary or ephemeral pools there is little, if any, emergent aquatic vegetation. Along with the algae, leaf litter from surrounding grasses or woods falling on the wetland soil forms the source of food energy for the invertebrates in spring. In more seasonally flooded and semipermanent wetlands the aquatic macrophytes provide a substrate for algae growth and a source of detritus as the plants die back into the wetland. Though herbivory does occur to some extent on some aquatic macrophytes by a few invertebrates, for example aquatic Lepidoptera larvae, crayfish and semiterrestrial beetles, the macrophytes are more available in senescence (Newman, 1991) as they decompose. Emergent vegetation contributes more to annual production than either submersed vegetation or epiphytic algae (Murkin, 1989).

The greatest input of macrophyte litter occurs in the spring (Davis and van der Valk, 1978). After the initial litter input, the processing of the detritus may be important in determining which invertebrates succeed in the wetland (Murkin, Kadlec and Murkin, 1991). There has been little study of detrital processing in wetlands, compared with the work of Cummins (1974) and others on detrital processing by aquatic invertebrates in streams. Mosquito larvae grow rapidly, to do so they browse on detritus and algae coatings using their mouth brushes to filter out particulate matter. They can pass live algae through their gut (J. Helgen, pers. obs.) and may be coprophagous (Nilsson, 1983).

Macroinvertebrate diversity and biomass may be greater in the vegetated areas of wetlands and in the emergent or mixed emergent areas as opposed to the submerged (open water) or floating-leaved vegetation areas (Clark, 1978). More permanent habitats have greater invertebrate richness than seasonal wetlands (Wiggins et al., 1980). No difference was seen between open water and emergent vegetation nektonic (free swimming) invertebrates in another study (Murkin et al, 1991).

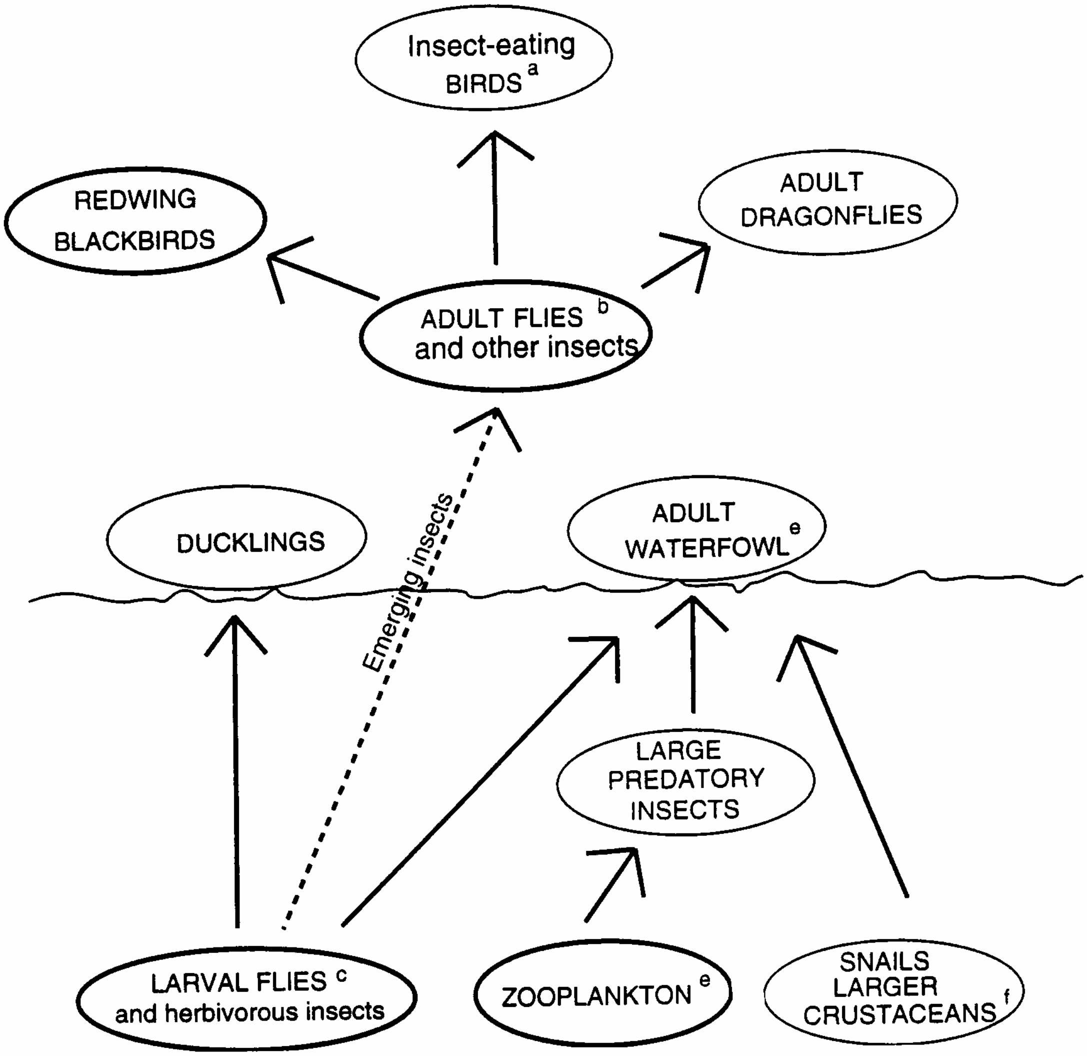
In the wetlands, the detritus and algae-feeding invertebrates, such as the zooplankton, many of the dipterans and crustaceans, are the prey for the invertebrate predators. Insects in wetlands especially the aquatic beetle larvae and adults, certain aquatic bugs, dragonfly and damselfly nymphs and leeches are the predominant predatory groups. They feed upon the prey species of invertebrates like the larvae of the chironomids (midges) and mosquitoes, the zooplankton and the larger crustaceans like amphipods and fairy shrimp. Certain leeches will feed largely on snails, others have a mixed diet including chironomids. The adult beetles and bugs (Coleoptera and Hemiptera) are able to fly between wetlands. The phantom midge larva, *Chaoborus*, consumes zooplankton and mosquito larvae

(Helgen, 1989). The carnivorous bladderwort, *Utricularia*, a plant common in semipermanent wetlands, consumes a similar diet.

The chironomids are very abundant in aquatic habitats, and as prey species, are a key component of the diet of a number of organisms including waterfowl (see Appendix III). In general, they are more abundant in the shallow vegetated littoral zone of lakes than in the deep zone (Wrubleski, 1987). Except for the predaceous, actively swimming Tanypodinae, most of the chironomids are microphagous, feeding on algae, detritus or both (Oliver, 1971). The life cycle of the egg to adult stage ranges from 10-12 days in the tropics to two years in the Arctic.

Figure 1 highlights some of the elements of wetlands food webs that were considered in the MMCD sponsored research. The lower level shows the primary consumers, the algae-detritus-feeding larval flies and insects, the herbivorous zooplankton and snails and the larger crustaceans. These groups ingest the detritus and algae and become the prey species for the next trophic level, the predators. The main prey species are the zooplankton, the non-predatory dipterans including the chironomids and mosquitoes, and the larger crustaceans such as amphipods, fairy shrimp and clam shrimp. The main predators in shallow wetlands are the invertebrates and some vertebrates: aquatic beetle larvae and adults, certain aquatic bugs, dragonfly and damselfly nymphs, leeches and salamander larvae. Waterfowl and ducklings consume invertebrates from wetlands (See Appendix III). The Northern Shoveler (*Anas clypeata*) specializes in filtering zooplankton, while most ducks feed on the macroinvertebrates. Insects emerging from the wetlands are consumed by birds, especially the birds like swallows, marsh and sedge wrens that feed within the wetland. Redwing blackbirds feed to some extent in the wetlands but also feed in the upland. The dragonflies are predators as nymphs in the water and as adults that emerge from the water.

Figure 1. Elements of the Wetland Food Web



Wetland food web showing some of the food chain based on the production of the detritus-feeding and/or herbivorous larval flies, zooplankton, snails and larger crustaceans. The arrows indicate the direction of energy flow in the food chain. The darker circles represent the groups examined most closely in the Wright County Long Term Experiment. The energy originates from algae, detritus, and decomposing vegetation.

---

[a] Swallows, marsh and sedge wrens.

[b,c] Chironomids, other midges and flies, mosquitoes (Dipterans).

[d] Dragonfly and damselfly nymphs, aquatic beetles and bugs.

[e] Zooplankton are a food source for filter-feeding Northern Shovelers.

[f] Including amphipods, clam shrimp, fairly shrimp, and crayfish.

APPENDIX III. An Overview of Selected Aspects of Duck Breeding Biology

Waterfowl, specifically ducks, are a public concern. What is known about duck–invertebrate relationships? It is well established that ducks eat wetland invertebrates, particularly chironomids or midges, during various times of the year. For most duck species, consumption of invertebrates is greatest during the reproductive period (see summaries in Krapu and Reinecke, 1992). The importance of invertebrates as a nutrient source during reproduction varies somewhat among species depending on species–specific reproductive strategies and whether one is concerned about lipids or about proteins. Recruitment among waterfowl may be particularly sensitive to the quantity and quality of available foods because their energy and nutrient requirements are large relative to other bird species (Krapu and Reinecke, 1992:1, Alisauskas and Ankney, 1992).

Nutrient Acquisition

Most female dabbling ducks obtain protein needed for egg production through intensive foraging on invertebrates after arriving on the breeding grounds, whereas timing of lipid acquisition is more variable (Krapu and Reinecke, 1992). Normally, Mallards return in the spring with enough stored lipids to begin nesting (Eldridge and Krapu, 1988, Krapu and Reinecke, 1992), but this does not appear to be the case for at least some other species (e.g., Wood Ducks, *Aix sponsa* – Drobney, 1982). Reproductive performance, particularly rate of re-nesting in species like Mallards(*Anas platythynchos*), Blue–winged Teal (*A. discors*), and Wood Ducks, depends on females acquiring sufficient proteinaceous food from the environment (Krapu et al., 1983, Krapu and Reinecke, 1992). Acquisition of adequate resources for egg–laying becomes more difficult as the spring advances because lipid reserves have become reduced and many available foods are low in lipids (Ankney and Afton, 1988, Alisauskas and Ankney, 1992). Lipids are needed for input to eggs as well as an energy source for the female.

Duck diets have been studied most during the reproductive period and less so at other times in the life cycle. Swanson et al. (1984/1985) noted that animal matter comprised from 72 to 99% of the diet of dabbling duck females during egg–laying. Of that total, Chironomidae (midges) were the dominant insect consumed by Blue–Winged Teal, (20% of diet), Northern Pintails (*A. acuta),* (20%), and Gadwalls (*A. strepera*), (17%). Two diving ducks, Ruddy Ducks (*Oxyura jamaicensis*), and Redheads (*Aythya americana),* have been reported to consume more than 70% and 60% chironomids, respectively (Tome, 1981, Woodin, 1987). Other insects, molluscs, Crustacea, and plant material comprised the remainder of the diet of most species.

Invertebrates are also important to duckling diets particularly during the first 15 days after hatching. Duck broods often move from wetlands with sparse numbers of chironomid larvae to those where larval densities are greater (Talent et al., 1982). More than half the diet of Mallard ducklings has been reported to be chironomids (Chura, 1961, Perret, 1962). The demand for invertebrate foods by breeding females and their ducklings extends from early spring until mid– to late summer.

Factors that influence an egg–laying or incubating female's foraging efficiency are especially important to nutrient acquisition given the constraints in daily time–budgets during egg–laying. This is one reason why disturbance of pairs on breeding territories is recognized as detrimental to nesting efforts. Time–budget studies have indicated that breeding females spend 40–91% of the day foraging during egg–laying (Krapu and Reinecke, 1992). Zicus and Hennes (1993) speculated that incubating Common Goldeneye (*Bucephala clangula*) females foraged more when foods were less available leading to a lower incubation constancy and longer incubation times (Zicus et al., 1995). Furthermore, Drobney and Fredrickson (1985) suggested egg–laying female Wood Ducks, with an average diet, that foraged 8 hr/day would need to find, capture, and consume a prey item every 3.0 to 5.5 sec. Obviously, factors affecting invertebrate availability and female foraging efficiency may be just as important to obtaining sufficient nutrients as are minimum quantities of biomass in a wetland.

Wetland Use

The use of wetlands by "breeding" or "nesting" ducks needs to be reviewed as it is sometimes misunderstood. Most duck species in the Midwestern U. S. can be classified into 2 broad groups (dabbling and diving). With some exceptions, patterns of wetland use are similar within a group. All species use wetlands for year round security and as a food source during most of the year. Most dabbling ducks establish their nests in upland sites that can be more than a mile from water. In contrast, most diving ducks construct nests in vegetation within the wetland basin. Species in both groups use wetlands for brood rearing and molting. The importance of small wetland basins with temporary and seasonal water regimes to breeding pairs is well known (Kaminski and Weller, 1992, Cowardin et al., 1995). Wetlands with deeper more permanent water are preferred by ducks for brood rearing provided there is ample vegetation in or around the basin (Baldassarre and Bolen, 1994). Biological and behavioral requirements of all species are such that several wetland basins having different degrees of water permanency are needed within each pair's home range. Consequently, the landscape makeup is important as few individual wetlands meet the total needs of breeding ducks (Kaminski and Weller, 1992).

Mallards, Blue–Winged Teal, and Wood Ducks are the most common species of ducks nesting in the Twin Cities Metropolitan Area of Minnesota. Behavioral spacing mechanisms during the nesting season vary among these species (Anderson and Titman, 1992). Mallard territories are quite large, include several wetlands for foraging, and will overlap spatially. Individual Mallard pairs are temporally intolerant of conspecifics, are mobile, and use many wetlands during the nesting season. In contrast, Blue–Winged Teal pairs usually occupy smaller non–overlapping territories with one or two wetlands from which they exclude all conspecifics . These spacing behaviors provide for the efficient use of the invertebrate foods found in smaller Type 1 and 2, and to a lesser extent, Type 3 wetlands.

As a consequence Type 1 and 2 wetlands support 9–16 times more breeding ducks on a per acre basis than do the larger more permanent Type 4 and 5 wetlands (Drewien and Springer, 1969, Mack 1991). Whether or not all wetlands in a landscape are used by breeding pairs in a particular year is difficult to assess. Surveys of wetland "occupancy" by breeding pairs are snapshots at a point in time. Breeding pair occupancy rates in Minnesota vary by species, wetland type, and degree of drought and range from 11–40% (Maxson and Pace 1989). Little brood rearing occurs on smaller temporary and seasonal wetlands. Duebbert and Frank (1984) estimated that >90% of duck brood use occurred on Type 3-4+ wetlands.

A Minnesota Landscape

We assembled data from several published sources and an unpublished Minnesota Department of Natural Resources (MDNR) radio–telemetry study of Mallards funded largely by the Reinvest in Minnesota (RIM) program. We used this information to depict generally the chronological relationships between breeding ducks, wetland types, and invertebrate foods in a Minnesota landscape. Average date of the last 1–inch snow depth where the telemetry study was conducted is the same as that of the Metropolitan area (Kuehnast et al., 1982). Thus, seasonal duck chronology also should be similar.

For many duck species including Mallards, overall nest success often averages less than 15%. Females make repeated nesting attempts until 25 to 35% of all females eventually hatch a nest (MDNR, unpublished data). Approximately 62% of Mallard egg–laying and 90% of incubation occurs after mid–May in western Minnesota (Figure 1). Nesting chronologies of Wood Ducks and Blue–winged Teal are somewhat later than Mallards (Bellrose, 1980) so a greater proportion of their nesting effort would occur after mid–May.

Mallard demographic data (Figure 2) weighted by food habits estimates gleaned from the literature (Table 1) suggest that utilization of invertebrate foods in Minnesota increases from early April (as ducks return), peaks first in mid– to late May (when egg–laying and incubation is greatest), and then begins increasing again in late June (when broods are hatching but nesting is declining) (Figure 3). Plant foods are utilized most in mid– and late summer when nesting has ceased and ducklings are maturing.

Because invertebrates have been identified in the literature as an important source of protein, we approximated their relative utilization from different wetland types in the Minnesota landscape. To do this, we assumed that the distribution of Mallard pairs and broods among wetland types reported in the literature reflected the use of various wetland types by ducks for foraging. This is a reasonable assumption because foraging is the primary activity of breeding ducks. For all wetland types, we also assumed that invertebrates in the diet with respect to breeding status were described by the Table 1 proportions. This assumption, although perhaps not strictly true, is also reasonable for our purpose. This exercise allowed us to estimate the chronological utilization of invertebrates from different wetland types based on the wetland makeup of the landscape and the reported distribution of pairs and broods (Figure 4a and 4b). For the Minnesota landscape, ephemeral wetlands (Types 1 to 3) are important as food sources primarily for pairs in May and June with the more permanent wetlands becoming important to females and their ducklings later in the season. These are the principles described throughout the waterfowl literature that were reviewed above.

Relevance of Food Web Changes

Many duck species have evolved in environments subject to periodic drought and related reductions in invertebrate foods. Failure of duck populations to prosper during drought years is well documented (Cowardin et al., 1985; Johnson and Grier, 1988). The location of water during drought generally shapes the distribution of breeding ducks (Johnson and Grier, 1988) and indirectly determines their reproductive success. During drought, the ability of egg–laying females to acquire needed resources is limited by the reduced availability of wetland invertebrates (Krapu et al., 1983). Invertebrate availability is reduced in two ways. First, fewer wetland basins contain water. Second, invertebrate densities in the remaining wetlands are reduced because important invertebrate habitat in the productive emergent vegetation zones has become dry (Swanson and Meyer, 1977). Food limitations negatively affect overall recruitment (Cowardin et al., 1985, Johnson and Grier, 1988) primarily by limiting re-nesting (Krapu et al., 1983; Krapu and Reinecke, 1992). The effect on recruitment is likely the result of the inability of females to obtain sufficient food resources efficiently in the time available for foraging.

Ducks as a group cope with reduced food during drought in different ways. Species such as Pintails or Blue–winged Teal move to areas with more favorable environmental conditions. In doing so, they may reduce the demands for food from the remaining wetlands. Others, such as Mallards or Gadwalls, are more philopatric (Johnson and Grier, 1988; Lokemoen et al., 1990). Those that are the most philopatric return to their natal areas and make a reduced breeding effort or fail to breed at all when conditions are unfavorable

(Krapu and Reinecke, 1992). From a fitness standpoint, these species have the advantage of being relatively long–lived and able to forgo nesting until conditions improve. Ducklings, on the other hand, have little recourse if wetland foods become inadequate during brood–rearing. Increased foraging time by ducklings may lead to increased mortality from a number of indirect causes (e.g., predation) (Hunter et al., 1984). Starvation might occur if food reductions are especially severe.

In summary, reproductive success of individual ducks will be affected by food web changes if 1) types of food needed by a breeding female or her ducklings are substantially reduced and 2) reductions in foods occur during egg–laying, incubation, or brood rearing. From a population standpoint, consequences will be most severe if 1) food reductions occur in many wetlands across a wide geographic area (depressed recruitment seen during drought years is an obvious example) and 2) reductions exist for many consecutive years.

Table 1. Diet composition of Mallard ducks, Wood Ducks, and Blue-winged Teal during the breeding season[1]

	Adults			
Food Items	Laying[2]	Incubating[3]	Brood Rearing[4]	Ducklings[5]
Chironomids	0.31	0.20	nd (0.20)	0.53 (0.16)
Other insects	0.12	0.08	nd (0.08)	0.12 (0.16)
Crustaceans	0.15	0.10	nd (0.10)	0.10 (0.16)
Gastropods	0.19	0.13	nd (0.13)	0.05 (0.16)
Other animals	0.05	0.03	nd (0.03)	0.05 (0.16)
Plant material	0.18	0.46	nd (0.46)	0.10 (0.16)

Notes

[1] Estimates were assembled from the following sources: adults--Krapu and Reinecke, 1992 (summary of many studies), ducklings--Chura, 1961, Perret, 1962.

[2] Includes prelaying females. Estimates represent an "average" for Mallards, Wood Ducks, and Blue-winged Teal. The best data exist for laying females.

[3] Few data exist for incubating Mallards, Wood Ducks, and Blue-winged Teal. Values for incubating females were derived by proportionately changing laying female values in a manner consistent with patterns reported for other, better studied species.

[4] There are no data for brood rearing females per se. They are likely quite similar to incubating females.

[5] Duckling data are for Mallards only up to 2 weeks of age. Values for older ducklings (in parentheses) are assumptions for modelling purposes.

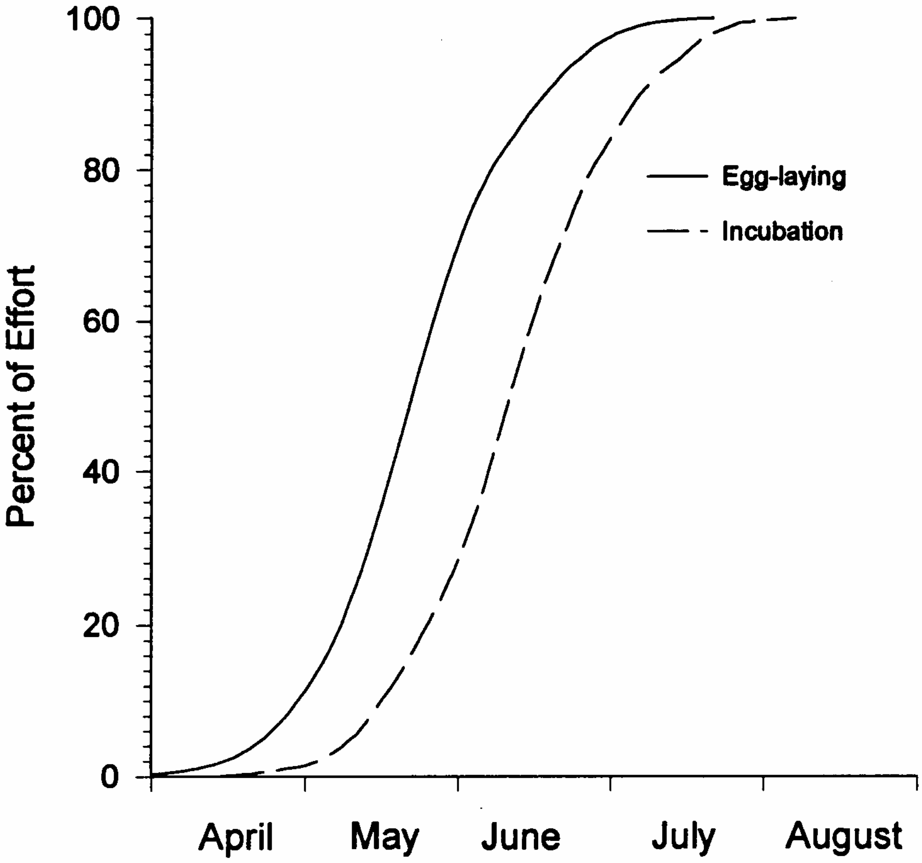


Figure 1. Average cumulative egg-laying and incubation effort by Mallard hens monitored with radio-telemetry in an unpublished Minnesota DNR study, 1990-92.

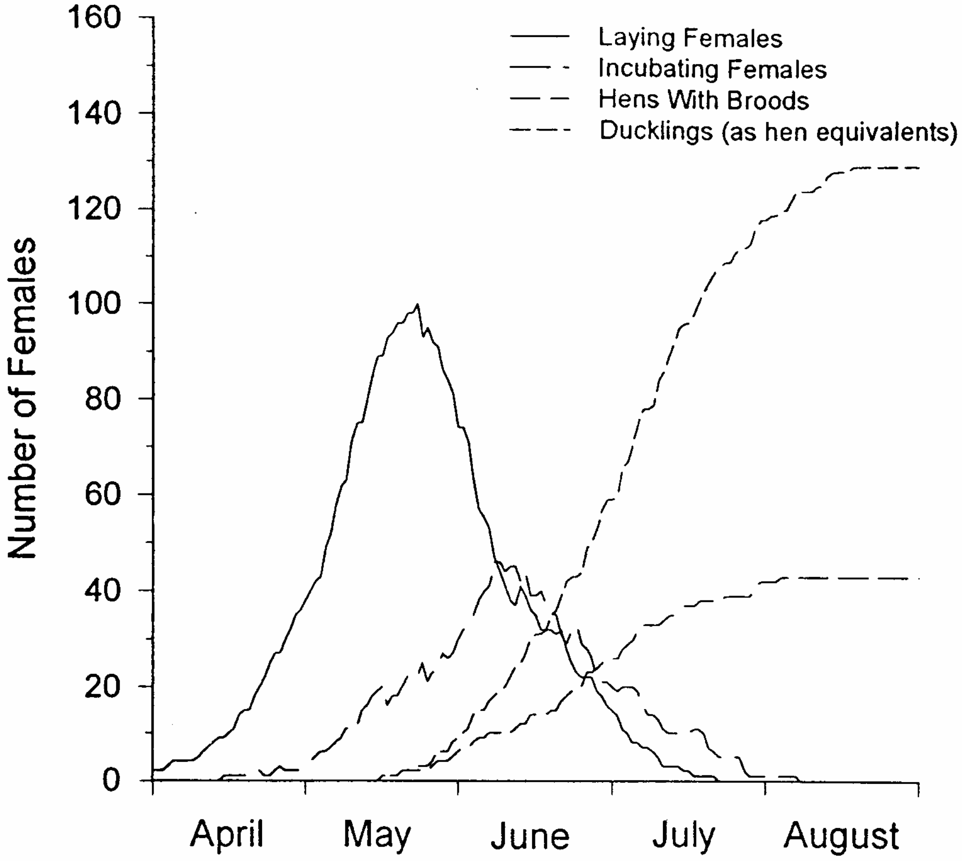


Figure 2. Mallard demographics determined in an unpublished radio-telemetry study conducted by the Minnesota DNR, 1990-92.

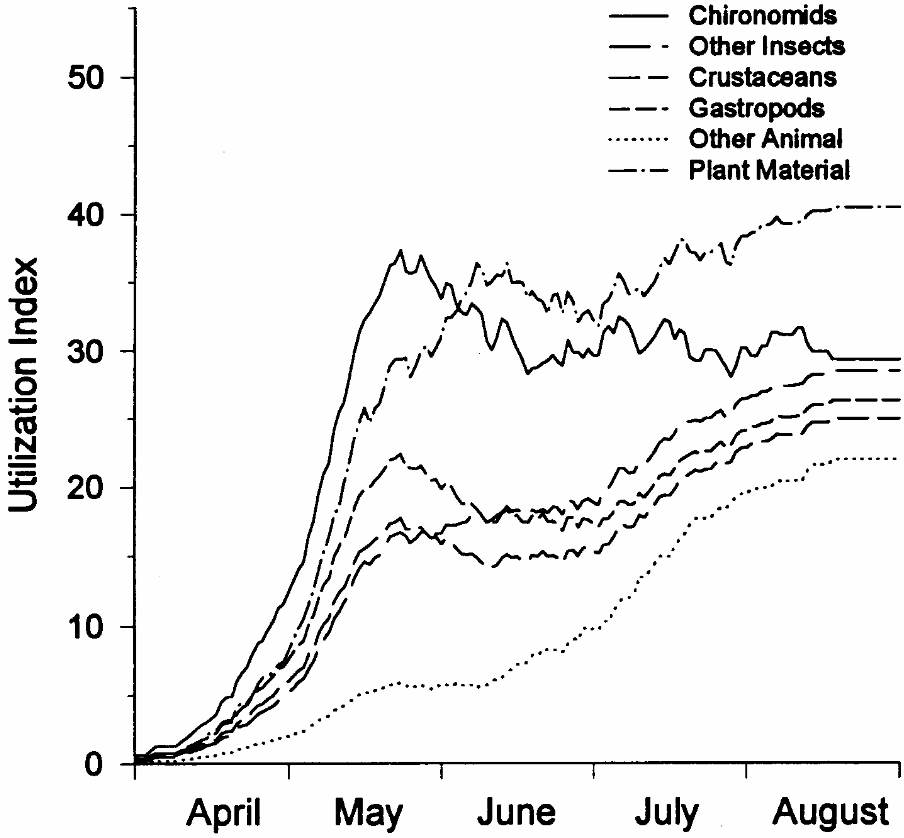


Figure 3. Chronological utilization of different foods by breeding female ducks and their ducklings modelled using the diet composition in Table 1 and Mallard demographics from an unpublished Minnesota DNR study.

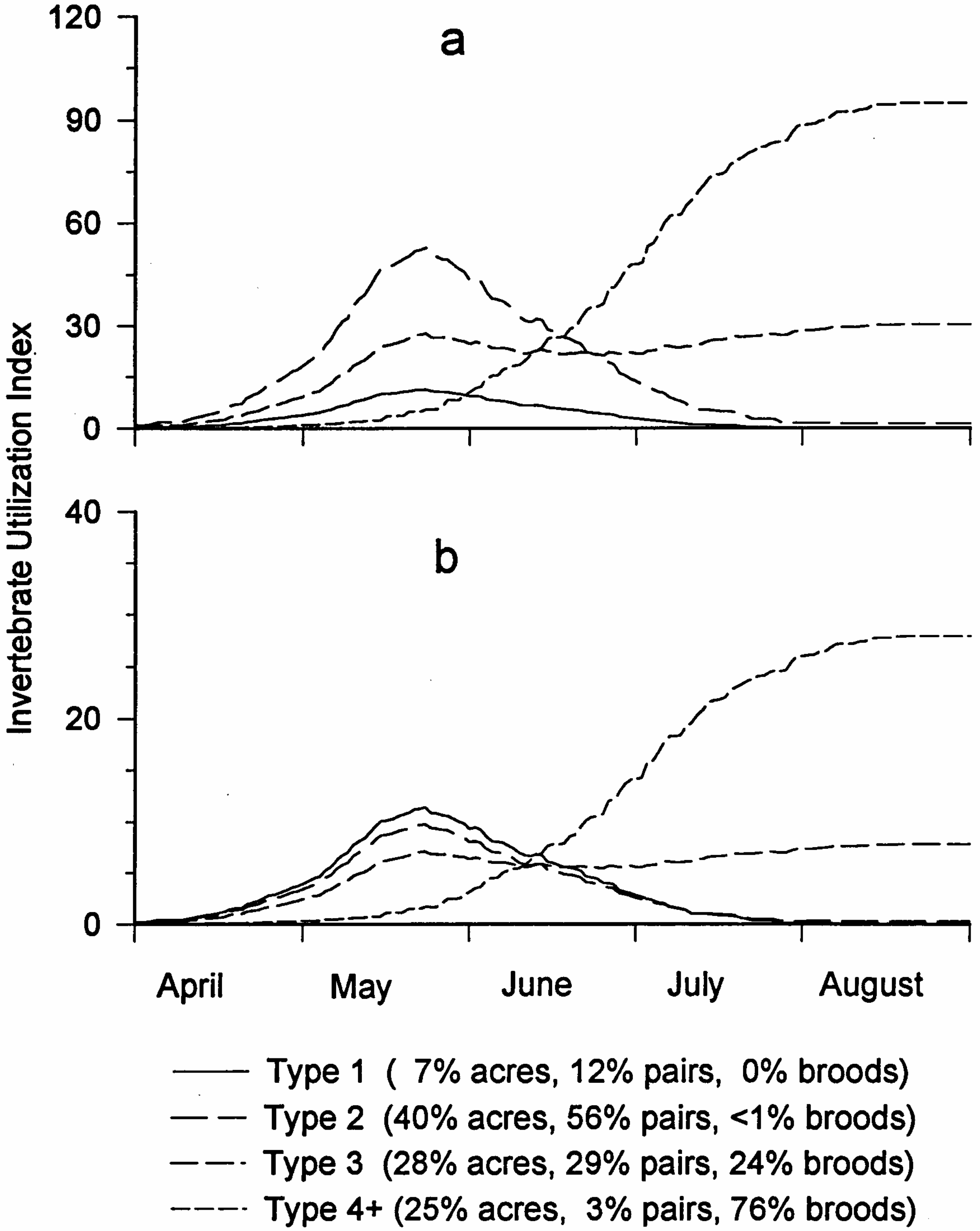


Figure 4. Invertebrate utilization from various wetland types in a Minnesota landscape (a) and on a unit-area basis (b). Curves modelled using the diet composition in Table 1 and Mallard demographics determined in an unpublished Minnesota DNR study.

Appendix IV: REFERENCES

Anonymous, 1985a. Petition by the National Audubon Society, Sierra Club, Defenders of Wildlife and Others, for an order directing the preparation of an environmental assessment on the Mosquito Control Program by an independent responsible governmental unit. [to the MN Environmental Quality Board]. August 1, 1985. 21 pp.

Anonymous, 1985b. A Response by the Metropolitan Mosquito Control District to a Petition for an order directing the preparation of an environmental assessment on the Mosquito Control Program by an independent responsible government unit.[to the MN Environmental Quality Board].October 18, 1985.27 pp. plus 14 pp. of appendices.

Ali, A., R. D. Baggs and J. P. Stewart. 1981. Susceptibility of some Florida chironomids and mosquitoes to various formulations of *Bacillus thuringiensis israelensis*. J. Econ. Entomol.74:672- 677.

Ali, A. 1991a Perspectives on management of pestiferous Chironomidae (Diptera), an emerging global problem. J. Amer. Mosq. Control Assoc. 7:260-281.

Ali A. 1991b. Activity of new formulations of methoprene against midges (Diptera: Chironomidae) in experimental ponds. J. Am. Mosq. Control Assoc. 7:616-620.

Alisauskas, R. T., and C. D. Ankney. 1992. The cost of egg laying and its relationship to nutrient reserves in waterfowl., p 30-61. In B. D. J. Batt et al. [eds.], Ecology and management of breeding waterfowl. University of Minnesota Press, Minneapolis.

Anderson, M. G., and R. D. Titman. 1992. Spacing patterns., p 251-289. In B. D. J. Batt et al. [eds.], Ecology and management of breeding waterfowl. University of Minnesota Press, Minneapolis.

Ankney, C. D., and A. D. Afton. 1988. Bioenergetics of breeding Northern Shovelers: diet, nutrient reserves, clutch size and incubation. Condor 90:459-472.

Baldassarre, G. A., and E. G. Bolen. 1994. Waterfowl ecolgy and management. John Wiley & Sons Inc. N.Y.

Bellrose, F. C. 1980. Ducks, geese, and swans of North America. Stackpole Books, Harrisburg, Pa.

Boley, R. A. 1992. Effects of methoprene on site fidelity and reproductive success of male Red-winged Blackbirds (*Agelaius phoeniceus*). M.S. thesis, University of Minnesota. vii + 68pp + two appendices.

Brazner, J.C., and R. L. Anderson. 1986. Ingestion and adsorption of *Bacillus thuringiensis subsp. israelensis* by Gammarus lacustris in the laboratory. Appl. and Environ. Microbiol. 52:1386-1390.

Brooke, et al., 1988. Final report to the Metropolitan Mosquito Control District on the effects of insect control agents, S- Methoprene and *Bacillus thuringiensis israelensis*, to certain aquatic organisms. Report to the Scientific Peer Review Panel of the Metropolitan Mosquito Control District.

Brown, P. W. and M. L. Hunter, Jr. 1984/85. Potential effects of insecticides on the survival of dabbling duck broods. J. Minn. Academy Science 50:41-45.

Charbonneau, C. S., R. D. Drobney and C. F. Rabeni. 1994. Effects of *Bacillus thuringiensis var. israelensis* on nontarget benthic organisms in a lentic habitat and factors affecting the efficacy of the larvicide. Environ. Toxicol. and Chemistry. 13:267-279.

Chapman, R. F. 1982. The Insects Structure and Function. 3rd Edition, Harvard University Press. Cambridge, Massachusetts. 919 pages.

Chilcott, C. N. and Wigley, P. J. 1993. Isolation and toxicity of *Bacillus thuringiensis* from soil and insect habitats on New Zealand. J. of Invert. Pathol. 61:244-247.

Chura, N. J. 1961. Food availability and preferences of juvenile Mallards. Trans. N. A. Wildl. Nat. Res. Conf. 26:121-133.

Clark, J. 1978. Fresh Water Wetlands: Habitats for aquatic invertebrates, amphibians, reptiles and fish. In Wetland Functions and Values: The State of our Understanding. Amer. Water Res. Assoc. Nov. 1978.

Cooper, J. A., C. M. Potter, and C. H. Welling. 1989. Mallard duckling growth and behavior in methoprene treated wetlands: 1989 final report. Univ. Minn., St. Paul, MN. Report to the Scientific Peer Review Panel of the Metropolitan Mosquito Control District.

Cowardin, L. M., D. S. Gilmer, and D. G. Jorde. 1985. Mallard recruitment in the agricultural environments of North Dakota. Wildl. Monogr. 92.

------, T. L. Shaffer, and P. M. Arnold. 1995. Evaluation of duck habitat and estimation of duck population sizes with a remote-sensing-based system. Nat. Biol. Serv. Biol. Sci. Rpt. 2.

Cummins, K.W. 1974. Structure and function of aquatic ecosystems. Bioscience 24: 631-641.

Cummins, K.W. 1973. Trophic relations of aquatic insects. Ann. Rev. Ent. 18:183-206.

Daborn, G.R. 1976. The life cycle of *Eubranchipus bundyi* (Forbes) (Crustacea: Anostraca) in a temporary vernal pond in Alberta. Can. J. Zool. 54:193-201.

Dadd, R. H. 1975. Alkalinity within the midgut of mosquito larvae with alkaline-active digestive enzymes. J. Insect Physiol. 21:1847-1853.

Davis, C.G. and A.G. van der Valk. 1978. The decomposition of standing and fallen litter of *Typha glauca* and *Scirpus fluviatilis*. Can. J. Bot. 56:662-675.

DeJong, M., and K. Rusterholz. 1989. The indirect effects of the larvicides methoprene and *Bacillus thuringiensis* on wetland bird populations. College of St. Thomas, St. Paul, MN. Report to the Scientific Peer Review Panel of the Metropolitan Mosquito Control District.

Drewien, R. C., and P. F. Springer. 1969. Ecological relationships of breeding Blue-winged Teal to prairie potholes. Saskatoon Wetlands Seminar. Can. Wildl. Serv. Rpt. Series No. 6.

Drobney, R. D. 1982. Body weight and composition changes and adaptations for breeding in Wood Ducks. Condor 84:300-305.

------, and L. H. Fredrickson. 1985. Protein acquisition: a possible proximate factor limiting clutch size in Wood Ducks. Wildfowl 36:122-128.

Duebbert, H. M., and A. M. Frank. 1984. Value of prairie wetlands to duck broods. Wildl. Soc. Bull. 12:27-34.

Dunham, L.L., Miller W.W. 1978. Methoprene. In: Analytical Methods for Pesticides and Plant Growth Regulators. Zweig, G., Sherma, J., eds. (Academic Press: NY): 10 (Chapter 11): 95-109.

DuPont C. and Boisvert, J. 1986. Persistence of *Bacillus thuringiensis* serovar. *israelensis* toxic activity in the environment and interaction with natural substrates. Water, Air and Soil Pollution, 29:425-438.

Eldridge, J. L., and G. L. Krapu. 1988. The influence of diet quality on clutch size and laying pattern in Mallards. Auk 105:102-110.

Essen, Van F. W. and Hembree, S. C. 1982. Simulated field evaluations with four formulations of *Bacillus thuringiensis* var. *israelensis* against mosquitoes: Residual activity and effect of soil constituents. Mosquito News. 42:66-72.

Flum, T. 1988. Ingestion of the bacterial mosquito and blackfly larvicide *Bacillus thuringiensis* var. *israelensis* by nymphs of the stonefly Pteronarcys dorsata. M.S. thesis. University of Minnesota, Duluth.

Fortin, C. 1988. Laboratory study of the impact of slow released methoprene used for the control of mosquito larvae. Report to the Scientific Peer Review Panel of the Metropolitan Mosquito Control District.

Fortin, C., D. Lapointe, and G. Charpentier. 1986. Susceptibility of Brook Trout (*Salvelinus fontinalis*) fry to a liquied formulation of *Bacillus thuringiensis* serovar. *israelensis* (Teknar) used for blackfly control. Can. J. Fish Aquat. Sci. 43:1667-1670.

Hastewo, S., Lay, B. W. and M. Ohba. 1992. Naturally occurring *Bacillus thuringiensis* in Indonesia. J. of Appl. Bacteriol. 73:108-113.

Helgen, J. C. 1989. Larval mosquitoes as vulnerable prey: Chaoborus predation. Can. J. Fish. Aquatic Sci. 46 (9): 1642-1650.

Henrick, C. A. 1982. Juvenile Hormone Analogs: Structure-Activity Relationships. IN Insecticide Mode of Action. Ed. Joel R. Coats. Academic Press.pp.315-402.

Hershey, A., Shannon, L. Whiteside, M., and Axler, R. 1990. Effects of methoprene and Bti (*Bacillus thuringiensis israelensis*) on non-target Invertebrates: A divided pond study. Final Report submitted to the Scientific Peer Review Panel of the Metropolitan Mosquito Control District.

Hunter, M. L. Jr., J. M. Witham, and H. Dow. 1984. Effects of a carbaryl-induced depression in invertebrate abundance on the growth and behavior of American Black Duck and Mallard ducklings. Can J. Zool. 62:452-456.

Johnson, D. G. and J. W. Grier. 1988. Determination of breeding distributions of ducks. Wildl. Monogr. 100:1-37.

Kaminski, R. M., and M. W. Weller. 1992. Breeding habitats of nearctic waterfowl relationship to nutrient reserves in waterfowl., p 568-589. In B. D. J. Batt et al. [eds.], Ecology and management of breeding waterfowl. University of Minnesota Press, Minneapolis.

Kantrud, H.A. et al. 1989. Prairie Basin Wetlands of the Dakotas: A community profile. Biol. Rep. 85 (7.28). Sept. 1989. U.S. Dept. Interior, U.S. Fish and Wildlife Service.

Knuth M.L. 1989. Determination of the insect growth regulator methoprene in natural waters by capillary gas-liquid chromatography. Chemosphere 18: 2275-2281

Kondo, S., Ohba, M. and T. Ishiii. 1995. Comparative susceptibility of chironomid larvae (Dopt., Chironomidae) to *Bacillus thuringiensis* serovar *israelensis* with special reference to altered susceptibility due to food difference. J. Appl. Ent. 119: 123-125.

Krapu, G. L., and K. J. Reinecke. 1992. Foraging ecology and nutrition., p 1-29. In B. D. J. Batt et al. [eds.], Ecology and management of breeding waterfowl. University of Minnesota Press, Minneapolis.

------, A. T. Klett, and D. G. Jorde. 1983. The effect of variable spring water conditions on Mallard reproduction. Auk 100:689-698.

Kuehnast, E. L., D. G. Baker, and J. A. Zandlo. 1982. Climate of Minnesota. Part XIII. Duration and depth of snow cover. Minn. Agr. Exp. Sta. Tech. Bull. 333.

Leclair, R. Charpentier, G. Pronovost, F. and S. Trottier. 1988. Progress report to the Metropolitan Mosquito Control District on the effects of the insect control agent, *Bacillus thuringiensis israelensis* (Bti) to some larval amphibian species. Report to the Scientific Peer Review Panel of the Metropolitan Mosquito Control District.

Lokemoen, J. T., H. F. Duebbert, and D. E. Sharp. 1990. Homing and reproductive habits of Mallards, Gadwalls, and Blue-winged Teal. Wildl. Monogr. 106.

Mack, G. D. 1991. The Mallard model handbook. U. S. Fish and Wildlife Service, Bismarck Wetland Habitat Office. Unpubl. Rpt. Revised by T. L. Shaffer and L. M. Cowardin.

Madder D.J., Lockhart W.L. 1980. Studies on the dissipation of diflubenzuron and methoprene from shallow prairie pools. Can. Entomol. 112: 173-179

Margalit, J. and Dean, D. 1985. The story of *Bacillus thuringiensis israelensis*. J. of Amer. Mosq. Cont. Assoc. 1:1-7.

Martin, P. and Travers, R. S. 1989. Worldwide abundance and distribution of Bacillus thuringiensis isolates. Appl. and Environ. Microbiol. 55:2437-2442.

Maxson, S. J., and R. M. Pace III. 1989. Summary and evaluation of Minnesota's waterfowl breeding population survey. Minn. Dep. Nat. Res. Minn. Wildl. Rep. 7.

Metropolitan Mosquito Control District 1994a Operational Review and Plans for 1995. Submitted to the Technical Advisory Board. March 1995.

Metropolitan Mosquito Control District 1994b. MMCD Self-Assessment of Performance.

Metropolitan Mosquito Control District, 1977. Environmental Impact Statement: Options for Control to the Year 2000

Metropolitan Mosquito Control District, 1985. Supplemental Environmental Impact Statement.

Mian, L. S. and Mulla, M. S. 1982. Biological and environmental dynamics of insect growth regulators (IGRs) as used against Diptera of public health importance. Residue Reviews 84:27-112.

Miura, T. and R. M. Takhashi. 1973. Insect development inhibitors. 3. Effects on nontarget aquatic organisms. J. Econ. Entomol. 66:917-922.

Mulla, M. S., G. Majori, and A. A. Arata. 1979. Impact of biological and chemical mosquito control agents on nontarget biota in aquatic ecosystems. Residue Reviews 71:121-173.

Murkin, H.R., Kadlec, J.A. and E.J. Murkin. 1991. Effects of prolonged flooding on nektonic invertebrates in small diked marshes. Can. J. Fish. Aquat. Sci. Vol 48:2355-2364.

Niemi, G. J., Axler, R., Hanowski, J. M., Hershey, A. E., Shannon, L. J. and M. C. Whiteside. 1990a. Evaluation of the effects of methoprene and Bti (*Bacillus thuringiensis israelensis*) on non-target species and communities in Metropolitan Mosquito Control District wetlands. Natural Resources Research Institute and the University of Minnesota- Duluth. Report to the Scientific Peer Review Panel of the Metropolitan Mosquito Control District.

Niemi, G. J., Hanowski, J. M., Hershey, A. E., Shannon, L. J. and M. C. Whiteside. 1990b. Evaluation of the effects of methoprene and Bti (*Bacillus thuringiensis israelensis*) on non-target species and communities: Historically treated sites in eastern Wright County. Natural Resources Research Institute and the University of Minnesota-Duluth. Report to the Scientific Peer Review Panel of the Metropolitan Mosquito Control District.

Niemi, G. J., R. P. Axler, J. A. Hanowski, A. E. Hershey, R. R. Regal, and L. J. Shannon, M. C. Whiteside. 1990c. Evaluation of the effects of methoprene and Bti (*Bacillus thuringiensis israelensis*) on non-target organisms: A summary of before- and after sites in western Wright County. Natural Resources Research Institute and the University of Minnesota-Duluth. Report to the Scientific Peer Review Panel of the Metropolitan Mosquito Control District.

Niemi, G. J., Axler, R. P., Barnidge, P., Hanowski, J. M. Hershey, A. E., Regal, R. R. and Shannon, L. J. 1992. Evaluation of the potential effects of methoprene and Bti (*Bacillus thuringiensis israelensis*) on non-target organisms: A summary of before-and-after sites in western Wright County for 1988, 1989, and 1990. Natural Resources Research Institute and the University of Minnesota-Duluth. Report to the Scientific Peer Review Panel of the Metropolitan Mosquito Control District.

Niemi, G. J., Axler, R. P., Hanowski, J. M. Hershey, A. E., Lima, A., Regal, R. R. and Shannon, L. J. 1994. Evaluation of the Potential Effects of Methoprene and Bti (*Bacillus thuringiensis israelensis*) on Wetland Birds and Invertebrates in Wright County, MN, 1988 to 1993. Natural Resources Research Institute and the University of Minnesota-Duluth. Report to the Scientific Peer Review Panel of the Metropolitan Mosquito Control District.

Nilsson, C. 1983. Coprophagy in larval *Culiseta bergrothi*. Hydrobiologia 98: 267-269.

Norland, R. L. and M.S. Mulla. 1975. Impact of Altosid®® on selected members of an aquatic ecosystem. Environ. Entomol. 4: 145-152.

Ohana, B., Margalit, J. and Z. Barak. 1987. Fate of *Bacillus thuringiensis* subsp. *israelensis* under simulated field conditions. Applied and Environ. Microbiol. 53: 828-831.

Oliver, D.R. 1971. Life history of the Chironomidae. In Smith, Ray F. and T. E. Mittler, eds, Annual Rev of Entomol. Vol 162:211-230.

Perret, N. G. 1962. The spring and summer foods of the common Mallard (*Anas platyrhynchos L.*) in south central Manitoba. M. S. thesis, Univer. of British Columbia, Vancouver, B. C.

Ravoahangimalala, O. J. F. Charles and J. Schoeller-Raccaud. 1993. Immunological localization of *Bacillus thuringiensis* serovar *israelensis* toxins in midgut cells of intoxicated Anopheles gambiae larvae (Diptera:Culicidae). Research in Microbiology 144:271-278.

Rodcharoen, J., Mulla, M. S. and J. D. Chaney. 1991. Microbial larvicides for the control of nuisance aquatic midges (Diptera:Chironomidae) inhabiting mesocosms and man-make lakes in California. J. Amer. Mosq. Assoc. 7:56-62.

Schaefer, C. H. and E. F. Dupras, Jr. 1973. Insect developmental inhibitors. 4, Persistence of ZR-515 in water. J. Econ. Entomol. 66:923.

Shaw, S. P. and C. G. Fredine, 1956. Wetlands of the United States: Their extent and their value to waterfowl and other wildlife. Fish and Wildlife Service, Circular 39. 67 pp.

Sjogren, R. D., D. P. Batzer, and M.A. Juenemann. 1986. Evaluation of methorene, temephos and *Bacillus thuringiensis* var. *israelensis* against Coquillettidia perturbans larvae in Minnesota. J. Amer. Mosq. Contr. Assoc. 2:276-279.

Snarski, V. M. 1990. Interactions between *Bacillus thuringiensis* subsp. *israelensis* and Fathead Minnows, *Pimpehales promelas* Rafinesque, under laboratory conditions. Appl. and Environ. Microbiology 56:2618-2622.

Swanson , G. A. 1984/1985.Invertebrates consumed by dabbling duck (Anatinae) on the breeding grounds. J. of the Minnesota Academy of Science 50(3):37-40.

Swanson, G. A., amd M. I. Meyer. 1977. Impact of fluctuating water levels onfeeding ecology of breeding Blue-winged Teal. J. Wildl. Manage. 38:396-407.

Talent, L. G., G. L. Krapu, and R. L. Jarvis. 1982. Habitat use by Mallard broods in south-central North Dakota. J. Wildl Manage. 46:629-635.

Tome, M. W. 1981. Reproductive bioenergetics of female Ruddy Ducks in Manitoba. M. S. Thesis. Univ. of Maine, Orono.

Trpis, M. and J.A. Chemanchuk. 1970. Effect of constant temperature on the larval development of *Aedes vexans* (Diptera: Culicidae). The Canadian Entomologist. Vol. 102: 1048-1051.

Vaishnav, D. D. and R. L. Anderson. 1995. Uptake and loss of *Bacillus thuringiensis* var. *israelensis* by *Daphnia magna* in laboratory exposures. Environ. Toxico. and Chem. 14:763-766.

Wiggins, G.B., R.J. Jackay, I.M. Smith. 1980. Evolutionary and ecological strategies of animals in annual temporary pools. Arch. Hydrobiol./Suppl. 58. 1/2: 97-206.

Woodin, M. W. 1987. Wetland selection and foraging ecology of breeding diving ducks. Ph. D. Dissertation. Univer. of Minnesota, St. Paul.

Wright J.E. 1976. Environmental and toxicological aspects of insect growth regulators. Environ. Health Perspect, 14:127-132.

Wrubleski, D. 1987. Chironomidae (Diptera) of peatlands and marshes in Canada. In, Rosenberg, D.M. and H.V. Danks, ed. Can. Mem. Entomol. Soc, No 140:141-161.

Yousten, A. A., Genthner, F. J. and E. F. Benfield. 1992. Fate of *Bacillus sphaericus* and *Bacillus thuringiensis* serovar *israelensis* in the aquatic environment. J. of Amer. Mosq. Control Assoc. 8: 143-148.

Zicus, M. C., and S. K. Hennes. 1993. Diurnal time budgets of breeding Common Goldeneyes. Wilson Bull. 105:680-685.

------, ------, and M. R. Riggs. 1993. Common Goldeneye nest attendance patterns. Condor 97:461-472.

APPENDIX V. Glossary of Terms

ANOVA: Analysis of variance, a statistical method for analysis of variance in response to two or more levels of a stimulus.

Benthic: Referring to the layer of detritus and soil at the bottom of a wetland.

Bioaccumulate: Acquiring a compound by feeding or adsorption.

Bioassay: Exposure of an animal or plant to measure the presence of a toxic substance.

Biomass: The weight of biological material in a given space.

Biorational: Characteristics of a toxic substance that permit its use against a pest species without affecting important non-target species, and which lead to its ready decomposition into harmless compounds.

Biota: Biological life forms.

Caddis Flies: An order of aquatic insects (Trichoptera), characterized by slender, mothlike adult insects. Larvae are found in many kinds of aquatic habitats, build unique cases, and are often active. The larvae are important foods for many aquatic and terrestrial animals.

CFU: Colony Feeding Units: A method of measuring the concentration of Bti or other bacteria. Samples are diluted and smeared on microbiological media. The number of colonies growing on the media is a measure of the bacteria in the original sample..

Conspecifics: Individuals that belong to the same species; e.g., two Mallard ducks.

Coprophagous: Feeding on dung or fecal material.

Delta-endotoxin: The toxin in Bti.

EC: Effective Concentration. The concentration of a substance necessary to cause an effect on a stated percent of a sample of an organism; e.g., slow growth, change in physiology, change in reproduction, or death.

Epiphytic: A characteristic of plants which grow on or are attached to another plant without deriving sustenance from the supporting structure: e.g., Spanish moss on a live oak.

Food Web: All the interconnecting food chains in a biological community.

Geometric Mean: A measure of central tendency in a set of observations that are not normally distributed. This is calculated by first converting the original values (x) to a log scale (usually $y = \log[x + c]$ where c is a small constant), computing the average Y of the y's, and then back-transforming the average from the log to the original arithmetic scale, i.e., Geometric mean = $[\text{antilog}\ \{Y\} - c]$. This manipulation of the data dampens the effect of the scores of very high or very low observations, which are common in biological measurements.

Herbivory: The eating of plants or algae by animals.

Holometabolous insects: Insects that undergo a complete metamorphosis, from eggs to larvae to pupae to adults.

Hydric Soils: Soils that are saturated with water and experience reducing as opposed to oxidizing conditions.

Instar: A form in the larval development of an insects between two successive moults.

ITU: International Toxic Unit. A measurement of the toxicity of a substance; e.g., Bti.

Larvae: Insects in their earliest stage of development, after they hatch and before they become a pupa.

Larvicide: A substance that is used to kill insects in their larval stage; e.g., Bti or methoprene. This is distinguished from adulticide, a substance that is used to kill the adult (e.g., resmethrin for adult mosquitoes).

LC: Lethal Concentration. The concentration of a substance necessary to cause death. The term is usually associated with a specific percentage, e.g., LC_{50} is the concentration that kills 50% of a population.

Log Series: In studies of toxicity, a series of test concentrations that is created through serial dilutions from a stock solution at the highest concentration. For example, a log (base 10) series of doses would result from diluting the first solution to one tenth strength (dose = 1/10 = 0.1) then again by another tenth (dose = 1/100 = .01) and so on to progressively lower concentrations.

Macroinvertebrate: Invertebrates larger than 3 mm in length, especially used with aquatic organisms.

Macrophyte: A large aquatic plant, as opposed to algae or phytoplankton, which can be microscopic.

Mayfield method: A research method used to estimate daily survival of bird nests or individual birds. It minimizes biases inherent in other techniques.

Mesocosm: An enclosure used in biological field research, in which barriers are placed within a given habitat, usually to compare the effects of different doses of a substance on organisms in different enclosures.

Metamorphosis: The physical transformation, more or less sudden, undergone by various animals during development after the embryonic stage, as of the larva of an insect to the pupa and the pupa to the adult, or as the tadpole to the frog.

Microgram (µ): One-one thousandth of a milligram; e.g., one gram of a chemical in a million liters of water. See ppb.

Microphagous: Refers to an animal that feeds on particles that are very small in comparison to its own size; e.g., zooplankton feeding on algae, or certain whales feeding on plankton.

Milligram (mg): One one-thousandth of a gram; e.g., one gram of a chemical in a thousand liters of water. See ppm.

Neonates: Newly hatched insects or zooplankton.

Non-target: An organism which is not intended to be harmed by an insecticide, but which lives in the same habitat as a pest species--in this case, mosquitos-- which is being killed by the insecticide.

Oviposition: The placement of eggs by an insect in a habitat.

Paraspore: Contains the toxin produced alongside the spore of Bti, during spore formation.

pH: A symbol for the negative log of the concentration of hydrogen ions in a solution, which represents the degree of acidity or alkalinity of that solution. pH7 is considered neutral; pH less than 7 are increasingly acidic; pH greater than 7 are increasingly alkaline.

Philopatric: Referring to the tendency of an individual to return to or stay in its home area.

Photodegradation: The process by which a substance is decomposed by sunlight, frequently to harmless subunits.

Photoisomerism: Change in chemical structure produced by exposure to light. The molecular bonds change, resulting in molecule(s) with altered conformation.

Polyolefin: A form of sheet plastic, resistant to tears and photodegradation.

Ppb: Part per billion; one part in a billion parts; e.g., one ppb would be the relationship between one microgram (μ) of chemical and a liter of water, or the approximate relationship between 15 inches and the distance of the Earth to the Moon.

Ppm: Part per million; one part in a million parts; e.g., one ppm would be the relationship between one milligram (mg) of a chemical and a liter of water, or the approximate relationship between 9 inches and the distance between Minneapolis and Fargo.

Protease: An enzyme that catalyzes the division by hydrolysis of the peptide bonds in a protein.

Proteinaceous: A descriptive word indicating a high protein content.

Pupa: An insect in the stage of development between the larval and adult form.

Substrates: An underlying object or material upon which an organism grows, or to which an organism is attached; e.g., rocks or plants.

Swales: Ground undulations in wetlands.

Taxon: Any taxonomic category; e.g., species, genus, variety.

Technical powder: The formulation of an insecticide in its purest form, before being mixed with inert ingredients, or being placed in a carrier.

Teratology: Study of deformed specimens.

Trophic level: One of the parts in a nutritive series in an ecosystem in which a group of organisms in a certain stage in the food chain secures food in the same general manner. The first lowest tropic level consists of Producers (green plants); the second of Herbivores (eaters of green plants); the third Carnivores (eaters of herbivores); and the fourth level Secondary Carnivores (eaters of smaller carnivores). Bacteria and fungi are in the Decomposer trophic level.

µg/l: Micrograms per liter. A term describing the amount of material in water. It is equal to a part per billion (ppb; see above).