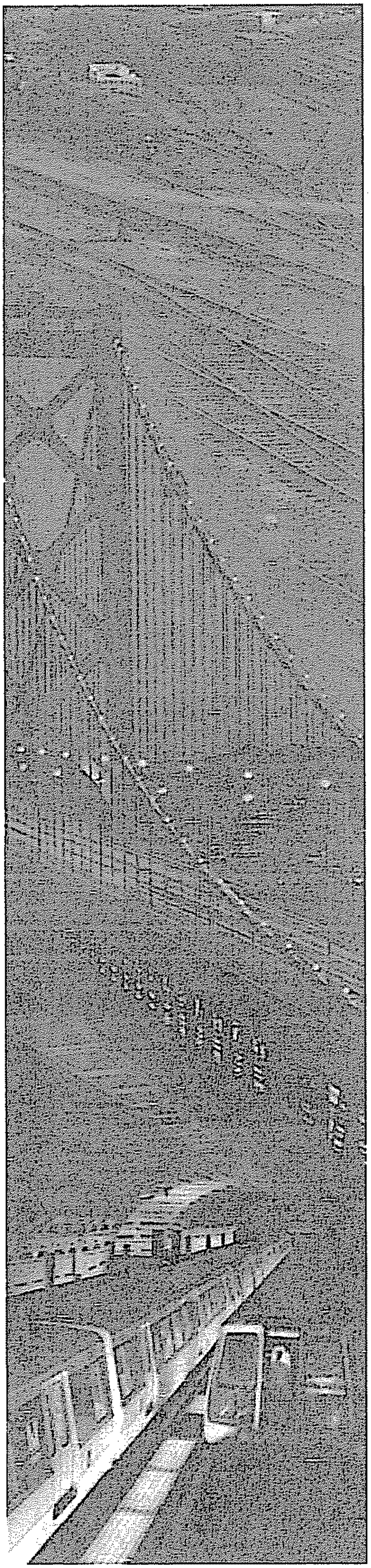

DCT 212

Potential of Retention Ponds to Produce Nuisance Mosquitoes and West Nile Virus Vectors

By

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FINAL REPORT ON SUPPLEMENTAL AGREEMENT NO. 1267-3 BETWEEN THE DELAWARE DEPARTMENT OF TRANSPORTATION AND THE UNIVERSITY OF DELAWARE, 4-15-2004 TO 4-14-2006.

PROJECT TITLE: POTENTIAL OF RETENTION PONDS TO PRODUCE NUISANCE MOSQUITOES AND WEST NILE VIRUS VECTORS. PART II: FIELD TRIALS FOR NON-PESTICIDAL, SELF-SUSTAINING CONTROL OF MOSQUITOES

Project Manager: Dr. Marianne Walch, NPDES Program, DelDOT
Investigator: Dr. Jack B. Gingrich, Department of Entomology and Wildlife Ecology, University of Delaware

Sponsorship and Disclaimer:
This work was sponsored by the Delaware Center for Transportation and was prepared in conjunction with the Delaware Department of Transportation and the U.S. Department of Transportation, Federal Highway Administration. The contents of this report reflect the views of the author who is responsible for the facts and accuracy of the data presented herein. The contents do not necessarily reflect the official views of the Delaware Center for Transportation or the Federal Highway Administration at the time of this publication. This report does not constitute a standard, specification, or regulation.

POTENTIAL OF RETENTION PONDS TO PRODUCE NUISANCE MOSQUITOES AND WEST NILE VIRUS VECTORS. II: FIELD TRIALS FOR NON-PESTICIDAL, SELF-SUSTAINING CONTROL OF MOSQUITOES

Dr. Jack B. Gingrich, Department of Entomology and Wildlife Ecology (ENTO 422114)

Final Report made for the Delaware Center for Transportation, April 1, 2005 – April 15, 2006, DE; Report to be forwarded to Dr. Marianne Walch, DelDOT.

1. Period Covered

This report covers the period of April 15, 2004 – April 14, 2006 and represents the final report that completes the preliminary research done on evaluation of various self-sustaining methods for control of mosquito larvae in retention ponds.

2. Year 1 Research. The work for the initial contract period (April 15, 2004 – April 14, 2005) has been published in a refereed journal, and the citation is listed below.

Gingrich, J.B., R.D. Anderson, G. M. Williams, L.L. O'Connor, and K.H. Harkins. 2006. Stormwater ponds, constructed wetlands, and other BMP's as potential breeding sites for West Nile virus vectors in Delaware during 2004. 2006. J. Amer. Mosq. Cont. Assoc. 22: In press.

A copy of this research is attached in Appendix 1.

3. Goals and Objectives for the Final Contract Period

Our overall goal for the research during the summer of 2005 was to test four presumably self-sustaining mosquito control methods in a range of retention ponds previously characterized for their mosquito abundance during 2003-2004. The methods to be used for larval mosquito control included solar pump aeration of ponds, Bactapur (a variant of *Bacillus subtilis*) treatment of ponds (with and without pump circulation), and alum (aluminum sulfate) treatment.

4. Methods Employed in the 2005 Research Season

We selected 30 ponds that were known from previous years' research to have fairly abundant mosquito numbers ranging from 0.1 to 20 larvae per dip. These ponds were located across all three counties. Ten other ponds, which were originally to be included in the project, had to be excluded from the study because of dryness, presence of killifish, or presumed treatment with pesticides. Ponds were selected in groups so that there four treatment groups of six ponds each, and one control group of six ponds each. An attempt was made to balance the ponds in terms of larval abundance for all treatment groups based on previous years' abundance results.

Pre-season assessments of ponds were conducted during the period of May 1, 2005 to June 12, 2005. This provided a total of three pretreatment assessments. Treatments of ponds began during the week of June 13-17, 2005 and extended through the third week of

September, 2006. Bactapur treatments were put in place once per month. Aerator treatments were continuous, as the aeration pump ran constantly. The first post-treatment evaluation of mosquito abundance was conducted during the week of June 24-30, 2005, and continued on a biweekly basis thereafter until the third week of September. This provided a total of six post-treatment evaluations.

We evaluated mosquito abundance at 5 subsites per pond, as in previous years (Gingrich et al. 2006). Using a standard 350-ml dipper, we took 20 dips per subsite, or 100 per pond. All larval mosquitoes were placed in 4-oz. Whirl-pak bags for return to the lab for identification to species whenever possible. A separate Whirl-pak bag was taken for phosphate analysis and returned in an ice pack-chilled cooler. Phosphates were analyzed in a LaMotte Colorimeter using a standardized reagent kit for phosphates. Other measurements taken of site were turbidity, chlorophyll a (as measured by a field fluorometer), temperature, pH, conductivity, percent shade, total dissolved solids, mosquito predators present, and estimated kinds and amounts of vegetation. Depths at the subsites and in the total pond were also obtained. All data was recorded on hand-coded data sheets, and returned to the lab. Following the completion of a given two-week cycle, data was entered into an Access database. Graphs showing critical relationships among variables were obtained using a query designed for the database. Whenever the depth of a subsite was zero (.i.e. dry), that part of the query would be automatically excluded from the analysis. Descriptive statistics, including means and standard errors, were constructed for datasets. Independent variables usually included chlorophyll a and phosphate concentrations at each time period, or during pretreatment vs. post-treatment periods. The principal dependent variable was larval abundance. Finally, an overall statistical assessment of the results was performed using analysis of variance with Tukey HSD (SPSS program).

The possibility of direct toxicity of alum to mosquitoes was a question we examined in the laboratory during September-October. We placed pond water containing 1st and 2nd instar *Ae. vexans* or *Culex restuans* larvae collected in the field into 8 X 11" rearing pans in a 27 C incubator in our lab. We then introduced alum at two doses into the pans to simulate field treatment. A second group of control pans contained mosquitoes but no alum. Mortality was assessed every 24 hours for 72 hours, and then continued up to one week post-treatment.

5. Results

Our first thought was to do an overall comparison of pretreatment vs. post-treatment results for each variable. In that way, we hoped to capture the effect of the various treatments on the variable. One figure each is presented for phosphates, chlorophyll a, and larval mosquito abundance (Figs. 1-3). As can be seen from Fig.1, alum produced the only significant reduction in phosphates. Both Bacta-pur treatments resulted in increased phosphate levels, while aerators and controls approximated one another.

As seen in Fig. 2, the only treatment to yield a reduction in chlorophyll a was the alum treatment. The other treatments showed either no difference in chlorophyll a (controls and aerators), or an actual increase (Bacta-pur treatments).

Fig. 1. Effects of five treatments on phosphate levels (ppm) as observed by comparing pre- and post-treated sample means (with standard errors).

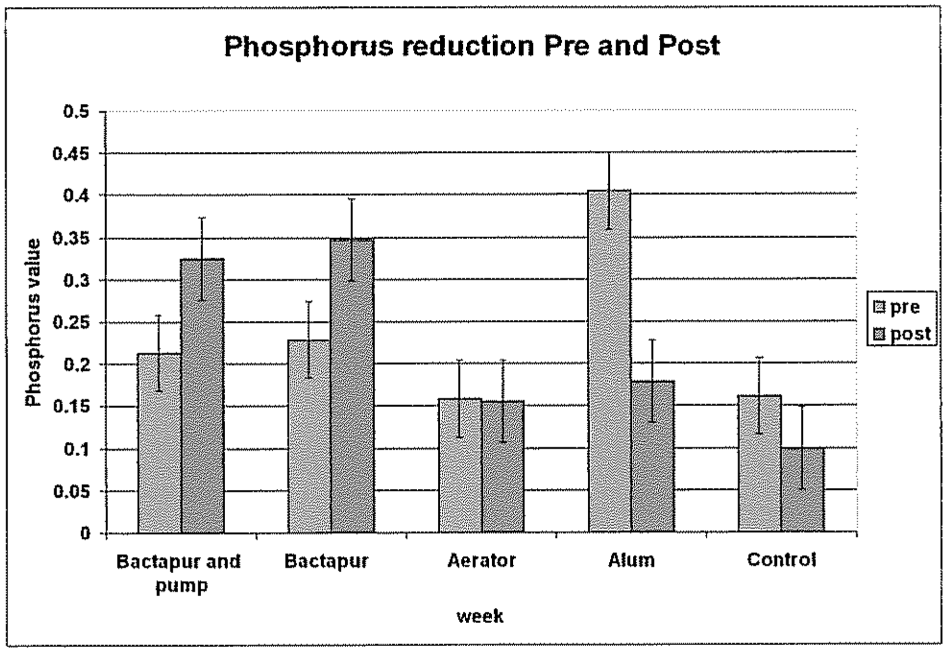


Fig. 2. Effects of five treatments on chlorophyll a concentrations (mg/dl) pre- and post treatment. Bars show means and standard errors.

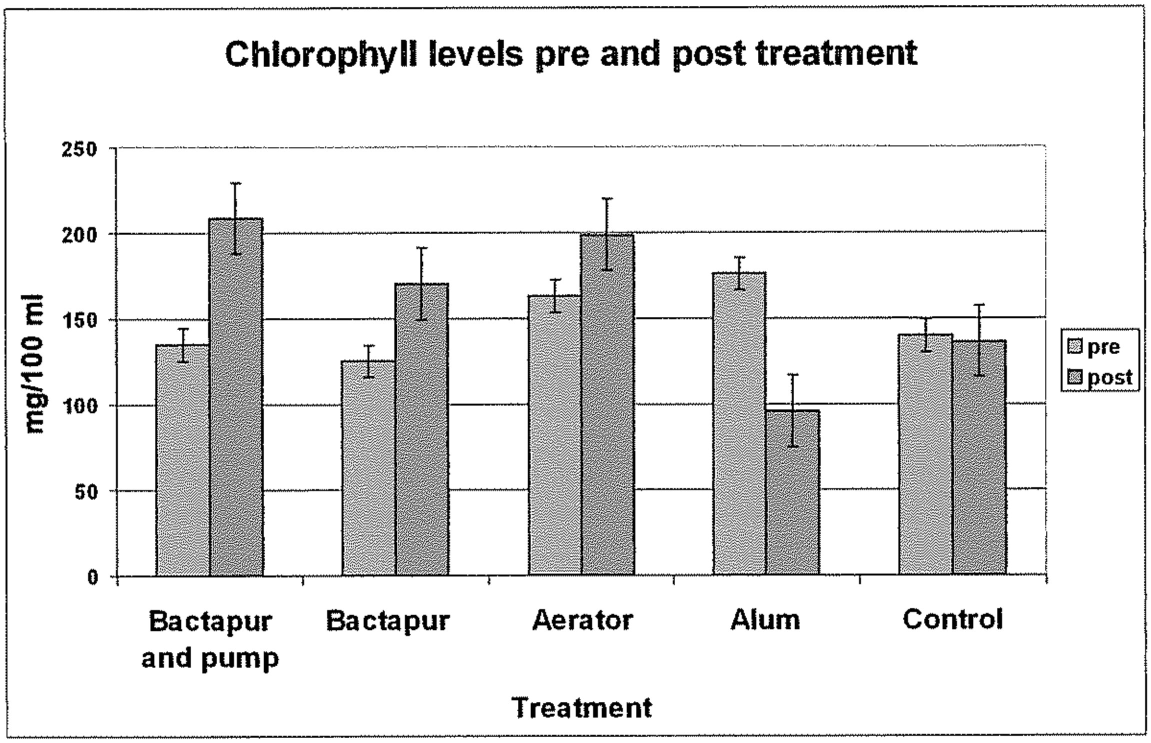
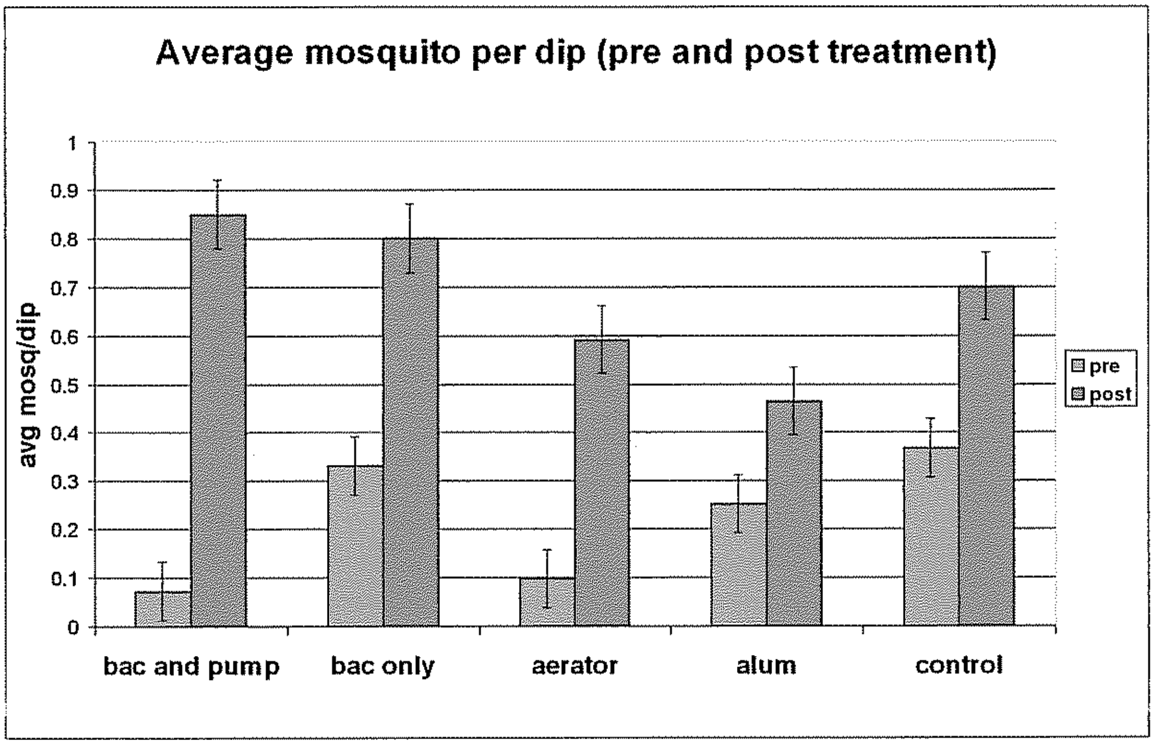


Fig. 3. Effects of five treatments on mean abundance of larval mosquitoes during pre- and post-treatment periods (standard errors are also indicated).



Larval mosquito abundance normally increases during the summer season, as can be seen for all treatments observed in Fig. 3. This represents normal seasonal increases following warmer temperatures. However, the treatment group with the smallest increase was the alum treatment, and this bears further analysis. Once again, Bacta-pur treatments far exceeded the controls.

We also had an interest in looking at variables throughout the season to see how long the treatments took to work and how long they appeared to operate. Phosphate and chlorophyll decreases occurred throughout the study period, except for the 8th biweekly treatment where there was a sudden decrease in chlorophyll, accompanied by a sharp increase in phosphates (Fig. 4). This corresponded to the most intensive part of the drought.

Chlorophyll a concentrations appeared to be low in the alum-treated group overall (Fig. 5), so this may be an unaccounted for source of variation. Phosphate levels were generally low except at the start of the season, and again in the 7th biweekly period. This could also have been a drought-related phenomenon.

Fig. 4. Season-long comparison of phosphate (ppm) and chlorophyll (mg/l) concentrations in the untreated control group.

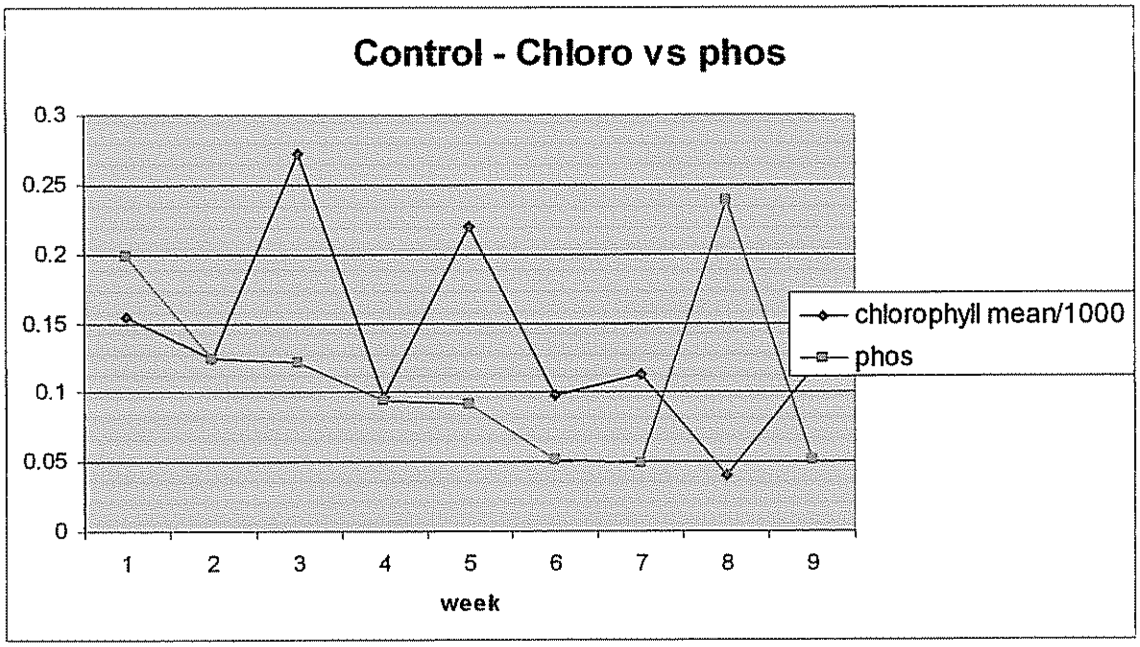
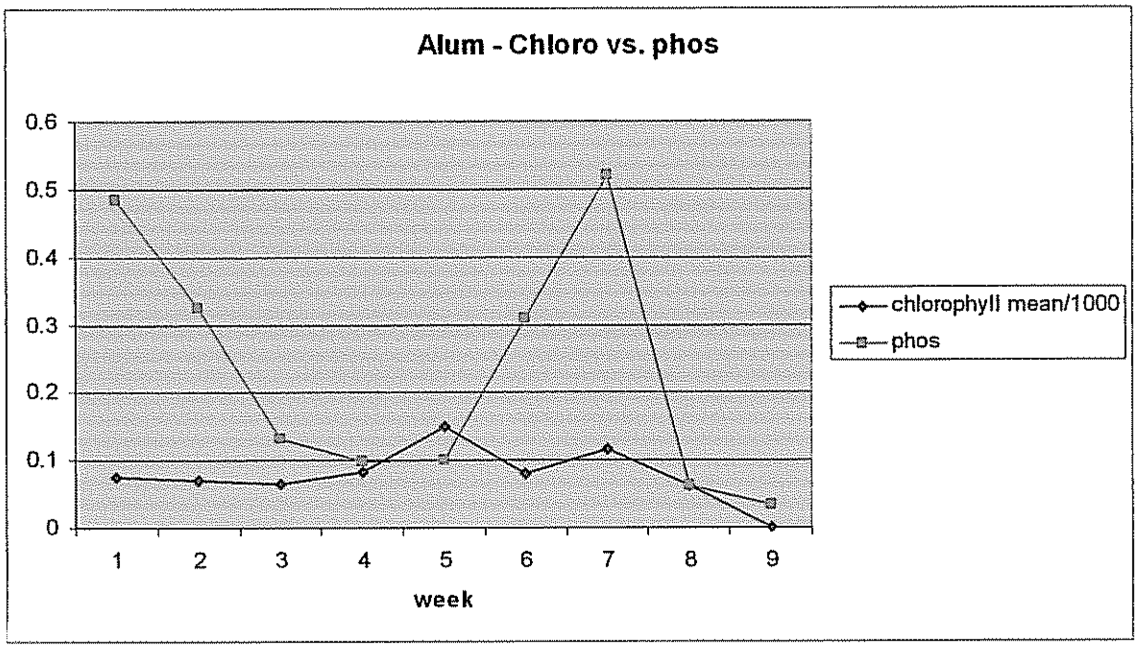


Fig. 5. Season-long concentrations of phosphates (ppm) and chlorophyll (mg/l) in alum-treated ponds.



Chlorophyll concentrations and phosphate concentrations were high overall in the aerator-treatment group (Fig. 6). There was a large rise in phosphate levels during the 7th biweekly period, with a corresponding steep decline in chlorophyll levels, somewhat similar to the control group. Chlorophyll levels appeared to rise following treatment in the Bacta-pur group, with a steep increase in phosphates in between two peaks of chlorophyll (Fig. 7). There was a big dcline in both values during the peak of the drought.

In comparing the relationship of mosquito abundance to chlorophyll a and phosphate levels, chlorophyll appeared to provide the better overall correlation. In the control group (Fig. 8) chlorophyll exhibited a series of descending peaks over the season, as did overall mosquito abundance. In the alum-treated group, Fig 9, both chlorophyll and abundance declined in tandem throughout the period following treatment. Bactapur treatment resulted in higher levels of mosquitoes and chlorophyll throughout the post-treatment period, while the aerator resulted in high levels of chlorophyll but erratic levels of mosquitoes.

In performing separate statistical analyses of chlorophyll and phosphates, we found statistical significance (F = 4.906, sig. at 0.001) among treatments, with only alum reducing chlorophyll a compared to other treatment groups. For phosphates, the differences were mixed, as was already seen in the graphs.

The possibility of direct toxicity of alum to mosquitoes was observed for up to 10 days post-treatment. Two separate tests indicated that alum was not directly toxic to mosquitoes, as there were no significant mortality differences between controls and alum treatments.

Looking just at suspected West Nile virus vector species, we note that in the pretreatment period, the alum treatment ponds had very high levels of *Ae. vexans*, while the Bactapur pump group had relatively high levels of *Culex pipiens* and *An. quadrimaculatus*. The aerator pump group had an overall low level of vector mosquito species during the pretreatment period. However, post-treatment data showed a significant change in these outcomes. The alum group had greatly reduced *Ae. vexans* abundance, while the Bactapur pump group displayed greatly increased *Ae. vexans* abundance. The levels of West Nile virus species abundance also increased in the Bactapur only group, with *Cx. pipiens* greatly advancing its numbers. The overall abundance of all mosquitoes jumped the most in the aerator pump group, Bactapur only, and control groups, while advancing least in the alum and Bactapur pump groups. All groups showed much greater numbers of non-vector species, specifically *Ur. sapphirina* and *Cx. territans*, during the post-treatment period. These species are generally found later in the season, are not human feeders, and not considered nuisance species. We should add that the aerator pump group had only one large peak of abundance (7/31/05), and that otherwise mosquito abundance in this group might have been comparable to the alum and Bactapur pump groups. This information was difficult to tease out statistically because only 4 of 6 ponds included in the aerator pump group could be retained for analysis. Two ponds were discarded from this dataset, one because of a flawed pump installation (Greystone Manor), and the other because of a total absence of mosquitoes in a pond (Dover Mall).

In comparing the findings of treatments on phosphate concentrations, chlorophyll a concentrations, and mosquito abundance, we saw excellent correspondence between the latter two, but more erratic results with phosphate concentrations and treatment groups. We are not quite sure how to interpret this outcome, except to suggest that we may need to adopt better handling methods of samples brought in from the field for phosphate analysis. The samples sometimes went through 1-2 days of variable temperatures from the time of collection to the time of analyses.

Fig. 6. Season-long concentrations of chlorophyll a (mg/l) and phosphates (ppm) in aerator-treated ponds.

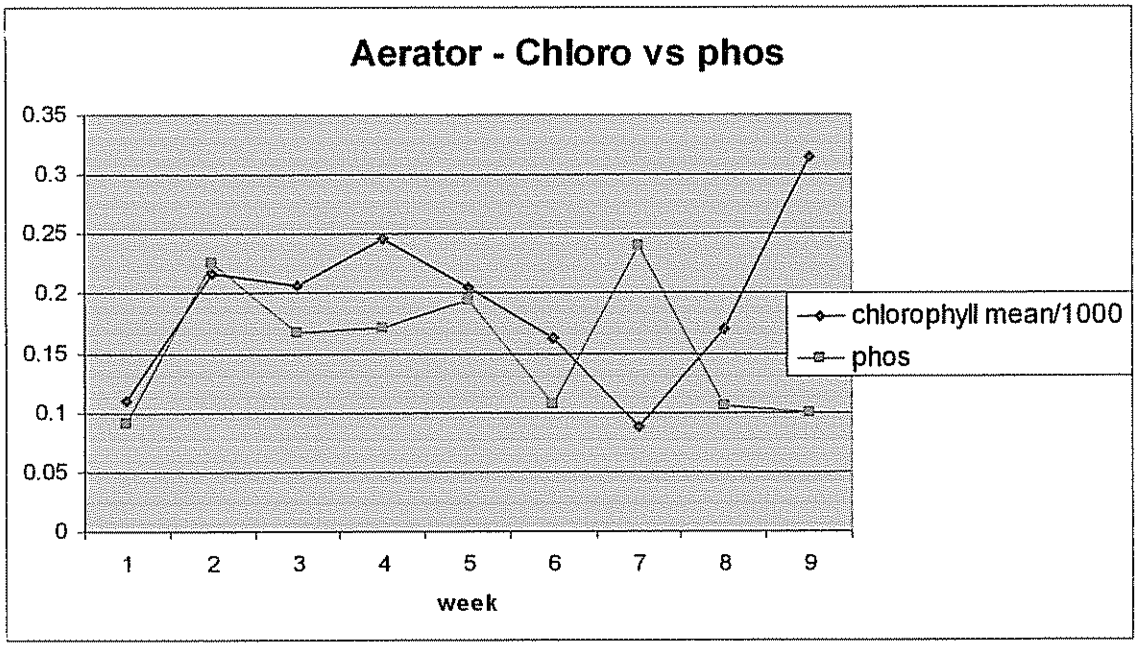


Fig. 7. Season-long concentrations of chlorophyll (mg/l) and phosphates (ppm) in Bactapur treated ponds.

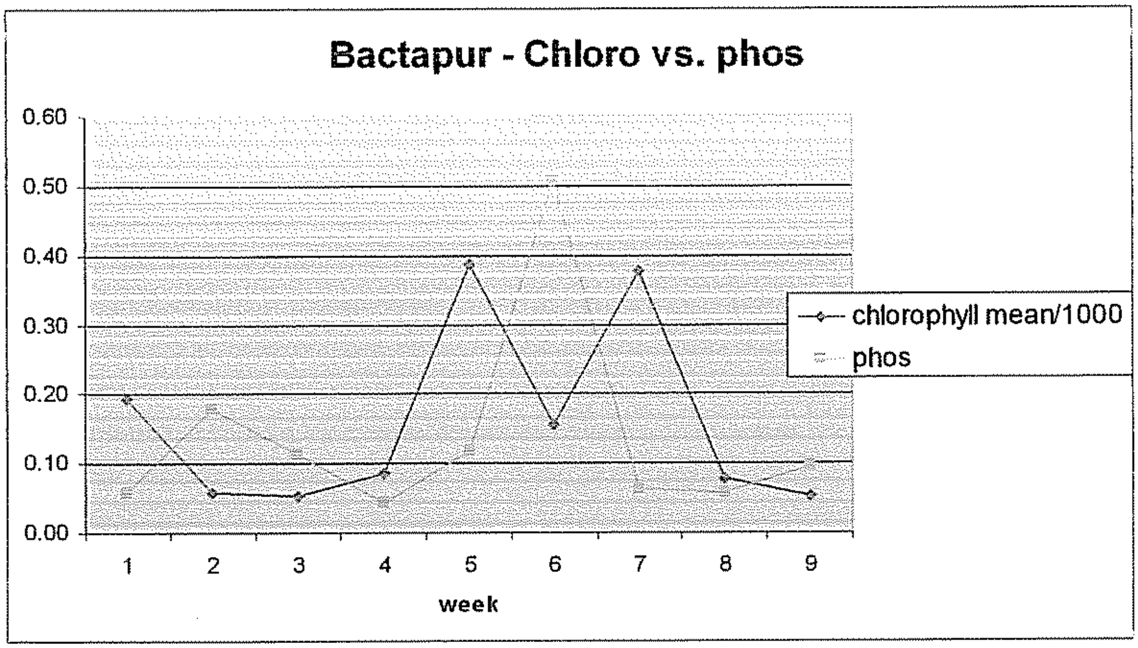


Fig. 8. Season-long concentrations of chlorophyll a (mg/l) and mosquitoes (mosquitoes per dip) in control ponds.

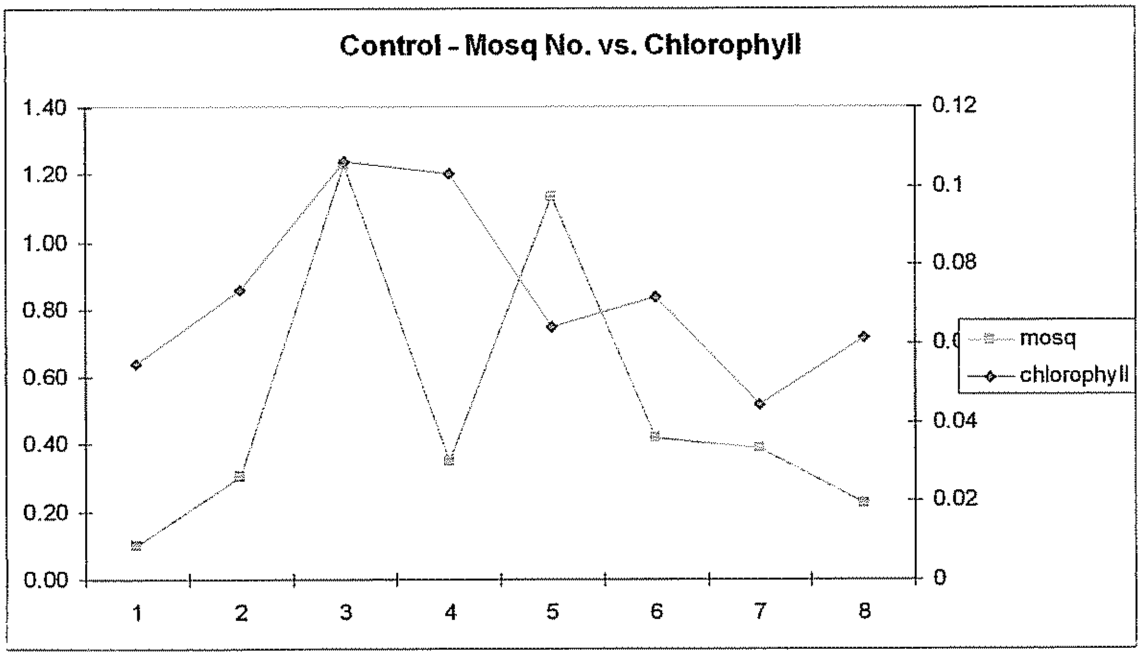
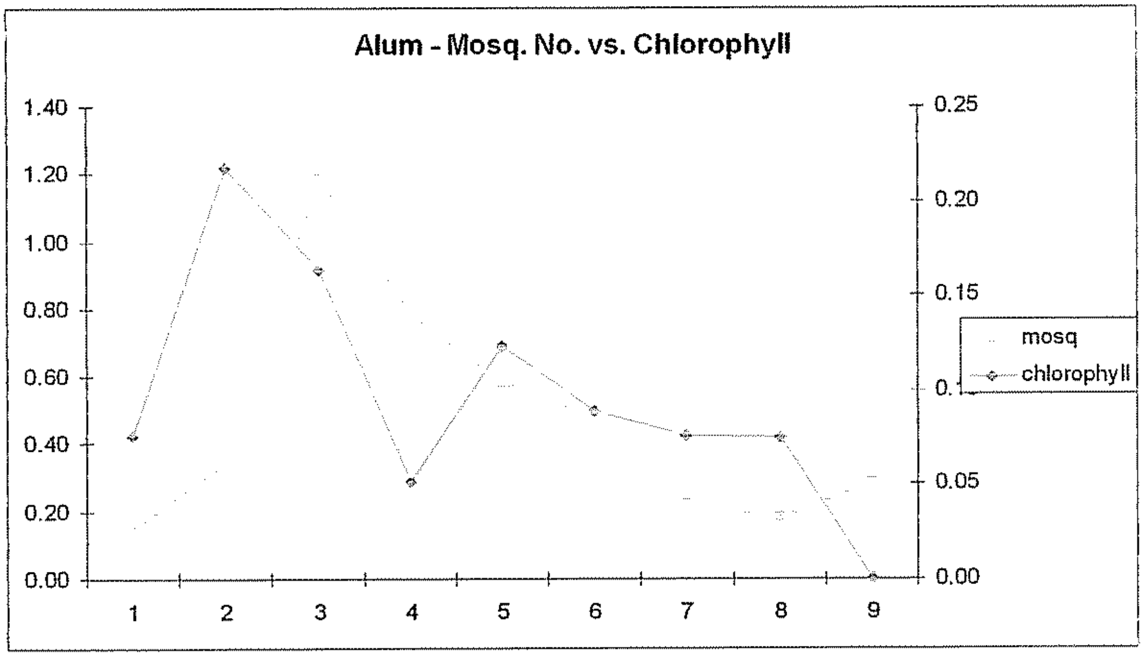


Fig. 9. Season-long concentrations of chlorophyll a (mg/l) and mosquitoes (mean number per dip) in alum-treated ponds.



5. Discussion

Although we made great efforts to minimize sources of variability, it appears that we were only partially successful. For things that we measured on site, which were basically everything except phosphates, we were successful in this endeavor. Measuring phosphates, however, may have been allowed higher variability because there we relied on prompt refrigeration of samples to preserve phosphate concentrations that reflected the situation at the time of collection. However, it appeared that there were some differences in the speed with which samples were placed into cold storage. We therefore feel that this is an area of improvement that will be required for the 2006 study.

Overall, we are satisfied that alum is having a very positive benefit in reducing mosquito abundance, and we remain confident that reduction of chlorophyll a, and probably phosphates, will be shown to be the primary indicators of the success of this treatment. This has, in fact been shown in other studies with alum (James et al. 1991). The direct toxicity effect of alum on mosquitoes appears to be nil, but there could well be a slowing of developmental processes. The reasons for the success of the alum treatment, therefore, are not clear at this point. Certainly, we have to consider that phosphate-dependent organisms may be involved, but that remains an open possibility for future research. Some of these concerns will need to be addressed in specific lab-based studies that allow for better control of variables.

It is also clear that the Bacta-pur treatment, as was mentioned by Duvall et. al. (2001), is not only not reducing mosquito abundance, but it is also not reducing chlorophyll a or bacterioplankton. The aerators, while appearing to improve somewhat upon the control group in terms of phosphate reduction and mosquito abundance, did not do so significantly. On these bases, we decided to eliminate the Bacta-pur and aerator treatment groups from future study in 2006.

One of the more important preliminary findings from these studies is that the most important West Nile vector species, i.e. *Cx. pipiens, Ae. vexans, and Cx. restuans,* appeared to be most affected by alum treatment, while the unimportant species, such as *Cx. territans* and *Ur. sapphirina*, were largely unaffected by this treatment. More details on these findings are going to be key areas of study for 2006 investigations.

6. Plans for 2006

Early in 2006, we will make collaborate with Dr. Marianne Walch and Mr. Randy Cole to lay out our summer of 2006 studies. Our initial plans are to intensify testing of the most successful treatment group, alum, against controls in 2006. We will want to increase the number of ponds in each group (control and alum) from 6 to at least 10 ponds so that we improve our chances of getting a statistically valid outcome, particularly with regard to phosphate reduction. Cutting the number of ponds from 30 to 20-24 will allow us to keep only our most consistent ponds, and eliminate some that are borderline in terms of size, depth, and condition. We will also want to look more carefully at the relationship between vector species and treatment type, and determine if certain species are more likely to be selectively impacted by alum treatment.

In terms of mode of action, we will be looking at comparing the effects of alum treatment of mosquito larvae with various kinds of food placed in rearing pans in the lab. We will be using bacterial and algal food, as well as various types of normal food substrates to makes these comparisons with and without alum treatments. During these studies, we will be hatching eggs of vector species such as Cx. Pipiens directly in the study pans, and measuring development time and survivorship across the life cycle ranging from first instar larvae to pupae.

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James, WF, JW Barko, and WD Taylor. 1991. Effects of alum treatment on phosphorus dynamics in a north-temperate researvoir. Hydrobiologica 215: 231-241.

Appendix 1. Publication of research of first year's research, April 15, 2004 – April 14, 2005.

Stormwater Ponds, Constructed Wetlands, and Other Best Management Practices as Potential Breeding Sites for West Nile Virus Vectors in Delaware during 2004

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STORMWATER PONDS AND BREEDING OF WEST NILE VIRUS VECTORS

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Abstract

We performed longitudinal surveys of mosquito larval abundance (mean mosquito larvae per dip) in 87 stormwater ponds and constructed wetlands in Delaware from June–September, 2004. We analyzed selected water quality factors, water depth, types of vegetation, degree of shade, and level of insect predation, in relation to mosquito abundance. The 2004 season was atypical, with most ponds remaining wet for the entire summer. In terms of West Nile virus (WNV) vectors, wetlands predominantly produced *Aedes vexans, Culex p. pipiens,* and *Cx. restuans.* Retention ponds generally produced the same species as wetlands, except that *Cx. p. pipiens* was more abundant than *Cx. restuans* in retention ponds. *Aedes vexans* and Cx. *salinarius* were the most abundant species in Conservation Restoration Enhancement Program (CREP) ponds. Sand filters uniquely produced high numbers of *Cx. restuans*, *Cx. p. pipiens*, and *Aedes j. japonicus*, a newly-invasive vector species. Sites that alternately dried and flooded, mostly detention ponds, forebays of retention ponds, and some wetlands, often produced *Ae. vexans*, an occasional WNV bridge vector species. Overall, seasonal distribution of vectors was bimodal, with peaks occurring during early and late summer periods. Ponds with shallow sides and heavy shade generally produced an abundance of mosquitoes, unless insect predators were abundant. Bright, sunny ponds with steep sides and little vegetation generally produced the fewest mosquitoes. The associations among mosquito species and selected vegetation types are also discussed.

KEY WORDS: Stormwater ponds, breeding sites, mosquito, West Nile virus

Introduction

With the West Nile virus (WNV) cases standing at 2,470 cases in 2004 (CDC, 2005), compared to 9,862 cases in 2003 (CDC, 2003), in many areas it appeared that the disease was temporarily in an interepidemic phase. However, in examining the zoonotic outbreaks in sentinel chickens and mosquito vector species, we found that zoonotic transmission in Delaware continued to be active, if not so widely as it was in 2003 (Williams and Gingrich, 2004, unpublished data). Following serious flooding associated with heavy storms during the summer of 2003-2004, particularly in New Castle County, it appears that one of the major programs to alleviate and prevent flooding in the area requires construction of greater numbers of retention ponds in flood-prone communities (Wilmington News Journal, 12/22/04). In addition, 1987 amendments to the Clean Water Act of 1972 required states to develop nonpoint source pollution management programs, resulting in the installation of many more stormwater ponds (BMP's) since that time (Copeland 2003). Since 2003, any new construction involving an area of 5,000 square feet or more began requiring a stormwater mitigation plan and a BMP of some sort (http://www.codemanage.com/norwalk/index.php?topic=18-18). The purpose of our study was to analyze the mosquito production potential of stormwater ponds, and ways to characterize these ponds, that we began in 2003.

Our primary objective for 2004 was to further evaluate and compare mosquito vector production and larval abundance at five different types of BMP's/wetlands, including: (a) Retention ponds; (b) detention ponds; (c) Conservation Enhancement and Preservation Program (CREP) ponds; (d) constructed wetlands; and (e) sand filters. Ancillary objectives included: (1) Determine the physical and biological attributes of good and poor mosquito habitats among the various types of stormwater catchments; (2) in areas where suspected vector species are present, determine the species associations present that might result in the sites being foci of virus activity; (3) determine seasonal factors that might influence mosquito breeding; and (4) determine additional biotic and abiotic factors that influence mosquito abundance. Stormwater ponds were selected in conjunction with input from the Delaware Department of Transportation (DELDOT), the Division of Soil and Water Conservation, Delaware Department of Natural Resources and Environmental Control (DNREC), and Mosquito Control in Milford and Glasgow (DNREC Division of Fish and Wildlife).

Materials and Methods

1. Initial Surveys, Research Plans, Database Development, Equipment, and Contracting

Preliminary surveys of ponds were conducted during March and April by our project staff. Surveys included visiting 87 stormwater ponds, created wetlands, sand filters, and CREP ponds across all three counties in the state. These ponds and wetlands supplemented 53 similar sites that we studied on a preliminary basis in 2003, and included most of the same sites from that year.

The breakdown of site types used in our 2004 study was as follows:

1. Retention ponds – 50

2. Detention ponds – 22
3. CREP's – 6
4. Created wetlands – 6
5. Sand filters - 3

Specific preliminary characteristics of ponds sought for our study were as follows:

a) Location on or near a state highway or housing development.
b) Having steep or shallow sides with vegetation on at least two sides of the shoreline.
c) Ponds with or without partial or heavy shade.
d) Absence of killifish fish (*Fundulus* spp.).
e) Ponds with or without abundant algae.
f) Ponds unaffected by agricultural runoff.

Materials and Methods

1. Initial Pond Surveys

During the initial spring survey of each pond, we conducted a perimeter and structural survey (inlets, outlets, forebays) to locate subsites of apparent likely mosquito activity. Information was obtained on pond size, overall depth, vegetation, and BMP design type. We also looked for killifish (*Fundulus* spp.), which were very clear in the spring, using mosquito dippers and observing for ripples signifying characteristic killifish activity. Ponds with such fish were excluded from the study. Six to ten subsites at each pond were dipped during the initial survey using a standard 350–ml dipper, with 10 dips per subsite obtained. Those with potential larval habitat characters were selected as subsites – regardless of whether or not larvae were actually collected during the preliminary survey. A total of five subsites were selected per pond, except for constructed wetlands and CREP sites, which had larger numbers of subsites selected commensurate with their size. Sand filters had 3-6 subsites selected per site, also depending on size.

2. Mosquito Collections

We collected mosquito larvae on a biweekly basis at each pond and subsite, with eight collections made during the season from June 1 to September 15, 2004. Twenty dips for mosquitoes were made per subsite, or 100 dips per pond, on average. All larval mosquitoes were placed in individual 4-oz. Whirl-pak bags (Bioquip, Inc. Rancho Dominguez, CA) for each subsite and then returned to the laboratory. Physical measurements of the water were taken at each subsite using a Combo-Pen (Hanna Instruments, Woonsocket, RI) and included pH, conductivity, total dissolved solids, and temperature. In addition, a light meter (Ex-Tech Instruments, Tampa, FL) was used to measure lux quantity at the water surface of each subsite. Unobstructed light readings were also measured at arm's length above head height in direct sun, and the percent reduction of sunlight was calculated by dividing the subsite surface lux reading by the unobstructed lux reading overhead, obtaining a percent, and subtracting it from 100. Shade (percent light reduction) created by vegetation at the water surface of subsites was categorized and indexed as follows: (1) Low = 0-29 percent reduction of light; (2) moderate = 30–59 percent reduction; (3) heavy = 60–89 percent reduction; and (4) deep shade = > 90 percent reduction.

Biotic factors at each subsite were also measured, including invertebrate predator numbers and types, major vegetation species present and estimated percent of water surface occupied by vegetation. We concurrently counted individuals from the following invertebrate predator groups as we performed our larval mosquito collections:

(1) Notonectidae – backswimmers
(2) Corixidae – water boatmen
(3) Carnivorous mosquitoes – *Toxorhynchites* and *Psorophora ciliata*
(4) Damselflies - suborder Anisoptera
(5) Nepidae – water scorpions
(6) Dragonflies – suborder Zygoptera
(7) Dytiscidae – predaceous diving beetles
(8) Hydrophilidae – predatory water beetles
(9) Gyrinidae – whirligig beetles
(10) Copepoda - copepods

At each subsite, we summed all of the above predators per 20 dips, and then assigned an index number for each collection. Predator indices were based on the total number of predators per subsite as follows: (1) None = 0; (2) 1-3 = 2; (3) 4-10 = 3; and (4) >10 = 4.

3. Preliminary Coordination Arrangements

Early in the year (April) we met with the Delaware Department of Natural Resources and Environmental Control (DNREC) staff from the State's two Mosquito Control sections. We provided them with a list and location of our expected sites, and requested that these be excluded from mosquito control actions. Also, we asked them not to seed these specific ponds with mosquito killifish.

4. Sampling Plan

Sampling of mosquito larvae began during the week of June 7, 2004. Mosquito larvae were collected from 87 sites (average 17 sites per person) per collection period. Each pond site consisted of 5 subsites, with 20 dips per subsite, for a total of 100 dips per site per collection. Constructed wetlands and CREP ponds generally had more subsites (up to 14), depending on their overall size. Eight collections were made on all 87 sites on a biweekly basis throughout the season, with the last collection made during the second week of September, 2004.

5. Laboratory Handling, Identification, and Data Handling

All collections were brought from the field to laboratory as indicated previously. Using stereozoom microscopes, larvae were identified at the third or fourth instars to species, using taxonomic keys of Darsie and Ward (1981). First and second instars were raised in 32-oz. mosquito breeders with funneled top until they reached the third or fourth instar. Pupae were identified to genus using a key by R.W. Lake (2001, personal communication). If adults emerged prior to identification, they were similarly identified using the taxonomic keys of Darsie and Ward (1981). *Aedes j. japonicus* was identified using figures and a description by Tanaka et al. 1979, while *Ae. albopictus* was identified using a 2001 key by B. A. Harrison (2001, personal communication). Those mosquitoes that were not identifiable to species were identified to genus. After identification, all species were enumerated and tabulated according to date, site, and subsite.

6. Preliminary Data Analyses

Preliminary analyses consisted of basic computation of the mean larval number (either by individual species or by all combined species) per dip for each site and subsite, then plotting them graphically against a number of variables. We also grouped ponds according to pond (BMP) type for purposes of these graphs. Basic ecological variables included in the analyses included pond category (retention, detention, etc.) steepness/shallowness of ponds (> or < 30°, percent of shade (percent light reduction) at subsites, predator index, pH, plant associations, total number of mosquitoes per site per dip, and vector species per site per dip. Vector species were considered those locally-occurring species rated at vector competence level 2-plus or greater as specified by Turell et al. (2005).

7. Statistical Analyses

The mean larval numbers per site were compared for each factor of interest (namely predator index, shade index, pond type, pond bank steepness, collection date) using a paired t-test ($P < 0.05$). We also analyzed for differences in vector species according to pond type. We used a one-way analysis of variance (ANOVA) provided in a statistical package from the SAS Institute (Cary, NC, 1994). Another SAS program was used to test for associations among species, including vegetation type and mosquito species. The procedure used was stepwise multiple regression from the SAS Institute (vs. 9.1, 2003).

Results

a. Basic Ecological Variables

1) Pond types – As we observed in 2003 (unpublished data), detention ponds produced more larval mosquitoes per dip than all other pond types. This was most striking during the second week of August, when the peak mosquito numbers were observed (Fig. 1). Retention ponds, CREP ponds, and constructed wetlands also produced peak numbers in August, but yielded no statistical differences.

2) Seasonal distribution – Combining mean larval numbers per dip for all 87 sites according to date collected, we observed a bimodal seasonal distribution similar to that observed in 2003 (Gingrich, unpublished data) for larvae and in 2001 for adults (Gingrich and Casillas 2004). Peak abundance of mosquitoes occurred during the weeks of 6/29/04 and 8/24/04, with the second peak being nearly double the size of the first peak (Fig. 2). It was noted that both seasonal peak dates for larvae in 2004 were about two weeks later than they were in 2003 (Gingrich, unpublished data).

3) Steepness of ponds – The sides of the pond were considered steep if the shoreline dropped at an angle of greater than 30 degrees, and not steep (shallow) if the angle was less than that. Steep retention ponds, on average, produced less than one-third the numbers of mosquitoes per dip that shallow ponds did. T-tests indicated differences between these two pond types at $P < 0.01$ (Fig. 3). This was shown consistently through most of the season. This difference was similar, but more pronounced, than what was observed in 2003 (Gingrich, unpublished data). Other types of ponds were excluded from this analysis because of insufficient numbers of steep ponds in the other pond categories.

4) Predators – Insect predator indices were used for computing this graph, as discussed in Materials and Methods. Mosquito numbers generally were inversely related to predator indices. Mosquito numbers were highest when predator numbers were zero, and low when indices were moderate or high (Fig. 4). This trend was also observed in 2003 (unpublished data).

5) Shade – As observed in Fig. 5, mosquito numbers were greatest when shade was deep (>90 %). Low shade indices were also associated with slightly higher mean mosquito numbers, while moderate and heavy shade produced statistically similar results. This apparent discrepancy between low shade and deep shade were resolved by comparing species composition according to the degree of shade (Table 1). Species that were frequently found in deep shade included *Ae. vexans, An. punctipennis, Cx. salinarius, Cx. territans,* and *Ur. sapphirina,* while *Cx. p. pipiens, Cx. restuans,* and *Ae. sollicitans* were found more often in ponds with low shade.

There appeared to be no large differences when low, moderate or heavily shaded areas were compared. These 2004 shade values were based on light meter reading ranges described in the Materials and Methods.

6) West Nile vector species according to pond type – Considering abundance and vector competence (Turell et al., 2001, 2005), five species are considered the most important vectors breeding in Delaware ponds, including *Aedes vexans, Ae.j. japonicus, Culex p. pipiens, Cx. restuans,* and *Cx. salinarius.* The most abundant potential WN virus vector was *Ae. vexans* in all four pond types (Table 2), but not in sand filters. However, *Ae. vexans* was much more abundant in CREP's and detention ponds than it was in retention ponds and constructed wetlands. On the other hand, *Cx. p. pipiens,* generally considered the most important overall WN vector in the mid-Atlantic area (Fonseca et al. 2004), was far more abundant in retention ponds than it was in other pond types, while *Cx. salinarius,* a fairly good vector, was most abundant in CREP's. *Culex restuans* was more abundant in detention ponds than it was in other pond types. *Aedes j. japonicus,* the newly invasive species of interest, was only present in low numbers in most ponds. Moreover, it was present in substantial numbers in sand filters, along with two other WN virus vectors, *Cx. p. pipiens* and *Cx. restuans.*

7) Association between WN vector species and vegetation – Looking at the 5 vector species discussed above, plus two minor potential vectors (*Ae. sollicitans* and *Ae. albopictus*), we found some associations between certain plant groups and mosquito species. On a preliminary basis, we observed that *Ae. vexans, Ae. sollicitans, Cx. p. pipiens*, and *Cx. salinarius* appeared to show some associations with certain vegetation types. Using stepwise multiple regression, *Ae. vexans* appeared very strongly associated with another vector species, *Ae. sollicitans* (P <0.0001), but not with any particular vegetation group. Moreover, *Ae. sollicitans* was strongly associated with *Ae. vexans*, but not with any vegetation group. *Culex p. pipiens*, however, appeared associated with loosestrife (*Lythrum* spp.), *Ludwigia* spp., and grasses (family Poaceae; P< 0.02) and *Cx. salinarius* (P< 0.0001), but only during the late season (August – September). *Culex salinarius* showed its strongest associations with *Cx. p. pipiens* (P< 0.0001*)* and *Phragmites* spp. (P<0.06), but only in the early season. There were not enough data on the other mosquito species and plant associations to perform statistical analyses.

8) Unexpected Species
Some species not normally found in artificial ponds or wetlands were collected. These included *An. barberi, Ae. albopictus, Ae. j. japonicus, Ae. triseriatus, Cs. melanura, Orthopodomyia signifera*, and *Toxorhynchites rutilus septentrionalis. In* reviewing the data from Table 2, with the exception of *Ae. j. japonicus* (n = 86), the numbers collected were very small - usually 4 or fewer.

Discussion

If you consider that the most prevalent mosquito species in ponds in 2004 was *Ae. vexans*, a floodwater mosquito, then it would not be too surprising that detention ponds, which are designed to only intermittently hold water, produce more potential vectors than retention ponds. This was also shown by Santana et al. (1994), although their terminology for detention and retention ponds was the opposite of today's conventional usage of those terms. Detention ponds normally dry out within 5 days, but most were holding water for at least 2 weeks at a time during our study, which easily permitted mosquitoes to mature, but still allowed floodwater oviposition sites to dry out and reflood to stimulate hatching. Bimodal seasonal peaks in production of mosquitoes appear to be the norm with mosquitoes in this region (Gingrich and Casillas, 2004). There is normally a burst of rainfall in the late spring that leads to a primary mosquito brood in June. Also, a second major brood is produced just prior to the onset of fall. Of course, there are generally several interim broods, but the late spring and early fall broods are usually the most consistent. The delay in the development of mosquito abundance peaks in 2004 by about two weeks compared to 2003 appeared to be driven by the cooler than normal average temperatures in 2004.

Steepness of ponds in this region appears to be inversely correlated with mosquito abundance. Shallow ponds provide several benefits to mosquitoes, including easier penetration of light for development of algae and diatoms (food sources), easier access to food for mosquitoes (quicker transit time from top to bottom of ponds), and more abundant hiding places among emergent plants (which are far more prolific in shallow ponds). Predators and shade are also major drivers of mosquito abundance. Scarcity of predators is a key to mosquito survival, while deep shade probably also affords some protection, assuming potentially poorer visibility to predators.

That *Ae. vexans*, a floodwater mosquito, was most abundant in ponds that exhibited intermittent dry periods (i.e. detention ponds) was as expected. The other associations between ponds and various species were less easily understood, and may have been more attuned to associations with dominant plants in the various pond types. *Ludwigia*, for example, occurred most often in retention ponds, and in turn this species favored *Cx. p. pipiens*. *Culex salinarius* was more abundant in ponds with loosestrife, grasses (Poaceae), duckweed and *Phragmites*, and these tended to be found primarily in permanent ponds or wetlands. *Culex restuans* has similar associations, and also a possible association with rushes (Juncaceae). These tended to occur in selected permanent ponds or wetlands with good shade. The association of *Ae. sollicitans* with rushes would seem to be related to the predominance of these groups in coastal wetlands.

Unexpected species found during the study deserve a few remarks. The finding of *Ae. j japonicus* is explained by the fact that it was found mostly in sand filters or in rocky forebays of stormwater ponds, both of which are highly suitable habitats. *Culiseta melanura*, which normally occurs in swamps, occurred in one of our constructed wetlands, and these wetlands often resemble swamps. For the most part, *An. barberi, Ae. albopictus, Ae. triseriatus, Or. signifera,* and *Tx. r. septentrionalis* occur in treeholes or artificial containers. It should be noted that in some constructed wetlands, tree stumps are placed in the water to enhance diversity. These tree stumps, which are emergent in the water, rot in the center and hold water. Occasionally they were included as a part of a subsite, and produced mosquitoes. In the early part of the year, prior to strict guidance from the senior author, some collections were also made from artificial containers if they were actually sitting in the pond or wetland being studied. Some of the container breeders, especially *Ae. albopictus,* were collected in these habitats. In any case, except for *Ae. albopictus*, the number collected were 4 or fewer, so the impact on the overall data was minimal.

In scrutinizing the 87 ponds to see which ones were high producers of mosquitoes, we found 23 ponds that were consistently breeding large numbers of larvae throughout most of the season. Among those were 2 CREP ponds, 2 wetlands, 5 detention ponds, 14 retention ponds, and 1 sand filter. Probably the most common characteristic of these highly productive ponds and wetlands was that many of them became seriously choked by vegetation during the course of the summer, to the point that eutrophication was probably occurring. Similar findings were reported by Santana et al (1994). With rare exception, the high mosquito-producing ponds were shallow, while the wetlands had isolated pools that were often separated from main bodies of standing water. Such isolated pools rarely had predators.

In order to fully explain the underlying reasons why these 23 ponds/wet sites have become high mosquito breeders, we will need to perform more replications of such pond types as sand filters, wetlands, and CREP ponds. The determination of the relevance of ecological and water quality variables to mosquito abundance will be even more challenging, but worth undertaking.

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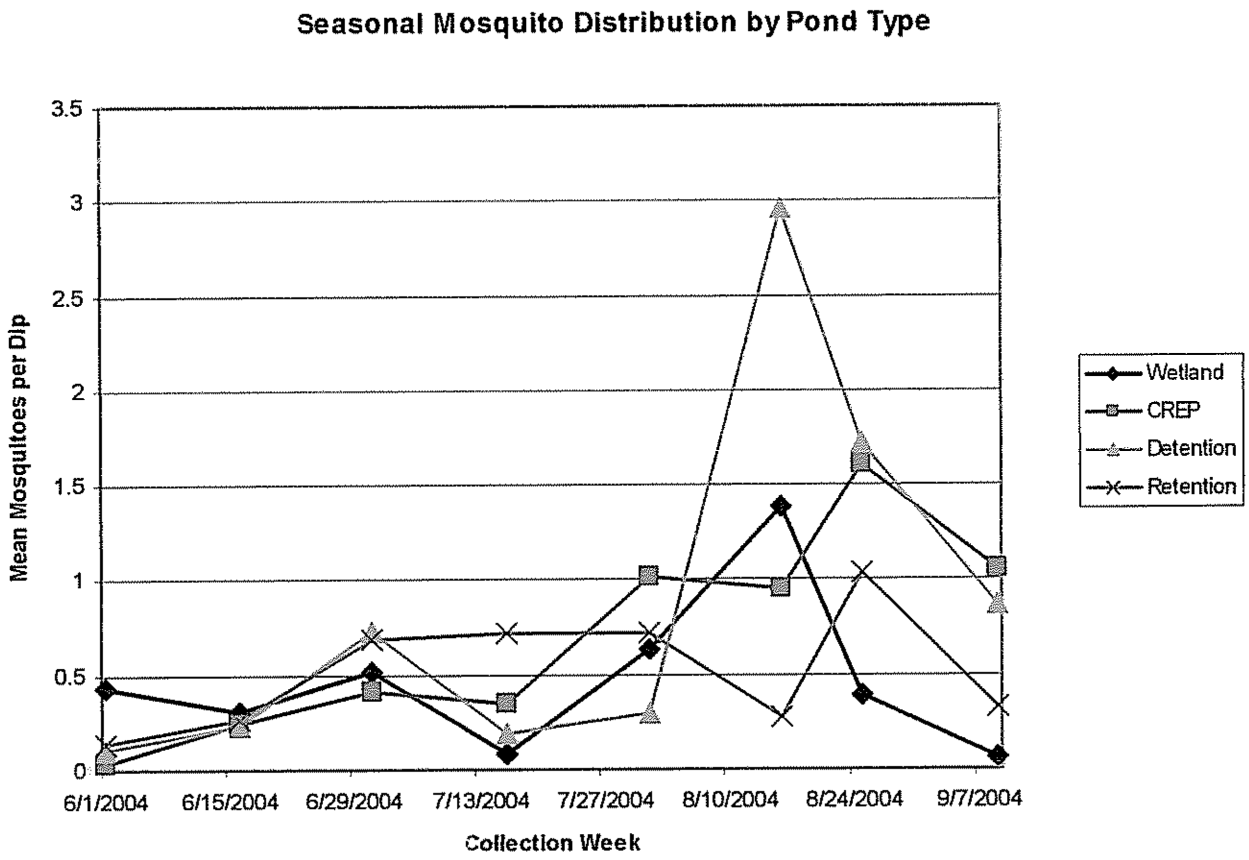

Fig. 1. Mean larval mosquitoes (all species) per dip over 16 weeks in 4 different pond types, including wetland ponds, CREP ponds, steep and shallow retention ponds, and detention ponds.

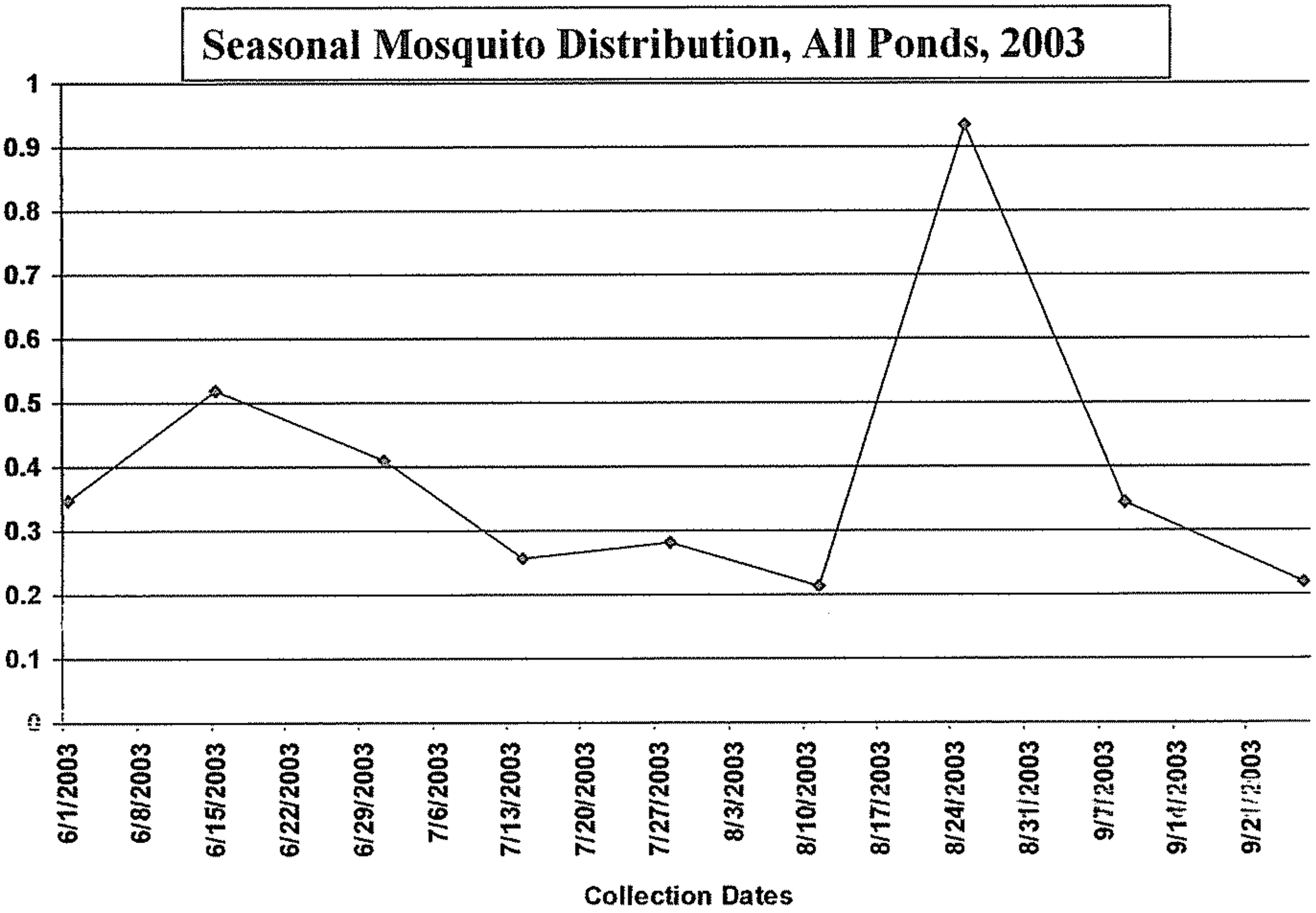

Fig. 2. Mean number of larval mosquitoes (all species) per dip at 45 ponds from June to September, 2003, throughout Delaware

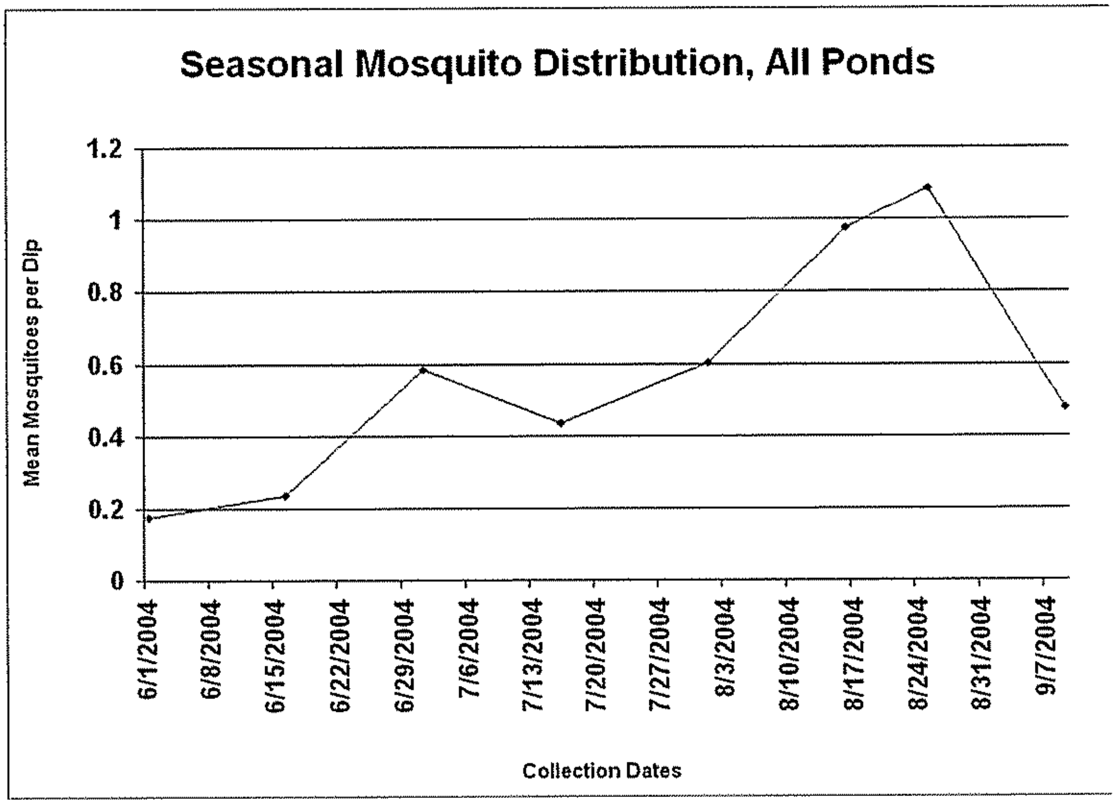

Fig. 3. Mean number of larval mosquitoes per dip at 87 ponds and sand filters from June to September, 2004, throughout Delaware. All species of mosquitoes collected were included in the means.

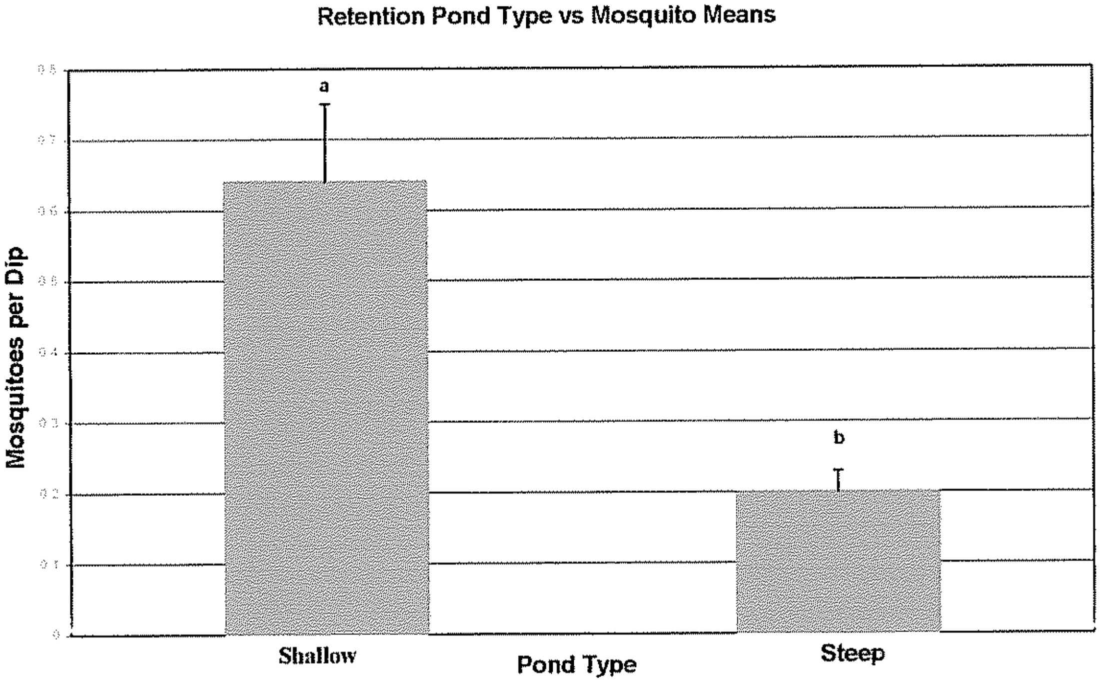

Fig. 4. Comparison of mean mosquitoes per dip (all species) in steep and shallow-sided retention ponds. Standard error bars with different letters are statistically different.

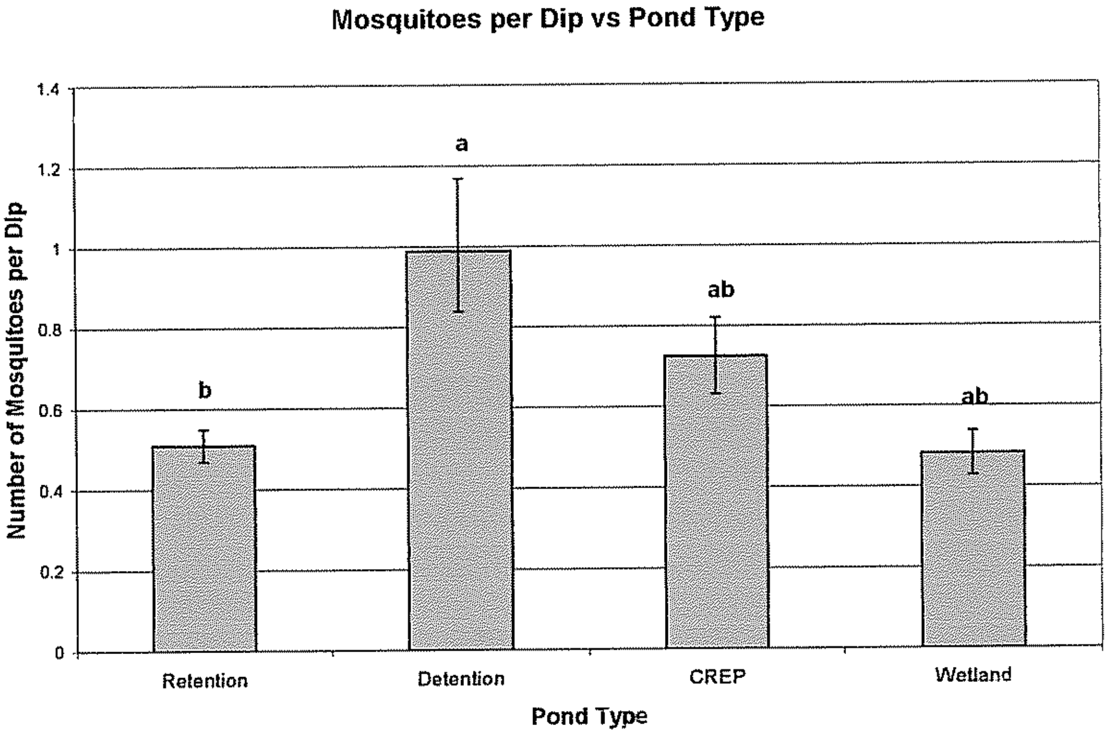

Fig. 5. Mean number of mosquitoes per dip (all species) in four different types of ponds – retention, detention, CREP, and constructed wetland. Standard error bars with different letters are statistically different.

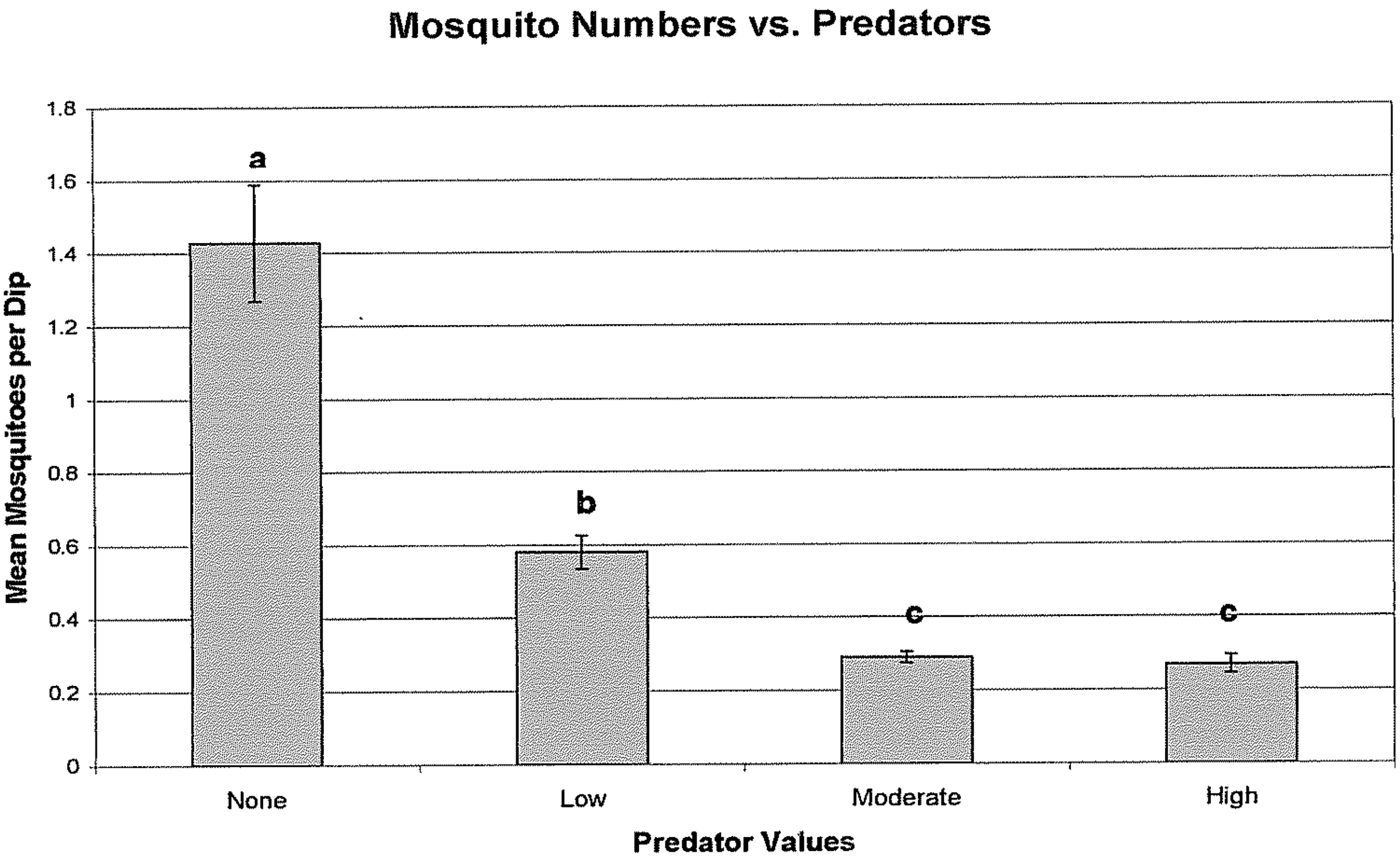

Figure 6. Mean number of larval mosquitoes per dip plotted against the index value of invertebrate predators per dip. None = 0, low = 1-3, moderate = 4-10, high = >10. Standard errors are shown on bars. Differing letters from bar to bar indicate significant statistical differences of P <0.05, while the same letters indicate no difference.

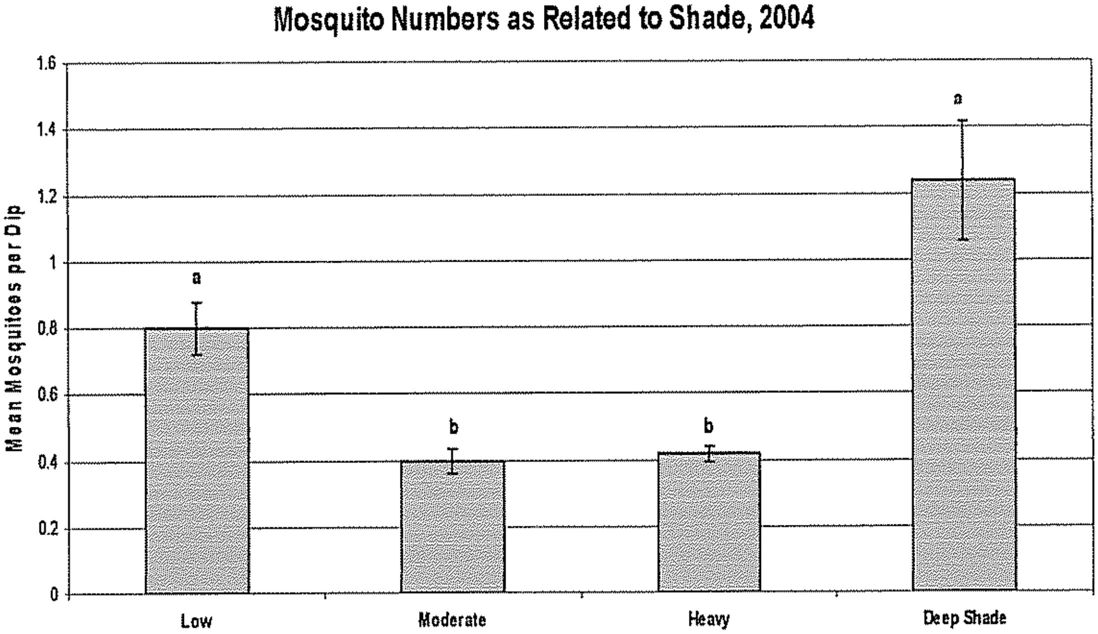

Figure 7. Mean larval numbers of mosquitoes per dip as related to shade index. Indices as follows: low = 0-29 % light reduction; moderate = 30-59 % reduction; heavy = 60-89 % reduction; and deep shade = >90 % reduction. Bars are shown with standard errors. Differing letters = statistical significance by t-test at $P < 0.05$., while the same letters indicate no difference.

Table 1. Common suspected West Nile virus vector species (*Aedes vexans, Culex pipiens, Cx. restuans,* and *Cx. salinarius*) are shown according to 5 pond types, including constructed wetlands, CREP ponds, detention ponds, retention ponds and sand filters. Means and standard deviations are shown, along with n-values. Values followed by differing letters within the same column are significant by ANOVA (P <0.05), while those with the same letters are not.

	Aedes vexans		***Culex p. pipiens***		***Cx. restuans***		***Cx. salinarius***		***Ae. j. japonicus***	
	n	mean ± std	n	mean ± std	n	mean ± std	n	mean ± std.	n	mean ± std
Wetlands	54	2.97 ± 7.45 a	5	1.33 ± 2.2 abc	7	1.04 ± 1.21 ac	13	0.57 ± 0.91 ab	2	0.15 ± 0.07 a
CREP	115	6.22 ± 14.27 a	3	0.13 ± 0.06 abc	2	0.98 ± 1.31 abc	5	3.16 ± 4.5 ab	0	---
Detention	113	5.27 ± 26.21 a	48	0.77 ± 1.2 bc	21	1.07 ± 2.56 bc	32	0.28 ± 0.41 b	0	---
Retention	156	3.69 ± 14.62 a	48	1.77 ± 3.97 bc	20	0.32 ± 0.34 b	54	0.66 ± 1.14 b	1	0.2 ± n/a
Sand Filters	2	0.08 ± 0.35 b	33	5.37 ± 9.34 a	5	8.96 ± 16.41 a	1	0.1 ± n/a	6	0.36 ± 0.32 a

Table 2. Total of larvae, sum of mean number of larvae per dip (cumulative basis) by species for 87 ponds, followed by separation of these summed collections into mean larvae per dip according to fo categories of shade. Deep shade = >90% light reduction; heavy shade = 60-89% light reduction; moderate shade = 30-59 % light reduction; low shade = 0=29 % light reduction.

Species	Total Larvae	Sum Larvae Per Dip	Deep shade	Heavy	Moderate	Low
Aedes albopictus	26	1.3		0.85	0.1	0.35
Aedes atlanticus	4	0.2		0.2		
Aedes atropalpus	1	0.05		0.05		
Aedes canadensis	1	0.05			0.05	
Aedes j. japonicus	86	4.3	0.75	0.25		3.3
Aedes sollictans	300	15	0.3	2.9	1.65	10.15
Aedes spp.	1007	50.35	3.5	3.55	16.9	26.4
Aedes sticticus	12	0.6		0.6		
Aedes sticticus	12	0.6		0.6		
Aedes taeniorhynchus	8	0.4				0.4
Aedes triseriatus	1	0.05			0.05	
Aedes vexans	29471	1478.95	599.5	296.6	235.9	346.95
Anopheles barberi	1	0.05	0.05			
Anopheles bradleyi	114	5.7	0.6	1.45	2.6	1.05
Anopheles crucians	130	7.45	1.2	2.8	1.35	2.1
Anopheles punctipennis	1294	66.7	28.05	17.2	13.95	7.5
Anopheles quadrimaculatus	1110	55.5	8.6	20.75	13.3	12.85
Anopheles spp.	278	13.9	3.65	2.95	4.9	2.4
Anopheles walkeri	92	4.65	2.15	1.6		0.9
Culex erraticus	341	17.05	5.8	2.8	6.95	1.5
Culex p. pipiens	5087	404.5	59.9	33.3	34.8	276.5
Culex restuans	1292	100.75	12.15	10.65	17.95	60
Culex salinarius	1486	74.35	22.65	23.15	20.3	8.25
Culex spp.	1393	94.35	24.35	11.6	14.35	44.05
Culex territans	10691	543.55	254.75	193.35	67.75	27.7
Culicine spp.	35	1.75		0.85	0.75	0.15
Culiseta melanura	2	0.1		0.05	0.05	
Orthopodomyia signifera	14	0.7		0.5	0.15	0.05
Psorophora ciliata	112	5.6	3	0.5	1.6	0.5
Psorophora columbiae	161	8.05	0.2	1.65	1.9	4.3
Psorophora ferox	20	1.05	0.15	0.55		0.35
Psorophora horrida	2	0.1				0.1
Psorophora howardii	81	4.05	0.1	0.75	0.3	2.9
Psorophora spp.	76	3.8	0.2	0.5	0.7	2.4
Toxorhynchites rutilus	4	0.2		0.05	0.15	
Uranotaenia sapphirina	1189	60.4	11.95	31.55	15.1	1.8

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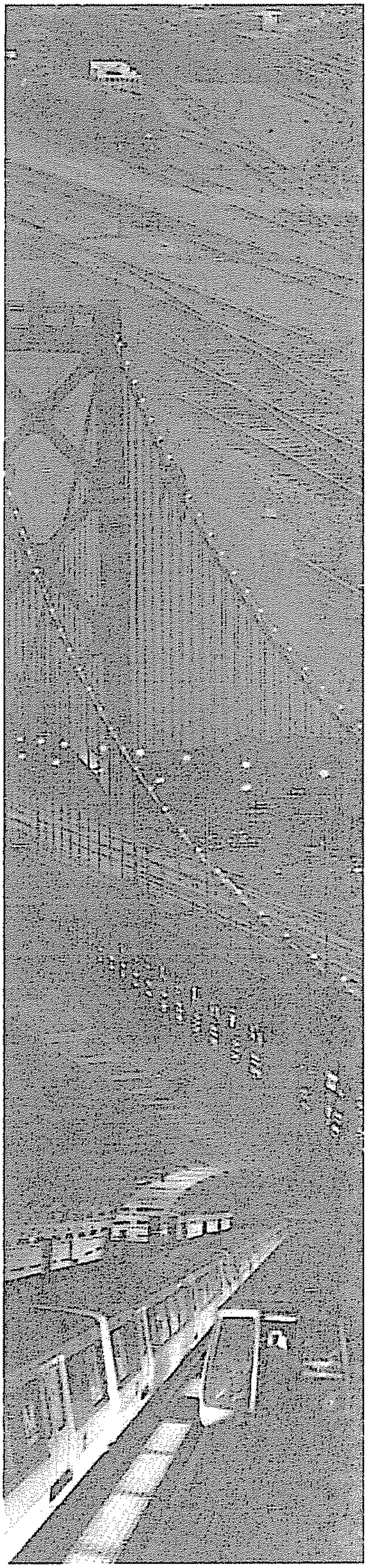

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