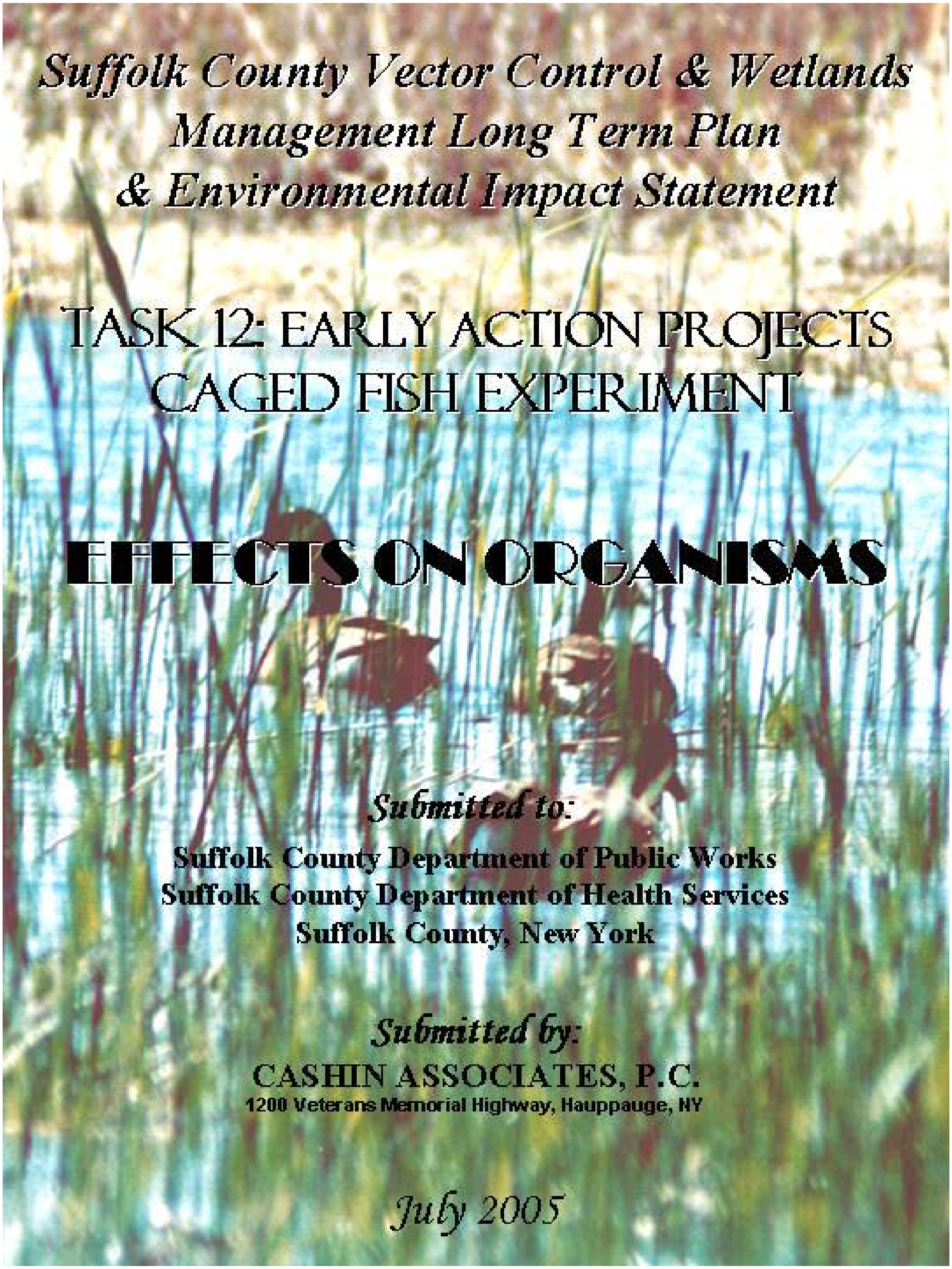

Suffolk County Vector Control & Wetlands Management Long Term Plan & Environmental Impact Statement

TASK 12: EARLY ACTION PROJECTS CAGED FISH EXPERIMENT

EFFECTS ON ORGANISMS

Submitted to:

Suffolk County Department of Public Works
Suffolk County Department of Health Services
Suffolk County, New York

Submitted by:

CASHIN ASSOCIATES, P.C.
1200 Veterans Memorial Highway, Hauppauge, NY

July 2005

SUFFOLK COUNTY VECTOR CONTROL AND WETLANDS MANAGEMENT LONG - TERM PLAN AND ENVIRONMENTAL IMPACT STATEMENT

PROJECT SPONSOR

Steve Levy
Suffolk County Executive

Department of Public Works
Charles J. Bartha, P.E.
Commissioner
Richard LaValle, P.E.
Chief Deputy
Leslie A. Mitchel
Deputy Commissioner

Department of Health Services
Brian L. Harper, M.D., M.P.H.
Commissioner
Vito Minei, P.E.
Director, Division of Environmental Quality

PROJECT MANAGEMENT

Project Manager: Walter Dawydiak, P.E., J.D.
Chief Engineer, Division of Environmental Quality, Suffolk County Department of Health Services

Suffolk County Department of Public Works, Division of Vector Control
Dominick V. Ninivaggi
Superintendent
Tom Iwanejko
Principal Environmental Analyst
Mary E. Dempsey
Biologist

Suffolk County Department of Health Services, Office of Ecology
Martin Trent
Acting Chief
Kim Shaw
Bureau Supervisor
Robert M. Waters
Bureau Supervisor
Laura Bavaro
Senior Environmental Analyst
Phil DeBlasi
Environmental Analyst
Jeanine Schlosser
Principal Clerk

SUFFOLK COUNTY LONG TERM PLAN CONSULTANT TEAM

Cashin Associates, P.C.	**Hauppauge, NY**
Subconsultants	
Cameron Engineering, L.L.P.	Syosset, NY
Integral Consulting	Annapolis, MD
Bowne Management Systems, Inc.	Mineola, NY
Kamazima Lwiza, PhD	Stony Brook University, Stony Brook, NY
Ducks Unlimited	Stony Brook, NY
Steven Goodbred, PhD & Laboratory	Stony Brook University, Stony Brook, NY
RTP Environmental	Westbury, NY
Sinnreich, Safar & Kosakoff	Central Islip, NY
Bruce Brownawell, PhD & Laboratory	Stony Brook University, Stony Brook, NY
Anne McElroy, PhD & Laboratory	Stony Brook University, Stony Brook, NY
Andrew Spielman, PhD	Harvard School of Public Health, Boston, MA
Richard Pollack, PhD	Harvard School of Public Health, Boston, MA
Masahiko Hachiya, PhD	Harvard School of Public Health, Boston, MA
Wayne Crans, PhD	Rutgers University, New Brunswick, NJ
Susan Teitelbaum, PhD	Mount Sinai School of Medicine, NY
Zawicki Vector Management Consultants	Freehold, NJ
Michael Bottini, Turtle Researcher	East Hampton, NY
Robert Turner, PhD & Laboratory	Southampton College, NY
Christopher Gobler, PhD & Laboratory	Southampton College, NY
Jerome Goddard, PhD	Mississippi Department of Health, Jackson, MS
Sergio Sanudo, PhD & Laboratory	Stony Brook University, Stony Brook, NY
Suffolk County Department of Health Services, Division of Environmental Quality	Hauppauge, NY

Primary research for this report was conducted by Anne McElroy of Stony Brook University, Robert Turner, and Christopher Gobler of Southampton College. This report was prepared by Anne McElroy of Stony Brook University. It was reviewed and edited by Cashin Associates (personnel including Elyse O'Brien and David J. Tonjes, PhD).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

N Raw dissolved oxygen data for 8/18/04 adulticide
O Raw dissolved oxygen data for 8/25/04 adulticide
P Raw dissolved oxygen data for 9/1/04 larvicide
Q YSI dates and measurements

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
CA	Cashin Associates, P.C.
DO	Dissolved oxygen
LC_{50}	Lethal concentration for 50% of organisms
LD_{50}	Lethal dose for 50% of organisms
NYSDEC	New York State Department of Environmental Conservation
SCDHS	Suffolk County Department of Health Services
SCVC	Suffolk County Vector Control
ULV	Ultra Low Volume
USGS	United States Geological Survey
USEPA	United States Environmental Protection Agency

ABSTRACT

During the summer of 2004, a caging study was conducted to assess potential effects of ultra low volume (ULV) application of Scourge® and Altosid® on estuarine fish and shrimp in several salt marshes in Suffolk County, NY. The study was part of a larger effort to assess the ecological risk of vector control activities in the county. Juvenile sheepshead minnows (*Cyprinodon variegatus*) and adult grass shrimp (*Palaemonetes pugio*) were deployed in cages placed in ditches or small creeks located within two sprayed and two reference marshes. Organisms were deployed in most cases the day before a spray event, and their survival was monitored daily for five days after six spray events. Temperature and dissolved oxygen at each site were monitored and recorded at 30-minute intervals throughout each experiment. Fish growth and shrimp prey capture ability were also assessed as sublethal endpoints in organisms retrieved from each experiment, and static renewal toxicity tests were performed on shrimp in the laboratory using water collected from each site 30 minutes after spraying. We did not consistently observe acute mortality or sublethal effects in organisms caged in sprayed versus unsprayed marshes, although transient periods of low dissolved oxygen at these sites may have influenced results obtained.

Purpose

Healthy wetland ecosystems are essential to estuarine and coastal ecology and are directly related to the well being of coastal populations. But, inhabitants of coastal regions are often inundated with mosquitoes due to the suitable nature of wetlands for mosquito breeding. A variety of vector control methods have been used to control mosquito populations. These have been intensified recently in response to public concern about the dangers of the West Nile Virus. The use of pesticides to control mosquito populations can be considered a source of chemical contamination that could potentially compromise the quality of wetland ecosystems. The purpose of this project was to determine whether aerial spraying of chemical adulticides (Scourge®) and larvicides (Altosid®) used in vector control is likely to have adverse effects on local fish and invertebrate populations in Suffolk County saltwater wetlands. Results obtained will support an informed risk assessment of aerial spraying. Although designed to specifically address questions related to the safety of vector control in Suffolk County, the resulting work is transferable to saltwater wetlands regionally and nationally.

BACKGROUND

In estuarine ecosystems, the grass shrimp *Palaemonetes pugio* is an important component of the food web, aiding in breakdown of detritus and acting as a prey species for higher trophic level organisms (Wirth et al., 2001). Due to their importance, this species has been widely used by the United States Environmental Protection Agency (USEPA) as an estuarine health indicator. Furthermore, marine crustaceans are reported to be extremely sensitive to pesticides, particularly to pyrethroid forms (Bradbury & Coats, 1989).

Salt marshes and similar wetlands provide suitable habitat for many species of wildlife, which often includes mosquitoes. Wetlands provide mosquitoes with standing water, a necessary habitat for the larval form of their lifecycle. Most efforts at reducing mosquito populations with insecticide applications target this more vulnerable larval stage. The most widely used larvicide in the eastern U.S. is Altosid®, a product containing 20 % S-methoprene, the toxic isomer. Methoprene is an insect growth regulator that is not toxic to the adult or pupal stage. Methoprene allows treated larvae to pupate, but prevents juvenile mosquitoes from successfully completing the final molt required to become an adult (Wirth et al., 2001).

Currently Altosid® is used most extensively to control mosquito larvae in Suffolk County, with another product, Scourge®, being used on a limited basis to combat adult mosquitoes when populations become a significant nuisance or West Nile Virus is detected. Scourge® is the trade name for an insecticide containing resmethrin, a pyrethroid, as its active ingredient. Pyrethroids are neurotoxins, and their effects can directly or indirectly cause mortality (Bradbury and Coats, 1989). Specifically, pyrethroids interfere with the ionic conductance of nerve membranes by prolonging the sodium current. This stimulates nerves to discharge repeatedly causing hyper-excitability in poisoned animals (Narahashi et al., 1998). Although Scourge® and Altosid® are intended to kill only insects, pesticide runoff or direct application to surface waters in marshes puts other organisms at risk, particularly marine crustaceans whose physiology is most similar to insects.

The acute toxicity of pyrethroids to non-target organisms is quite high, as compared to that of methoprene, with crustaceans generally being more sensitive to pyrethroids than fish (Clark et al., 1989). Cypermethrin LD_{50}s for brown trout, carp and rainbow trout were found to be 1.2, 0.9 and 0.5 µg/L, respectively (Stephenson, 1982). Coats et al. (1989) described the pyrethroid toxicity for crustaceans to be well below 1 µg/L. Cripe (1994) observed LC_{50}s for pink shrimp, *Penaeus duorarum*, to be as low as 0.012 µg/L in static tests using fenvalerate, which is two orders of magnitude lower than LC_{50}s observed for fish. For resmethrin, Rand (2002) observed 96-h LC_{50}s for freshwater fish, sheepshead minnows (*Cyprinodon variegatus*), and pink shrimp (*P. duorarum*) to be 2.36-15.0, 11.0 and 1.3 µg/L, respectively. The toxicity of resmethrin and methoprene to larval lobsters (*Homarus americanus*) was examined by Zulksoky et al. (submitted). Under continuous exposure in flow-through systems, 48 and 96 h LC_{50}s were reported to be in the range of 0.10-0.20 µg/L, while methoprene (a mixture of both S and R isomers) was not toxic at the highest concentration tested, 10 µg/L. Wirth et al. (2001) also failed to observe toxicity of methoprene to *P. pugio* at 1 mg/L concentrations, while Brown et al., (1996) reported a 96-h LC_{50} of 14.32 µg/L for S-methoprene (the more toxic isomer) with the estuarine shrimp, *Leander tenicornis*.

In addition to acute toxicity, pyrethroids and other pesticides are known to have deleterious sublethal effects on reproduction, growth, and behavior in aquatic organisms (Coats et al., 1989). Wirth et al. (2002) observed a decrease in the overall appearance of gravid females over time at a South Carolina site following sublethal, chronic endosulfan exposure. *Gammarus pulex*, a freshwater amphipod, exposed to 0.05 µg/L of esfenvalerate for 1 h resulted in the disruption of reproducing pair formation, release of eggs or offspring from the brood pouch and delays in reproduction even after transference to clean water (Cold and Forbes, 2004). DeGuise et al. (2004) did not observe acute mortality in adult lobsters at methoprene and resmethrin concentrations of 221 and 1 µg/L, respectively; however, phagocytosis (the ability of phagocytes to ingest bacteria or other foreign bodies) was significantly decreased at 33 and 0.01 µg/L respectively of these pesticides. Ross et al. (1994) observed a negative impact on juvenile fish growth after exposure to methoprene. The ability of *P. pugio* to locate and capture food (brine shrimp) was negatively affected by sublethal exposure to sediment from environmental sites exposed to industrial and municipal discharges, oil spills and leachate from landfills (Perez and

Wallace, 2004). Sublethal pesticide effects are important measures of pesticide toxicity due to the fact that they can indirectly lead to organism death by lowering reproductive output or the ability of an organism to locate and capture prey, and thus grow. Potential ecological effects of pesticide exposure are, in fact, likely to be underestimated by short term LC_{50}s.

During the summer of 2003, investigators from Southampton College conducted a study to evaluate the effects of mosquito spraying on salt marshes on Long Island (Southampton College, 2004). Cages of juvenile sheepshead minnows (*Cyprinodon variegates*) were placed in two test marshes both before and after aerial application (Altosid® was sprayed in Oakdale and Scourge® was sprayed in Mastic/Shirley), and in coastal areas that were not sprayed and used as reference sites (Old Fort Pond and Flanders). Decreased growth rates were observed in one of two sites within the marsh sprayed with Scourge®, and decreased growth rates and reduced survival were observed at both sites within the marsh sprayed with Altosid®. This study suggested that further work on the effects of aerial spraying of Suffolk County marshes was necessary.

Project Description

The protocol originally proposed by New York State Department of Environmental Conservation (NYSDEC) envisioned assembling a team to maintain test organisms ready to go within 24 hours notice to respond to helicopter spraying events as implemented by Suffolk County Vector Control (SCVC) as part of its normal operations. After careful consideration, the research team determined that such a plan would involve unnecessary costs and would not provide adequate lead time to find and test locations suitable for the conduct of caged studies. Therefore, during the months of June and July a number of marshes on the south shore of Long Island were assessed for their suitability as test sites for the caging study. Access, likely spray history and the presence of ditches maintaining sufficient water to support caged organisms throughout a tidal cycle were our primary selection criteria. Once identified as a suitable location, cages containing both fish and shrimp were deployed for a series of days to access survival. Midway throughout this process, personnel at NYSDEC informed us that they would not approve a scientific permit to spray adulticide from helicopters to support the study. They did agree however to consider issuing a permit to upgrade what would have usually been an operational ground application of adulticide to a helicopter spray in select areas, providing no environmental impact of spray or research activities was likely. Approval to conduct this study on Gilgo Island, Fireplace Neck and the Mastic Shirley area was eventually obtained. Havens Point and Flax Pond were chosen as reference marshes, being the only nearby marshes with adequate access to marsh creeks that were not routinely sprayed with pesticides and showed good survival of caged organisms in trial deployments.

In addition to evaluation of the potential impacts of aerial pesticide application described below, other members of the team working on this project also assessed related parameters. Notably, the effectiveness of pesticide application on adult and larval mosquitoes was assessed by Suffolk County Department of Health Services (SCDHS), SCVC personnel, and the concentration of pesticides in the water immediately surrounding the cages and in nearby sediment was assessed jointly by a team from Stony Brook University (Brownawell et al.), the United States Geological Survey (USGS) (Terracciano et al.) and SCDHS (Waters et al.). Their results will be reported elsewhere.

METHODS

Cage Deployment

The original experiment plan called for marsh applications of both adulticide and larvicide on two separate occasions. During each spray we hoped to simultaneously evaluate caged organisms at spray sites and at nearby reference sites that were not sprayed. This would allow the study to be duplicated both in space and in time. However, due to restrictions imposed by NYSDEC on approved spray areas and the limited availability of suitable reference sites, only four sites were suitable for this study. These sites, Timber Point and Johns Neck as spray sites and Havens Point and Flax Pond as reference sites, are shown in Figure 1. Due to the limited number of operational adulticide sprays that were thought to be justifiable last summer, we were only able to evaluate adulticide spraying at the Johns Neck spray site. The study was additionally confounded by low oxygen and high temperature during test deployments. Due to delays in securing approval to allocate funds for this project, fully monitored tests did not commence until August, and extended into the first week of September when spraying operations ceased for the season. Although we had achieved good survival of caged organisms at our test sites prior to this period in preliminary studies, survival at both reference and test sites was sometimes diminished during the period of the fully monitored caging study. The sequence and characteristics of experimental deployments are detailed in Table 1.

Cages

This study utilized a modification of Plexiglas traps designed and successfully deployed in other studies (Scott et al. 1999) for crustaceans, and simple plastic buckets with mesh inserts similar to those used in 2003 by Southampton College for the fish. The crustacean cages consisted of a rectangular Plexiglas cage with a fitting top (15 X 3 X 3 inches) where shrimp are housed in 14 individual compartments, each open to ambient water on two sides and the top through 2 mm window screen. These chambers were placed into larger wire mesh cylinders for predator deterrent. The fish cages consisted of plastic pails with removable lids lined with 1 mm Nitex mesh screen and featuring cutouts on the sides and bottom to allow adequate water circulation. Three cages for each species were deployed at each site with 14 and 20 individuals per cage for shrimp and fish, respectively. Both types of cages were tethered to bricks on the bottom and suspended from floats to allow them to move with the tides and maintain animals at a fixed distance (3-6 inches) below the surface of the water. Floats were attached so as to not interfere with direct deposition of aerosol material to the top of the mesh portions of the cages.

Test Organisms

The sheepshead minnow (Cyprinodon variegatus) and the grass shrimp (Palaemonetes pugio) were used as representative marine fish and crustacean species in this study. The grass shrimp is a common salt marsh resident on Long Island. Furthermore, crustaceans typically are most sensitive to pesticides, thereby making a good test organism (Clark et al., 1989). Sheepshead minnows were chosen as a fish species because they are commonly used in toxicity studies and were used in a pilot study on mosquito spraying on LI conducted by Southampton College in 2003 (Southampton College, 2004; Wirth et al., 2001). The use of young fish allowed growth rates during the experiment to be measured, providing a sublethal measure of effect not available using adult fish as a test species. The fish were purchased from Cosper Inc., a professional bioassay company, prior to each experiment, ensuring identical age and similar health, size, and genetic characteristics.

All test animals were held and maintained either at the Flax Pond Marine Laboratory in Stony Brook (shrimp) or Southampton College's Marine Station in Southampton (fish) prior to use.

Organism Assessment

The study plan called for both shrimp and fish to be deployed the day before a spray event and their survival checked immediately prior to spraying. Shrimp and fish survival were observed daily for the duration of each spray experiment (4 or 5 days). Dead organisms were removed so as not to deleteriously influence the continued survival of live organisms. Length of juvenile fish was measured prior to deployment in the field and after retrieval at the end of each experiment to determine relative growth rates. Shrimp surviving field exposure were brought back to the laboratory and their prey capture rates were determined in feeding trials with live brine shrimp. The ability to locate and successfully capture prey is an excellent measure of locomotor and sensory ability that has been shown to be compromised by sublethal exposure to contaminants found in urban estuaries (Perez and Wallace, 2004). Prey capture response studies were performed on a large proportion of field spray survivors following the completion of each spray experiment. If no (or few) survivors were left at a particular site, individuals used in the static tests were used instead. Large dissection bowls 20 cm in diameter were filled with 1 L of ultra-violet sterilized and filtered Flax Pond seawater and experiments were run for one hour. At the beginning of the experiment, five brine shrimp were placed in the center of all bowls, and every 15 minutes the number of prey consumed was observed and replenished. At the end of the hour, average prey capture rate was calculated and compared among groups.

In addition to the measurements taken on field-collected organisms, static tests were also conducted on reference shrimp exposed to water collected just after each spray event. Approximately 30 minutes following each spray event, 4 L of water from both spray and control sites was collected in dark bottles for static survival tests. Small dissection bowls were filled with 150 mL of water collected from each site, and shrimp acclimated to laboratory conditions were placed one per bowl in six bowls per site for the duration of each spray experiment. The pesticide/water solution in the bowls was replenished once daily, and at this time, shrimp survival was observed. These tests provided an independent test of the toxicity of surface waters immediately after spraying that would not be influenced by site specific water quality factors or cage failure or loss.

Water Quality

Data on water temperature and dissolved oxygen (as % saturation) were collected for the duration of each experiment. WTW 340i dissolved oxygen meters with Durox® probes were used to obtain continuous oxygen data in percent. Dissolved oxygen meters monitored and recorded these variables at 30-minute intervals, and YSI Model 85 readings were taken during daily survival assessments measuring salinity, temperature and dissolved oxygen measurements with which to compare meter readings. A USEPA model based on actual time to death data resulting from exposure to low oxygen was used to determine whether or not diurnal hypoxia observed at many sites during the field study was in itself a significant cause of toxicity (USEPA, 2000).

Statistical Analyses

Toxicity data from each test analyzed by analysis of variance (ANOVA) with post hoc means tests. P-values of 0.05 or less were considered significant. To account for pre-spray mortality, survival data on day 4 was corrected to the percentage of organisms surviving after 1 day of deployment, but pre-spray. These corrected survival data were then subjected to an arcsin transformation prior to application of ANOVA. When the data set was completely balanced with respect to numbers of sprayed and non-sprayed sites we were able to conduct a nested two-way ANOVA to evaluated spray related effects. When data from a site was lost, usually due to high (>80%) pre-spray mortality, one-way ANOVAs were performed.

Description of Spray Events and Measurements and Samples Taken

A large number of personnel cooperated to carry out this study, involving a large suite of measurements taken during the course of each experiment. The time course of these measurements, including those described in this report, is outlined in Table 1.

RESULTS

Survival of Caged Fish and Shrimp

Corrected survival of shrimp and fish deployed at each site is shown in Figures 2-7. The approximate time of the spray event is depicted in each figure. Due to mortality observed frequently after deployment in the field, but before pesticide spraying occurred, post spray survival data was normalized to survival after one day in the field to better assess subsequent changes in survival due to spraying. If survival post deployment was less than 20%, no correction was made and the data were excluded from presentation in these figures. For reference, figures showing uncorrected survival are shown in the Appendix, as are tables showing the numerical survival data from each experiment and the results of the statistical analyses.

As described in Table 1, five sets of deployments occurred where both shrimp and fish cages were deployed, dissolved oxygen (DO) and temperature measured and chemical analysis conducted. In addition, a preliminary fish experiment was conducted on 7/19 where only fish survival and growth were measured. Due to the late arrival of the DO/temperature meters, there is no continuous record of dissolved oxygen during this experiment.

7/20 Larvicide at Timber Point and Johns Neck with Havens Point and Old Fort Pond as reference sites – Figure 2: This preliminary study only examined toxicity in caged fish and did not examine post deployment, pre spray mortality. Two-way nested ANOVA did reveal spray related effects ($p=0.030$) but site-specific tests showed that Johns Neck was the only site showing significantly reduced survival as compared to all other sites including Timber Point.

8/3 Larvicide Spray at Johns Neck and Timber Point with Flax Pond and Havens Point as reference sites – Figure 3: Two-way nested ANOVA of the fish data did not reveal significant spray related differences ($p= 0.059$), although mortality in the sprayed sites tended to be higher. Shrimp survival at Timber Point was less than 20% after their first night in the field (assessed a few hours after spraying); therefore these data were not included in the statistical analysis, or in the figure showing corrected data. One-way ANOVA showed significant differences between shrimp mortality at the other three sites, with mortality at Johns Neck significantly higher than that observed at Flax Pond.

8/10 Larvicide Spray at Johns Neck and Timber Point with Flax Pond and Havens Point as reference sites – Figure 4: Due to the poor survival at Timber Point during the 8/3 spray study, cages were moved to deeper water in this and subsequent experiments at that site. Two-way nested ANOVA showed no significant spray related effects (p=0.09), although there were significant differences between sites with Haven's Point showing increased mortality with respect to Flax Pond or Timber Point. Two-way nested ANOVA of the shrimp data did indicate a significant increase in mortality at sprayed sites (p=0.050). But individual site comparisons also showed increased mortality at Haven's Point as compared to Flax Pond and Timber Point, and at John's Neck as compared to Timber Point and Flax Pond.

8/18 Adulticide Spray at Johns Neck with Havens Point as a reference site – Figure 5: One-way ANOVA did not show significant differences between mortality at the sprayed and non-sprayed sites for fish, although shrimp mortality was significantly elevated at John's Neck as compared to Havens Point (p=0.0005). It should be noted that larvicide was sprayed at Johns Neck just after animal cages were deployed and about 24 hours before adulticide was sprayed at these sites.

8/25 Adulticide Spray at Johns Neck with Havens Point as a reference site – Figure 6: Due to the continued high mortality in the ditch at Johns Neck prior to spraying, cages were also deployed in additional locations at Johns Neck prior to this spray event. In addition to placing cages 6 inches below the surface in the ditch as had been done previously, cages of fish and shrimp were also deployed in the main channel at the entrance to this ditch, and cages of fish were deployed right at the surface in the ditch at Johns Neck and at Havens Point. For both fish and shrimp, survival was excellent in the cages placed in the channel at the end of the ditch at Johns Neck, with significant mortality observed at the Johns Neck ditch site as compared to the Johns Neck channel or Havens Point sites for both species. Significant mortality was also observed at Havens Point as compared to the Johns Neck channel site for fish. It should be noted that in this experiment that organisms were deployed the same day as the spray, so there was no opportunity to assess pre-spray mortality.

9/1 Larvicide at Timber Point with Havens and Johns Neck Channel used a reference site – Figure 7: Only Timber Point was sprayed on this date, but cages were deployed both at Havens Point and Johns Neck for comparison. For fish, significantly greater mortality was observed at Johns Neck ditch or at Havens Point as compared to Timber Point. No significant differences were observed in shrimp mortality, although it should be noted that shrimp were not deployed at the Johns Neck ditch site during this event.

Diel Patterns of Dissolved Oxygen and Temperature

Data on the patterns of DO measured by probes deployed on one cage at each site are shown in Figures 8-12. The actual DO and temperature data obtained is shown in tabular form in the Appendix. The missing values in some cases are due to operator error or probe malfunction. At the time of sampling, DO, temperature and salinity were also measured using a YSI meter and probe. In general, the field deployed recording DO/temperature meters agreed well with the YSI probes. These data are shown in tabular form in the Appendix.

It can be seen from the plots that significant diel variation was observed at all sites. Often DO crashed twice a day at Johns Neck, early in the morning, and late in the evening. Periods of low DO were most pronounced in the ditch at Johns Neck and at the first site at Timber Point (used in the 8/4 deployment). As both the severity of hypoxia and its duration are important determinants of low DO toxicity, a time to death approach developed by the USEPA (USEPA, 2000) was used to estimate when we could expect to see toxicity resulting solely from periodic DO fluctuations. With the help of Glen Thursby (one of the principal developers of the USEPA approach), we applied this approach to data obtained for adult *P. pugio* to determine on which days and at which sites toxicity due to DO alone could be expected. Two criteria were examined, hypoxia likely to exceed the LC_{50} for shrimp (<5% saturation for 1 or more hours or <7 % saturation for 4 or more hours during any 24 hr period) and hypoxia likely to exceed the no observable effects threshold for shrimp (<9% saturation for 2 or more hours during any 24 hr period). Unfortunately, time to death data, in response to controlled hypoxic conditions, were not available for the sheepshead minnows. As the juvenile fish used in our study appeared to be less sensitive to low DO (there were several cases where all the shrimp died, but reasonable fish survival was observed), using the critical values available for the shrimp seemed appropriate.

Using these criteria, toxicity due to low DO alone could have been expected to contribute to mortality in fish at Johns Neck and Timber Point during the 8/3 spray study, at Havens Point and Johns Neck Ditch during the 8/18 and 8/25 spray studies, and on some days (9/1, 9/3, and 9/4) at Havens Point and on all days at the Johns Neck ditch location during the last spray study.

Laboratory Static Renewal Shrimp Toxicity Studies

Four day static renewal toxicity tests conducted in the laboratory on grass shrimp exposed to water collected at the depth of the cages 30 minutes after spraying indicated no toxicity associated with exposure to this water from the spray sites (Table 2).

Growth in Caged Fish

Length measurements taken on fish prior to and after field deployments are shown in Table 3. Growth in this fish was relatively small with large variability between groups. As there were no obvious trends in fish growth, statistical analysis was not conducted.

Prey Capture Studies

Shrimp surviving field deployment were also tested for their ability to capture and consume live adult brine shrimp in the laboratory. In cases where there were insufficient numbers of surviving shrimp from the field deployment, shrimp exposed to water collected from the field in the static renewal toxicity tests were assessed. The results of these experiments are shown in Table 4. No significant differences between sites was observed except during the 9/1 larvicide spray at Timber Point where shrimp deployed at Johns Neck showed significantly lower prey capture ability when compared to shrimp deployed at either Havens or Timber Point ($p<0.002$).

DISCUSSION

The caging study described above, along with the components reported elsewhere (chemical analysis of spray deposition and spray effectiveness), represents a comprehensive examination of the acute effects of aerial spraying of Altosid® and Scourge® in coastal salt marshes. Studies examining both the biological effects and chemical fate of real operational sprays of these pesticides under similar conditions are generally not available in the published literature.

The original plan for this study called for all field work to be conducted prior to the beginning of August to avoid anticipated low DO events that are more prevalent during the hottest period of the summer. Unfortunately, due to many delays in obtaining permission to conduct the study, this was not possible. Preliminary data on caged fish and shrimp survival at all sites showed good survival during July. However, by the time the fully replicated study was performed, this was not the case. Periodic low DO was prevalent at the ditch site at Johns Neck and the ditch site at Timber Point used during the 8/3 spray event (the cages were moved into more open water for subsequent spray events). Later in August, and for the early September spray, low DO was also a problem at our Havens Point reference site. These problems with low DO compromised our ability to detect toxicity that may have been due to pesticide exposure.

During this study, we evaluated larvicide spraying on four occasions (7/20, 8/3, 8/10, and 9/2). During the first spray, reduced fish survival was observed at Johns Neck, as compared to Timber Point, which was also sprayed, and Havens Point and Old Fort Pond, reference sites. Unfortunately, no DO measurements were taken, so we do not know whether or not low DO could have been a factor at Johns Neck. The reduced survival for shrimp observed at Johns Neck and Timber Point, as compared to the reference sites at Havens Point and Flax Pond during the larvicide spray on 8/3 could be attributed to low DO alone. During the 8/10 spray, reduced survival was observed both at Johns Neck and at Havens Point, even though low DO should not have been a problem. During the 9/1 spray, Timber Point, the only site sprayed, showed the best survival of all the sites evaluated at that time. Finally, in the static renewal studies conducted in the laboratory using water collected 30 minutes post spray at each site, excellent survival was observed in shrimp exposed to water from the spray sites. Taken all together, these data do not present consistent evidence of toxicity due to Altosid® application when comparing sprayed to non-sprayed sites.

The absence of acute mortality due to larvicide exposure is not terribly surprising in this study. The active ingredient in Altosid, S-methoprene, acts as a hormone mimic and inhibits molting. As such, in short term tests it would not be expected to kill adult shrimp, which molt only infrequently during the exposure period, nor juvenile fish. As discussed in the introduction, data from the literature also shows that S-methoprene had very limited acute toxicity to crustaceans and fish.

We were only able to follow two Scourge® sprays during this study, one on 8/18, and one on 8/25, both at Johns Neck only. We had hoped to also study adulticide spray impacts at approved sites in Gilgo State Park, but none occurred during the study period. Unfortunately, low DO was persistent enough at both the Johns Neck ditch site and at Havens Point during these sprays to contribute to mortality. In the first study, enhanced fish mortality was observed only at Havens Point, whereas enhanced shrimp mortality was observed at Johns Neck. In the second adulticide spray, organisms were also caged in the creek at Johns Neck. There was no evidence of increased mortality at the Johns Neck creek site, while significant mortality was again observed at Havens Point. Finally, no evidence of reduced survival was observed in static tests with shrimp performed in the laboratory following these adulticide applications.

Although the periodic low DO observed at some sites during the main portion of this study seriously compromised data interpretation, taken all together, the results of this study indicate that operational sprays of either Scourge® or Altosid® in south shore marshes in Suffolk County are not causing significant acute mortality or even sublethal effects on growth or prey capture ability to two non-target species, the grass shrimp and sheepshead minnow. The abundance of wild fish and shrimp observed at these sites is consistent with this conclusion. However, we did not evaluate long-term effects on these and potentially other more sensitive salt marsh organisms. Furthermore, it is important to note that daily excursions of DO at these sites present a severe physiological stress to marsh organisms, particularly those not able to rapidly move away from waters with reduced oxygen. The combination of periodic low DO in addition to potential exposure to stress from anthropogenic chemicals such as pesticides may be a challenge to resident organisms. It remains possible that more subtle changes in offspring fitness or community structure or function may result from repeated pesticide application. The excellent survival observed in organisms caged at the Timber Point and Johns Neck creek site as compared to organisms placed only meters away in shallower ditch sites emphasizes the importance how minor changes in the physical characteristics of an area can influence survival.

The conclusions of this study are in contrast to those observed in a similar study conducted during the late summer of 2003 by Southampton College (Southampton College, 2004). There are several factors that probably contributed to these differences. In the 2003 study, 10 day old fish were used. We had originally planned to use fish of similar age in the 2004 study, but during preliminary experiments, trouble both with fish survival and with fish escaping from the cages led us to use older juvenile fish. Although the older fish generally did well in our cages, they did not grow as fast as the younger fish. Even in our control marshes, significantly positive fish growth was not always observed, making reductions in fish growth potentially due to pesticide exposure almost impossible to detect. The Southampton College study in 2003 only evaluated two spray events, one at each marsh, whereas in the present study six applications of Altosid® and two applications of Scourge® were evaluated. Furthermore, an additional test species, the shrimp, and additional test endpoints were evaluated, and probably most importantly, DO and temperature were recorded for the duration the organisms were deployed in the field. Based on results obtained in 2004, it is clear that low DO contributed significantly to mortality of the caged organisms. There is no way to know how DO may have affected the results of the 2003 study, as continuous DO recordings were not made. However, the reduced survival, most likely due to low DO, observed at the Mastic/Shirley site and even our reference site at Havens Point in 2004 indicates that DO could easily also have been a problem in 2003.

ACKNOWLEGEMENTS

The authors wish to acknowledge the help of many people who made this work possible. Robin Barnes and Brian Gibbins were responsible for carrying out the study, each logging thousands of miles on their personal cars, and tramping out to set, monitor and retrieve cages and samples at all hours of the day and night. Robin also contributed significantly to data analysis and presentation. In addition, personnel at SCVC, the USGS, Cashin Associates (CA), and Bruce Brownawell's laboratory at Stony Brook University, and students in Chris Gobler's and Robert Turner's laboratory at Southampton College provided invaluable help in carrying out this project.

REFERENCES

Bradbury, S. P. and J. R. Coats. 1989. Comparative toxicology of the pyrethroid insecticides. Review of Environmental Contamination and Toxicology, 108. 133-177.

Brown, M. D., Thomas, D., Watson, K., Greenwood, J. G., and B. H. Kay. 1996. Acute toxicity of selected pesticides to the estuarine shrimp *Leander tenuicornis* (decapoda: palaemonidae). Journal of the American Mosquito Control Association, 12(4). 721-724.

Cold, A. and V. E. Forbes. 2004. Consequences of a short pulse of pesticide exposure for survival and reproduction of *Gammarus pulex*. Aquatic Toxicology, 67. 287-299.

Clark, J. R., Goodman, L. R., Borthwick, P. W., Patrick, J. M., Cripe, G. M. and P. M. Moody. 1989. Toxicity of pyrethroids to marine invertebrates and fish: a literature review and test results with sediment-sorbed chemicals. Environmental Toxicology and Chemistry, 8. 393-401.

Coats, J. R., Symonik, D. M., Bradbury, S. P., Dyer, S. D., Timson, L. K. and G. J. Atchison. 1989. Toxicology of synthetic pyrethroids in aquatic organisms: an overview. Environmental Toxicology and Chemistry, 8. 671-679.

Cripe, G. M. 1994. Comparative acute toxicities of several pesticides and metals to *Mysidopsis bahia* and postlarval *Penaeus duorarum*. Environmental Toxicology and Chemistry, 13(11). 1867-1872.

DeGuise, S., Maratea, J. and C. Perkins. 2004, November. Toxicity of pesticides used to control mosquitoes in the American lobster, *Homarus americanus*. Paper presented at the Fourth SETAC World Congress, Portland, Oregon.

USEPA. 2000. Ambient aquatic life water quality criteria for dissolved oxygen (saltwater): Cape Cod to Cape Hatteras. U.S. Environmental Protection Agency, Report EPA-822-R-00-012.

Hook, S.E, and R. F. Lee. 2004. Genotoxicant induced DNA damage and repair in early and late developmental stages of the grass shrimp *Palaemonetes pugio* embryos as measured by the comet assay. Aquatic Toxicology, 66. 1-14.

Narahashi, T., Ginsburg, K. S., Nagata, K., Song, J. H. and H. Tatebayashi. 1998. Ion channels as targets for insecticides. Neurotoxicology, 19(4-5). 581-590.

Perez, M. H. and W. G. Wallace. 2004. Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Environmental Contamination and Toxicology, 46. 81-89.

Rand, G. M. 2002. Hazard assessment of resmethrin: I. effects and fate in aquatic systems. Ecotoxicology, 11. 101-111.

Ross, D. H., Cohle, P., Blasé, P. R., Bussard, J. B., and K. Neufeld. 1994. Effects of the insect growth regulator (s)-methoprene on the early life stages of the fathead minnow, *Pimehhales promelas* in a flow through laboratory system. Journal of the American Mosquito Control Association, 10. 211-221.

Scott, G.I., Fulton, M.H., Moore, D.W. Sirth, E.F., Chandler, G.H., Key, P.B., Daugomah, J.W., Strozier, E.D., Clark, J.R. Lewis, M.A., Finley, D.B., Ellenberg, W., and K. J. Karnaky Jr. 1999. Assessment of risk reduction strategies for the management of agriculture nonpoint sources pesticide runoff in estuarine ecosystems. Toxicology and Industrial Health, 15. 200-213.

Southampton College Technical Report 05-04. 2004. The effects of pesticide application on *Cyprinodon variegatus* (Sheepshead Minnows) in salt marsh ecosystems on Long Island.

Stephenson, R. R. 1982. Aquatic toxicology of cypermethrin. I. acute toxicity to some freshwater fish and invertebrates in laboratory tests. Aquatic Toxicology, 2. 175-182.

Wirth, E. F., Lund, S. A., Fulton, M. H. and G. I. Scott. 2002. Reproductive alterations in adult grass shrimp, *Palaemonetes pugio*, following sublethal, chronic endosulfan exposure. Aquatic Toxicology, 59. 93-99.

Wirth, E. F., Lund, S. A., Fulton, M. H. and G. I. Scott. 2001. Determination of actute mortality in adults and sublethal embryo responses of *Palaemonetes pugio* to endosulfan and methoprene exposure. Aquatic Toxicology, 53. 9-18.

Zulkosky, A. M., Ruggieri, J. P., Terracciano, S. A., Brownawell, B. J., and A. E. McElroy. Submitted. Acute toxicity of resmethrin, malathion, and methoprene to larval and juvenile American lobsters (Homarus americanus) and analysis of pesticide levels in surface waters after Scourge™, Anvil™ and Altosid™ application. Submitted to Journal of Shellfish Research.