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EFFECTS OF ULTRA-LOW VOLUME PYRETHRIN, MALATHION, AND PERMETHRIN ON NONTARGET INVERTEBRATES, SENTINEL MOSQUITOES, AND MOSQUITOFISH IN SEASONALLY IMPOUNDED WETLANDS

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ABSTRACT. Wildlife managers are concerned that insecticides used to control mosquitoes could suppress invertebrates on which wildlife feed. We assessed whether ultra-low volume (ULV) applications of pyrethrin, permethrin, and malathion for control of adult mosquitoes reduced macroinvertebrate abundance and biomass or killed mosquitofish in seasonal wetlands in California. Pyrethrin was applied over 3 seasonal wetlands on Sutter National Wildlife Refuge (NWR), and malathion or permethrin were each applied over 2 seasonal wetlands on the Colusa NWR. Three control wetlands were used per site. We measured aquatic macroinvertebrate abundance and biomass before and after insecticide application and compared the survival of mosquito larvae held in sentinel cages. At Colusa, we also used mosquitofish as sentinels, caged adult mosquitoes over the wetlands to test for pesticide efficacy and drift, and sampled night-flying insects using ultraviolet light traps. Results showed no detectable reductions in the abundance or biomass of aquatic macroinvertebrates in treated wetlands. Larval mosquitoes showed high survival in all areas. All adult mosquitoes died when caged over wetlands treated with malathion or permethrin, but all survived in controls. All mosquitofish survived. Flying insect abundance decreased after insecticide application in both treated and control wetlands but rebounded in 48 h. Results indicated that ULV applications of these insecticides to control adult mosquitoes are unlikely to have substantial effects on the aquatic insects or fish in seasonal wetlands.

KEY WORDS Aquatic macroinvertebrates, California, insects, malathion, mosquito control, mosquitofish, permethrin, pyrethrin, seasonal wetlands

INTRODUCTION

Seasonal wetlands support many species of wildlife that are ecologically, economically, and aesthetically important. Unfortunately, wetlands also produce pestiferous mosquitoes that transmit pathogens to humans, wildlife, and livestock (Eldridge 1989). Mosquito control agencies attempt to reduce disease and pest problems by suppressing mosquito populations, sometimes using methods that can bring wildlife managers into conflict with these agencies. Three general strategies exist for mosquito control. Source reduction is management or reduction of mosquito breeding habitat (see Carlson et al. 1991, Batzer and Resh 1992, for recent reviews). Wildlife managers must sometimes provide oversight of source reduction to preserve or improve wildlife habitat. Larviciding (killing larval mosquitoes) and adulticiding (killing adult mosquitoes) may be accomplished with broad-spectrum insecticides, although larvae can sometimes be controlled with predators or more specific bacterial agents (see Mulla 1994 for a review). Some broad-spectrum insecticides are toxic to fish and all are toxic to insects. Many varieties of wildlife depend on aquatic and terrestrial insects for food, either directly or indirectly through the food chain. Whether insecticides impact fish and invertebrates depends on the quantity, frequency, and method of pesticide application. This research focuses on the nontarget effects of adulticiding using ultra-low volume (ULV) aerosol applications of 3 insecticides.

The objective of our study was to assess the effects of ULV application of 3 pesticides on nontarget invertebrate populations and caged mosquitoes and mosquitofish (*Gambusia affinis*) in seasonal wetlands. The pesticides, pyrethrin, permethrin, and malathion, are broad-spectrum insecticides that are used to control adult mosquitoes in many areas of North America. These insecticides feature relatively low toxicity to terrestrial vertebrates and rapid breakdown (Smith and Stratton 1986, Smith 1987, Mulla 1994). However, considerable concern exists that application of these insecticides could suppress the aquatic insects and other macroinvertebrates that birds and other wildlife use for food. All 3 are also toxic to fish (pyrethrin and permethrin: Smith and Stratton 1986, Coats et al. 1989; malathion: Smith 1987).

Field studies are necessary to assess the effects of these insecticides because the physical and biological complexity of wetlands may influence the level of exposure of organisms to the insecticides. Laboratory studies provide a valuable standardized way of comparing the relative toxicities of chemicals. However, most laboratory tests are conducted indoors in simple arenas using clean water, under conditions where the concentrations of insecticide often exceed field exposures by orders of magnitude (Clark et al. 1989, Day 1989, Hill 1989). Also,

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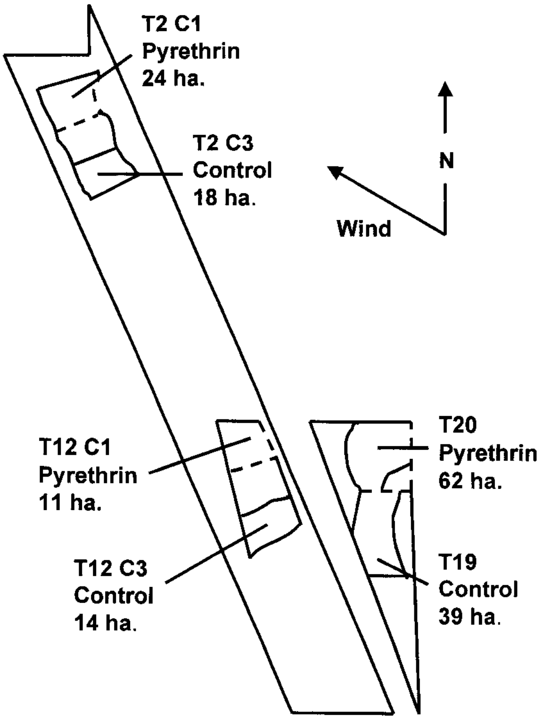

Fig. 1. Map of a site on the Sutter National Wildlife Refuge, Sutter County, California, showing locations and dimensions of wetlands used for assessing the effect of ultra-low volume pyrethrin application on aquatic macroinvertebrates in June 1996. Broken borders represent spray routes.

insecticides may become more toxic in field situations, if field conditions are stressful to the organisms (Coats et al. 1989). Behavioral differences between organisms in the field and the laboratory can also alter insecticide exposure.

MATERIALS AND METHODS

Study areas: Study sites were managed seasonal wetlands in the Central Valley of California. Managed and natural wetlands are the primary overwintering area for over 60% of the waterfowl in the Pacific flyway (Frayer et al. 1989). The studies were conducted in the Sutter and Colusa National Wildlife Refuges (NWRs), which lie in the historic floodplain of the Sacramento River. The wetlands are intentionally flooded each year in early fall to provide habitat for overwintering migratory waterfowl. They remain covered with standing water during winter and are drained in spring each year.

When dry, the wetlands contain resting stages of aquatic organisms such as zooplankton, algae, and aquatic insects, including mosquito eggs. When the wetlands are flooded large broods of *Aedes melanimon* Dyar mosquitoes are produced, and subsequently wetlands may produce *Culex tarsalis* Coquillett. Both species may transmit viruses that cause disease in humans and animals, and they are significant biting pests (Richards 1956, Hardy 1987, Reeves 1990, Jensen and Washino 1991).

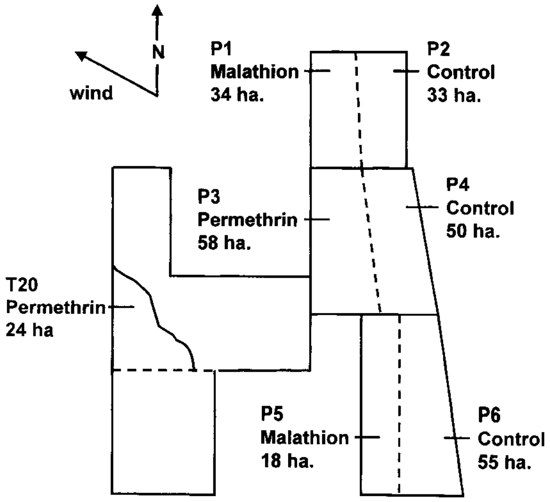

Fig. 2. Map of a site on the Colusa National Wildlife Refuge, Colusa County, California, showing the locations and dimensions of wetlands used for assessing the effect of ultra-low volume application of malathion or permethrin on aquatic macroinvertebrates and fish in September and October 1996. Broken borders represent spray routes.

Dominant vegetation in Sutter NWR study wetlands consisted of Bermuda grass (*Cynodon dactylon*), joint grass (*Paspalum distichum*), water grass (*Echinochloa* sp.), smartweeds (*Polygonum* sp.), cocklebur (*Xanthium* sp.), and sprangletop (*Leptochloa* sp.). Dominant vegetation was similar on the Colusa NWR but also included stands of bulrushes (*Scirpus* sp.) and cattails (*Typha* sp.).

Experiments: We performed large-scale pesticide applications over entire seasonally impounded wetlands. These applications were identical to operational pest control in the area. Although the large scale of the study limited the number of replicates, it also maximized the realism of the tests and minimized the chance that effects would be obscured by immigration of invertebrates from untreated areas within wetlands.

We measured the effect of ULV application of pyrethrin on nontarget organisms using 3 pairs of treated and control wetlands on the Sutter NWR during a June 1996 irrigation for cocklebur control (Fig. 1). We tested permethrin and malathion in 2 sets of paired treatment and control wetlands each on the Colusa NWR in September 1996 (Fig. 2). Site P2 at Colusa served as the control for both T20 and P1 because it was flooded at the same time, and no nearby control was available for T20. The 1st test occurred before a very diverse nontarget community had developed in the wetlands. We tested the likely effect of pyrethrin on nontarget aquatic insects by using mosquito larvae as sentinel organisms and by extensively sampling those insects that colonized early. This test was important because waterfowl forage in the wetlands during irrigations and pesticide applications are common because of development of *Ae. melanimon.* The 2nd test occurred 2 wk after wetlands were flooded, during early autumn when mosquito control activities were most frequent.

On the Sutter NWR, all wetlands were completely inundated to depths of 30–50 cm 3–5 days before pyrethrin application. On the Colusa NWR, all wetlands were flooded to depths of 30–50 cm 2 wk before permethrin and malathion were applied. No pesticides were applied during the 9 months before our study to help ensure the development of a normal nontarget community.

In all tests, control wetlands were upwind from treatment wetlands to prevent insecticide drift over controls. The Sutter–Yuba Mosquito and Vector Control District (SYMVCD) applied pyrethrin synergised with piperonyl butoxide (Pyrocide™ 5%, McLaughlin Gormley King Co., Minneapolis, MN) to the Sutter NWR wetlands at a calibrated rate of 0.118 liters/min at 16 kph using a truck-mounted Becomist ULV sprayer (Becomist Systems) at dusk on June 11, 1996. The ULV droplets were 1–31 μm in diameter with a mean of 14.3 μm. Winds were from the southeast at less than 3.2 kph. Using the same calibrated equipment, the SYMVCD and Colusa Mosquito Abatement District applied permethrin (Biomist™, Clarke Environmental Mosquito Management, Inc., Roselle, IL) and malathion (Cythion™, American Cyanamid Co., Wayne, NJ) at rates of 0.148 liters/min and 0.236 liters/min, respectively, at 16 kph truck speed on the Colusa NWR at dusk on September 30, 1996. Pyrethrin was applied on the Sutter NWR between 2005 and 2126 h on June 11, 1996, with winds from the southeast at 1.6–3.2 kph. During application, air temperature decreased from 28.6 to 25.2°C with a temperature inversion of 1.3–2.4°C. Malathion and permethrin were applied over the wetlands on the Colusa NWR between 1916 and 1937 h on September 30, 1996. During application, air temperature decreased from 22.7 to 21.3°C with an inversion of 0.4–0.7°C and wind from the east-southeast at 3.3–5 kph.

We collected pre- and posttreatment surface water samples from each treatment and control wetland for insecticide analysis. Samples consisted of 3 pooled 0.333-liter samples of surface water, collected within 2 h before and within 1 h after insecticide application, from sites 5, 10, and 15 m from the spray route. Samples were kept in a dark refrigerator and analyzed <24 h after collection by the toxicology section of the California Veterinary Diagnostic Laboratory System–Davis.

The layout of wetlands at Sutter NWR allowed us to separate different treatments by an untreated impoundment in 2 sites, but this was not possible in the 3rd site or Colusa NWR (Figs. 1 and 2). We therefore avoided sampling from the south end of those wetlands where materials could overlap. We performed a test for insecticide drift into control areas at Colusa. We set out caged adult mosquitoes in both treatment and control wetlands, which allowed us to monitor the efficacy of insecticide application as well as to detect whether spray drifted into control areas in amounts lethal to mosquitoes. Cages containing 20–31 counted adult female *Ae. melanimon* were set out at 1 m above the water surface, 10, 15, and 20 m from the spray route in treated wetlands and <3, 10, and 15 m from the spray route in controls. Cages in control wetlands were closer to the spray route to detect possible insecticide contamination along the wetland edge because application trucks drove along this edge. Cages were set out 1 h before insecticide application and were collected 2 h later. Mortality was assessed 24 h after exposure.

Mosquito larvae were used as indicator organisms to gauge the effects of the insecticides on aquatic organisms. This type of test is more sensitive than collecting samples using sweep nets because there is virtually no sampling error when known numbers of sentinels are used. Mosquito larvae are especially good sentinels because strains are available that are known to be sensitive to these materials (these are maintained for tests of pesticide resistance), and they spend most of their time at the air–water interface where deposition of ULV insecticides occurs. We caged larvae in cylindrical floating predator-exclusion cages that had screened sides to allow water exchange with the flooded wetlands (cage dimensions = 20 cm diameter × 14 cm deep). Cage tops were removed during insecticide applications and replaced thereafter. In each wetland of the Sutter NWR pyrethrin study, we set out 2 cages of 25 wild *Ae. melanimon* larvae, plus 2 cages of 25 larvae from a known insecticide-susceptible laboratory colony of *Cx. tarsalis,* before insecticide application. Only *Cx. tarsalis* larvae were used for the permethrin and malathion trials because wild *Ae. melanimon* larvae were unavailable. We monitored survivorship daily for 7 days after exposure, until nearly all larvae had either died or pupated.

In the permethrin and malathion trials, we also placed 2 predator-exclusion cages containing 4 mosquitofish per cage in each treated and control wetland. We initiated a similar experiment during the pyrethrin trial, but it failed because the fish were stressed during transport to the site and were dead or moribund on arrival.

We sampled aquatic macroinvertebrates in each wetland using D-ring aquatic sweep nets (mesh size 1 mm, length of flat side 30.5 cm; Bioquip Inc., Gardena, CA). During the Sutter NWR pyrethrin study, we collected organisms along 4 transects of 30 standardized sweeps of approximately 1 m along the bottom of each wetland. Daily sampling commenced 3–5 days before insecticide application and continued until wetlands were drained 7 days postapplication. Macroinvertebrate abundance and diversity were substantially higher during the Colusa NWR permethrin and malathion trials, which allowed us to reduce the number of sweep net collections per wetland to 2 transects of 10 sweeps in each wetland. We made collections on days 1, 4, and 5 before treatment and 1, 3, 5, 7, and 14 days after insecticide application. We sorted and identified the organisms in sweep net collections to order or family and counted them. The organisms were then pooled, dried to a stable weight during 72 h in a drying oven, and weighed. Effects of insecticides were only expected for the 1st few days posttreatment, but later samples were collected in case it was necessary to track the recovery of the community.

Centers for Disease Control and Prevention (CDC) ultraviolet light traps were used to monitor flying insect abundance in treatment and control wetlands during the permethrin and malathion trials. Traps were placed at 1 m in height on poles 25 m from the edge in each treatment and control wetland. Six 24-h collections were made from each wetland, 3 before insecticide application (on September 24–25, 25–26, and 26–27), and 3 afterward (September 30–October1, October 1–2 and 2–3).

We used repeated-measures analysis of variance (ANOVA) to test the hypothesis that macroinvertebrate abundance or biomass in treated wetlands decreased relative to control wetlands. Data were ln transformed before analysis. Repeated-measures analysis improves accuracy by providing better estimates of the mean and variance of samples within each wetland. This analysis can improve power somewhat because more samples are used in the analysis, although the actual power gained depends on how much significance tests must be corrected for autocorrelation among samples from different dates within sites (Von Ende 1993). This analysis is presented because it potentially maximizes power. We also conducted exploratory ANOVAs on the differences between pre- and posttreatment abundances and biomass. These results are not presented because they were similar to the repeated-measures results, whether or not we used location of sites as a blocking factor.

The survivorships of larval mosquitoes were arcsine transformed and analyzed using ANOVA. Data points were the averaged proportion surviving in the 2 buckets per species per impoundment. Survivorships of adult mosquitoes and mosquitofish were not analyzed statistically because no variance in outcome within treatments occurred (survival was either 0 or 100%).

RESULTS

Pyrethrin and permethrin were not detected in pre- and posttreatment water samples from treatment and control wetlands. The detection limit for these materials was 0.02 ppm. Malathion was detected only in the posttreatment water samples from both malathion-treated wetlands, at 0.006 ppm.

Table 1. Repeated-measures analysis of variance on the abundance and biomass of benthic invertebrates collected in a test of the nontarget effects of ultra-low volume pyrethrin.[1]

Source	df	MS	F	*P*
	Abundance			
Between subjects				
Treatment	1	2.43	0.77	0.43
Error	4	3.17		
Within subjects				
Day	8	0.43	0.57	0.53
Day × treatment	8	0.29	0.39	0.62
Error	32	0.75		
	Biomass			
Between subjects				
Treatment	1	11.52	3.19	0.15
Error	4	3.609		
Within subjects				
Day	8	0.97	0.66	0.55
Day × treatment	8	0.63	0.42	0.68
Error	32	1.475		

[1] df, degrees of freedom; MS, mean square; F, F statistic; P, Greenhouse–Geisser corrected probability.

Aquatic macroinvertebrate abundance and diversity were initially low in the newly flooded wetlands on the Sutter NWR. Only aquatic beetles (Dytiscidae and Hydrophilidae), snails (Gastropoda), water boatmen (Corixidae), mayfly nymphs (Ephemeroptera), and *Cx. tarsalis* larvae were present. *Aedes melanimon* larvae were initially abundant but were excluded from analysis because they emerged as adults before pyrethrin was applied.

No detectable postapplication decrease occurred in the total abundance or biomass of invertebrates in treated wetlands (Table 1 and Fig. 3). Such differences would appear as significant date × treatment interactions in a repeated-measures ANOVA, indicating that treatments and controls diverged through time. We also performed exploratory ANOVAs on each taxon separately but found no statistically significant differences. Table 2 presents mean abundances of the 3 most abundant taxa, Coleoptera larvae, snails, and corixids, in treated and control wetlands before and after pyrethrin application.

Macroinvertebrate abundance and diversity were greater during the permethrin and malathion trials. Abundant taxa included midges (Chironomidae), damselfly and dragonfly nymphs (Odonata), mayfly nymphs, water boatmen, and snails. Backswimmers (Notonectidae) and beetles were widespread, but less common. Macroinvertebrate abundance did not decrease in treated wetlands relative to controls after insecticide application, although a significant effect of sampling date on invertebrate abundance oc-

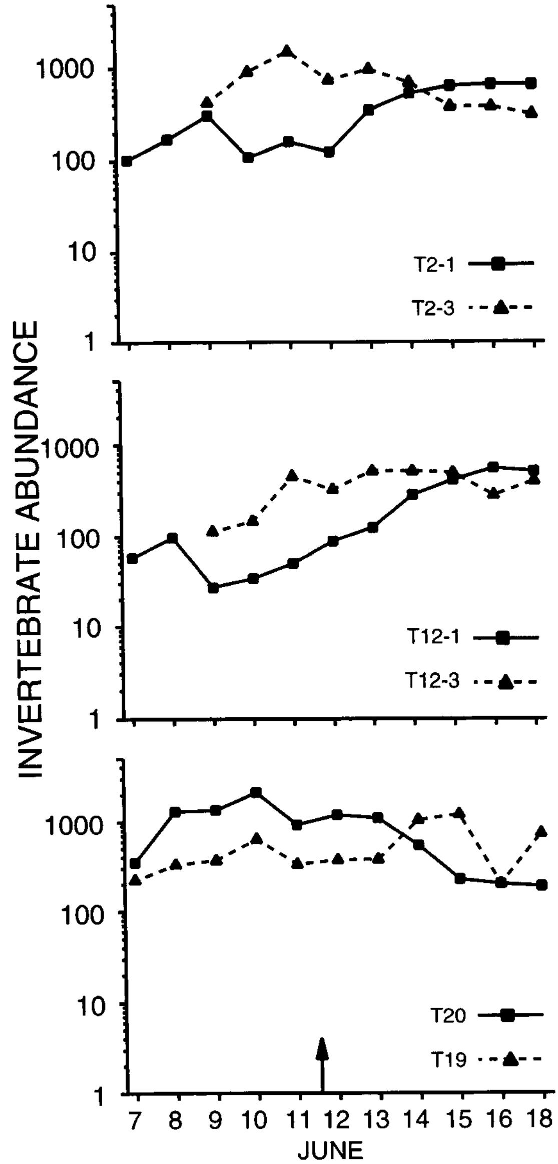

Fig. 3. Time series of the total number of aquatic macroinvertebrates collected in sweep nets from 3 paired pyrethrin-treated and control wetlands in Sutter County, California, during June 1996. Solid lines indicate treated wetlands; dashed lines are controls. An arrow indicates when pyrethrin was applied.

curred (Table 3). Temporal changes might be expected due to natural processes such as colonization, reproduction, and mortality. Abundance fluctuated, but apparent concordance occurred between fluctuations in paired treatment and control wetlands (Figs. 4 and 5). Exploratory ANOVAs showed no effects of treatments on individual taxa, and most trends were toward increased abundances after treatment, as would be expected if invertebrates continued to reproduce or colonize (Table 4).

Table 2. Mean pre- and postapplication abundances of the 3 most abundant invertebrate groups in a study designed to detect the effects of pyrethrin on the abundance of aquatic macroinvertebrates in irrigated wetlands on the Sutter National Wildlife Refuge, California. Each mean represents 3 sites where invertebrates were collected in 4 transects of 30 D-ring net sweeps per site; sample counts were averaged over 3 sampling days immediately before or after the spray date.

		Prespray		Postspray	
Taxon	Treatment	Mean	SD	Mean	SD
Coleoptera larvae	Pyrethrin	25.4	16.3	69.7	24.8
Coleoptera larvae	Control	17.6	10.5	61.8	35.8
Gastropoda	Pyrethrin	464.7	725.2	294.0	383.2
Gastropoda	Control	268.0	175.8	353.7	143.6
Corixidae	Pyrethrin	41.2	58.9	39.9	50.9
Corixidae	Control	58.1	48.2	65.0	63.0

For example, midge abundances increased severalfold in all wetlands during the 1st 3 days after insecticide application.

There were no detectable effects of insecticides on the survivorship of mosquito larvae in any of the trials (pyrethrin: F = 0.703, df = 1,8, *P* = 0.426; permethrin: F = 0.245, df = 1,3, *P* = 0.655; malathion: F = 2.637, df = 1,3, *P* = 0.203; Table 5). In the pyrethrin trial, wild-collected *Ae. melanimon* larvae had higher survival than the colonized strain of *Cx. tarsalis,* as might be expected if the wild larvae were already acclimatized to field conditions (species effect: F = 7.661, df = 1,8, *P* =

Table 3. Repeated-measures analysis of variance on the abundance and biomass of benthic invertebrates collected in a test of the nontarget effects of ultra-low volume permethrin and malathion.[1]

Source	df	MS	F	*P*
	Abundance			
Between subjects				
Treatment	2	0.58	0.77	0.93
Error	4	7.62		
Within subjects				
Day	8	1.24	4.49	0.001
Day × treatment	16	0.29	1.05	0.44
Error	32	0.28		
	Biomass			
Between subjects				
Treatment	2	3.82	0.50	0.64
Error	4	7.65		
Within subjects				
Day	8	0.23	0.97	0.47
Day × treatment	16	0.13	0.56	0.89
Error	32	0.23		

[1] df, degrees of freedom; MS, mean square; F, F statistic; P, Greenhouse–Geisser corrected probability.

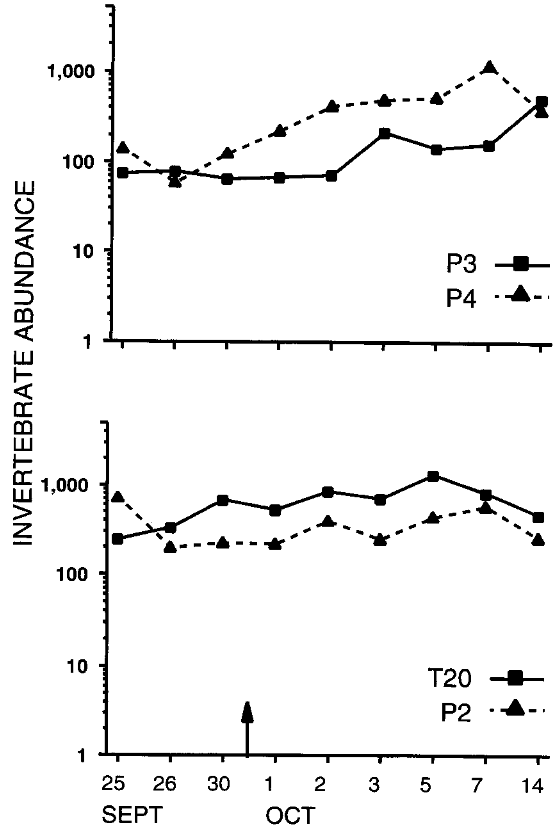

Fig. 4. Time series of the number of organisms collected each day in aquatic sweep net collections from 2 pairs of permethrin-treated and control wetlands in Colusa County, California, during September and October 1996. Solid lines indicate treated wetlands; dashed lines are controls. The arrow indicates when permethrin was applied.

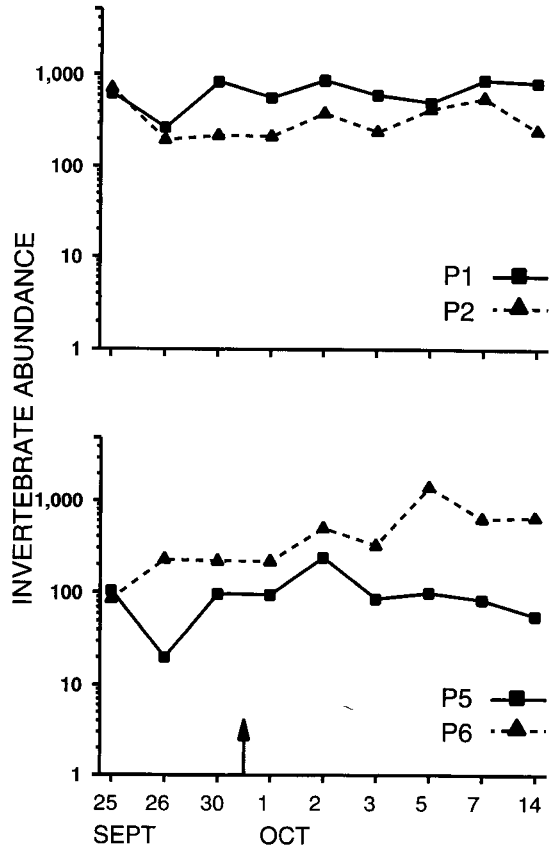

Fig. 5. Time series of the total number of aquatic macroinvertebrates collected in sweep nets from 2 pairs of malathion-treated and control wetlands in Colusa County, California, during September and October 1996. Solid lines indicate treated wetlands; dashed lines are controls. An arrow indicates when malathion was applied.

0.024). No species × treatment interaction was found. Most mosquito larvae successfully completed larval development and pupated within 7 days after insecticide application. Pyrethrin, permethrin, and malathion are fast-acting insecticides and should kill mosquito larvae within a few hours, so the 24-h mortality data presented in Table 5 should indicate any mortality differences due to insecticides, with minimal variance due to natural mortality. However, we also report survival at 7 days to rule out delayed effects.

All caged adult mosquitoes placed in permethrin- and malathion-treated impoundments died within 24 h, but all mosquitoes caged over the adjacent control impoundments survived, indicating that the pesticide cloud did not pass over our sampling area in the controls. All mosquitofish survived and seemed to be healthy in every wetland regardless of treatment, during the 7 days after insecticide exposure.

A repeated-measures ANOVA showed that numbers of night-flying insects collected varied significantly by day but not by treatment (treatment effect: $F = 0.366$, $df = 2,4$, $P = 0.714$; day effect: $F = 27.384$, $df = 5,20$, $P = 0.001$; day × treatment interaction: $F = 0.354$, $df = 10,20$, $P = 0.798$). We observed a marked decrease in flying insect abundance on October 1, the night that the insecticides were applied, but abundance rebounded within 24 h and subsequent counts were similar to those before the insecticides were applied (Fig. 6). Insects collected in the light traps included Diptera (Chironomidae, Culicidae, Tipulidae, and others), Lepidoptera, Coleoptera, and a few Hemiptera.

DISCUSSION

This study did not detect decreases in the biomass or abundance of aquatic invertebrates in seasonal wetlands due to ULV applications of pyrethrin, permethrin, or malathion. Total numbers of aquatic insects showed similar fluctuations in treated and control wetlands. Although we had only 2 or 3 replicates per treatment because of the realis-

Table 4. Mean pre- and postapplication abundances of the 4 most abundant aquatic macroinvertebrate groups collected in a study to detect the effects of malathion and permethrin on the biota of seasonal wetlands in Colusa County, California. Mean abundances were calculated over 3 sampling days and are from 2 sites per treatment. Invertebrates were collected in 2 transects of 10 D-ring net sweeps per site.

		Prespray		Postspray	
Taxon	Treatment	Mean	SD	Mean	SD
Chironomidae	Malathion	70.1	38.1	143.3	23.8
Chironomidae	Control	35.3	22.9	121.4	73.2
Chironomidae	Permethrin	61.7	83.2	204.5	215.7
Chironomidae	Control	27.3	11.5	146.6	108.8
Ephemeroptera	Malathion	49.3	63.9	43.1	58.8
Ephemeroptera	Control	56.1	7.9	40.3	30.2
Ephemeroptera	Permethrin	61.7	83.2	204.5	215.7
Ephemeroptera	Control	27.3	11.5	146.6	108.8
Odonata	Malathion	187.8	253.3	213.6	297.3
Odonata	Control	57.5	5.0	73.8	7.9
Odonata	Permethrin	13.3	7.7	20.8	3.5
Odonata	Control	36.0	35.4	63.4	22.5
Corixidae	Malathion	5.9	4.8	3.3	3.3
Corixidae	Control	90.5	116.2	18.8	4.0
Corixidae	Permethrin	66.4	87.7	61.4	90.0
Corixidae	Control	111.6	86.4	42.9	38.1

tically large spatial scale of the study, we believe that our results are robust for several reasons. We collected large numbers of invertebrates on each sampling date, so the resolution of the data should be good (data for individual taxa are noisier because of the lower numbers). No clear decreases occurred in invertebrate abundance after insecticide application and abundances increased in many cases, so it is unlikely that an effect would materialize given more statistical power. Survival of sentinel mosquito larvae was uniformly high, and this is particularly compelling evidence that the insecticide applications did not kill aquatic insects because the larvae are known to be sensitive to the pesticides. In addition, larvae were caged at the air–water interface where contamination should have been highest.

Table 5. Percent survivorship of *Aedes melanimon* and *Culex tarsalis* larvae in seasonal wetlands exposed to ultra-low volume adulticide applications of pesticides and in untreated wetlands on national wildlife refuges (NWRs) in California. Effects of pyrethrin were tested on Sutter NWR, Sutter Country, in June 1996 and permethrin and malathion were tested on Colusa NWR, Colusa County, in September 1996.[1]

Location Species	Treatment	*n*	24 h (% ± SD)	168 h (% ± SD)
Sutter NWR				
Aedes melanimon	Pyrethrin	3	93 ± 7	89 ± 8
	Control	3	96 ± 4	81 ± 8
Culex tarsalis	Pyrethrin	3	87 ± 2	73 ± 3
	Control	3	86 ± 2	72 ± 2
Colusa NWR				
Culex tarsalis	Permethrin	2	83 ± 1	67 ± 21
	Malathion	2	95 ± 1	83 ± 4
	Control	3	92 ± 7	86 ± 8

[1] n, sample size, where samples were the mean percent survival of 2 cages of 25–31 larvae per species, per wetland; SD, is standard deviation.

The lack of detectable mortality of aquatic invertebrates probably resulted from low exposure to the insecticides. The absence of detectable levels of pyrethrin or permethrin in the water samples and the low concentrations of malathion in posttreatment water samples indicate that little insecticide was deposited in the water.

All sentinel mosquitofish survived, probably because of the low concentration of pesticide. The malathion concentration was an order of magnitude below the median lethal concentration dosage producing acute toxicity in fish (e.g., mosquitofish, rainbow trout; Shao-nan and De-fang 1996). Other studies have also shown that insecticides applied via ULV techniques have low deposition rates (Tietze et al. 1994, Knepper et al. 1996, and references therein). The ULV aerosol spray equipment produces very small droplets that are distributed over a wide area by wind, and deposition is also limited because some droplets evaporate before deposition (Lofgren 1970).

The physical properties of 2 of these insecticides may have contributed to low exposure of nontarget organisms. Permethrin and pyrethrin are pyrethroids, which are lipophilic, readily adsorb to plant surfaces and small particles, and break down rapidly in water and sunlight (Coats et al. 1989, Hill 1989). Adsorption of insecticide to particles and vegetation in the water may reduce its availability to macroinvertebrates and fish. The bottoms of our study sites were covered with herbaceous vegeta-

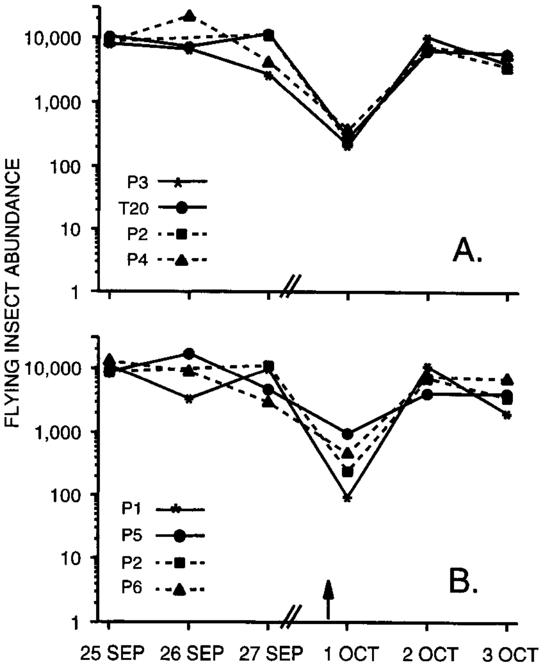

Fig. 6. The total number of flying insects collected each night in Centers for Disease Control and Prevention (CDC) ultraviolet light traps in paired pesticide-treated and control wetlands in Colusa County, California, during September and October 1996. Solid lines indicate treated wetlands; dashed lines are controls. An arrow indicates when pesticides were applied. (A) Permethrin. (B) Malathion.

tion, and all sites had scattered patches of emergent plants. Organic particles were evident in the water.

Most previously published field studies of the effects of these insecticides on aquatic macroinvertebrates are of forestry or agricultural applications, and some of these studies have shown loss of nontarget organisms. Studies of permethrin in silviculture or agriculture have found either no effect on aquatic organisms (Frank et al. 1991), transient behavioral changes but no mortality (Werner and Hilgert 1992), or significant behavioral change and mortality when permethrin was added directly to the water or applied so as to cause heavy deposition (see Smith and Stratton 1986 for a review; see also Kreutzweiser and Kingsbury 1987, Sibley et al. 1991, Helson et al. 1993). Malathion has been shown to decrease amphipod populations when poured directly into the water (Crane et al. 1995). However, application rates for controlling adult mosquitoes are much lower than rates for controlling pest insects in agriculture or silviculture. For example, the product label for the formulation of malathion we used shows that it can be applied at up to 1.17 liters/ha (16 oz/acre) to control insects on food crops, but the maximum rate for mosquito control is 0.29 liters/ha (4 oz/acre). Our results suggest that the lower application rates and method of application used in mosquito control limits nontarget effects.

Evaluating the effects of permethrin and malathion on night-flying insects was difficult. The insecticides killed all adult mosquitoes caged over treated impoundments but all survived in controls. However, counts of insects in light traps decreased in both treated and control areas on the night of insecticide application. Two explanations for this result are possible. First, a rise in wind speed may have caused a drop in light trap catches for all sites. Faster winds decrease insect flight activity and trap counts (Harling 1968, Mizutani 1984, McGeachie 1989). Wind speed rose 1 h after the last insecticide application (10 mph vs. 2–6 mph on other nights). This should not have interfered with insecticide deposition because it occurred after the insecticide cloud had been moved through the study area by slower wind. Second, substantial mortality in treated wetlands could account for decreased abundance in control wetlands if insects dispersed readily among wetlands. In either case, the number of insects caught in light traps rebounded within 48 h, indicating that flying insect populations were resilient because of either renewed activity or immigration and continued emergence of adults.

In this area of California, ULV adulticides are applied in the early evening after sunset, during the period of maximum mosquito flight activity. This is intended to maximize the effectiveness of insecticide application while limiting exposure of diurnally active flying insects, which are then resting and less likely to contact the suspended droplets, although the droplets may contact at least some resting insects. The loss of night-flying insects could affect bats and other nocturnal insectivores. We did not measure day-flying insects; however, other studies have examined the effects of daytime applications of ULV malathion on breeding birds, which are dependent on invertebrates. These studies found no effects of the insecticide on the fledging success and weights of nestling birds (blue tits, Pascual 1994; sage thrashers and Brewer's sparrows, Howe et al. 1996). In both studies, malathion was applied at 2 or more times the label rate for mosquito control.

Our results indicate that effective control of adult mosquitoes can be accomplished near wetlands using ULV applications of pyrethrin, permethrin, and malathion without substantially reducing the amount of aquatic macroinvertebrates available to foraging wildlife, or killing fish. We hope that this paper will help promote good communication about mosquito control methods between wildlife managers and mosquito abatement agencies.

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REFERENCES CITED

Batzer, D. P. and V. H. Resh. 1992. Macroinvertebrates of a California seasonal wetland and responses to experimental habitat manipulation. Wetlands 12:1–7.

Carlson, D. B., P. D. O'Bryan and J. R. Rey. 1991. A review of current salt marsh management issues in Florida. J. Am. Mosq. Control Assoc. 7:83–88.

Clark, J. R., L. R. Goodman, P. W. Borthwick, J. M. Patrick, G. M. Cripe, P. M. Moody, J. C. Moore and E. M. Lores. 1989. Toxicity of pyrethroids to marine invertebrates and fish: a literature review and test results with sediment-sorbed chemicals. Environ. Toxicol. Chem. 8: 393–401.

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

Crane, M., P. Delaney, S. Watson, P. Parker and C. Walker. 1995. The effect of malathion 60 on *Gammarus pulex* (L.) below watercress beds. Environ. Toxicol. Chem. 14:1181–1188.

Day, K. 1989. Acute, chronic and sublethal effects of synthetic pyrethroids on freshwater zooplankton. Environ. Toxicol. Chem. 8:411–416.

Eldridge, B. F. 1989. Presidential address: the best of times and the worst of times. J. Am. Mosq. Control Assoc. 5:307–310.

Frank, R., K. Johnson, H. E. Braun, C. G. Halliday and J. Harvey. 1991. Monitoring air, soil, stream, and fish for aerial drift of permethrin. Environ. Monit. Assess. 16:137–150.

Frayer, W. E., D. D. Peters and H. R. Pywell. 1989. Wetlands of the California Central Valley, status and trends 1939 to mid 1980's. U.S. Fish and Wildlife Service, Portland.

Hardy, J. L. 1987. The ecology of western equine encephalomyelitis virus in the Central Valley of California, 1945–1985. Am. J. Trop. Med. Hyg. 37(Suppl.): 18S–32S.

Harling, J. 1968. Meteorological factors affecting the activity of night flying macro-Lepidoptera. Entomologist 101:83–93.

Helson, B. V., N. J. Payne and K. M. S. Sundaram. 1993. Impact assessment of spray drift from silviculture aerial applications of permethrin on aquatic invertebrates using mosquito bioassays. Environ. Toxicol. Chem. 12: 1635–1642.

Hill, I. R. 1989. Aquatic organisms and pyrethroids. Pestic. Sci. 27:429–465.

Howe, F. P., R. L. Knight, L. C. McEwen and T. L. George. 1996. Direct and indirect effects of insecticide applications on growth and survival of nestling passerines. Ecol. Appl. 6:1314–1324.

Jensen, T. and R. K. Washino. 1991. Assessment of the biological capacity of a Sacramento Valley population of *Aedes melanimon* to vector arboviruses. Am. J. Trop. Med. Hyg. 44:355–363.

Knepper, R. G., E. D. Walker, S. A. Wagner, M. A. Kamrin and M. J. Zabik. 1996. Deposition of malathion and permethrin on sod grass after single, ultralow volume applications in a suburban neighborhood in Michigan. J. Am. Mosq. Control Assoc. 12:45–51.

Kreutzweiser, D. P. and P. D. Kingsbury. 1987. Permethrin treatments in Canadian forests. Part 2: impact on stream invertebrates. Pestic. Sci. 19:49–60.

Lofgren, C. S. 1970. Ultralow volume application of concentrated insecticides in medical and veterinary entomology. Annu. Rev. Entomol. 15:321–342.

McGeachie, W. J. 1989. The effects of moonlight illuminance, temperature and wind speed on light trap catches of moths. Bull. Entomol. Res. 79:185–192.

Mizutani, M. 1984. The influences of weather and moonlight on the light trap catches of moths. Appl. Entomol. Zool. 19:133–141.

Mulla, M. S. 1994. Mosquito control then, now, and in the future. J. Am. Mosq. Control Assoc. 10:574–584.

Pascual, J. A. 1994. No effects of a forest spraying of malathion on breeding blue tits (*Parus caeruleus*). Environ. Toxicol. Chem. 13:1127–1131.

Reeves, W. C. 1990. Epidemiology and control of mosquito-borne arboviruses in California, 1943–1987. California Mosquito and Vector Control Association, Sacramento, CA.

Richards, C. S. 1956. *Aedes melanimon* and related species. Can. Entomol. 88:261–269.

Shao-nan, L. and F. De-fang. 1996. Correlation between biochemical parameters and susceptibility of freshwater fish to malathion. J. Toxicol. Environ. Health 48:413–418.

Sibley, P. K., N. K. Kaushik and D. P. Kreutzweiser. 1991. Impact of a pulse application of permethrin on the macroinvertebrate community of a headwater stream. Environ. Pollut. 70:35–55.

Smith, G. J. 1987. Pesticide use and toxicology in relation to wildlife: organophosphorus and carbamate compounds. U.S. Fish and Wildl. Serv. Resour. Publ. 170. Springfield, VA.

Smith, T. M. and G. W. Stratton. 1986. Effects of synthetic pyrethroid insecticides on nontarget organisms. Residue Rev. 97:93–120.

Tietze, N. S., P. G. Hester and K. R. Shaffer. 1994. Mass recovery of malathion in simulated open field mosquito adulticide tests. Arch. Environ. Contam. Toxicol. 26: 473–477.

Von Ende, C. N. 1993. Repeated-measures analysis: growth and other time-dependent measures, pp. 113–137. *In:* S. M. Scheiner and J. Gurevitch (eds.). Design and analysis of ecological experiments. Chapman and Hall, New York.

Werner, R. A. and J. W. Hilgert. 1992. Effects of permethrin on aquatic organisms in a freshwater stream in south-central Alaska. J. Econ. Entomol. 85:860–864.