EFFECTS OF MOSQUITO ABATEMENT PESTICIDES ON VARIOUS LIFE STAGES OF COMMERCIALLY IMPORTANT SHELLFISH AQUACULTURE SPECIES IN THE SOUTH

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PROJECT OBJECTIVES

1. Review the various mosquito abatement pesticides utilized in the Southern United States near the major shellfish hatchery and nursery facilities and select a larvicide and adulticide of most concern, based on application data and available toxicity data, for further bioassay testing.

2. Use standard toxicity testing protocols to assess the potential impacts of the mosquito control larvicide and adulticide pesticides of most concern on larval and post-set (1-3 mm) hard clams, *Mercenaria mercenaria*, and Eastern oysters, *Crassostrea virginica*.

3. Disseminate information to shellfish hatchery operators and agencies responsible for mosquito abatement through SRAC Fact Sheets and meetings.

ANTICIPATED BENEFITS

Developing (larval and juvenile) shellfish may be subject to many environmental and biological stressors, including predation, disease, abiotic variables (temperature, dissolved oxygen, salinity, pH), and chemical contamination. Mosquito control pesticide applications often coincide with both the location of shellfish hatcheries and nurseries, as well as the season(s) when sensitive early life stages are occurring. Shellfish aquaculture facilities are concerned with mosquito spraying activities as they may impact their source water and thereby their larval or juvenile offspring or their algal cultures, potentially causing significant mortality. Currently, toxicity data for mosquito control chemicals and larval clams and oysters are limited (Table 1).

This Southern Regional Aquaculture Center (SRAC)-supported project established the concentrations required to cause mortality in early molluscan life stages, and enables us to predict whether these chemicals will have adverse effects on coastal shellfish operations or for that matter, native field populations. The potential impacts of this work are increased knowledge of the effects of mosquito control chemicals on molluscan shellfish, as well as improved management strategies for competing uses of coastal resources.

Table 1. Summary of existing organophosphate and pyrethroid toxicity data on *M. mercenaria* and *C. virginica*.

Active Ingredient	Chemical Class	Species	Life Stage/Size	Toxicity (µg/L)	Source
Chlorpyrifos	Organophosphate	*C. virginica*	Juvenile	270 (96h EC50)	Mayer, 1987
Chlorpyrifos	Organophosphate	*C. virginica*	Embryo	2000 (48h EC50)	Mayer, 1987
Malathion	Organophosphate	*C. virginica*	Juvenile	>1000 (48h EC50)	Mayer, 1987
Malathion	Organophosphate	*C. virginica*	Juvenile	>1000 (96h LC50)	Mayer, 1987
Naled	Organophosphate	*C. virginica*	Juvenile	590 (96h EC50)	Mayer, 1987
Dichlorvos	Organophosphate	*C. virginica*	Juvenile	>1000 (96h EC50)	Mayer, 1987
Dichlorvos	Organophosphate	*C. virginica*	Adult	89,000 (96h LC50)	Jones and Davis, 1994
Dichlorvos	Organophosphate	*C. virginica*	Adult	31,620 (96h LC50)	Bolton-Warberg *et al.*, 2007
Permethrin	Pyrethroid	*C. virginica*	Embryo	>1000 (48h EC50)	Mayer, 1987
Cypermethrin	Pyrethroid	*C. virginica*		370 (96h EC50)	Werner & Moran, 2008
Bifenthrin	Pyrethroid	*C. virginica*	Embryo	285 (48h EC50)	Werner & Moran, 2008
Cyfluthrin	Pyrethroid	*C. virginica*		2.69 (96h LC50)	Werner & Moran, 2008
Deltamethrin	Pyrethroid	*C. virginica*		8.2 (96h EC50)	Werner & Moran, 2008

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Review the various mosquito abatement pesticides utilized in the Southern United States near the major shellfish hatchery and nursery facilities and select a larvicide and adulticide of most concern, based on application data and available toxicity data, for further bioassay testing.*

Organophosphates used in adult mosquito control include malathion, fenthion, naled, and chlorpyrifos. Some of the pyrethroids used in mosquito control include: permethrin, resmethrin, sumithrin, lambda-cyhalothrin, esfenvalerate, tralomethrin, deltamethrin, cyfluthrin, bifenthrin, cypermethrin, and etofenprox. Commercial and government applicators decide which chemical to use based on several factors such as efficacy (determined by field trials), mosquito species sensitivity, safety, and cost. The pesticide choice is made by each individual mosquito control agency and varies with location because of differences in mosquito species and application requirements. Mosquito species may develop resistance to a given insecticide over time, rendering it less effective and necessitating a change in the chemical used.

To collect information regarding what types of chemicals are being used to control adult and larval mosquitoes, we conducted a survey (internet and phone) of the following southeastern coastal counties: Charleston, Beaufort, and Georgetown counties of SC; Cataret County, NC; Chatham County, GA; Brevard, Charlotte, Collier, Lee, Manatee, Sarasota, Citrus, St. Lucie, and Seminole counties of FL.

The survey revealed that permethrin, resmethrin, sumithrin, and naled accounted for the majority of chemicals used to control adult mosquitoes. The same survey revealed that methoprene-based products (Altosid) were the most common larvicide applied. Eleven of the 14 southeastern counties surveyed reported using naled for mosquito control. Permethrin was applied by 50% of the 14 counties surveyed. The extensive

use of resmethrin to control mosquito populations in New York City coincided with massive lobster mortality in Long Island Sound, and concerns over resmethrin toxicity to estuarine organisms remains (Zulkosky et al., 2005).

We selected permethrin, resmethrin and naled (dibrom) as the adult mosquito control compounds for toxicity evaluation based on their frequency of use in our coastal county survey (Table 2), number of registered products, and preliminary testing data. These chemicals represent two different classes of mosquito control compounds (permethrin and resmethrin are pyrethroid insecticides, whereas naled is an organophosphate insecticide). By including two different classes of chemical, we were able to compare organism sensitivity across chemical groups and identify the compounds of most concern to shellfish species. Methoprene, a juvenile growth hormone mimic, was tested as the larvicide in this project. In target invertebrates, the principal mechanism of action of pyrethroids is disruption of sodium channel function in the nervous system, whereas the toxicity of OPs is produced through the inhibition of certain cholinesterase enzymes of the nervous system (Klaassen, 1996). Juvenile growth hormone mimics interfere with insect maturation and prevent the insect from reaching the adult stage (Klaassen, 1996).

Table 2. Survey (June, 2011) of mosquito control chemicals applied in southeastern coastal counties.

County	State	Adulticides Used	Active Ingredient	Larvicides Used	Active Ingredient
Beaufort	SC	Anvil 10+10 ULV	sumithrin	Agnique MMF	monomolecular film
		Biomist 30+30 ULV	permethrin	Altosid Briquets	methoprene
		Evoluer 30-30 ULV	permethrin	Altosid Liquid Conc.	methoprene
		Suspend SC	deltamethrin	Altosid Pellets	methoprene
				Altosid SBG	methoprene
				Altosid XR Briquets	methoprene
				Altosid XR-G	methoprene
				Aquabac XT	Bti
				Aquabac 400-G	Bti
				Bactimos Pellets	Bti
				GB-1111	petro-hydrocarbons
				Summit Bti Briquets	Bti
				VectoBac 12AS	Bti
Charleston	SC	Zenivex	etofenprox	Agnique MMF	monomolecular film
		Kontrol 30-30	permethrin	VectoBac AS	Bti
				Altosid XR Briquets	methoprene
		Anvil 10+10	sumithrin	Altosid SR20	methoprene
		Trumpet	naled (dibrom)	30-day Altosid Pellets	
		5 EC	malathion		
Georgetown	SC		permethrin		methoprene
		Trumpet	naled (dibrom)		Bti
		Fyfanon	malathion		
Chatham	GA	Scourge	resmethrin	Altosid	methoprene
		Trumpet	naled (dibrom)	Golden Bear	petro-hydrocarbons
				Agnique MMF	monomolecular film
				mineral oil	petro- hydrocarbons
Brevard	FL	Trumpet	naled (dibrom)	Vectolex CG	Bti
				GB-1111	petro-hydrocarbons
				Altosid SBG	methoprene

Charlotte	FL	Trumpet	naled (dibrom)	VectoBac 12AS	Bti
		Anvil 10+10	sumithrin	Altosid XR-G	methoprene
Collier	FL	Trumpet	naled (dibrom)	Aquabac XT	Bti
		Zenivex	etofenprox	Altosid Briquets	methoprene
St. Lucie	FL	Biomist 3+15	permethrin	Agnique MMF	monomolecular film
		Permanone 30+30	permethrin	Altosid 30-d	methoprene
		Anvil 10+10	sumithrin	Altosid XR Briquets	methoprene
		Duet	prallethrin+ sumithrin	Altosid XR-G	methoprene
		Scourge 4+12	resmethrin	Summit Bti Briquets	Bti
		Zenivex	etofenprox	Vectobac G & CG	Bti
				Natular T30	Spinosyn
				Vectolex WSP	Bti
Seminole	FL	Permanone - RTU	permethrin	Agnique MMF	monomolecular film
		Aqua-Reslin	permethrin	Altosid 30-d	methoprene
		Trumpet	naled (dibrom)	Altosid Liquid Conc.	methoprene
				Altosid Pellets	methoprene
				Altosid XR Briquets	methoprene
				Aquabac 200-G	Bti
				Aquabac XT	Bti
				Natular T30	Spinosyn
				Natular XRG	Spinosyn
				Natular XRT	Spinosyn
				VectoBac 12AS	Bti
				Vectobac G& CG	Bti
				Vectolex WDG	Bti
Lee	FL	Trumpet	naled (dibrom)	Abate 4E	temephos
		Fyfanon	malathion	Vectolex CG	Bti
				GB-1111	petro-hydrocarbons
				Altosid	methoprene
Manatee	FL	Trumpet	naled (dibrom)	Abate 4E	temephos
			permethrin	Altosid	methoprene
		Anvil 10+10	sumithrin	Vectolex CG	Bti
Sarasota	FL	Anvil 10+10	sumithrin	5% Skeeter Abate	temephos
		Trumpet	naled (dibrom)	Agnique MMF	monomolecular film
				GB-1111	petro- hydrocarbons
				Altosid Pellets	methoprene
				Altosid XR Briquets	methoprene
				Altosid XR-G	methoprene
				Vectolex CG	Bti
				Aquabac XT	Bti
				Aquabac 200-G	Bti
Citrus	FL	Trumpet	naled (dibrom)	Abate 4E	temephos
		Anvil 10+10	sumithrin	Vectolex CG	Bti
		Zenivex	etofenprox		Bs
		Duet	prallethrin+ sumithrin	Natular XRG	Spinosyn
				Natular XRT	Spinosyn

				VectoBac 12AS	Bti
				Vectobac G& CG	Bti
				Altosid XR Briquets	methoprene
Carteret	NC	Aqua-Reslin	permethrin	Altosid Pellets	methoprene
				Altosid XR Briquets	methoprene

Objective 2. *Use standard toxicity testing protocols to assess the potential impacts of the mosquito control larvicide and adulticide pesticides of most concern on larval and post-set (1-3 mm) hard clams,* Mercenaria mercenaria*, and Eastern oysters,* Crassostrea virginica.

Shellfish aquaculture facilities are concerned that environmental risk assessments of mosquito control pesticides do not include enough larval and small post-set molluscan shellfish information in the overall data available for assessments. The Eastern oyster, *Crassostrea virginica* forms living subtidal and intertidal reefs in many Atlantic and Gulf coast estuaries. Hard clam populations also are critical species in the ecosystems where they naturally occur. At present, hard clam (*Mercenaria mercenaria*) aquaculture occurs in more Eastern U.S. states than any other native bivalve species under culture. Hence these two species are ideal candidates for focused research relating to impacts from spraying for mosquitoes. Specifically, there are few data available regarding the effects of mosquito control compounds on larval and juvenile forms of clams and oysters.

Larval Clams and Oysters

Newly hatched *M. mercenaria* were supplied by Bay Shellfish Co., Terra Ceia, FL; and newly hatched *C. virginica* were supplied by Fishers Island Oyster Farm (Fishers Island, NY). Larvae were transferred to 1 L flasks and acclimated to laboratory conditions (25°C temperature, 20 ppt salinity, 16h light:8h dark photoperiod) over a 72 h period. The larvae were fed *Isochyrsis galbana* daily, and natural seawater changes were made every 48 h.

Aqueous 96 h static bioassays were performed with the larval and juvenile life stages of each test species (US EPA, 1996; Mayer, 1987). Test conditions were 25 °C, 16h light:8h dark photoperiod, and 20 ppt salinity. Seawater used in the experiments was supplied to the laboratory from the Charleston Harbor estuary, filtered (5 μm), UV-sterilized, activated carbon filtered, and diluted with deionized water to adjust salinity.

Larval shellfish exposures to the mosquito control pesticides were conducted based on methods of Finnegan et al. (2009). Veliger larvae were exposed in 24-well plates pre-coated with hydrophilic gel to minimize chemical binding. The plates were soaked overnight in deionized water before use. One 7 day-old larva (approximate size 100-150 μm) was added per well in 2 mL test solution. The larvae were fed 12,000 cells/mL *I. galbana*. The plates were sealed with Parafilm to minimize evaporation, and placed on orbital shakers (150 rpm) in an environmental chamber. Pesticide stock solutions were prepared in 100% HPLC grade acetone, and the doses were administered to obtain a final acetone concentration of 0.1% in each treatment and control. The upper insecticide concentration used for testing was set at 10 mg/L (approximate limit of solubility). There were three replicate well plates per treatment (n=72 larvae per treatment), and there were five pesticide treatment concentrations plus a control.

Each day of the 96 h static-renewal exposure, the larvae were examined using a dissecting microscope. Mortality was noted and water quality parameters were measured (salinity, temperature, pH, and dissolved oxygen). Larvae were transferred to new plates containing fresh exposure media and *I. galbana*. Median lethal concentrations (LC50) with 95% confidence intervals were determined using the Trimmed-Spearman-Karber method (Hamilton et al., 1977).

Post-Set Juvenile Clams and Oysters

Acute (96 h) tests were conducted using 1-3 mm post-set clams and oysters. Static renewal toxicity tests were performed using the same pesticide stock preparations as described above. Tests consisted of a control and 5 treatment concentrations, with three replicates per treatment. Juveniles were exposed in 16 oz. glass jars with Teflon-lined lids containing 180 mL of test media. Thirty juvenile clams or oysters were added to each replicate jar. Aeration was supplied through a glass pipette inserted through a hole in the lid. The bioassays were conducted at 20-ppt salinity (5 μm filtered seawater), 25 ºC, and a 16-h light: 8-h dark photoperiod in an environmental chamber. Temperature, salinity, dissolved oxygen, and pH were measured and the test media was renewed daily. Juveniles were not fed during the 96 h exposure. Mortality (indicated by gaping, lack of response to stimuli, and/or shell closure for more than two minutes) was determined and a 96 h LC50 was calculated. Significant differences ($p<0.05$) between LC50 values for different species, life stages, and test chemicals were determined using the LC50 ratio test (Wheeler, 2006).

In order to assess the effects of these insecticides in juvenile clams and oysters over a longer exposure, we conducted 21 d chronic static renewal tests with each chemical. Every three days, the test solutions were renewed, the organisms were fed, and water quality and mortality were assessed. After 21 d, an LC50 was calculated, and wet and dry weights (dried 48 h at 60 °C) were determined. The shell maximum length, maximum width, and shell area were then determined using an Olympus dissecting microscope, a digital camera, and Image Pro Plus image analysis software.

RESULTS

Acute testing

Larval clams (7 d old) were most sensitive to the pyrethroid, resmethrin, with a 96 h LC50 of 1.92 mg/L. The next most toxic compound was the juvenile growth hormone mimic, methoprene, with a 96 h LC50 of 2.40 mg/L. The pyrethroid, permethrin, yielded a 96 h LC50 of 6.81 mg/L, whereas the organophosphate chemical, naled had a 96 h LC50 of 8.03 mg/L. Larval clams were significantly more sensitive to methoprene than the other species and life stages tested.

Larval oysters (7 d old) were less sensitive to resmethrin, permethrin, and methoprene than larval clams, with 96 h LC50 values greater than the highest concentration tested (10 mg/L). They showed similar sensitivity to the organophosphate chemical, naled, with a 96 h LC50 of 6.75 mg/L.

Juvenile clams were significantly less sensitive to resmethrin and methoprene compared to larval clams, with 96 h LC50 values of 7.79 mg/L and >10 mg/L, respectively. The juvenile clam 96 h LC50 values for permethrin and naled were 9.66 mg/L and 3.44 mg/L, respectively.

Juvenile oysters were similar in sensitivity to oyster larvae and clam larvae for the organophosphate compound, naled (96 h LC50 of 6.80 mg/L). Juvenile oysters had 96 h LC50 values greater than the highest concentration tested (10 mg/L) for resmethrin, permethrin and methoprene.

A summary of acute toxicity in larval and juvenile clams and oysters is shown in Tables 3 and 4. In general, larval clams were the most sensitive species and life stage tested. Larval clams were significantly more sensitive to resmethrin and methoprene than juvenile clams. Larval clams were significantly more sensitive to resmethrin, permethrin, and methoprene than larval or juvenile oysters.

Table 3. Acute toxicity in clams. Values are LC50 (mg/L) with 95% confidence interval. Asterisks indicate significant differences between larval and juvenile values for same species determined using LC50 ratio test (= 0.05).

	Resmethrin	Permethrin	Naled	Methoprene
Larval	1.59* (0.97 – 2.58)	6.81 (4.88 – 9.51)	8.03 (3.73 – 17.28)	2.40* (1940 – 2980)
Juvenile	7.79* (6.46 – 9.40)	9.66 (7.07 – 13.20)	3.44 (0.83 – 14.36)	>10*

Table 4. Acute toxicity in oysters. Values are LC50 (mg/L) with 95% confidence interval. Asterisks indicate significant differences between larval and juvenile values for same species determined using LC50 ratio test (= 0.05).

	Resmethrin	Permethrin	Naled	Methoprene
Larval	> 10	> 10	6.75 (4.17 – 10.94)	> 10
Juvenile	> 10	> 10	6.80 (5.93 – 7.79)	> 10

Chronic testing

Juvenile clams and oysters did not exhibit much additional mortality after 21 d exposure to resmethrin than the acute 96 h exposure. Clam and oyster acute values (7.79 mg/L and >10 mg/L, respectively) were similar to 21 d chronic values (7.77 mg/L and 9.06 mg/L, respectively). The chronic permethrin exposure had a significant effect on mortality for both clams and oysters. The toxicity values decreased from 9.66 mg/L to 0.80 mg/L for clams, and from >10 mg/L to 4.06 mg/L for oysters. Methoprene also exhibited an increase in toxicity with chronic exposure, with clam LC50 values decreasing from >10 mg/L to 0.83 mg/L and oyster LC50 values decreasing from >10 mg/L to 1.30 mg/L.

A summary of acute toxicity in larval and juvenile clams and oysters is shown in Tables 5 and 6. In general, increased length of exposure led to an increase in mosquito control insecticide toxicity to juvenile clams and oysters. The only compound that did not cause much increase in toxicity from 96 h to 21 d was resmethrin. The compound that had the most increased toxicity was methoprene; approximately 12 fold more toxic after 21 d than 96 h.

Juvenile clam and oyster growth was also affected by chronic exposure to most of the insecticides tested. In general, clam shell area was smaller after exposure to the mosquito control pesticides tested, with the exception of resmethrin (Figure 1). Clam shell weight was reduced by chronic exposure to the mosquito control pesticides tested, with resmethrin having the smallest effect on weight and methoprene having the largest effect (Figure 2). Oyster shell area was significantly reduced by all the compounds tested (Figure 3) and oyster shell dry weight was also significantly reduced by all the compounds tested (Figure 4).

Table 5. Acute vs. chronic toxicity in juvenile clams. Values are LC50 (mg/L) with 95% confidence interval. Asterisks indicate significant differences between acute and chronic values determined using LC50 ratio test (□ = 0.05).

	Resmethrin	Permethrin	Naled	Methoprene
Acute (96 h)	7.79 (6.46 – 9.40)	9.66* (7.07 – 13.2)	3.44 (0.83 – 14.36)	>10*
Chronic (21 d)	7.77 (5.03 – 12.01)	0.80* (0.63 – 1.01)	2.32 (1.31 – 4.10)	0.83* (0.63 – 1.09)

Table 6. Acute vs. chronic toxicity in juvenile oysters. Values are LC50 (mg/L) with 95% confidence interval. Asterisks indicate significant differences between acute and chronic values determined using LC50 ratio test (□ = 0.05).

	Resmethrin	Permethrin	Naled	Methoprene
Acute (96 h)	> 10*	> 10*	6.80* (5.94 – 7.79)	>10*
Chronic (21 d)	9.06* (7.40 – 11.09)	4.06* (3.26 – 5.07)	1.01* (0.57 – 1.79)	1.30* (1.05 – 1.61)

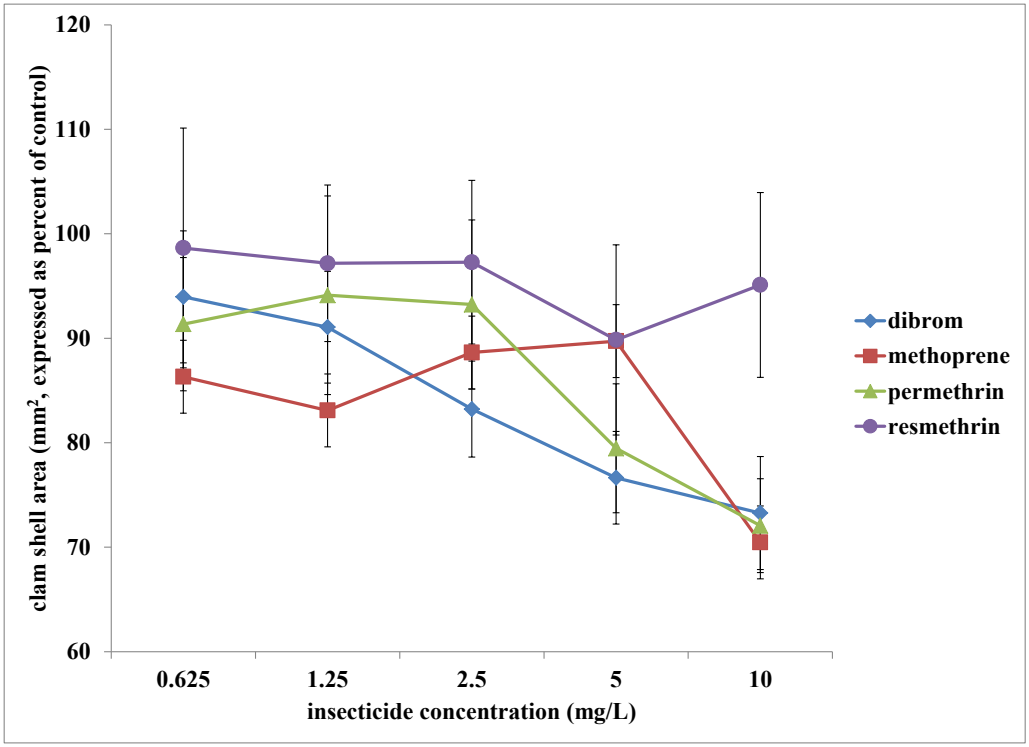

Figure 1. Effect of insecticide exposure on juvenile clam shell area after 21 d exposure. Data are average and standard error for each treatment expressed as percent of control.

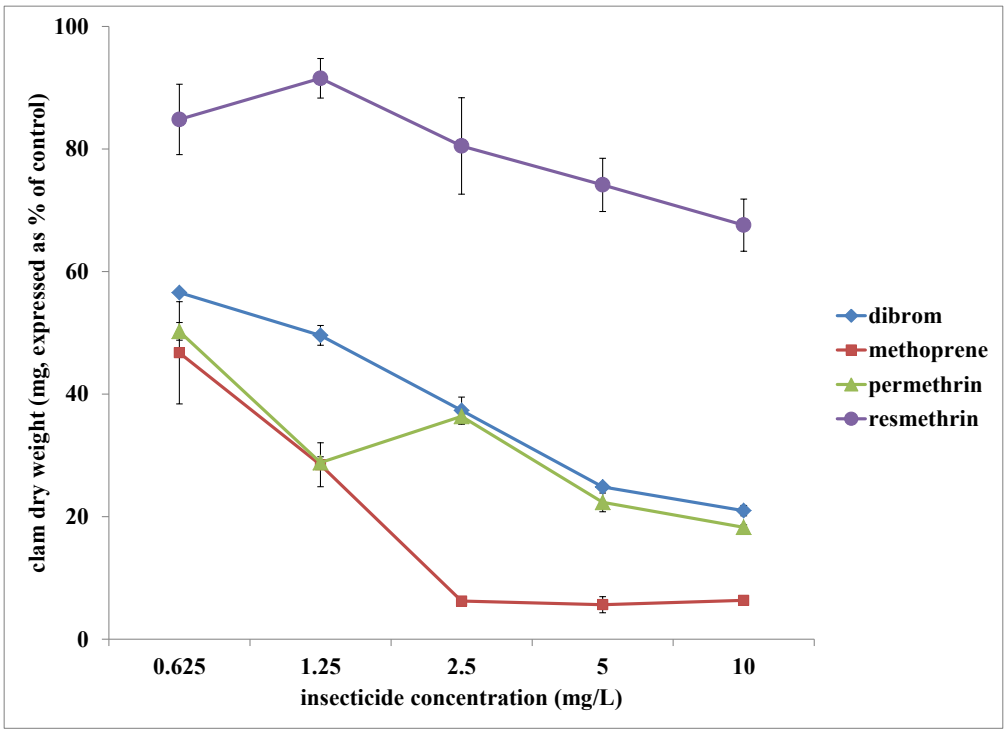

Figure 2. Effect of insecticide exposure on juvenile clam dry weight after 21 d. Data are average and standard error for each treatment expressed as percent of control.

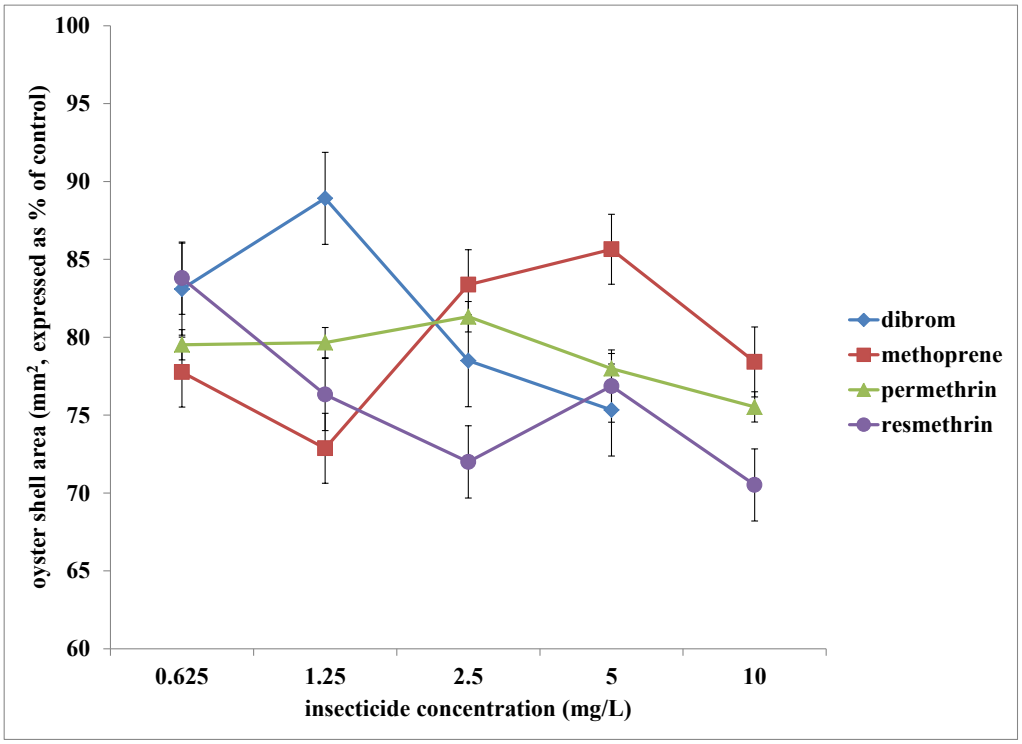

Figure 3. Effect of insecticide exposure on juvenile oyster shell area after 21 d exposure. Data are average and standard error for each treatment expressed as percent of control.

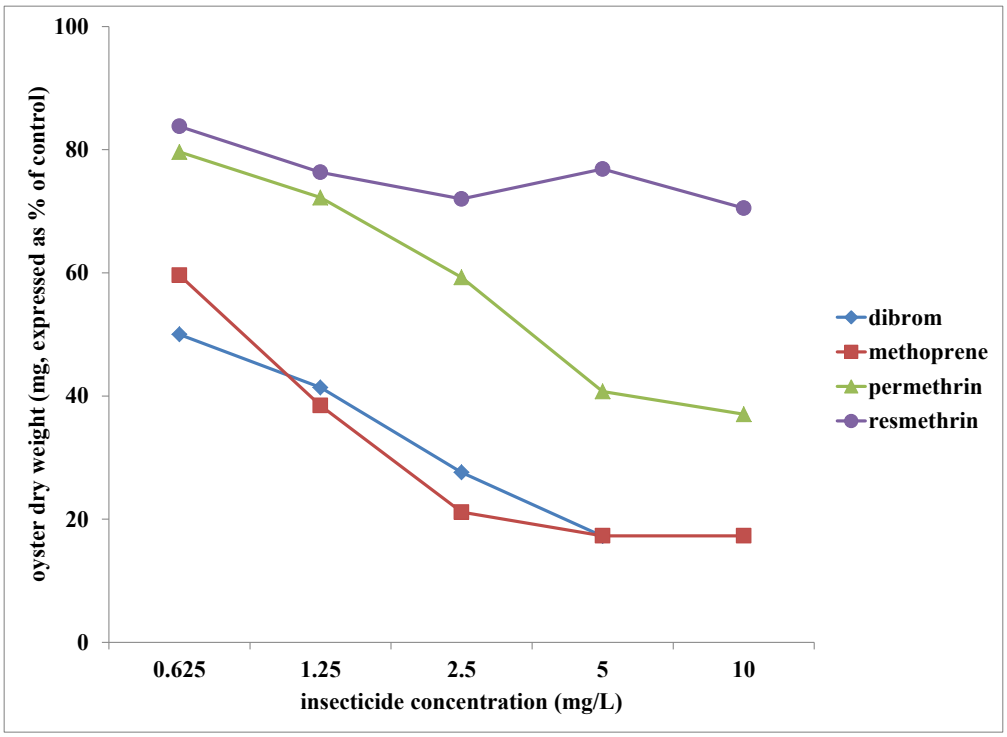

Figure 4. Effect of insecticide exposure on juvenile oyster dry weight after 21 d. Data are average and standard error for each treatment expressed as percent of control.

Resmethrin significantly decreased clam weight ($p=0.0230$), but only at the highest concentration (10 mg/L). Clam shell area was not affected ($p=0.0665$). Resmethrin concentrations ≥ 1.25 mg/L significantly decreased oyster weight ($p<0.0001$). The shell surface area was significantly smaller at all resmethrin concentrations compared to the control ($p<0.0001$).

Clam shell weight was significantly reduced at all permethrin concentrations tested ($p<0.0001$). A significant decrease in clam shell area was also detected ($p=0.0002$), at 5 mg/L and 10 mg/L permethrin. Permethrin significantly decreased oyster weight and shell area at all concentrations tested ($p<0.0001$).

Naled had a significant effect on oyster weight ($p=0.0004$), with all concentrations showing reduced weight compared to the control. Naled concentrations ≥2.5 mg/L had significantly smaller shell area than the control ($p<0.0001$) for both oysters and clams. Clam weight was significantly reduced at naled concentrations ≥1.25 mg/L ($p=0.0032$).

Methoprene caused a significant decrease in oyster weight at all concentrations tested ($p<0.0001$), and oyster shell area was significantly smaller than the control ($p<0.0001$) at all concentrations except for 5 mg/L methoprene. Clam weight was reduced at methoprene concentrations ≥0.625 mg/L ($p=0.0055$). The effect of methoprene on clam shell area was variable, with a significant effect detected ($p<0.0001$), but only at concentrations of 0.625, 1.25, and 10 mg/L.

Risk assessment

The data generated from the toxicity tests can be used to assess the risk to shellfish populations. Using the threshold concentrations established for the four insecticides tested, we prepared an environmental risk assessment specifically for clams and oysters and the selected mosquito control applications.

Application data were obtained from the pesticide product labels, which were then used to calculate the maximum potential concentration (µg/L) in 12" of surface water. This is a worst-case scenario estimate, and assumes all the product enters the water and is uniformly mixed. We then calculated a risk quotient, which is determined as the estimated water concentration/96 h LC50 value. In this simplified assessment if the risk quotient is greater than one, then a toxic effect is expected to occur (Suter, 1995).

The maximum application rate of resmethrin in a mosquito control product (Scourge® 18 + 54) is 0.007 lbs. of active ingredient (AI)/acre, yielding a maximum potential concentration of resmethrin in 12" of surface water of 0.00257 mg/L. Using the lowest 96h LC50 value found in this study (1.59 mg/L, larval clams), the risk quotient is 0.0016.

For permethrin, the maximum application rate of a mosquito control product (Permanone® 30-30) is 0.007 lbs. AI/acre, which equates to a maximum potential concentration of permethrin in 12" of surface water of 0.00257 mg/L. Using the lowest 96h LC50 value found in this study (6.81 mg/L, larval clams), the risk quotient is 0.0004.

Naled is used at a maximum application rate of 0.1 lbs. AI/acre in the mosquito control product Trumpet® EC, which equates to a maximum potential concentration of naled in 12" of surface water of 0.03677 mg/L. Using the lowest 96h LC50 value found in this study (3.44 mg/L, juvenile clams), the risk quotient is 0.0107.

The maximum application rate of methoprene in a mosquito control product (Altosid) is 0.13 lbs AI/acre, yielding a maximum potential concentration of methoprene in 12" of surface water of 0.0478 mg/L. Using the lowest 96h LC50 value found in this study (2.40 mg/L, larval clams), the risk quotient is 0.0199.

The estimated risk quotients for resmethrin, permethrin, naled and methoprene are all less than one, indicating that use of these compounds for mosquito control is unlikely to cause direct mortality to *M. mercenaria* and *C. virginica*. The risk quotients are at least 100 times below the predicted effects thresholds for the most sensitive species and life stage identified in this study, larval clams.

Objective 3. *Disseminate information to shellfish hatchery operators and agencies responsible for mosquito abatement through SRAC Fact Sheets and meetings.*

A summary of these findings has been presented at various regional scientific meetings and research community forums. In addition to this report, the data are also part of a College of Charleston graduate student thesis, and will be prepared for publication in a peer-reviewed scientific journal.

A summary of these findings was presented to members of the South Carolina Coastal Pesticide Advisory Committee (includes representatives from the SC Department of Natural Resources, US Fish and Wildlife Service, SC Department of Health and Environmental Control, National Ocean Service, US Environmental Protection Agency, SC Sea Grant, Clemson University Department of Pesticide Regulation, The Citadel, Charleston, Georgetown and Beaufort county mosquito control programs, South Carolina Coastal Conservation League, US Department of Agriculture, and local golf course resort community managers).

IMPACTS

While we were able to demonstrate significant effects of mosquito control insecticide exposure on molluscan growth and survival, the insecticide concentrations that were found to be detrimental to clams and oysters in this study are much higher than concentrations they are likely to encounter in the field. Environmental factors such as turbidity, sunlight, and microbial interactions all serve to enhance chemical breakdown and decrease bioavailable fractions of these compounds.

National monitoring data by the U.S. Geological Survey detected permethrin in 12% of surface water samples collected, with a maximum concentration of 5.60 x 10^{-4} mg/L (USGS 2005). Targeted monitoring of the Florida Keys National Marine Sanctuary after mosquito control spraying detected permethrin in canal surface water at a maximum concentration of 9.4 x 10^{-3} mg/L, naled was detected once at 1.9 x 10^{-4} mg/L, and dichlorvos (breakdown product of naled) was detected at concentrations up to 5.6 x 10^{-4} mg/L (Pierce et al. 2005). Bolton-Warberg et al. (2007) did not detect naled in water samples following two spray events in Charleston, South Carolina, but detected dichlorvos at 0.21 mg/L. Zulkosky et al. (2005) detected resmethrin in 50% of the samples taken from Long Island, New York within an hour of spray events with a maximum concentration of 9.8 x 10^{-4} mg/L.

The results of this study provide toxicity threshold values for commonly-used mosquito control insecticides to early molluscan life stages. These data enable us to predict that the use of these chemicals in mosquito control operations should have a low risk of adverse effects on coastal clam and oyster operations and native bivalve populations. However misuse of the products or combined application of multiple products could lead to greater risk to these estuarine species. Furthermore, additional research has demonstrated that other estuarine organisms such as fish and shrimp are more sensitive to insecticides than molluscs; therefore, in order to protect all coastal resources, careful use of mosquito control insecticides is recommended.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Garcia, R. 2012. Effects of hypoxia and low pH on mosquito pesticide toxicity in two commercial shellfish species. M.S. Thesis, College of Charleston Graduate Program in Marine Biology, Charleston, SC.

Garcia, R., DeLorenzo, M.E. Effects of hypoxia and low pH on mosquito pesticide toxicity in two commercial shellfish species. June 11, 2012, Harbor Branch Oceanographic Institution, Fort Pierce, FL.

Garcia, R., Chung, K.W., Key, P.B., Burnett, L.E., Coen, L.D., DeLorenzo, M.E. Effects of hypoxia and low pH on mosquito pesticide toxicity in two commercial shellfish species. Southeastern Estuarine Research Society Meeting, April 13, 2012, Beaufort, NC.

Garcia, R., Chung, K.W., Key, P.B., Burnett, L.E., Coen, L.D., DeLorenzo, M.E. Effects of hypoxia and low pH on mosquito pesticide toxicity in two commercial shellfish species. Carolinas Society of Environmental Toxicology and Chemistry Meeting, March 30, 2012, Aiken, SC.

The graduate student funded by this project presented the results of this study at two regional scientific society meetings; Carolinas Society of Environmental Toxicology and Chemistry and the Southeastern Estuarine Research Society. The data are being incorporated in to this student's College of Charleston master's thesis, and will be prepared for publication in a peer-reviewed scientific journal.

Results at a Glance……..

The insecticides, resmethrin, permethrin, and naled, caused decreases in larval and juvenile clam and oyster survival and growth, but at higher concentrations than they would be likely to encounter in the environment as a result of mosquito control spraying applications.

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