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Acute Toxicity of Permethrin to Four Populations of Ovigerous Grass Shrimp, *Palaemonetes pugio* Holthuis

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The dagger-blade grass shrimp *Palaemonetes pugio* Holthuis (Crustacea Decapoda) is distributed in coastal regions all along the eastern United States (Anderson 1985). This omnivore occupies an important role in estuarine ecosystems by enhancing nutrient recycling turnover rates and facilitating bacterial decomposition, as well as serving as prey for larger carnivores (Buikema et al. 1980). Recent concern over the spread of the West Nile Virus has led to increased spraying of insecticides around Chesapeake Bay in hopes of reducing populations of the vector – various species of mosquitoes (Centers for Disease Control, 2006). Permethrin is a pyrethroid insecticide that has been disseminated within the Chesapeake Bay watershed (Maryland Department of Agriculture 2006). Because permethrin is highly toxic to non-target organisms, especially crustaceans (Clark et al. 1989), and is capable of lingering in the environment for extended periods before degradation (Wauchope et al. 1992, Ismail and Kailasam 2002), there is some concern that it may be negatively affecting populations of grass shrimp and other Crustacea in Chesapeake Bay. Recent attempts to collect grass shrimp for laboratory work at locations around the Delmarva Peninsula previously known to have an abundance of shrimp have sometimes ended in failure (Carson and Merchant, 2005; this study).

Permethrin is a synthetic version of pyrethrum, a chlorinated hydrocarbon produced by the chrysanthemum plant to ward off insect infestation (NPTN, 2006). Its molecular weight is 391.31 and it has the formula $C_{21}H_{20}Cl_2O_3$. Effective on all growth stages, permethrin quickly paralyzes the insect nervous system without causing morphological damage. Because permethrin has a relative density of 1.2 (water = 1.0) and is relatively insoluble in water (0.2 mg/L at 20° C, 50 µg/L in seawater), formulation is usually required (EXTOXNET 2006). Permethrin and other chlorinated hydrocarbons are soluble in fats and adsorb strongly to inorganic particles. Because inorganic particles are likely to settle to the seafloor, marine substrates may act as reservoirs for permethrin (Clark 1986). The Virginia and Maryland Departments of Agriculture currently uses a preparation called Biomist 30 + 30, composed of ground ULV (ultra low volume) concentrate diluted in mineral oil. ULV sprayers dispense very fine aerosol droplets that stay airborne to kill flying insects on contact. Permethrin and piperonyl butoxide act as the active ingredients in this spray; these two

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compounds have a synergistic relationship. Application cannot occur within 100 feet of open water where fish are known to dwell (Maryland Department of Agriculture 2006). However, other organisms are distributed in these waters, regardless of fish absence or presence, and are at risk. Application error, such as spraying in wind, during precipitation, or in other conditions that favor drift, elevates risk of non-target organisms encountering pesticide.

The purpose of the present study was to determine and compare the median lethal concentration of the insecticide permethrin on four different populations of ovigerous *P. pugio* taken from two locations in Chesapeake Bay and one on the "outer" (i.e., Atlantic) coast. We sought to relate any significant differences in acute toxicity between populations to morphological features (length, biomass) of the shrimp, environmental conditions (salinity, temperature) and differences in pesticide exposure.

MATERIALS AND METHODS

In this study, a population is defined as a group of individuals of one species occupying a specific location at a specific time. Sites of shrimp collection for this study were chosen for four reasons; (1) grass shrimp are known to inhabit each area in large numbers (Knowlton et al. 1994; Gallin 2002), (2) each site is located at equivalent latitude with respect to each other on Chesapeake Bay and Delmarva Peninsula, eliminating photoperiod effects between each population, (3) each site also has the potential to receive differing levels of insecticide contamination, and (4) each site has a different ambient salinity, making it possible to examine what effects salinity may have on LC_{50} estimates. The three sites are: (1) Chincoteague Channel in the town of Chincoteague, VA, (2) public boat landing at Messongo Creek near Saxis, VA, and (3) Point Lookout State Park, MD. All specimens were collected at high tide. Efforts were also made to collect specimens from each site at the same time of day. Individuals used in this study were selected at random from those that were between 1-2 cm in carapace length. Ovigerous females were chosen to eliminate potential sex effects. Moreover, this stage in the life history of *P. pugio* is a critical determinant of the population's contribution to larval recruitment (Bauer 2004).

All stock organisms underwent a four-day holding period prior to testing in 25 ppt water at 22° C on a 14:10 L:D cycle, then held for an additional two days (EPA Committee 1974). Test organisms were allowed to feed on detritus and macroalgae during the holding period; external food was not added during the bioassay. Shrimp experienced gentle aeration while in the holding tank and for the duration of each test. All holding solutions and test solutions had unionized ammonia (NH_3) values less than 20 ppb. Shrimp from each of the three sites underwent a 48-hour LC_{50} bioassay with exposure to permethrin in six of the following different concentrations: 0.072, 0.12, 0.20, 0.33, 0.55, 0.925, 1.54, and 2.56 ppb. Concentrations were in a geometrically spaced series with a range wide enough so that experimental high levels yielded at least 90% kills and low levels yielded 10% kills, and each subsequent level had a toxicant concentration at least

60% of the next highest level (American Public Health Association 1995, Tietze et al. 1995). Populations of stock organisms from each of the three sites were exposed to six of the eight test solutions of various toxicant concentrations, as well as a control solution (sea water) and a sham (5 mL ethanol in sea water). Control bioassays took place in dilution water alone. For each of the six bioassays of differing concentrations, and the control and sham, there were three identical replicates, each housing a minimum of ten shrimp. Living organisms were counted and dead organisms were removed and preserved in 95% ethanol at times 1.5, 3, 6, 12, 24, and 48 hours after the beginning of each bioassay. Death was defined as the lack of movement or reaction to gentle prodding. At the conclusion of all bioassays, organisms were weighed to determine biomass and carapace length (tip of rostrum to thoraco-abdominal junction) measured.

To determine if there were any effects of time of collection, an additional experimental bioassay was carried out for the Saxis, VA population. This site's bioassay was repeated because it represented the middle salinity condition. In addition, the Saxis bioassay was the first performed, and so repeating it at the completion of all other sites would allow for the longest duration between replicates. Bioassay mortality data were evaluated by probit analysis (EPA Probit Analysis Program Used for Calculating LC/EC Values, Version 1.5) to establish LC_{50} values, along with their 95% confidence intervals. Data were also transformed (probit transformation of the proportion dead vs. log concentration of pesticide) and evaluated by graphical interpolation using linear regression analysis, according to the Litchfield-Wilcoxon method (Litchfield and Wilcoxon 1949). Graphical analysis was performed to check for reasonableness of the computer analysis, and to determine where the EPA program could not be used. Data were transformed to help make the dose-response curve more linear. Significant differences between LC_{50} values were tested for using the formula $f_{1,2} = anti\log\sqrt{(\log f_1)^2 + (\log f_2)^2}$, where f = factor for 95% confidence limits of LC_{50}. If the ratio of the compared LC_{50} values is greater than $f_{1,2}$, the LC_{50} values are significantly different (American Public Health Association 1995).

RESULTS AND DISCUSSION

Linear regression analysis for all populations is summarized in Figure 1. LC_{50} estimates and their 95% confidence intervals for all populations are presented in Table 1. Population mean body length and biomass with standard deviation (SD) are also presented. The confidence intervals for Pt. Lookout, MD shrimp did not overlap any of the confidence intervals for the other populations, while confidence intervals for the remaining populations overlapped each other, and regression slopes for the Saxis bioassays are virtually identical.

Pair-wise comparisons of population LC_{50} ratio and $f_{1,2}$ support a similar portrayal of significance/nonsignificance (Table 2). LC_{50} estimates made using the EPA's probit analysis program agreed with those determined graphically (some data did

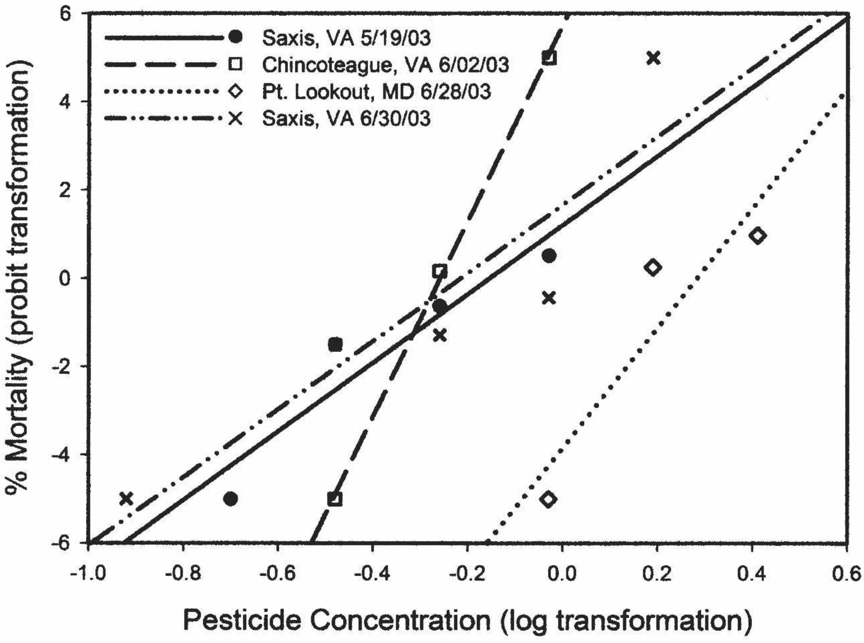

Figure 1. Linear regression analysis for acute toxicity tests with different populations of *P. pugio* ovigerous females exposed to permethrin in static tests at 22°C. Note relative shift of Pt. Lookout, MD population and similarity of the two Saxis, VA populations.

not fit the EPA model and were omitted from such analysis). Both populations analyzed (Pt. Lookout and Saxis 5/19/03) had non-overlapping confidence intervals (Table 1). In addition, the LC_{50} ratio > $f_{1,2}$ for this pair-wise comparison indicated a significant difference. LC_{50} estimates for Pt. Lookout were significantly different from all other LC_{50} estimates for the other test populations. Pearson Product-moment correlation analysis revealed that there was no significant correlation between LC_{50} estimate and ambient salinity, ambient temperature, shrimp length, or shrimp biomass (Table 3).

In decapod Crustacea, the gills appear to be the major organ involved in permethrin absorption, as they function prominently in the continuous absorption of ions for osmotic regulation and they are the most permeable part of the integument (Waterman 1960). Once in the body, fat-soluble (log K_{ow}=6.1, Bromilow et al. 2003) permethrin may bioaccumulate (BCF=1900, Schimmel et al. 1983) in the hepatopancreas (Gonzáles-Baró et al. 1997), which has been shown to be the major site of pyrethroid metabolism (Johnston and Corbett 1986, Kargin et al. 2001). Permethrin and other synthetic pyrethroid insecticides are known to affect the opening and closing kinetics of the sodium channel, important in neuron impulse transmission. Changes in the depolarization of neuronal cell membranes have been found to be related to toxicity (Soderlund et al. 2002). A failure of normal depolarization can lead to a failure of synaptic transmission.

Table 1. 48 hr. LC_{50} estimates with 95% confidence intervals (CI) for acute toxicity tests with different populations of *P. pugio* ovigerous females exposed to permethrin in static tests at 22°C.

Population	Body length (mean±SD; mm)	Biomass (mean±SD; g)	LC50 (95%CI; ppb) Graphical Interpolation	EPA Probit Analysis
Pt. Lookout, MD 6/28/03	1.433±0.0927	0.525±0.088	1.908 1.609~2.262	1.627 1.436~1.844
Saxis, VA 6/30/03	1.426±0.161	0.559±0.129	0.604 0.449~0.814	-- --
Saxis, VA 5/19/03	1.397±0.126	0.484±0.105	0.731 0.541~0.998	0.733 0.63~0.891
Chincoteague, VA 6/02/03	1.340±0.084	0.421±0.065	0.548 0.495~0.608	-- --

Table 2. LC_{50} estimate comparisons for significance. In each comparison, the first listed population is $site_1$ and the second listed is $site_2$, each with their respective f value. LC_{50} ratio values are created by dividing the larger LC_{50} value within the comparison by the smaller. Asterisks (*) indicate site 1,2 are significantly different.

	Graphical interpolation			
Comparison ($site_1$ x $site_2$)	f_{site1}	f_{site2}	$f_{1,2}$	LC_{50} ratio
Saxis 6/30/03 x Saxis 5/19/03	1.35	1.35	1.53	1.21
Saxis 6/30/03 x Pt. Lookout	1.35	1.19	1.42	3.16*
Saxis 6/30/03 x Chincoteague	1.35	1.11	1.38	1.1
Saxis 5/19/03 x Pt. Lookout	1.35	1.19	1.42	2.61*
Saxis 5/19/03 x Chincoteague	1.35	1.11	1.38	1.09
Pt. Lookout x Chincoteague	1.19	1.11	1.23	3.48*
	Probit analysis			
Comparison ($site_1$ x $site_2$)	f_1	f_2	$f_{1,2}$	LC_{50} ratio
Saxis 5/19/03 x Pt. Lookout	1.22	1.13	1.26	2.22*

Table 3. Pearson Product-moment correlation matrix between abiotic conditions during collection, LC_{50} estimates, and morphological characteristics of test organisms. Asterisks (*) indicate a significant correlation.

	Salinity	Temperature	LC_{50}	Length	Biomass
Salinity	1	-0.847	-0.692	-0.972*	-0.889
Temperature		1	0.656	0.850	0.878
LC_{50}			1	0.518	0.367
Length				1	0.966*
Biomass					1

Moreover, failure of neuron impulse conduction is consistent with the final symptoms of poisoning, which are prostration and/or paralysis.

The evolution of a population of grass shrimp more capable of tolerating permethrin seems plausible. Numerous other arthropod species have developed resistance to permethrin (see Hemingway et al. 2004 for review). Pyrethroid resistance has been attributed to reduced neural sensitivity and enhanced metabolism in many insects (Soderlund et al. 2002).

Differences in LC_{50} values between populations, as determined in the present study, may have been the result of variations in proximity to spraying and to the frequency and duration of these events. However, spraying data for our study locations do not support this. Chincoteague Mosquito Control has implemented a daily spraying regimen for peak months (May through September). There is a history of spraying with permethrin and chlorpyrifos at Pt. Lookout State Park during similar times of the year (Carson and Merchant 2005). The Virginia Department of Agriculture and the town of Saxis do not spray near our Saxis collection site routinely, but permethrin is applied to nearby agricultural land by farmers in May. Thus, all tested shrimp populations received some exposure to permethrin during summer months, but measurements of ambient concentrations at our sites are required to confirm differences in *in situ* doses. Our data provide a general measure of the sensitivity of a ubiquitous inhabitant of Chesapeake Bay and illustrate the variability between geographically close but distinct populations of the same species.

The greater tolerance of permethrin (as measured by LC_{50}), by shrimp from Pt. Lookout State Park compared to shrimp populations from Saxis and Chincoteague, VA, may be a reflection of an acquired resistance to this insecticide. This resistance could have developed from more frequent sublethal exposure caused by nearby pesticide application. However, more detailed records concerning pesticide application, determination of permethrin levels in water samples and biota, and more extensive sampling of shrimp populations are needed to test this hypothesis.

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REFERENCES

American Public Health Association (1995) Toxicity. In: Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association. Washington. pp. 1-73

Anderson G (1985) Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Gulf of Mexico) – grass shrimp. U. S. Fish Wildl. Serv. Biol. Rep. 82(11.35) U. S. Army Corps of Engineers, TR EL-82-4. 19 pp

Bauer RT (2004) Remarkable shrimps; adaptations and natural history of the carideans. University of Oklahoma Press, Norman. 282 pp

Bromilow RH, Evans AA, Nicholls PH (2003) The influence of lipophilicity and formulation on the distribution of pesticides in laboratory-scale sediment/water systems. Pest Manage Sci 59: 238-244

Buikema AL Jr., Niederlehner BR, Cairns J Jr. (1980) Use of grass shrimp in toxicity tests. In: Buikema AL Jr., Cairns J Jr., (eds.) Aquatic Invertebrate Bioassays, ASTM STP 715 American Society for Testing and Materials. Philadelphia. pp. 155-173

Carson ML, Merchant H (2005) Predator-prey interactions: a laboratory study of the behavior of two species of grass shrimp (*Palaemonetes pugio* Holthius and *P. vulgaris* Holthius) and the killifish (*Fundulus heteroclitus* Linnaeus). J Exp Mar Biol 314: 187-201

Centers for Disease Control. CDC West Nile Virus Background. 13 June 2006. CDC. 21 June 2006. http://www.cdc.gov/ncidod/dvbid/westnile/index.htm

Clark RB (1986) Marine pollution. Clarendon Press, Oxford. 215 pp

Clark JR, Goodman LR, Borthwick PW, Patrick JM, Jr., Cripe GM, Moody PM, Moore JC, Lores EM (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

EPA Committee on Methods for Toxicology Tests with Aquatic Organisms (1974) Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. National Water Quality Laboratory. U. S. EPA-660/3-75-009. U.S. Environmental Protection Agency. Corvallis. 67 pp

EXTOXNET. Pesticide Information Profiles. June 1996. Extension Toxicology Network (EXTOXNET). 21 June 2006. http://extoxnet.orst.edu/pips/permethr.htm

Gallin RB (2002) Effects of salinity and temperature on survival and behavior of two sympatric species of grass shrimp, *Palaemonetes pugio* and *Palaemonetes vulgaris*, and implications of these effects on their distribution at Pt. Lookout State Park, Maryland. Ph.D. dissertation, The George Washington University, Washington. 152 pp

Gonzáles-Baró, MR, Garda H, Pollero R (1997) Effect of fenitrothion on hepatopancreas microsomal membrane fluidity in *Macrobrachium borellii*. Pest Biochem Physiol 58: 133-143

Hemingway J, Hawkes NJ, McCarroll L, Ranson H (2004) The molecular basis of insecticide resistance in mosquitoes. Insect Biochem Mol Biol 34:653-665

Ismail BS, Kailasam K (2002) Measurement and prediction of permethrin persistence in six Malaysian agricultural soils. Australian J Soil Res 40: 817-826

Johnston JJ, Corbett MD (1986) The uptake and *in vivo* metabolism of the organophosphate insecticide fenitrothion by the blue crab, *Callinectes sapidus*. Toxicol Appl Pharmacol 85:181

Kargin F, Dönmez A, Çoğun HY (2001) Distribution of heavy metals in different tissues of the shrimp *Penaeus semiculatus* and *Metapenaeus monocerus* from the Iskenderun Gulf, Turkey: seasonal variations. Bull Environ Contam Toxicol 66:102-109

Knowlton RE, Khan RN, Arguin PM, Aldaghlas TA, Sivapathasundram R (1994) Factors determining distribution and abundance of Delmarva grass shrimp (*Palaemonetes* spp.). Virginia J Sci 45: 231-247

Litchfield JT, Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments. J Exp Theory 96: 99-110

Maryland Department of Agriculture. Insecticides for Mosquito Control in Maryland 2005 Recommendations. Maryland Department of Agriculture. 21 June 2006. http://www.mda.state.md.us/plants-pests/mosquito_control/insecticides_for_mosquito_control_md.php

NPTN. Permethrin. September 1997. National Pesticide Telecommunications Network. 21 June 2006. http://npic.orst.edu/factsheets/permethrin.pdf

Schimmel SC, Garnas RL, Patrick JM Jr., Moore JC (1983) Acute toxicity, bioconcentration and persistence of AC222,705, benthiocarb, chlorpyrifos, fenvalerate, methyl parathion and permethrin in the estuarine environment. J Agric Food Chem 31: 104-113

Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D, Stevens JT, Weiner ML (2002) Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. Toxicology 171: 3-59

Tietze NS, Hester PG, Shaffer KR (1995) Acute effects of Permanone ® 31-66 (permethrin-piperonyl butoxide) on nontarget minnows and grass shrimp. J American Mosquito Control Assoc 11: 476-479

Waterman TH (1960) The physiology of Crustacea. Vol. 1: Metabolism and Growth. Academic Press, New York. 670 pp

Wauchope RD, Buttler TM, Hornsby AG, Augustijn-Beckers PWM, Burt JP (1992) SCS/ARS/CES pesticides properties database for environmental decision making. Rev Environ Contam Toxicol. 123: 1-155