The Lethal and Sublethal Effects of Aldicarb on the Estuarine Grass Shrimp, *Palaemonetes pugio.*

By

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Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Biology

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November, 1996

Blacksburg, Virginia

Keywords: Aldicarb, Grass shrimp, Acetylcholinesterase, Behavior, *Palaemonetes pugio,* toxicity

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(Abstract)

Estuaries, an important facet of coastal regions, are highly productive natural systems, frequently acting as drainage basins for various pollutants such as agricultural runoff. The estuarine grass shrimp, *Palaemonetes pugio*, has been shown to be a sensitive indicator of pesticide exposure. In this study, emphasis was placed on the quantification of the lethal and sublethal effects of aldicarb on three different life stages of *P. pugio*. Acute 96-h toxicity tests were conducted with newly hatched larvae, 22-d old larvae and adult grass shrimp to determine lethal toxicant ranges. LC_{50} values were 85.0 ug/L for newly hatched larvae, 70.7 ug/L for 22-d old larvae and 125.4 ug/L for adults.

The impact of aldicarb on specific neurological functions in the grass shrimp was examined using an acetylcholinesterase assay. Acetylcholinesterase (AChE) activity was reduced in the larvae after exposure to acute concentrations of aldicarb. Mean whole-body AChE activity for the newly hatched and 22-d larvae was significantly lower from the controls ($P=0.009$). Conversely, mean whole body AChE activity in the adult shrimp was not significantly different from the controls at any concentration ($P=0.401$),

although there was a trend towards reduced activity at 50 and 100 ug/L exposures.

A behavioral study was conducted to examine the ability of adult grass shrimp to detect and avoid aldicarb-treated seawater. Behavioral responses were measured in a modified steep gradient chamber. A partition divided the chamber, creating three distinct areas: 1) seawater 2) aldicarb-treated seawater and 3) mixing.

There were significant differences between the control and exposed adult shrimp in the amount of time spent in the mixing area ($P<0.05$). Aldicarb exposed shrimp spent, on the average, 20% more time in the mixing area than the controls. Additionally, exposed shrimp spent significantly more time facing downstream, away from the toxicant source ($P<0.05$). Exposed shrimp displayed increasing hyperactivity and attempted to jump out of the chamber.

Dedicated in loving memory of Audrey Lou Dvorak and Harold Breakiron

Acknowledgements

I am deeply indebted to my committee for their support, patience and guidance during this project. Special thanks goes to Dr. Arthur Buikema, Jr. for chairing my committee and providing constructive criticism on this manuscript. I am extremely grateful to my parents, Robert and Sharon Dvorak, for their continued encouragement and for giving me this opportunity. I have also been blessed with two wonderful sisters - thanks for putting up with me! There are many people who helped me during this journey - Ray Ebbett, Scotty Bolling, Dave Balfour, Stuart Lynde, Brian Ward, Abby Campbell, Rhonda Wilhite, Cathy Light, Judy Alls and Mary Schaeffer - I couldn't have done this without all of you. I would like to give particular thanks to Dr. Suzanne Braunschweig for her friendship and invaluable advice.

I would also like to thank Drs. Geoff Scott, Mike Fulton and Pete Key of the National Marine Fisheries Service, Charleston Laboratory for their helpful suggestions and for the use of their facilities. I am especially grateful to my husband and best friend Shon for believing in my abilities and always encouraging me to follow my dreams. Lastly, I would like to recognize Boadi and Kiwi for their wonderful companionship over the years.

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INTRODUCTION

Estuaries are an important feature of coastal regions and are one of the most highly productive natural systems, capable of sustaining a wide variety of organisms. Estuaries serve as a feeding ground, migratory route, nursery area and refuge for many fish and invertebrate species, along with being a location for coastal fisheries (McLusky 1981). The value and importance of estuaries have only recently been fully realized, partly due to the degradation and loss of resources within these systems. Pesticides enter coastal estuaries through agricultural runoff, spills, industrial discharges, spray drift, domestic sewage and/or atmospheric deposition. Agricultural runoff is a major concern in coastal areas due to the close proximity of agricultural lands to estuarine drainage basins. For example, in the Southeast and Gulf coast regions of the USA, agriculture may comprise anywhere from 36-75% of the coastal land surface area (Pait, 1989). Estuaries are a pollutant sink and they have experienced declining integrity and productivity. Contaminants trapped in an estuary may bind with the surface microlayer, the particulate matter, or may settle out to the bottom. The potential for pesticides to persist in the aquatic environment is affected by 1) the trapping capabilities and flushing rate of an estuary, and 2) the chemical and physical properties of the pesticide itself.

Estuarine organisms have been shown to be particularly susceptible to pesticide contamination since they presumably experience greater exposure to

agricultural runoff which, after application to crops throughout regional drainage basins, collects and concentrates in estuarine waters (McKenney and Hanmaker, 1984). Inputs of agricultural pesticides into receiving waters have often resulted in fish kills, decreased fisheries productivity and shellfish closure. Pesticides may also impact an organism's development, reproduction, food capturing capabilities and ability to avoid predation. Impacts, in general, appear more likely to occur near the site of application, during the growing season and/or after a heavy rainfall when pesticides have the greatest potential to enter into aquatic systems (Willis and McDowell, 1982). The specific objectives of this study were to assess the lethal and sublethal effects of aldicarb, a carbamate insecticide, on the estuarine grass shrimp, *Palaemonetes pugio.*

CARBAMATES

In the last twenty years, insecticide use patterns have shifted away from organochlorine compounds toward carbamate, organophosphorus, and synthetic pyrethroid compounds. The development of the carbamates began with the discovery of the calabar bean. The calabar bean was traditionally used as an ordeal poison in West Africa. The Egbo rulers of Nigeria were the first to use an extract of the Calabar bean in their witchcraft trials (Marshall, 1985). The Calabar bean was taken to Scotland in the 1800's for further study. In 1863 the toxin was identified, but it was not until 1925 that the molecular configuration of the toxin was discovered (Marshall, 1985). The actual synthesis and

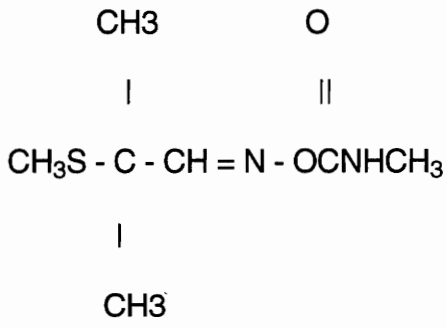
commercialization of the carbamate pesticides began in the 1950's. The first major carbamate to be produced was carbaryl, also known under the trade name Sevin (Marshall, 1985). By 1971, over 25 million kg were being produced annually in the United States.

Carbamate pesticides act as potent acetylcholinesterase inhibitors. Acetyl choline (ACh) is a neurotransmitter that conducts impulses across nerve endings. Once ACh has carried a nerve signal, it is removed from the synapse by the enzyme acetylcholinesterase (AChE), thereby clearing the way for the next signal. When AChE is inhibited, it can no longer hydrolyze acetyl choline. Therefore, acetyl choline remains in high concentrations and the nerve endings become overloaded with signals and locked in stimulation (Marshall, 1985; WHO, 1986). This causes continuous muscle contractions and, unless relieved, paralysis and death. Carbamate insecticides do not permanently attach to the enzyme, thus the rate of reactivation of the carbamylated enzyme is relatively rapid.

ALDICARB

Aldicarb, (Figure 1) a carbamate ester, is a white crystalline solid, moderately soluble in water, and susceptible to oxidation and hydrolysis (WHO, 1991). Aldicarb is a systemic insecticide that is applied to soil for control of certain insects (esp. aphids, white flies, leaf miners, & soil-dwelling insects), mites and nematodes. Aldicarb was originally registered in the USA for cotton and potatoes in 1970 and 1974, respectively (WHO, 1991). Soil application is

Chemical Structure:



2-methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxime

Molecular Formula: $C_7H_{14}N_2O_2S$

Relative Molecular Mass: 190.3

Melting Point: 100°C

Boiling Point: unknown, decomposes above 100°C

Solubility (20°C): 6g/litre of water; 40% in acetone;
35% in chloroform; 10% in toluene

Log Octanol/Water Partition Coefficient: 1.359

Figure 1. Identity, physical and chemical properties of aldicarb (WHO, 1991).

used for a wide range of crops such as coffee, bananas, onions, potatoes, maize, tobacco, sorghum, citrus (grapefruit, lemons, limes, oranges), cotton, dry beans, pecans (southeast only) and sugarcane (Louisiana only) (Willoughby, 1989; WHO, 1991). Wherever aldicarb is used, the active ingredient is applied to the soil at a rate of 4.5 kg/ha for citrus and 0.45 to 1.3 kg/ha for most other crops (Marshall, 1985). Granular formulations of aldicarb are used commercially due to the hazards associated with handling it. Aldicarb is applied to crops several times a year and has been detected in runoff at a level of 12 µg/L, which has been shown to be lethal to some crustacean and fish species (Scott, personal communication). In 1988, the EPA estimated that between 2140 and 2346 metric tons of aldicarb were applied annually in the United States (US EPA, 1988a).

ECOLOGICAL FATE

Generally speaking, several factors influence the biodegradation of carbamates in soil. These include volatility of the chemical, soil type, soil moisture, adsorption, pH, temperature and photo-decomposition (WHO, 1986). Carbamates can be metabolized by microorganisms, plants, and animals. Aldicarb, and its metabolites, are absorbed by plants from the soil and translocated into roots, stems, leaves and fruit (Ware, 1989). Aldicarb is mobile in water and is acutely toxic to mammals, birds, estuarine/marine animals and freshwater organisms. It has been estimated that aldicarb is at least 250 times more toxic to mammals than DDT, and 80 times more potent than carbaryl

(Sevin™) (Marshall, 1985). Aldicarb is distributed throughout tissues by systemic circulation, but is not known to bioaccumulate in tissues (WHO, 1991).

Exposure of non-target terrestrial organisms to aldicarb is usually through the ingestion of contaminated food or through accidental ingestion of aldicarb granules as reported in some birds and mammals (WHO, 1991). Bunyan et al. (1981) conducted an extensive field study sampling invertebrates, birds and mammals inhabiting an area around an aldicarb treated sugar beet field. The field had been treated with aldicarb granules at 1.12 kg/ha. High residue levels were found in partridges, blackbirds and several small mammals. A secondary hazard involved the ingestion of aldicarb contaminated earthworms by various bird species. Low residues of aldicarb were also found in various herbivores, having ingested plants that had taken up aldicarb. The authors hypothesized that exposure to aldicarb could potentially be widespread and therefore have negative impacts on non-target organisms.

Aldicarb is metabolized by both oxidative pathways and hydrolytic processes. Oxidation results in compounds which are active cholinesterase inhibitors, while hydrolysis produces compounds of little or no insecticidal activity or toxicity to non-target organisms (National Research Council - Drinking Water and Health, 1977).

Hydrolysis of aldicarb, which inactivates the insecticide, is pH dependent. The half-life of aldicarb can vary from as little as a few minutes at a pH > 12 to as long as 560 days at a pH of 6 (reported using distilled water) (WHO, 1991).

Quraishi (1972) studied the persistence of aldicarb in the field. Field water treated with aldicarb in the laboratory at 100,000 μg/L resulted in residues of aldicarb and its metabolites of 400 μg/L after 11 months. The field treated water was stored at 16-20°C and exposed for 507 hours to sunlight.

Aldicarb, initially thought to be incapable of contaminating groundwater, has been found in groundwater supplies. Factors such as the amount of organic matter and moisture in a field greatly affect the leaching of aldicarb into aquatic ecosystems. Aldicarb has been found in groundwater in New York, Wisconsin and Florida (WHO, 1991). The USEPA groundwater team has reported aldicarb contaminated groundwater in 22 states (WHO, 1991). During the application season of 1984 in 30 counties in Florida, 1850 metric tons of aldicarb were used on citrus fruits (WHO, 1991). Although no residues of aldicarb were found in community water systems, traces of aldicarb, aldicarb sulfoxide, and aldicarb sulfone were found in the Calloosahatchee River indicating that aldicarb has the potential to leach into surrounding surface waters (WHO, 1991).

TOXICITY

Aldicarb is probably the most toxic of the commonly used insecticides (Gill et al., 1990) and based on results of acute oral and dermal toxicity studies was labelled as extremely hazardous (WHO, 1991). The USEPA has rated aldicarb as a category 1 insecticide (extremely toxic). Many pesticides, while not as persistent as the organochlorine compounds, are just as toxic to

nontarget estuarine species (Pait et al., 1992). In the 1980's, 150 fish pesticide-related kill incidences were reported in coastal waters (Key, 1995). The 1991 organophosphate fish poisonings in southern Louisiana were among the most massive in history (Williams, 1993). Conservative estimates placed the 1991 losses at 1 million fish, not counting the loss of crabs, crawfish, shrimp and other smaller invertebrates. In certain cases, the use of carbamate insecticides has been shown to cause significant reduction in non-target organisms (WHO, 1991).

Freshwater Organisms

The acute toxicity of aldicarb to freshwater organisms varies a great deal. The 96-h LC_{50} values for different fish species ranged between 52 and 242 µg/L depending on temperature and water hardness (WHO, 1991). LC_{50} values for the water flea, *Daphnia laevis,* ranged between 70 and 1124 µg/L depending on the life stage of the organism (WHO, 1991).

Pickering and Gilliam (1982) conducted flow-through early life stage toxicity tests with aldicarb on the fathead minnow (*Pimephales promelas*). Although 78 µg/L did not effect survival and growth, 156 µg/L was lethal to larvae/juveniles exposed for 30 days posthatch. The 96-h LC_{50} value was 137 µg/L and the MATC (maximum acceptable toxicant concentration) was estimated to be between 78 and 156 µg/L.

In another study with a more tolerant freshwater fish species, *Barbus conchonius* (a freshwater species), pathological deformations of the vital organs

were observed when the fish were exposed to a concentration of 806.6 µg/L of aldicarb in hard water and 3,300 µg/L in soft water. Aldicarb appeared to produce a wide spectrum of histopathological impairments in the fish (Pant and Kumar, 1984).

Estuarine and Marine Organisms

Aldicarb has also been shown to be extremely toxic to several estuarine and marine species. Estuarine crustaceans are generally more sensitive to aldicarb than fish. For juvenile and adult *Mysidopsis bahia,* the 96-h LC_{50}'s were 13 and 16 µg/L, respectively. The 96-h LC_{50} for adult pink shrimp (*Penaeus duorarum*) was 12 µg/L and 72 µg/L for adult white shrimp (*Penaeus stylirostris*) (Mayer, 1987). Mayer (1987) reported a 96-h LC_{50} of 170 µg/L and 41 µg/L in juvenile and adult sheepshead minnows (*Cyprinodon variegatus*), respectively. In other estuarine and marine fish, 96-h LC_{50}'s were reported in a range of 80 µg/L for adult pinfish (*Lagodon rhomboides*), 100 µg/L for juvenile snook (*Centropomus undecimalis*) and 200 µg/L for adult spot (*Leiostomus xanthurus*) (Mayer, 1987). Landau and Tucker (1984) evaluated the toxicity of aldicarb in juvenile common snook (*Centropmus undecimalis).* The 36-h median lethal concentration of aldicarb in snook embryo/larvae bioassay was 40 µg/L. At 250 and 500 µg/L, eggs had a granulated yolk and larvae were more contorted at death.

SELECTION OF TEST ORGANISM

Grass shrimp were chosen as the test organism in this study for several

reasons. First, invertebrates have been shown to be more sensitive to pesticide exposure than fish, particularly insecticidal exposure (Hansen, Schimmel, and Keltner, 1973). Second, grass shrimp are important in the estuarine food web, serving as a major food source for many commercial fish species. They are also an important predator in the regulation of meiofauna (Bell, 1978; Berg and Sandifer, 1984). Third, grass shrimp constitute a significant biomass in estuaries, and are instrumental in transporting nutrients between various trophic levels (Welsh, 1975). Fourth, grass shrimp have been used extensively in toxicity tests due to their sensitivity to various pollutants and because of their importance in estuarine ecosystems (Tyler-Schroeder, 1978; McKenney and Hanmaker, 1984; Anderson, 1985; Clark et al., 1987). Fifth, grass shrimp are cosmopolitan in estuaries and marshes, ranging along the entire Eastern seaboard to the Gulf of Mexico and they play a dominant role in the energy cycles of tidal marsh estuaries (Welsh, 1975). Grass shrimp are abundant where turbidity is relatively high. This is particularly evident in habitats where water currents tend to keep sediments suspended, such as in shallow tidal creeks or near river mouths (Anderson, 1985). In less turbid habitats, these shrimp inhabit the shallows surrounding the emergent aquatic macrophytes. Grass shrimp are adapted to the low-oxygen environment of the emergent estuarine habitat and thus are able to escape predators and competition and develop large populations (Buikema and Cairns, 1980).

In relation to other species of *Palaemonetes*, *P. pugio* represents an

intermediate stage in transition from salt to fresh water. *P. pugio* is able to tolerate low salinity levels and adults can regulate blood sodium over a wide range of salt concentrations. However, newly hatched larvae require brackish to marine water (Knowlton and Kirby, 1984). In the laboratory, larval development is rapid and reproductive size is attained within two months (Buikema, Cairns, and Hall, 1976). Larvae are planktonic and feed upon zooplankton, algae and detritus. Depending on the species and environmental conditions, there may be from 3 to 11 morphologically distinct stages during larval development. The final larval stage metamorphoses into a postlarva at 18 to 22 days of age and closely resembles the adult. Juvenile *P. pugio* reach sexual maturity when they are 1.5 to 2 months of age and are about 15 to 18 mm long. The average life span for *P. pugio* is about 1 to 2 years.

P. pugio has proven to be useful in bioassay testing not only because of their sensitivity to various toxicants, but also because they are easily cultured in the laboratory and can be used in flow-through life cycle toxicity studies (Tyler-Schroeder, 1978). The ecological importance of *P. pugio*, coupled with their usefulness in bioassay testing has therefore led to its selection as the test species for this research.

RESEARCH CONSIDERATIONS

Aquatic organisms have the greatest potential to experience environmental impact from pesticide usage (Clark et al., 1987; Baughman et al., 1989) and seem to bioaccumulate pesticides more readily than do terrestrial

organisms (Willis and McDowell, 1982). In assessing the impacts of pesticides on nontarget organisms, previous studies have focused mainly on lethal toxicant effects through the use of chronic and acute toxicity tests (Livingston, 1977; Murty, 1986; Clark, 1988; Baughman, 1989; Moore, 1989).

However, pesticides are generally present in aquatic ecosystems at levels much below those concentrations that cause mortality. Organisms that survive exposure to sublethal concentrations of pesticides may exhibit changes in their behavior, physiology and ability to learn (Farr, 1977; Kraus and Kraus, 1986). The ability of an organism to detect and avoid harmful concentrations of a toxicant is an ecologically important behavior by which an organism can adapt to, or compensate for, potentially lethal changes in its' environment (Giattina and Garion, 1983). To date, the available information on the sublethal effects of insecticides on nontarget species, particularly invertebrates, is limited.

As a step to further understanding the impacts of certain insecticides on invertebrates, measurement of AChE activity and inhibition at different life stages could be a useful indicator of sub-lethal toxicant effects to the nervous system. AChE activity has been found in the whole body of the freshwater shrimp, *Pararya australiensis* (Abdullah et al., 1994); the hepatopancreas of the brown shrimp and the oyster, *Crassostrea virginica* (Chambers et al., 1979); in the brain and ventral ganglion of the blue crab, *Callinectes sapidus* (Habig et al., 1986); the muscle of the shrimp, *Palaemon serratus* (Galgani and Bocquene, 1990) and in the nerve tissue of the prawn, *Metapenaeus*

monoceros (Reddy and Rao, 1988).

As an assessment tool, the display of preference-avoidance responses by organisms has been shown to be a sensitive indicator of the sub-lethal effects of various toxicants (Giattina and Garion, 1983). Although fish avoidance behavior studies (Giattina and Garton, 1983; Cherry and Cairns, 1982; Heath, 1995) have been well documented, limited information is available on crustaceans. The avoidance response of *Palaemonetes pugio* to DDT, endrin, chlorpyrifos (Dursban), malathion, carbaryl (Sevin) and 2,4-D was evaluated by Hansen, Schimmel, and Keltner (1973). The authors suggested that the shrimp were extremely vulnerable to insecticidal exposure because the shrimp were unlikely to avoid pesticide polluted water.

In a study by Chu and Lau (1994), the effects of diazinon, malathion, and paraquat on the feeding responses of the shrimp, *Metapenaeus ensis,* to chemical stimuli was investigated. At pesticide concentrations lower than the reported LC_{50} values, the shrimp displayed inhibited feeding behaviors. These authors concluded that at sub-lethal concentrations of pesticides, the long-term growth and survival of the organism could be impacted.

Farr (1977) found that the grass shrimp, *Palaemonetes pugio,* became more susceptible to predation after exposure to sublethal concentrations of ethyl or methyl parathion. Similarly, Tagatz (1976) found that the shrimp, *P. vulgaris,* displayed significantly different predator avoidance responses when exposed to the insecticide Mirex (cyclodiene insecticide). These studies show

that behavior displayed by organisms could be used as sensitive assays for determining the impact of sub-lethal concentrations of toxicants on various aquatic species.

RESEARCH OBJECTIVES

In a report published by the World Health Organization (1991), the findings of numerous studies on the toxicity of aldicarb was summarized. However, there was no data on the toxicity of aldicarb on *P. pugio,* even though this organism has been shown to play a dominant role in the energy cycles of estuaries, in addition to being the major prey item for many commercially important fish species. In this study, emphasis was placed on the quantification of behavioral responses, enzyme activity and mortality rates of different life stages of *P. pugio* exposed to aldicarb.

The specific hypotheses to be tested in this study included the following:

1) Adult *P. pugio* detect and avoid lethal and sublethal concentrations of aldicarb contaminated water.

2) Young *P. pugio* are more sensitive to aldicarb than the adults, therefore toxicity decreases with age.

3) Aldicarb inhibits acetylcholinesterase activity in a dose dependent manner and can be used as a sublethal indicator of insecticide exposure.

MATERIALS AND METHODS

TEST ORGANISM

Adult grass shrimp were collected by dipnet from Leadenwah Creek (Figure 2), a tidal tributary of the North Edisto River, in South Carolina. This site is considered uncontaminated and has been extensively studied (Scott et al., 1990). Grass shrimp were transported back to the laboratory where they were cultured and maintained in a 220 L re-circulating glass tank.

Shrimp were cultured and maintained in Instant Ocean (Aquarium Systems, Mentor, OH) artificial seawater made with distilled water. Water temperature was maintained at 22 to 25° C and was aerated with airstones and circulated through the chamber using Aqua Clear 201 and 301 pump heads (Rolf C. Hagen, Inc., Mansfield, MA). Salinity was maintained at 24 ppt because this was a natural level previously found in the field. The optimal salinity levels for this species range between 20 and 25 ppt. Dissolved oxygen was kept around 6.0 mg/L and the pH of the water was maintained at 8. During Spring/Summer a 14-L:10-D light cycle regime was maintained for breeding and maintenance stock. The light cycle was progressively reduced to 10L:14D during Fall/Winter to mimic natural seasonal changes. Adult shrimp were fed a combination of Wardley's Shrimp Pellets™, Argent Hatchfry Encapsulon™ powdered food, and newly hatched *Artemia* (Buikema et al., 1980).

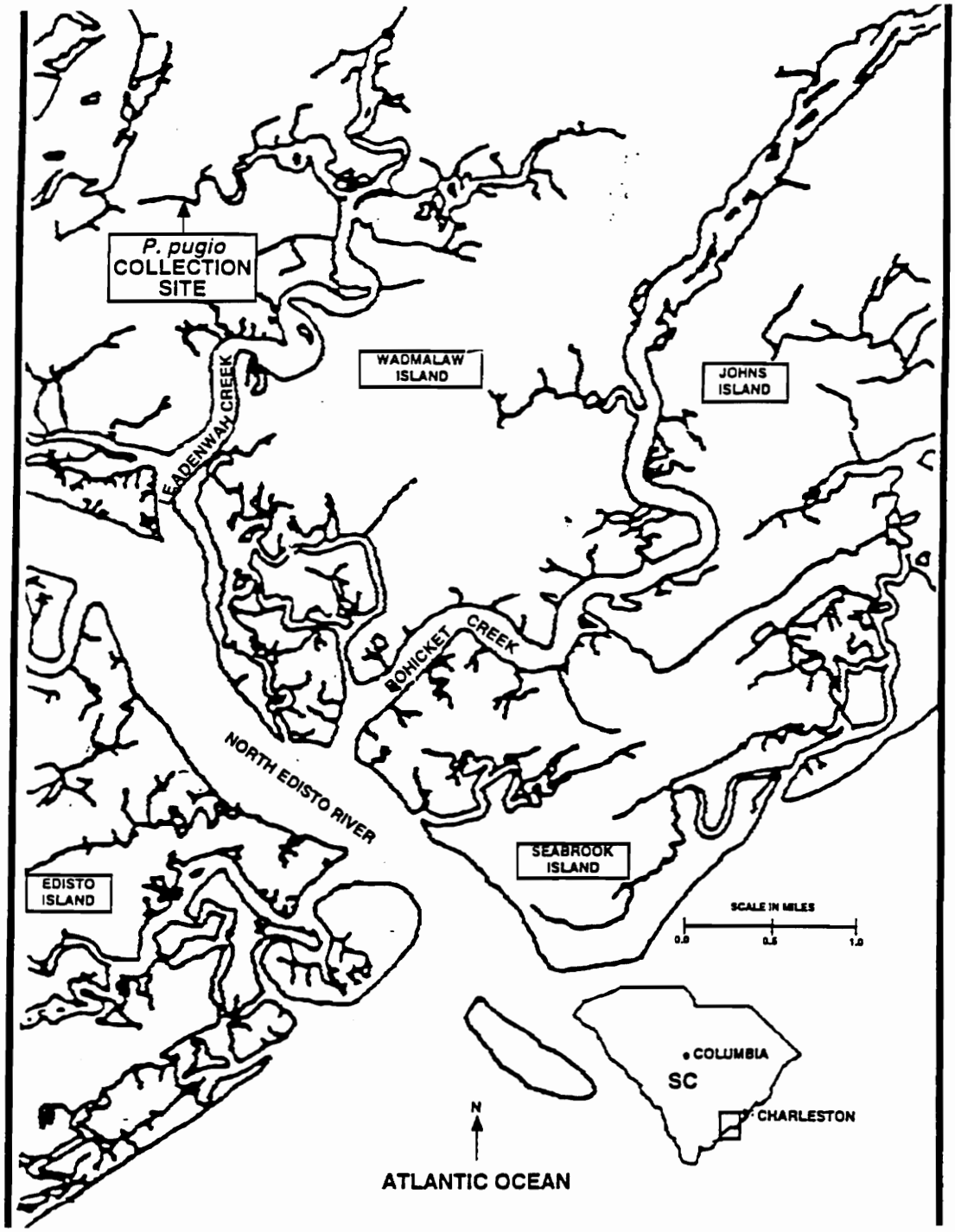


Figure 2. Map of *Palaemonetes pugio* collection site (North Edisto River in S.C., USA).

To collect larvae, gravid females were placed in mesh breeding chambers (mesh size: 2 x 3 mm) in a 20 L glass aquarium. This mesh allowed the larvae to escape and avoid predation by the females. Larvae used for bioassays were reared in 4 L glass aquaria until they reached testing age (newly hatched or 22-d old). Larvae were fed a diet of Argent Hatchfry Encapsulon™ and newly hatched *Artemia*. Larvae used for testing were pooled from the broods of at least 10 females to better represent a natural population.

TOXICITY TESTS

Because sensitivity to pesticides can change during different developmental life stages (Key, 1995; Mayer, 1987), the acute 96-h toxicity of aldicarb to three life stages was determined. These different age groups were newly hatched larvae, 22-d old larvae, and adult (carapace length = 2.5 cm) grass shrimp. Technical grade aldicarb (99.0% pure) was used in all tests (ChemService, West Chester, PA).

Determination of Standards

Prior to initiating the toxicity tests, a degradation study was conducted to determine the degradation rate of aldicarb over a 5-d period and also to verify stock toxicant concentrations of 2 mg/L and 100 µg/L . A Shimadzu high performance liquid chromatograph was used to analyze aldicarb stocks. Stock solutions were processed through a C18 reverse phase columns (column size: 4.6 ml x250 ml) at 42°C, with a flow rate of 1 ml/min. The initial stock solutions were diluted with deionized water (pH=7) to 0.004 µg/L and analyzed against

the following standards:

SET 1 standards (µg/L): 0.001 Lannate, 0.001 Aldicarb, 0.001 Sevin, and 0.002 Carbofuran.

SET 2 standards (µg/L): 0.002 Lannate, 0.002 Aldicarb, 0.002 Sevin and 0.004 Carbofuran.

Three separate runs were performed (standards and aldicarb stock solutions) on each testing day. There was no detectable degradation after 1 to 3 days and after 5 days there was 10% degradation. Because of degradation, test solutions were changed daily. These concentrations were not verified because of the expense of the procedure involved and the extreme handling hazards associated with aldicarb.

Range Finding Toxicity Tests

A series of range-finding 96-h bioassays were performed to determine the toxicity of aldicarb. Results from the range-finding tests were used to define definitive test concentrations. During each toxicity test, water chemistry analyses were performed before and after each water change. Salinity was measured using a SeaTest (Aquarium Systems, Mentor, OH) plastic hydrometer. Dissolved oxygen and pH were measured using a YSI Model 54A oxygen meter (Yellow Springs Instrument Co, Yellow Springs, OH) and a Fisher Accumet 10 pH meter (Pittsburgh, PA), respectively. Toxicity tests were conducted in a Sherer Model Gel 44 environmental chamber. Environmental conditions for all tests were maintained at 22°C, a 14L:10D light cycle and 24

ppt salinity.

Static Renewal Toxicity Tests

Static renewal toxicity tests (96-h) were conducted under the same test conditions described previously for the range-finding tests. The newly hatched larvae (48 to 72-h old) were exposed in 400-mL pyrex culture dishes. Each culture dish contained 200 ml of test solution and a partial water change of the test solution was conducted every 24 hours. Larvae were fed 0.5 mL of Argent Platinum Grade™ *Artemia* at the beginning of the test and after each water change. Larvae were randomly placed in the containers with three replicates for each of six aldicarb concentrations used and one control (N=30 organisms/treatment). These procedures and conditions were repeated for 22-d old larvae. The following toxicant concentrations were used: 6.25, 12.5, 25, 50, 100 and 200 µg/L .

Adult grass shrimp (average carapace length 2.5 cm) were exposed in 4-L glass aquaria, for each of the six aldicarb concentrations and one control (N = 30 organisms/treatment). Each aquarium contained 4 L of test solution, with a partial water change made every 24 hours. Environmental conditions were the same as those described for larvae. The following concentrations of aldicarb were used: 12.5, 25, 50, 100, 150 and 200 µg/L .

BEHAVIORAL STUDY

The ability of an organism to detect potentially lethal changes in its environment is an ecologically important one. The ability of adult grass shrimp

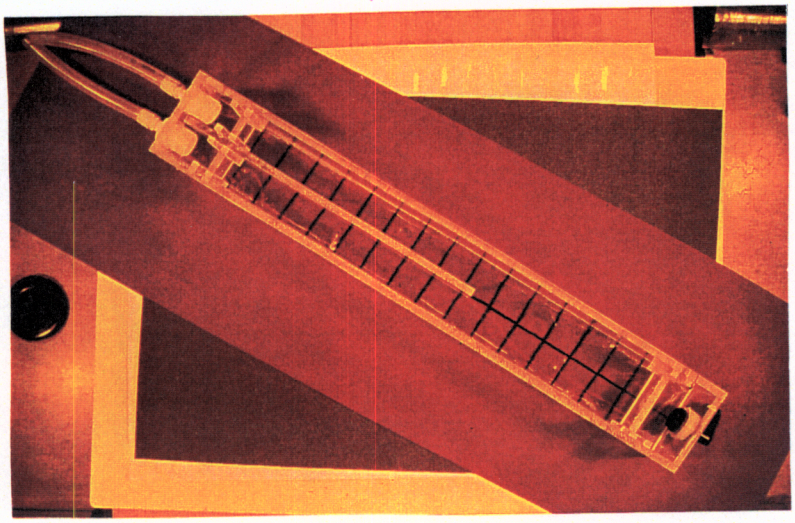
to detect lethal and sublethal concentrations of aldicarb treated seawater was determined in this study. The following behaviors were measured:

1) ability of the animal to detect and avoid aldicarb treated seawater
2) percent time spent in each area of the chamber.
3) directional orientation of the shrimp in the chamber.

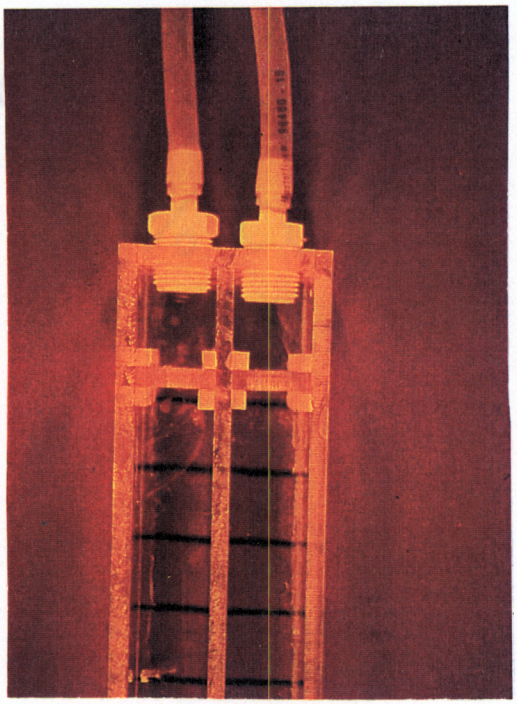
Behavioral responses were measured in an 45 x 7.5 x 6 cm modified steep gradient plexiglass chamber (Figure 3). The chamber was closed except for two inlet plastic fittings and a single outlet fitting, located on opposite ends of the chamber near the base of the plexiglass sides. One inlet delivered seawater and the other delivered aldicarb treated seawater. The outlet carried seawater from the chamber into a drainage bucket. A partition divided the area between the two inlet valves (35 x 1.25 x 3.8 cm), which allowed the shrimp to make a distinct choice between the following three areas of the chamber: aldicarb treated seawater (under test conditions), seawater, or a mixing area where the two solutions converged. Removable overflows (2.5 cm ht. for outlet and 3 cm ht. for inlet) were positioned at each end of the chamber so that the water moved uniformly (i.e. as a "wall") in and out of the chamber. Seawater with and without aldicarb was pumped through the chamber at a rate of 500 ml/min using a Masterflex peristaltic pump (Cole-Palmer, Chicago IL, Model# 7520-10) fitted with size 18 heads and Masterflex size 18 tubing. Each of the three areas of the chamber was comprised of approximately ten 2.5 x 2.5 cm squares.

Before testing, a study was conducted to determine if mixing would occur

Overview



Inlet



Outlet

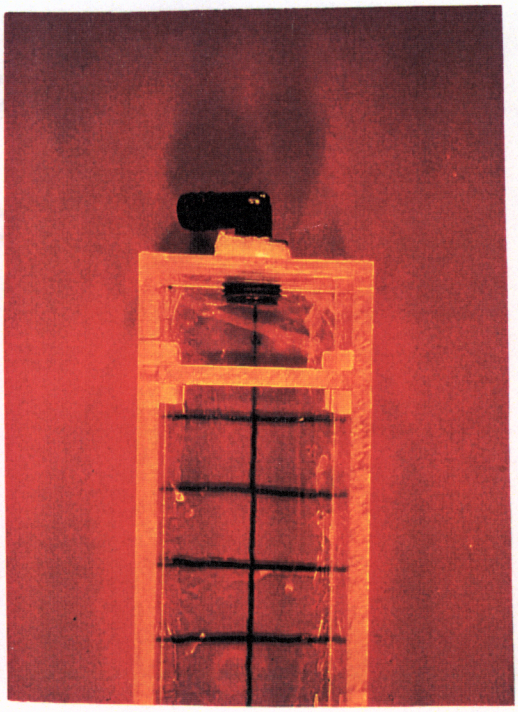


Figure 3. Diagram of behavioral chamber used to test responses of adult grass shrimp to aldicarb.

between the two sides of the chamber. Methylene blue dye was introduced through one side of the chamber and plain seawater was introduced on the other side. Fifteen minutes (3 min longer than behavioral test trials) after the dye had been introduced, a water sample was taken every 5 cm on both sides of the chamber to determine if mixing had occurred between the two sides. The samples were analyzed in a Perkin-Elmer Lambda 3B UV/VIS Spectrophotometer and read at a wavelength of 656 nm. Results showed that no mixing occurred until 2.5 cm past the partition.

The aldicarb concentrations used in the behavioral study were 0, 25.0 and 125.0 μg/L , which were the LOEC (lowest observable effect concentration) and LC_{50} values as determined by the toxicity studies. There were 10 behavior trials (N=4 animals/trial) per test concentration. Each trial was twelve minutes in duration.

Artificial seawater was used in the behavioral study and maintained at 24 ppt salinity and 25°C. Salinity was measured using a SeaTest plastic hydrometer (Aquarium Systems, Mentor, OH). The water was constantly aerated and dissolved oxygen was measured using procedures descibed earlier. To partially eliminate observer influence, the sides of the behavior chamber were covered by white cardboard. A white backround was chosen so that the animals could be observed and videotaped. Black plastic was suspended around the outside of the cardboard to minimize the penetration of

overhead fluorescent lighting. Adult shrimp were introduced into the chamber at the outlet overflow and allowed to acclimate in pesticide-free seawater for ten min prior to testing. Clear Plexiglass covers were placed over the chamber to prevent the shrimp from jumping out.

Shrimp activity was recorded for 12 min using a Hi Band 8 mm Sony camcorder. While viewing the video tapes, a stopwatch was used to record the amount of time individual shrimp remained in a particular area of the chamber. Times for individual shrimp were pooled and averaged for each trial to determine the mean percent time spent in each area. Shrimp orientation was quantified as the amount of time 1 shrimp/trial spent facing in a given direction. The orientation of the shrimp was noted at 1 min intervals. Observations were pooled and averaged for each test condition to determine the mean percent time spent facing a given direction. Additionally, physiological/behavioral condition (swimming or escape/telson-reflex) in the shrimp was noted.

ACETYLCHOLINESTERASE ASSAY

Newly hatched and 22-d old larval grass shrimp used for AChE analysis were exposed to aldicarb in 400 mL pyrex culture dishes with 200 mL of test solution per container. Larvae were obtained from the same breeding colony as those used for the toxicity study. A control and four concentrations of aldicarb were used: 12.5, 25, 50, and 100 µg/L . There were three replicates of ten animals each for each test concentration and control (N = 30 animals per concentration).

The animals were exposed for 24-h under the same test conditions as

described above. After 24-h exposure, the remaining live larvae (10-15 larvae/sample) and adult grass shrimp (2-3 adults/sample) were pooled, wrapped in acetone-rinsed aluminum foil and frozen for subsequent analysis. Shrimp were pooled to provide sufficient tissue for analysis. Whole body AChE activity was measured using a continuous assay described by Ellman (1961) and modified by Fulton (1989). Each newly hatched larval tissue sample was homogenized using a Potter-Elvehjem glass homogenizer with a teflon pestle. Tissue samples were homogenized on ice in 50 mM of AChE buffer (Tris-HCl buffer @ pH 8.1) at 5 mg/mL. 22-d old larval tissue was homogenized at 1 mg/L due to the larger size of the organism. Adults were homogenized at 20 mg/L . For each homogenate sample, three subsamples were assayed. A fourth subsample contained 10 mM of eserine (a plant alkaloid that blocks cholinesterase) to provide a blank for nonenzymatic hydrolysis of the substrate. For newly hatched, 22-d old larvae, and adults the following procedure was used to assay AChE activity:

1) Each test tube received 1.425 mL of Tris-HCl buffer.

2) 15 µl of 100% EtOH was added to all tubes except the eserine blanks, which contained 15 µl of $1x10^{-3}$ M eserine.

3) 75 µl of homogenate was added to all test tubes.

4) Test tubes were then vortexed at 2 min intervals and incubated at 37°C in a water bath for 15 min.

5) After the 15 min incubation period, 967 µl of homogenate was taken

from the test tube and added to a cuvette containing 33 µl of DTNB (0.87% 5,5' - dithiobis - [2-nitrobenzoic acid]), a color reagent.

6) 10 µl of the substrate (75 mM acetylthiocholine) was added to the cuvette.

7) The cuvette was covered with parafilm, inverted to mix, placed in a Spectronic 601^{R} spectrophotometer and read continuously for 1 min at a wavelength of 412 nm.

This procedure was repeated for each sample. Whole body AChE activity was recorded as nmol product formed/mg wet wgt/minute.

DATA ANALYSIS

Median lethal concentrations (LC_{50}) with 95% confidence intervals (CI) were determined using the Trimmed Spearman-Karber Method (Hamilton et al., 1977). The LOEC was estimated as the lowest concentration causing mortality. The no observable effect concentration (NOEC) was the concentration causing no mortality. Analysis of variance was used to determine if significant ($P \leq 0.05$) group differences existed for AChE activity and behavioral responses in shrimp exposed to aldicarb. An All Pairwise Multiple Comparison Procedure (Dunnett's Method) was used to determine if significant differences occurred between the control and treatments (Gad and Weil, 1988) for AChE activity and behavioral responses. Linear regression was used to analyze how AChE activity was affected by aldicarb and to determine if a significant correlation existed between the two variables.

RESULTS

96-h TOXICITY TESTS

The 96-h LC_{50}'s for grass shrimp exposed to aldicarb were 85.0 µg/L for newly hatched larvae, 70.7 µg/L for 22-d old larvae and 125.4 µg/L for adults (Figure 4). The NOEC values were 6.25 µg/L for newly hatched and 22-d old larvae and 12.5 µg/L for adults (Table 1). The LOEC's ranged from 12.5 mg/L for newly hatched and 22-d old larval shrimp to 25.0 µg/L for adults.

ACETYLCHOLINESTERASE ASSAY

Newly hatched

Mean whole body AChE activity in newly hatched larval shrimp was significantly different from mean AChE activity in the control larvae (P=0.001). AChE activity in the newly hatched larvae was significantly (P<0.05) reduced after 24-h in the 12.5, 50.0, and 100.0 µg/L concentrations, but not in the 25.0 µg/L concentration (Figure 5). There was a negative relationship between AChE activity and aldicarb exposure for newly hatched larvae (P=0.0004).

22-d old larvae

Mean whole body activity for 22-d old larvae was significantly reduced from mean AChE activity in the control larvae (P=0.009). AChE activity was significantly reduced (P<0.05) after 24-h at 50.0 and 100.0 µg/L (Figure 6). There was a negative relationship between AChE activity and aldicarb exposure (P=0.0002).

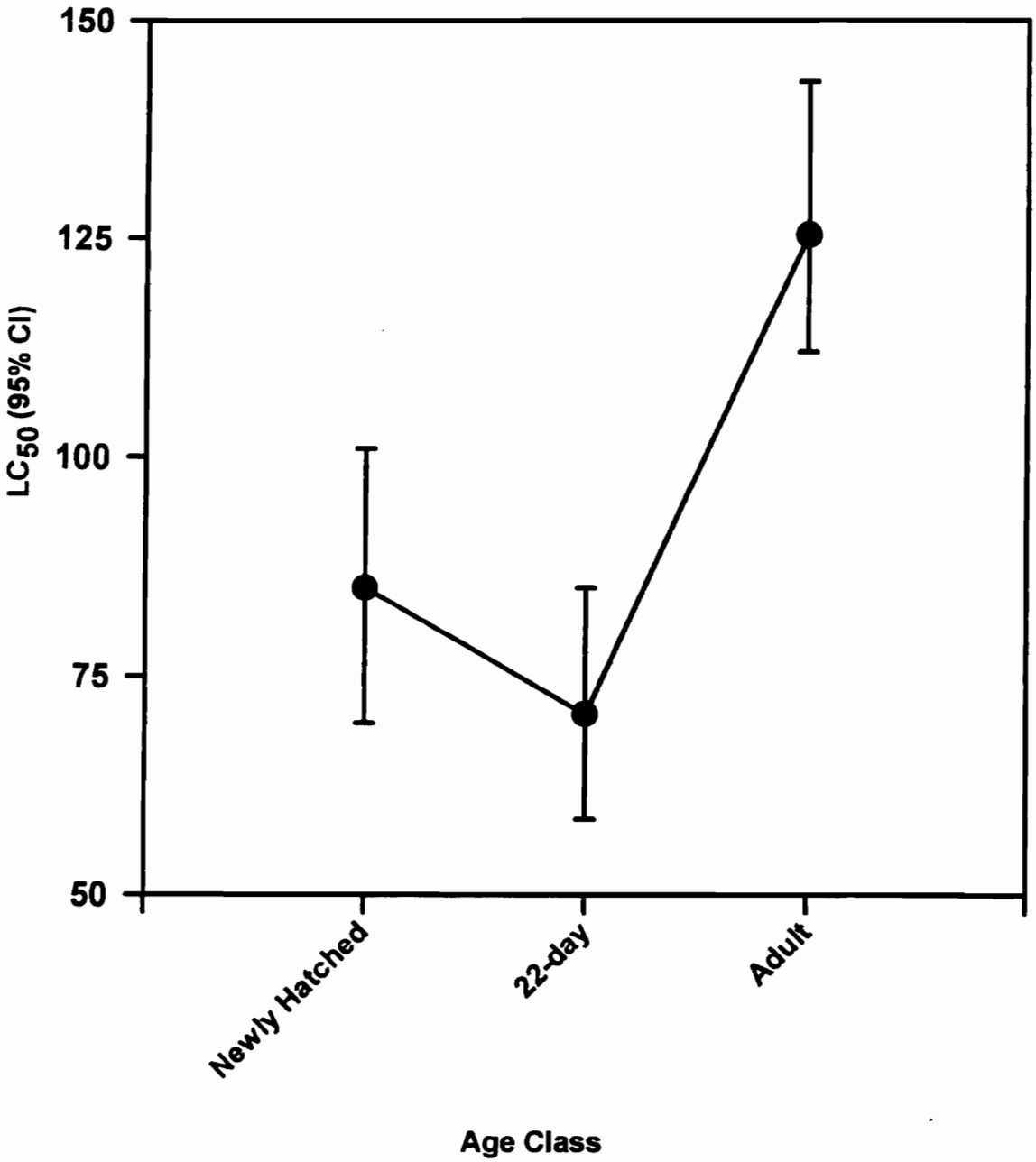


Figure 4. LC50 values for three life stages of *Palaemonetes pugio*.

Table 1. LC_{50}, NOEC and LOEC 96-h toxicity values for newly hatched larvae, 22-d old larvae, and adult *Palaemonetes pugio* (N = 30 animals/concentration).

Life Stage	**LC_{50} (μg/L) [95% CI]**	**NOEC (μg/L)**	**LOEC (μg/L)**
Newly hatched larvae	85.0 [71.6 -100.9]	6.25	12.50
22-d old larvae	70.7 [58.7 - 85.1]	6.25	12.50
Adult	125.4 [110.0 -143.0]	12.5	25.0

* LC_{50}=lowest concentration causing 50% mortality; LOEC=lowest observable effect concentration; NOEC=no observable effect concentration.

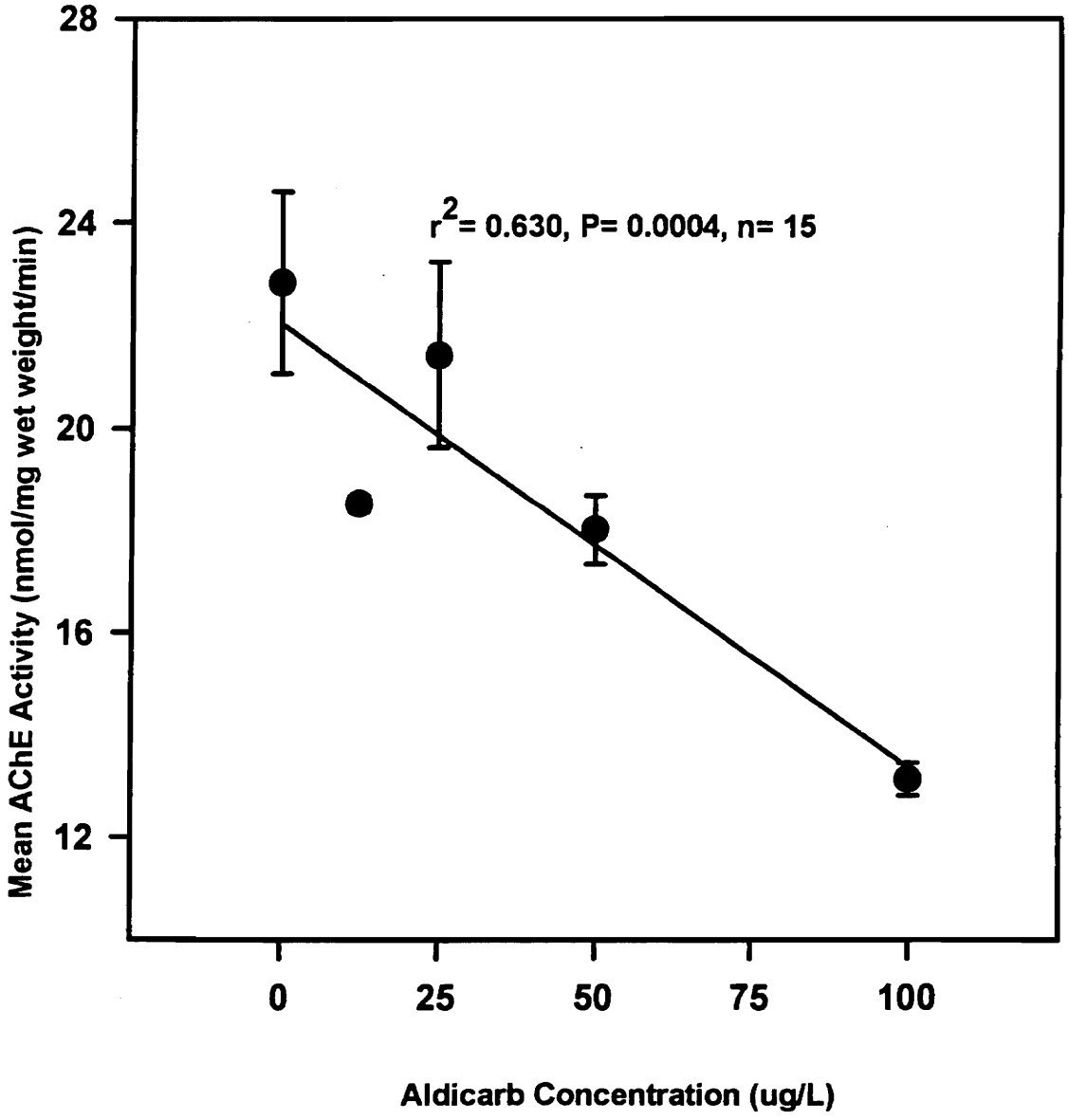


Figure 5. Linear regression of mean acetylcholinesterase (AChE) activity in newly hatched larval grass shrimp after 24-h exposure to different aldicarb concentrations.

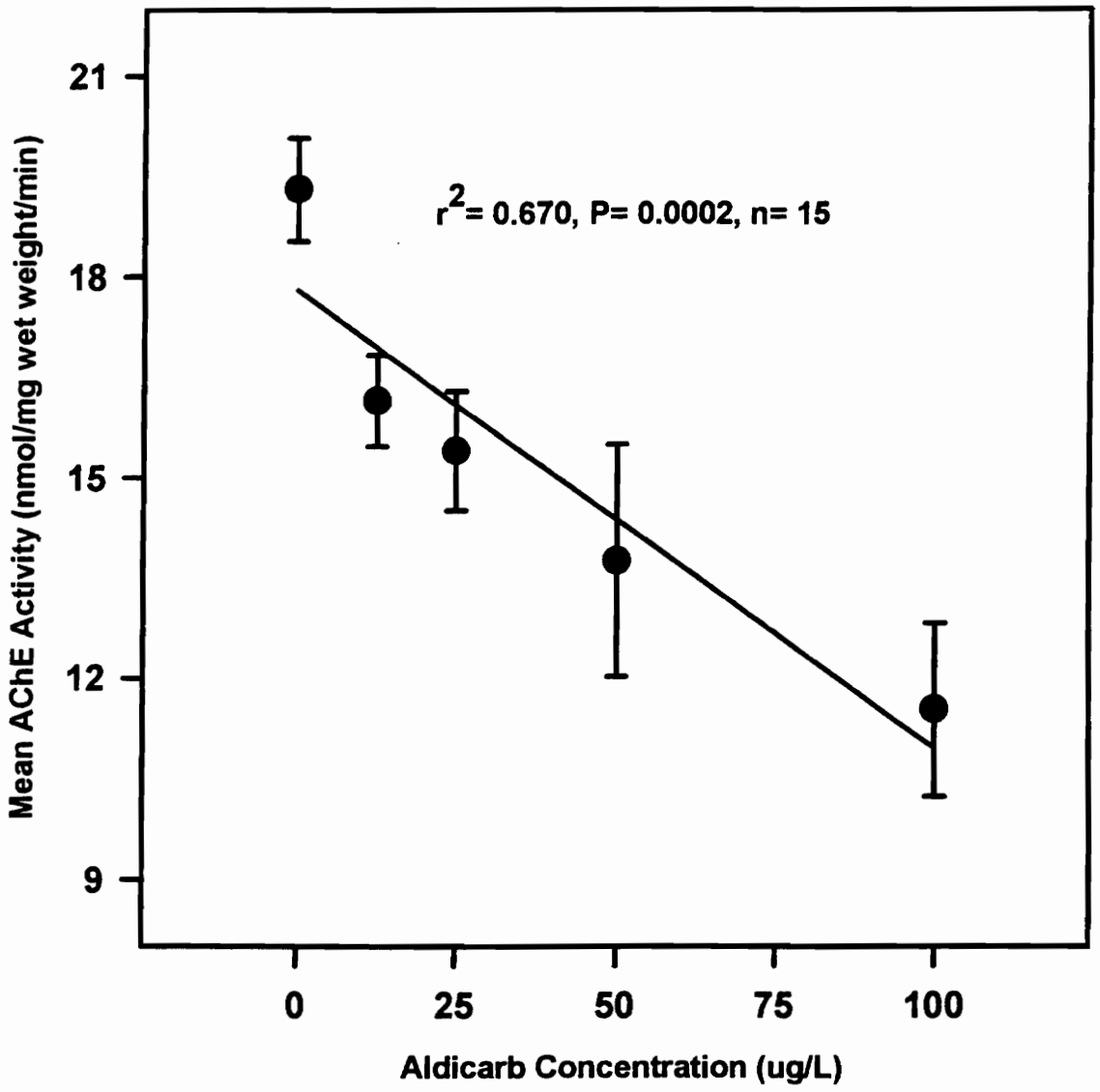


Figure 6. Linear regression of mean acetylcholinesterase (AChE) activity in 22-day old grass shrimp after 24-h exposure to different aldicarb concentrations.

Adults

Mean whole body AChE activity for adult shrimp (Figure 7) was not significantly different from the control at any concentration (P=0.401). There was no significant relationship between AChE activity and aldicarb concentration.

A reliable estimate of the 24-h EC_{50} (Effective concentration causing 50% inhibition of AChE) for larval and adult grass shrimp exposed to aldicarb could not be obtained with the design used in this study. At 100 µg/L, AChE inhibition was no greater than 41% for the newly hatched larvae, 39% for the 22-d old larvae and 11% for the adults. At concentrations higher than 100 µg/L, shrimp mortality was near 100% for 22-d and newly hatched larvae, thereby leaving an insufficient amount of tissue for later analysis.

BEHAVIOR

Evaluation of the ability of the adult grass shrimp to avoid aldicarb treated seawater at the LC_{50} and LOEC values was based on observations that under control conditions the shrimp would enter each area of the three areas of the chamber with equal frequency. Under test conditions, control shrimp showed no preference for the seawater area or the aldicarb-treated seawater area. However, control shrimp did display a preference for the mixing area. Therefore, control shrimp did not enter all areas of the chamber with equal frequency as expected.

In comparing the control trials to the exposure trials, there was a

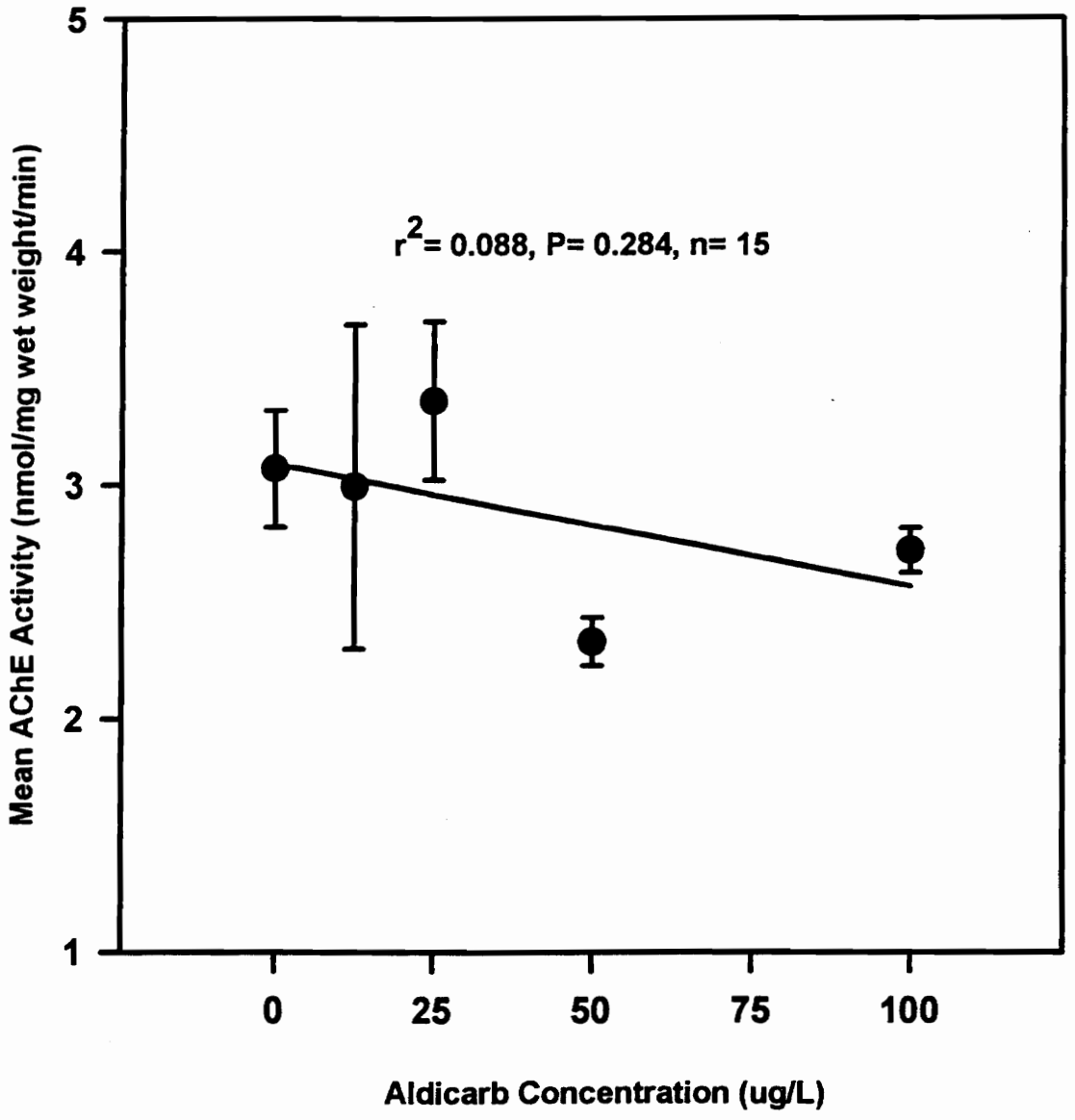


Figure 7. Linear regression of mean acetylcholinesterase (AChE) activity in adult grass shrimp after 24-h exposure to different aldicarb concentrations.

significant difference ($P<0.05$) between the time spent in areas by the control shrimp and the time spent by exposed shrimp in the same corresponding area (Figure 8). On the average, exposed shrimp spent 20% more time in the mixing area than did the control shrimp.

There was also a significant difference in the mean percent time spent by grass shrimp facing downstream, away from the flow of the water ($P<0.05$). Control grass shrimp spent 34% of their time facing downstream. At 25 and 12 µg/L concentrations, the grass shrimp spent 69% and 73% of the time facing downstream, respectively (Figure 9).

Other behavioral observations such as flight-type reactions were noted. As aldicarb concentrations increased, grass shrimp exhibited increasing hyperactivity and would swim into the outlet overflow. Additionally, exposed shrimp displayed telson-reflex behaviors in an attempt to jump out of the behavioral chamber.

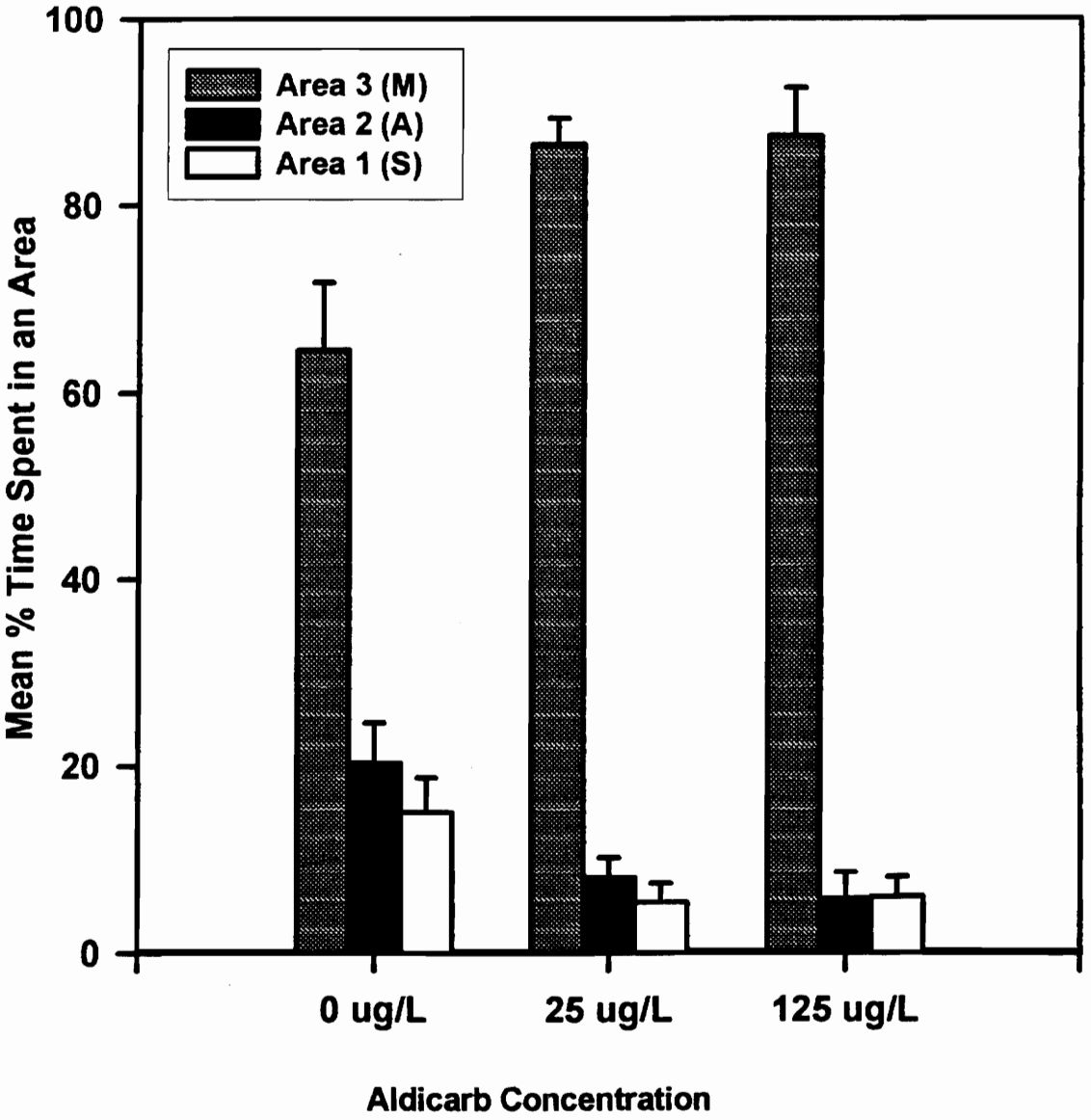


Figure 8. Mean % time spent in each area of the behavioral chamber at 0, 25 and 125 ug/L of aldicarb (A1 = seawater; A2 = Aldicarb-treated seawater; A3 = Mixing).

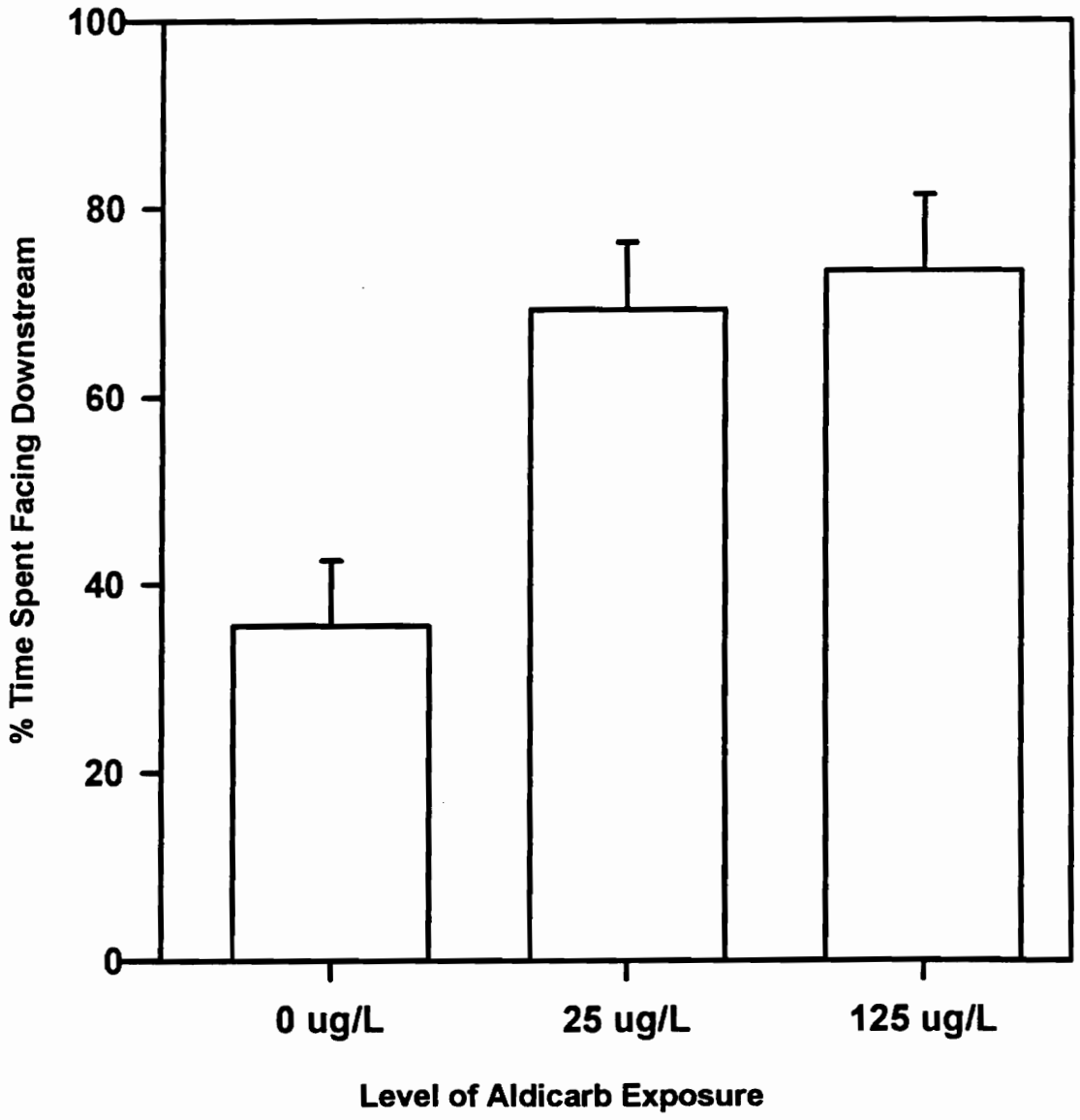


Figure 9. Mean time (%) spent facing downstream (180^{O} to stream flow).

DISCUSSION

ACUTE TOXICITY TESTS

Larval grass shrimp proved to be more sensitive to aldicarb than the adults after 96-h exposure. Other studies have also shown larval organisms to be more sensitive to pesticides than adults (Mayer, 1987; Cripe, 1994; Key, 1995). In toxicity studies done with larval crabs and sand shrimp, adults were 1 to 2 orders of magnitude more tolerant (Conner, 1972; Armstrong et al., 1976). Cripe (1994) found that larval pink shrimp were much more sensitive to total cadmium or fenvalerate than were the juveniles and adults. Crustaceans are particularly sensitive during molting and, since more frequent molting occurs during larval development, sensitivity to pesticides should be greater for these early life stages (Key, 1995). Co-occurrence of molting and mortality due to toxicant exposure has been observed in other studies. Death at molting has been seen previously in exposures of the crustacean *Penaeus kerathurus* (Forskal) to chlorine (Sargolia and Scarano, 1979) and in juvenile pink shrimp exposed to Aroclor™ 1254 (Nimmo et al., 1971).

In this study. the newly hatched larvae were slightly less sensitive to aldicarb than were the 22-d old shrimp, but the differences were not significant. After 96 hours, the LC_{50} was 70.71 µg/L for 22-d old larvae and 85.06 µg/L for newly hatched larvae. The onset of metamorphosis to the postlarval form usually occurs between 18 to 22-d and this may account for the apparent

increased sensitivity of the juvenile shrimp. The period just before metamorphosis is a crucial time and death occurring even under natural conditions is not uncommon (Tyler-Schroeder, 1978). Key (1995) noticed similar results when exposing 18-d and newly hatched *Palaemonetes pugio* to azinphosmethyl.

In other aldicarb studies with related crustaceans, Mayer (1987) reported an LC_{50} of 13 µg/L and 16 µg/L for 1-d old juvenile and adult mysid shrimp (*Mysidopsis bahia*), respectively. Mayer (1987) reported a similar 96-h LC_{50} of 12 µg/L for adult pink shrimp (*Penaeus duorarum*). Juvenile white shrimp (*Penaeus stylirostris)*, exhibited greater tolerance with an 96-h LC_{50} of 72 µg/L (Mayer, 1987). This is comparable to the LC_{50} of 70.71 µg/L for the 22-d old shrimp reported for this study. In reviewing the results of other acute toxicity studies with estuarine crustaceans, Mysidae and Penaeidae were generally the two most sensitive families (Tagatz, 1979; Goodman, 1988; Cripe, 1990, 1994). The results of these bioassays indicate that there is a wide range in the sensitivity of crustaceans to aldicarb and that sensitivity may vary with age.

ACETYLCHOLINESTERASE ASSAY

The inhibition of acetylcholinesterase in crustaceans is a function of the uptake, accumulation and metabolism of the toxicant. Crustaceans have been used extensively in determining lethal parameters of toxicants, but the use of sub-lethal parameters such as acetylcholinesterase inhibition has been limited (Edwards and Fisher, 1991). Previous studies have indicated that AChE

inhibition is a useful indicator of contaminant stress and exposure (Coppage and Matthews, 1974; Zinkyl et al., 1987; Key, 1995). Inhibition of AChE activity has been used as an indicator of toxicity in the brains of spot, pinfish, Atlantic croaker, sheepshead minnow and in the ventral nerve cord of the pink shrimp (Coppage and Matthews, 1974). Coppage and Matthews (1974) found that mean reductions in AChE activity of about 80% resulted in fish and pink shrimp mortalities. Zinkl et al. (1987) noted similar results in the rainbow trout, *Oncorhyncus mykiss*, after exposure to carbaryl. Minimum AChE depression that caused mortality was 61% in fish exposed to 0.25 µg/L of carbaryl. Fish allowed to recover in uncontaminated water had brain AChE activities that were only slightly lower than the controls.

Sublethal effects of AChE were noted in this study. There was a consistent decline in AChE activity as toxicant concentrations increased. For newly hatched larvae there was 30% and 41% inhibition at 50 and 100 µg/L , respectively. The 22-d old shrimp displayed similar inhibitions of 29% and 39% at 50 and 100 µg/L , respectively. In comparison to the newly hatched larvae, AChE levels in the 22-d old larvae were slightly lower for all concentrations and the control. Further testing at higher concentrations may have increased AChE inhibition in the shrimp, however at concentrations above 100 µg/L the shrimp were either moribund or dead.

Although there was no significant AChE inhibition at any toxicant concentration for the adult shrimp, there was a trend toward reduced activity at

50 and 100 µg/L exposures. AChE activity was higher in the 25 µg/L exposure than the control, but the difference was not significant. This could be a compensatory response induced by insecticide exposure. Hormesis is a compensatory response utilized by some organisms exposed to low and/or brief toxicant exposures (Davis and Svendsgaard, 1990). However, as exposure levels increase, an organisms ability to compensate is eventually overcome by the toxicant, as seen in this study. Hormetic effects have been reported in fish, coelenterates, and polychaetes (Laughlin et al., 1981).

Many marine organisms may have the ability to compensate for potentially harmful changes in their environment for short durations or when exposed to low toxicant concentrations. Key (1995) noted similar results in the grass shrimp when exposing them to chlorpyrifos. Repetto et al. (1988) reported an increase in AChE activity in the red crayfish, *Procambarus clarkii*, when exposed to low levels of trichlorfon. However, at higher concentrations AChE levels decreased.

In this study, shrimp were exposed to a high concentration of 100 µg/L of aldicarb; a level shown to be toxic to the grass shrimp. However, an EC_{50} (effective concentration causing 50% inhibition) for larval and adult grass shrimp could not be obtained for reasons not fully understood. In order to achieve 50% AChE inhibition in the tiger shrimp, *Penaeus japonicus*, Rompas et al. (1989) had to increase fenitrothion concentrations to 50 times higher than the 24-h LC_{50} value. In the same study, diazinon concentrations had to be

increased to 300 times the 24-h LC_{50} in order to achieve 50% inhibition in the tiger shrimp (Rompas et al., 1989).

In addition to AChE inhibition, it is probable that other factors contributed to the toxicity of aldicarb to the grass shrimp. In this study, aldicarb caused AChE inhibition, but the sequence of the inhibition was unclear. Because of their small size, whole body analysis had to be performed on the grass shrimp, and no specific target tissues could be pinpointed. Other factors essential to shrimp function and survival such as ventilation of the gills could be inhibited by aldicarb, but were not apparent in the AChE assay.

Low levels of AChE inhibition can cause physiological and behavioral impairments in fishes (Coppage et al., 1975; Gill et al., 1990; Key, 1995), but no data are available for invertebrates. Gill et al. (1990) examined the hemopathological changes in the rosy barb (*Puntius conchonius* Hamilton) when exposed to aldicarb. At sublethal concentrations, the agent caused statistically significant polycythemia, along with an increase in hemoglobin. The authors concluded that the affected fish suffered from immunodepression, which could lead to decreased disease resistance, inability to secure food, and physiological impairments. In another study, the carbamate carbaryl lowered RBC counts, hemoglobin, and hematocrit in *Tilapia mossambica* (Koundiya and Ramamurthi, 1979). Christensen et al. (1982) found that carbamates, along with being strong AChE inhibitors, also affect lipase *in vitro*.

Zinkyl et al. (1991), observed hyperactivity and loss of equilibrium in

rainbow trout exposed to carbaryl. Adverse behavioral responses due to AChE inhibition may therefore prove lethal for reasons other than direct poisoning.

BEHAVIORAL STUDY

All organisms react to adverse environmental conditions through a series of responses ranging from subtle metabolic adjustments to avoidance behavior to death. Behavioral responses have been shown to be useful indicators of toxicant effects on aquatic organisms. The clam *Macoma baltica* (Stekoll et al. 1980) and the fiddler crab *Uca pugnax* (Krebs and Burns, 1977) exhibited altered burrowing behavior when exposed to petroleum hydrocarbons. Mirkes et al. (1978) showed that cadmium and mercury affected the swimming behaviors in larvae of the mud crab *Eurypanopeus depressus*. Larvae of the copepod, *Eurytemora affinis*, exhibited reduced swimming velocities in response to copper and cadmium. Copper exposure caused the larvae to become hyperactive (Sullivan et al., 1983). Additionally, increased copper concentrations decreased predator avoidance abilities of the lavae in the presence of both larval striped bass and mysid shrimp.

Studies with *P. pugio* have been conducted as well. Hansen et al. tested the avoidance response of grass shrimp to DDT, endrin, malathion, chlorpyrifos (Dursban) 2,4-D and carbaryl (Sevin). The shrimp did not avoid any of the five pesticides, although they did avoid the herbicide 2,4-D. Bathalmus (1977) showed that the avoidance response to electric shock of *P. pugio* was significantly impaired when exposed to mercuric chloride. At 560 µg/L of

cadmium, locomotion in the grass shrimp was reduced (Hutcheson et al., 1985). Farr (1977) demonstrated that *P. pugio* became more susceptible to predation after exposure to sublethal concentrations of ethyl or methyl parathion. In a similar study, Tagatz (1976) showed that *P. vulgaris* displayed significantly different anti-predator responses after exposure to the insecticide Mirex.

In the present study, as aldicarb concentrations increased, more shrimp moved into the mixing area and increasingly faced downstream, away from the chemical source. Grass shrimp preference for the mixing area and downstream orientation may be natural responses or may in part be due to the chamber design, effect of current, and/or toxicant effects.

To some extent, the results may have been an artifact of the chamber design. In the control trials the shrimp spent 65% of their time in the mixing area. The width of the mixing area was twice as wide as the delivery channels and the grass shrimp may have preferred the additional space.

Water flow rate through the chamber may have also affected the results. Water flowed through the behavioral apparatus at 500 ml/min, which created a subtle current through the chamber. Field studies have shown that the movement and distributional patterns of the grass shrimp may be influenced by tidal cycles. Grass shrimp in tidal creeks migrate seaward or drift (downstream) with the current during ebb tides and migrate upstream into tidal creeks during incoming tides (Anderson, 1985). This natural behavioral response to current could have been a factor in causing the shrimp to spend more time in the mixing

area than in the other areas of the chamber. A static control would address whether or not current was a factor affecting the behavioral response of the shrimp.

The ability of an organism to detect lethal and sublethal changes in its environment is an ecologically important one (Kraus and Kraus, 1986). As stated previously, as aldicarb concentrations increased, the amount of time spent in the mixing area increased, as did the amount of time the shrimp spent facing downstream. Exposed shrimp spent, on the average, 20% more time in the mixing area than the control shrimp. It was observed that as dosage increased the shrimp became hyperactive, would display telson-reflex behaviors in an attempt to jump out of the chamber and would repeatedly swim into the plexiglass overflow at the opposite end of the chamber (away from the chemical source). These animals are highly mobile by nature, and therefore may have been attempting to swim away from the affected area. The behavior displayed by the shrimp could have been a negative (moving away) chemotactic response.

SUMMARY

This study provides basic information on the lehtal and sublethal toxicity of aldicarb to three life stages of *P. pugio.* Acute toxicity tests demonstrate that aldicarb is highly toxic to all life stages of grass shrimp. Larval shrimp were more sensitive to aldicarb than were the adults. Newly hatched larvae were slightly more tolerant to aldicarb than the 22-d old larvae.

The inhibiton of AChE activity in larval and adult shrimp generally occurred in a dose dependent manner. However, inhibition was more pronounced in the larvae. Mean whole body AChE activity for the newly hatched and 22-d old larvae was significantly lower from the mean AChE activity in the controls. Conversely, mean whole body AChE activity in the exposed adults was not significantly different from the controls. An EC_{50} could not be obtained under the design of this study. Grass shrimp may not have been able to survive higher levels of AChE inhibition after aldicarb exposure. Other factors not apparent in the AChE assay, such as behavioral, neurological and physiological modifications, may have reduced grass shrimp survival.

Initial grass shrimp preference for the mixing area, as seen in the control trials, may have been natural responses to the movement of current through the chamber or a behavioral preference for the larger width of the mixing area. As aldicarb concentrations increased, grass shrimp spent significantly more time in the mixing area, facing downstream away from the flow of the toxicant.

Additionally, exposed shrimp exhibited hyperactivity and escape behaviors.

Due to the lethal and sublethal toxicity of aldicarb to the larval life stages, use of this insecticide near coastal waters should be viewed with caution. Additional studies with varied estuarine species should be conducted to fully assess how chemicals, such as aldicarb, affect the estuarine ecosystem. Future studies should expose adult grass shrimp to higher aldicarb concentrations to discover if AChE inhibition increases. Further information on AChE inhibition is needed to determine if carbamates, such as aldicarb, are causing detrimental effects in the environment. Additional behavioral studies are also needed to further elucidate coping mechanisms utilized by organisms when exposed to toxicants. Equally important are studies that will enable agriculturalists to minimize potential impacts and discharges of pesticides into estuaries, while maintaining crop productivity. Without proper management and regulation of pesticides in coastal regions, valuable commercial fish species and ecologically important organisms such as *P. pugio* may be detrimentally affected.

LITERATURE CITED

Abdullah, A.; A. Kumar and J. Chapman. 1994. Inhibition of acetytlcholinesterase in the australian freshwater shrimp (*Paratya australiensis*) by profenofos. Environ. Tox. Chem. 13:1861-1866.

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. Rep. 82(11.35) U.S. Army Corps of Engineers, TREL-82-4. 19 pp.

Armstrong, D.A.; M.G. Stephenson and A.W. Knight. 1976. Acute toxicity of nitrite to larvae of the giant Malaysian prawn, *Macrobrachium rosenbergii.* Aquaculture. 9: 39-46.

Barthalmus, G.T. 1977. Behavioral effects of mercury on grass shrimp. Mar. Poll. Bull. : 87-90.

Baughman, D.S.; D.W. Moore and G.I. Scott. 1989. A comparison and evaluation of field and laboratory toxicity tests with fenvalerate on an estuarine crustacean. Eviron. Tox. Chem. 8: 417-429.

Bell, A.V. and B.C. Coull. 1978. Field evidence that shrimp predation regulates meiofauna. Oecologia. 35: 141-148.

Berg, A.V. and P.A. Sandifer. 1984. Mating behavior of the grass shrimp *Palaemonetes pugio* Holthius. Journal of Crustacean Biology. 4(3): 417-424.

Buikema, Jr., A.L.; J. Cairns, Jr. and L.W. Hall. 1976. Grass shrimp invertebrate bioassay for refinery effluent. Center for Environmental Studies. Virginia Polytechnic Institute and State University.

Buikema, Jr., A.L.; B. Niederlehner and J. Cairns, Jr. 1980. Use of grass shrimp in toxicity tests. Pp. 155-173. In: Aquatic invertebrates bioassays ASTM STP 715. A.L. Buikema, Jr. and J. Cairns, Jr., eds. American Society for Testing and Materials.

Bunyan, P.J.; M.J. Van Den Heuvel, P.I. Stanley and E.N. Wright. 1981. An intensive field trial and a multi-site surveillance exercise on the use of aldicarb to investigate methods for the assessment of possible environmental hazards presented by new pesticides. Agro. Ecosyst. 7: 239-262.

Christensen, G.; D. Olsen and B. Riedel. 1982. Chemical effects of the activity of eight enzymes: a review and a discussion relevant to environmental monitoring. Environ. Research. 29: 247-255.

Chambers, J.; J. Heitz, F. McCorkleand J. Yarbrough. 1979. Enzyme activities following chronic exposure to crude oil in a simulated ecosystem. Environ. Research. 20: 247-255.

Cherry, D.S. and J. Cairns. 1982. Biological monitoring part V - preference and avoidance studies. Water Res. 16: 263.

Chu, K. and P. Lau. 1994. Effects of diazinon, malathion, and parquat on the behavioral response of the shrimp, *Metapenaeus ensis*, to chemoattractants. Bull. Environ. Contam. Tox. 53: 127-133.

Clark, J.R.; J.M. Patrick,J.C. Moore and E.M. Lores. 1987. Waterborne and sediment source toxicities of six organic chemicals to grass shrimp (*Palaemonetes pugio*) and Amphioxus (*Branchiostoma caribaeum*). Arch. Environ. Contam. Toxicol. 16: 401-407.

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

Conner, P.M. 1972. Acute toxicity of heavy metals to some marine larvae. Mar. Pollut. Bull. 3: 190-192.

Coppage, D. and E. Matthews, G. Cook and J. knight. 1975. Brain acetylcholinesterase inhibition in fish as a diagnosis of environmental poisoning by malathion, 0,0-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate. Pest. Biochem. Physiol. 5: 536-542.

Coppage, D. and E. Matthews. 1974. Short-term effects of organophosphate pesticides on the cholinesterase of estuarine fishes and pink shrimp. Bull. Environ. Contam. Tox. 11(5): 483-488.

Cripe, G.M. and C.R. Cripe. 1990. Comparative acute sensitivities of selected estuarine and marine crustaceans to toxic substances. EPA 600/x-90/358. U.S. Environmental Protection Agency. Gulf Breeze, Florida.

Cripe, G. 1994. Comparative acute toxicities of several pesticides and metals to *Mysidopsis bahia* and postlarval *Penaeus duorarum*. Environ. Tox. Chem. 13: 1867-1872.

Davis, J. and D. Svendsgaard. 1990. U-shaped dose-response curves: their occurrence and implications for risk assessment. J. Tox. Environ. Health. 30: 71-83.

Edwards, C. and S. Fisher. 1991. The use of cholinesterase measurements in assessing the impacts of pesticides on terrestrial and aquatic invertebrates. Pp. 256-275. In: Cholinesterase-inhibiting insecticides: their impact on wildlife and the environment. P. Mineau, ed. New York: Elsevier Science Publishing Company Inc.

Ellman, G. K. Courtney, V. Andreas, Jr. and R. Featherstone. 1961. A new and colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7: 88-95.

Farr, J. 1977. Impairment of antipredator behavior in *Palaemonetes pugio* by exposure to sublethal doses of parathion. Trans. Fish. Soc. 1: 287-290.

Fulton, M. 1989. The effects of certain intrinsic and extrinsic variables on the lethal and sublethal toxicity of selected organophosphorus insecticides in the mummichug, *Fundulus heteroclitus* under laboratory and field conditions. PhD. Dissertation. University of South Carolina.

Gad, S. and C.Weil. 1988. Statistics and experimental design for toxicologists. Caldwell, NJ: Tellford Press.

Galgani, F. and G. Bocquene. 1990. In vitro inhibition of acetylcholinesterase from four marine species by organophosphates and carbamates. Bull. Environ. Contam. Tox. 45; 243-249.

Giattina, J.D. and R.R. Garton. 1983. A review of the preference-avoidance responses of fishes to aquatic contaminants. Residue Review. 87: 44-90.

Gill, T.; J. Pande and H. Tewari. 1990. Hemopathological changes associated with experimental aldicarb poisoning in fish (*Puntius conchonius* Hamilton).

Goodman, L.; D. Hansen, G. Cripe, D. Middaugh and J. Moore. 1985. A new early life stage toxicity test using the California grunion (*Leuresthes tenuis*) and results with chlorpyrifos. Ecotox. Environ. Safety. 10: 12-21.

Habig, C. and r. DiGiulio. 1991. Biochemical characteristics of cholinesterases in aquatic organisms. New York: Elsevier Science Publishers.

Hamilton, M.; R. Russo and R. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Tech. 11: 714-719.

Hansen, D.; S. Schimmel and J. Keltner, Jr. 1973. Avoidance of pesticides by grass shrimp (*Palaemonetes pugio*). Bull. Environ. Cont. Tox. 9: 129-133.

Heath, A.G. 1995. Behavior and Nervous System Function *in* Water Pollution and Fish Physiology. CRC Press, Boca Raton, FL. Chap. 12.

Hutcheson, M.; D.C. Miller and A.Q. White. 1985. Respiratory and behavioral responses of the grass shrimp *Palaemonetes pugio* to cadmium and reduced dissolved oxygen. Mar. Biol. 8: 59-66.

Key, P. B. 1995. The lethal and sublethal effects of malathion, azinphosmethyl and chlorpyrifos exposure on the grass shrimp, *Palaemonetes pugio*,

with emphasis on larval life cycle pulse exposures. PhD. Dissertation. University of South Carolina.

Knowlton, R.E. and D.F. Kirby. 1984. Salinity tolerance and sodium balance in the prawn *Palaemonetes pugio* Holthius, in relation to other Palaemonetes spp. Comp. Biochem. Physiol. 77A (3): 425-430.

Koundiya, P.R. and R. Ramamurthi. 1979. Hematological studies in *Sarotherodon mossambica* (Tilapia) exposed to lethal (LC_{50} 48 hr) concentration of sumithion and sevin. Curr. Sci. 48: 877-879.

Kraus M.L. and D.B. Kraus. 1986. Differences in the effects of mercury on predator avoidance in two populations of grass shrimp. Marine Environ. Research. 18: 277-289.

Krebs, C.T. and K.A. Burns. 1977. Long term effects of an oil spill on populations of the salt marsh crab *Uca pugnax.* Science. 197: 484-487.

Kuhr, R.J. and H.W. Dorough. 1976. Carbamate insecticides: Chemistry, biochemistry, and toxicology. Cleveland, Ohio, CRC Press, Inc.

Landau, S. and J.W. Tucker, Jr. 1984. Acute toxicity of EDB and aldicarb to young of two estuarine fish species. Bull. Environ. Contam. Tox. 33: 127-132.

Laughlin, R.; J. Ng and E. Guard. 1981. Hormesis. a response to low environmental concentrations of petroleum hydrocarbons. Science. 3111: 705-707.

Livingston, R.J. 1977. Review of the current literature concerning the acute and chronic effects of pesticides on aquatic organisms. CRC Critical Reviews in Environmental Control. 325.

Marshall, E. 1985. The rise and decline of Temik. Science. 229(4720): 1369-1371.

Mayer, F. 1987. Acute toxicity handbook of chemicals to estuarine organisms. EPA/600/8-87/017.

McKenney, C. and D, Hanmaker. 1984. effects of fenvalerate on larval development of *Palaemonetes pugio* (Holthius) and on larval metabolism during osmotic stress. Aquatic Tox. 5: 343-355.

McLusky, D.S. 1981. The estuarine ecosystem. John Wiley and Sons, publishers.

Mirkes, D.Z.; W.B. Vernberg and P.J DeCoursey. 1978. Effects of cadmium and mercury on the behavioral responses and development of *Eurypanopeus depressus* larvae. Mar. Biol. 47:143-147.

Murty, A.S., 1986. Toxicity of pesticides to fish. Vol. 2. CRC Press. Boca Raton, FL.

National Research Council. 1977. Drinking water and health. National Academy of Science. p. 637.

Nimmo, D.R.; R.R. Blackman, A.J. Wilson, Jr. and J. Forester. 1971. Toxicity and distribution of Aroclor^{R} 1254 in the pink shrimp, *Penaeus duorarum*. Mar. Biol. 11: 197-197.

Pait, A; A. DeSouza and D. Farrow. 1992. Agricultural pesticides in coastal areas: a national summary. Rockville, MD: Strategic Environmental assessments Division, ORCA/NOS/NOAA. 112pp.

Pant, S.C. and S. Kumar. 1981. Toxicity of temik for a freshwater teleost, *Barbus conchonius* Hamilton. Experientia (Basel). 37: 1327-1328.

Pickering, D.J. and W.T. Gilliam. 1982. Toxicity of aldicarb and fonofos to the early life stage of the fathead minnow. Arch. Environ. Contam. Toxicol. 11: 699-702.

Quraishi, M.S. 1972. Edaphic and water relationships of aldicarb and its metabolites. Canadian Entomol. 104(3): 1191-1196.

Reddy, S. and R. Rao. 1988. *In vivo* recovery of acetylcholinesterase activity from phosphamidon and methylparathion induced inhibition in the nervous tissue of penaeid prawn (*Metapenaeus monoceros*). Bull. Environ. Contam. Toxicol. 40: 752-758.

Repetto, G.; P. Sanz and M. Repetto. 1988. *In vivo* and *in vitro* effect of triclorfon on esterases of the red crayfish *Procambarus clarkii*.

Rompas, R.; K. Kobayashi, Y. Oshima, N. Imada. K. Yamato and Y. Mitsuyasa. 1989. Relationship between toxicity and acetylcholinesterase inhibition of some thiono- and oxo- form organophosphates in tiger shrimp larvae at different stages. Nippon Suisan Gakkaishi. 55 (4): 669-673.

Saroglia, M.G. and G. Scarano. 1979. Influence of molting on the sensitivity to toxics of the crustacean *Penaeus kerathurus* (Forskal). Ecotoicol. Environ. Saf. 3: 310-320.

Scott, G.I.; M. Fulton, D. Moore, G. Chandler, P. Key, T. Hampton, J. Marcus, K. Jackson, D. Baughman, A. Trim, L. Williams, C. Louden and E. Patterson. 1990. Agricultural insecticide runoff effects on estuarine organisms: correlating laboratory and field toxicity testing with ecotoxicological biomonitoring. U.S. National Marine Fisheries Service. CR-813138-02-1. 511 pp.

Stekoll, M.S.; L.E. Clement and D.G. Shaw. 1980. Sublethal effects of chronic oil exposure on the intertidal clam *Macoma baltica*.Mar. Biol. 77: 299-306.

Sullivan, B.K.; E. Buskey, D. C. Miller and P.J. Ritacco. 1983. Effects of copper and cadmium on growth, swimming and predator avoidance in *Eurytemora affinis* (Copepoda). Mar. Biol. 77: 299-306.

Tagatz, M.; P. Borthwick, G. Cook and D. Coppage. 1974. Effects of ground applications of malathion on salt-marsh environments in northwestern Florida. Mosq. News. 34: 309-312.

Tyler-Schroeder, D. 1978. Bioassay procedures for the ocean disposal permit program. EPA 600/9-78-010. Gulf Breeze, Florida.

U.S. EPA. 1988a. Aldicarb special review technical support document. Wasington, DC, US Environmental Protection Agency, Office of Pesticides and Toxic Substances.

Ware, G. 1989. The pesticide book. Fresno, CA: Thompson Publications.

Welsh, B. 1975. The role of grass shrimp, *Palaemonetes pugio*, in a tidal marsh ecosystem. Ecology. 56: 513-530.

Williams, T. 1993. Hard news on soft pesticides. Audubon. 95(2): 30-40.

Willis, G. and L. McDowell. 1982. Pesticides in agricultural runoff and their effects on downstream water quality. Environ. Tox. Chem. 1: 267-279.

Willoughby. 1989. Farm Chemicals Handbook: Meister Publishing Co.

World Health Organization. 1991. Aldicarb. 121: 130 pp.

World Health Organization. 1986. Carbamate pesticides: a general introduction. 64: 137 pp.

Zinkyl, J.G.; P.J. Shea, R.J. Nakamoto and J. Callman. 1987. Brain cholinesterase activity of rainbow trout poisoned by carbaryl. Bull. Environ. Contam. Toxicol. 38: 29-35.

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M.S., Biology, Virginia Polytechnic Institute and State University, Blacksburg,Virginia. November, 1996. Thesis title: The Lethal and Sublethal Effects of Aldicarb on the Estuarine Grass Shrimp, *Palaemonetes pugio*.

B.S., Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. May, 1991. * Minor: Political Science.

Professional Experience:

Graduate Teaching Assistant, Dept. of Biology, Virginia Polytechnic Institute and State University. 1994 - 1996

Graduate Researcher, Dept. of Biology, Virginia Polytechnic Institute and State University. 1993 - 1996

Laboratory Technician, Dept. of Biology, Virginia Polytechnic Institute and State University. 1992-1994

Laboratory Technician, Ecosystem Simulation Laboratory, Virginia Polytechnic Institute and State University. 1991-1992

Special Recognition:

Tuition Scholarship, VPI & SU, Academic Year 1994-1995, 1995-1996, second summer session 1995, first summer session 1996.

Instructional Fees Scholarship, VPI & SU, 1994.

Elected as an associate member of Sigma Xi (The Scientific ResearchSociety),1996.

Professional Organizations:

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Presentations:

Dvorak-Grantz, A.L. The Lethal and Sublethal Effects of Aldicarb on the Grass Shrimp, *Palaemonetes pugio*. Virginia Academy of Sciences, 1996.

Research Grants:

Principal Investigator: The Lethal and Sublethal Effects of Aldicarb on the Grass
Shrimp, *Palaemonetes pugio*. Sigma Xi, The Scientific Research Society. $500.00. February 1995.

Biology Departmental Research Grant. $500.00. March, 1995.

Research Experience:

Freshwater acute and chronic toxicity testing (static and flow-through):

Ceriodaphnia dubia
Pimephales promelas
Daphnia magna

Saltwater acute and chronic toxicity testing:

Mysidopsis bahia
Palaemonetes pugio
Menidia beryllina
Fundulus heteroclitus
Cyprinodon variegatus

Artificial estuarine simulation systems:

assisted in construction of systems
maintained simulation systems
cultured estuarine organisms
performed water chemistry

Field biomonitoring:

Benthic invertebrate sampling

Fish sampling

Behavioral testing:

evaluated the effect of aldicarb on the preference/avoidance behavior of *Palaemonetes pugio*

Culturing Experience:

Saltwater organisms:

Mysidopsis bahia

Palaemonetes pugio

Menidia beryllina

Fundulus heteroclitus

Cyprinodon variegatus

Freshwater organisms:

Ceriodaphnia dubia

Daphnia magna

Pimephales promelas

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