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AGRICULTURAL INSECTICIDE RUNOFF EFFECTS ON ESTUARINE ORGANISMS: CORRELATING LABORATORY AND FIELD TOXICITY TESTS, ECOPHYSIOLOGY BIOASSAYS, AND ECOTOXICOLOGICAL BIOMONITORING

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16. ABSTRACT

This study compared in situ, field and laboratory toxicity testing results for several insecticides (azinphosmethyl - an organophosphate; endosulfan - an organochlorine, and fenvalerate - a synthetic pyrethroid) with ecotoxicological biomonitoring results from the macropelagic, estuarine tidal creek community in pristine habitats and in areas receiving significant insecticide runoff from agriculture. Field studies were conducted over a four-year period (1985-88) at several coastal field sites on Wadmalaw (Leadenwah Creek) and Johns (unnamed tidal creek near Kiawah Island) Island, coastal sea islands located just south of Charleston, South Carolina. Results indicated that laboratory and field toxicity testing and biomonitoring methodologies should be integrated to provide holistic environmental risk assessments for pesticides. Laboratory toxicity tests provide the initial bench mark for estimating toxic effects. In situ, field toxicity tests provide a mechanism to validate initial laboratory tests and expand their design to test differences in formulations, life history stages, pulsed versus continuous dose, salinity interactions, and pesticide mixtures for more realistic estimates of effects of field exposures. Application of this method in the environmental risk assessment for three classes of pesticides (organochlorines-endosulfan, pyrethroids-fenvalerate, and organophosphates-azinphosmethyl) has been demonstrated in assessing the effects of nonpoint source agricultural runoff on sensitive estuarine tidal creek fauna in South Carolina. Over a three year period of study, the integration of this approach has provided significant data to assist environmental regulators trying to control recurrent problems of agricultural runoff effects in Leadenwah Creek and other areas of the state. Future studies should be expanded to broaden our understanding of the usefulness of this integrated approach in better assessing pesticide runoff in other aquatic ecosystems throughout the U.S.

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Agricultural Insecticide Runoff Effects On Estuarine Organisms: Correlating Laboratory and Field Toxicity Tests, Ecophysiology Bioassays, and Ecotoxicological Biomonitoring

by

G.I. Scott[1,3], M.H. Fulton[1], M.C. Crosby[2], P.B. Key[1], J.W. Daugomah[1], J.T. Waldren[1], E.D. Strozier[1], C. J. Louden[3], G.T. Chandler[3],T.F. Bidleman[3], K.L. Jackson[3], T.W. Hampton[4], T. Huffman[3], A. Shulz[3], and M. Bradford[3]

[1]U.S. National Marine Fisheries
Southeast Fisheries Science Center
Charleston Laboratory
Charleston, SC 29422-0607

[2]U.S. National Oceanic and Atmospheric Administration
National Ocean Survey
Office of Estuarine Sanctuaries and Reserves
1825 Connecticut Avenue, N.W. Room 714
Washington, DC 20235

[3]University of South Carolina
School of Public Health
Columbia, SC 29208

[4]Agency for Toxic Substance
Disease Registry
Atlanta, GA

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Office of Research and Development
Gulf Breeze, FL 32561

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EXECUTIVE SUMMARY

The primary objective of this study was to evaluate and compare *in situ* field and laboratory toxicity tests and ecophysiology bioassays (specific - brain AChE inhibition and nonspecific-O_2 consumption, NH_4 excretion, and O/N ratios) for several insecticides (azinphosmethyl - an organophosphate; endosulfan - an organochlorine, and fenvalerate - a synthetic pyrethroid) with several field ecotoxicological biomonitoring approaches (block seining and push netting) for assessing the macropelagic estuarine tidal creek community. Studies were conducted in pristine habitats (reference or control = CTL Site) and in two agricultural areas, one highly managed [Integrated Pest Management (IPM), Best Management Practices (BMP), and retention ponds] for control of nonpoint source pesticide runoff (Treatment site or Exposure site 1 = TRT Site) and a second unmanaged (no IPM, BMP, or retention ponds) site (Kiawah or Exposure site 2 = KWA Site). Field and laboratory studies were conducted over a two year period (1989-90) on coastal sea islands (Wadmalaw and Johns Island) located just south of Charleston, South Carolina.

Parameters measured included:

1) Laboratory ecophysiology bioassays with mummichogs exposed to azinphosmethyl for determination of specific (enzyme AChE) and nonspecific (bioenergetic metabolism - O_2 consumption, NH^4 excretion, and O/N ratios) biomarkers of exposure effects,:

2) *In situ* field toxicity tests with adult mummichogs (*Fundulus heteroclitus*), juvenile tidewater silversides (*Menidia berylina*), juvenile Penaeid shrimp (*Penaeus aztecus, Penaeus setiferus* and *Penaeus duorarum*), adult mysid shrimp (*Mysidopsis bahia*), and adult grass shrimp (*Palaemonetes pugio*) at the CTL, TRT, and KWA sites during periods of fair weather and following significant runoff events;

3) *In situ* ecophysiology bioassays with adult oysters (*Crassostrea virginica*) and mummichogs using both specific (enzyme AChE) and nonspecific (i.e., bio-energetic metabolism) biomarkers of exposure/effects at CTL, TRT and KWA sites during periods of fair weather and following significant runoff events;

4) Pesticide (azinphosmethyl, endosulfan, and fenvalerate) levels in surface waters (ng/L), sediments (ug/kg) and oysters (ug/kg) at the CTL, TRT and KWA sites along with transboundary measurements from tomato field at the KWA and adjacent tidal creeks at Seabrook Island;

5) Daily and continuous (i.e., every 15 minutes) measurements of water temperature (°C), salinity (ppt), dissolved oxygen (mg/L), pH, and relative water depth (M) at the CTL, TRT, and KWA sites before and after periods of significant pesticide runoff;

6) Biomonitoring (monthly or bimonthly block seining) of the macropelagic tidal creek community at the CTL and TRT sites;

7) Additional biomonitoring (monthly push netting) of grass shrimp (*Palaemonetes pugio*) populations at the CTL, TRT and KWA sites;

8) Statistical analysis of all data using both parametric and nonparametric procedures including calculation of laboratory and field derived LC_{50} and EC_{50} values, between site comparisons, and regression analysis (linear and logistic).

Results indicated that during 1989 there were four to five days of significant (> 1.27 cm/day) rainfall during the peak of the vegetable crop growing season (May - June) which resulted in significant runoff of azinphosmethyl, endosulfan and fenvalerate at the unmanaged agricultural site at KWA. Discharge of this runoff resulted in significant mortality to all caged toxicity test species at the KWA Site. Additionally, two fish kills were observed at this site. Significant inhibition of brain AChE in surviving mummichogs was noted along with uptake of insecticides (i.e., endosulfan) by oysters. All toxicity observed in field toxicity tests were the result of pesticide exposure, as a physicochemical water quality parameters (salinity, pH, and dissolved oxygen) remained at levels within the zone of capacity adaptation or tolerance for the crustacean and fish species tested. Transboundary movement (movement away from the original point of discharge) of insecticide runoff some two river miles (4.5 Km) during one tidal cycle (12-14h) was noted at the KWA Site, which resulted in additional impacts to juvenile fish in adjacent tidal creeks.

At the TRT Site, where agricultural management practices were in place (BMP, IMP, and retention ponds), pesticide discharges were greatly reduced. Only elevated levels of fenvalerate (65 - 93 ng/L) were observed, which caused significant toxicity among caged grass shrimp and penaeid shrimp. All grass shrimp and penaied shrimp toxicity resulted from pesticide exposure, as physicochemcial water quality parameters (i.e., dissolved oxygen) remained within normal limits of tolerance. There was no significant toxicity observed in other deployed bioassay species. Additionally, ecotoxicological biomonitoring indicated significant decreases in field populations (43%) of grass shrimp at the TRT Site. Oysters were exposed to fenvalerate (15 - 93 ng/L) and extremely low salinity conditions which

caused altered Q_{10} adjusted respiration (increased), nitrogen excretion rates (increased) and O/N Ratios; however, these slight changes in energetic metabolism did not translate into major changes in body component indices (condition index) and parasite infection intensity. While these results clearly demonstrated the potential of oyster ecophysiology measurements to assess NPS pesticide runoff effects, it is important to note the significance of co-factors such as low salinity exposure, that may occur concomitantly with pesticide exposure and confound interpretation of results. Results from oyster ecophysiology studies indicate the need for controlled laboratory studies to confirm that observed exposure response relationships with pesticides in the field are not influenced by concomittant changes in physiochemical water quality parameters such a low salinity.

At the CTL or reference site, only background levels of endosulfan (< 10 ng/L) were observed. There was extremely high survival in all species deployed in field toxicity tests, along with normal bioenergetic metabolism in oysters and brain AChE in fish. Biomonitoring indicated very high population densities of grass shrimp, mummichogs and other fish/shellfish species similar to population densities measured earlier (1985-88) at this site.

Results from 1990 indicated only two days of significant (>1.27cm/day) rainfall occurred during the peak of the vegetable crop growing season (May - June) which resulted in only slight runoff of azinphosmethyl (<DL - 62ng/L) at the KWA Site and fenvalerate (<DL - 123ng/L) at the TRT Site. The 1990 study period was an unusually dry period compared with results from 1989, characterized by relatively low rainfall, little, if any significant insecticide runoff, and generally high survival in fish and crustacean species deployed in acute toxicity tests at each field site. Additionally, there were no significant sublethal effects observed in field bioassays assessing specific (brain AChE) biomarkers in mummichogs and nonspecific (bioenergetic metabolism) biomarkers in oysters. Ecotoxicological sampling (block seining) indicated that the macropelagic community of fish and crustaceans at the TRT Site recovered from previous insecticide impacts (1985-89), with population densities for grass shrimp, mummichogs and other species often exceeding densities measured at the reference or CTL Site. Additionally, there were no significant differences detected in grass shrimp populations in comparisons of the CTL, TRT and KWA Sites using push netting procedures. Comparisons of grass shrimp population differences using block seining and push netting procedures indicated very consistent agreement, as 86% of the conclusions reached in statistical comparisons were the same using both methods. An error rate of 7 - 14% may be expected using the push net method as a surrogate method of ecotoxicological assessment. While this may result in a slight decrease in statistical

precision, sampling of more replicates/site may enhance the statistical power using this method.

Ecotoxicological sampling (block seining and push netting) during 1989-90 was confined to CTL, TRT, and KWA Sites. No sampling upstream or downstream was conducted to evaluate spatial distribution of impacts to macropelagic fauna. The labor intensive method of block seining while thorough and effective, is time consuming and cost prohibitive to spatially characterize a watershed; however, the use of pushnetting provides a time and cost effective method for assessing spatial impacts within a watershed and should be employed in future studies. Additional studies of benthic communities, marine mammals, sea turtles and bird populations in agriculturally influenced watersheds should be conducted to fully understand the ecological impacts of pesticide runoff.

Comparing results between the two agricultural sites for 1989-90, clearly indicates the importance of various management strategies (BMP, IPM, and retention ponds) at the TRT Site in significantly reducing pesticide risk from vegetable farming to adjacent estuarine tidal creek nursery habitats. During a relatively dry year, (i.e. 1990) these management practices were not necessary for protecting the environment, given the small amount of rain and resulting runoff observed. During a relatively wet year, such as 1989, it was evident that these management strategies greatly minimized pesticide impacts at the TRT Site, especially when compared to results for 1985-88, prior to implementation of these practices.

Further analysis of field and laboratory results from this study have indicated:

1) Significant agreement between field and laboratory toxicity tests for azinphosmethyl, endosulfan, and fenvalerate for grass shrimp (*P. pugio*) and mummichogs (*F. heteroclitus*);

2) The field-derived LC_{50} for azinphosmethyl was 0.95 ug/L (CL = 0.86 - 1.05 ug/L) versus 96h laboratory, static renewal (SR) LC_{50} ranging from 0.97 - 1.05 ug/L (CL = 0.77 - 1.24 ug/L) in *P. pugio*.

3) The field-derived LC_{50} for *P. pugio* exposed to endosulfan was 0.28 ug/L (CL = not calculated) versus 96h laboratory, SR LC_{50} ranging from 0.25 - 1.01 ug/L (CL = 0.14 - 1.43 ug/L) in adults and 0.39 ug/L (CL = 0.27 - 0.58 ug/L in zoeae.

4) The field-derived LC_{50} for *P. pugio* exposed to fenvalerate was 0.060 ug/L (CL = 0.050 - 0.070 ug/L) versus 96h laboratory, SR LC_{50} valves ranging from 0.052 - 0.060 ug/L (CL = 0.037 - 0.097 ug/L) in adults and 0.007 - 0.020 ug/L (CL = 0.005 - 0.031 ug/L) in zoeae.

5) The field-derived LC_{50} for *F. heteroclitus* exposed to azinphosmethyl was >7.002 versus 96h laboratory SE LC_{50} ranging from 28.00 - 36.95 ug/L (CL = 20.23 - 48.24 ug/L). Also the field derived lowest observable effect concentration (LOEC) was 7.00 ug/L versus a 96h laboratory, SE no observable effect concentration (NOEC) of 4.95 ug/L.

6) The field derived LC_{50} for *F. heteroclitus* exposed to endosulfan was 0.12 ug/L (CL = not calculated) versus 96h laboratory, SR LC_{50}s ranging from 1.29 - 1.45 ug/L (CL = 1.21 - 1.59 ug/L) for adults and 0.23 ug/L (CL= 0.14 - 0.40 ug/L) for juveniles. Most field population impacts were in juvenile *F. heteroclitus*, which resulted in field derived values more closely resembling juvenile laboratory toxicity test results.

7) The field derived LC_{50} for *F. heteroclitus* exposed to fenvalerate was 0.100 ug/L (CL = 0.090 - 0.110 ug/L) versus 96h, SR laboratory LC_{50} values ranging from 1.63 - 2.86 ug/L (CL = 1.080 - 4.060 ug/L) for adults and 2.67 ug/L (CL = 1.670 - 4.260 ug/L for juveniles. The wide disparity between field and laboratory test results for *F. heteroclitus* exposed to fenvalerate, may have resulted from potential low salinity (<10ppt) pesticide mixture (endosulfan, fenvalerate and azinphosmethyl) interactions. Laboratory toxicity tests indicated that fenvalerate was 1.75 times more toxic to adult *F. heteroclitus* at 5ppt salinity than at 20ppt. Similarly, laboratory studies conducted with other juvenile estuarine fish [*Menidia menidia* (LC_{50} = 0.31 ug/L, CL = 0.21 - 0.40 ug/L) and *Mugil cephalus* (LC_{50} - 0.58 ug/L, CL = 0.41 - 1.00 ug/L)] reported similar LC_{50} values for fenvalerate.

8) Significant agreement between field and laboratory derived EC_{50} estimates of brain AChE inhibition in mummichogs exposed to azinphosmethyl;

9) The 24h laboratory-derived EC_{50} based upon brain AChE in *F. heteroclitus* exposed to azinphosmethyl was 0.90 ug/L versus field derived EC_{50} values ranging from 0.63 ug/L (based upon 24h post rain pesticide insecticide

concentrations) to 1.53 ug/L (based upon peak insecticide concentrations). A field derived EC_{50} value of 1.13 ug/L was determined using the average insecticide concentration measured during each rain event. The most significant limiting factor affecting correlations between field and laboratory validations for biomarkers such as AChE may be in the accuracy of the characterization of field pesticide concentrations. These results suggest, based upon differences between peak, post rain and 24h, post rain sampling, average measured EC_{50} values for azinphosmethyl AChE inhibition varied by a factor of 2.42. For field validation determinations, it is important to report maximum, minimum and average pesticide concentrations measured in all field studies to accurately characterize field exposure concentrations. It is interesting to note that the 24h field derived EC_{50} of 1.13 ug/L, which was derived, based upon the average post rain concentration only varied from the laboratory derived EC_{50} of 0.90 ug/L by a factor of 1.25.

10) Significant sublethal (increased respiration and increased nitrogen excretion rates) effects of pesticide (fenvalerate and azinphosmethyl/endosulfan) runoff on adult oysters (*Crassostrea virginica*) ecophysiology;

11) Significant sublethal effects (reduced nitrogen excretion) from mummichogs exposure to azinphosmethyl;

12) Significant correlation between block seining and push netting approaches for assessing ecotoxicology effects of pesticide runoff in macropelagic fauna;

13) Ecological recovery of the macropelagic faunal community at the highly managed agricultural site (TRT Site) following construction of retention ponds there to reduce nonpoint source agricultural runoff into estuarine tidal creeks; and;

14) Insecticide runoff and related toxicological impacts to estuarine organisms were greatly reduced at the highly managed agricultural site when compared to the unmanaged site.

Results from this study have indicated that the application of field and laboratory testing for both lethal (acute toxicity) and sublethal (ecophysiology) pesticide effects when coupled with ecotoxicological biomonitoring provides an integrated method for holistic environmental risk assessment for pesticides. Laboratory toxicity and ecophysiology studies

provide the initial bench marks for lethal and sublethal effects for each pesticide studied. The range between lethal and sublethal endpoints determined in the laboratory indicates the boundary for potential and/or realized field impacts. Field toxicity tests and ecophysiological studies provide a mechanism to validate initial laboratory studies and to expand their design (pulsed vs. continuous dose; low vs. high salinity; the interaction of pesticide mixtures; and adult vs. other life history stages) to better interpret field results. Ecotoxicological biomonitoring provides an independent mechanism to confirm the validity of both laboratory and field toxicity tests and in some instances (i.e. reproductive impairment) ecophysiology bioassays.

Application of BMP, IPM, and retention ponds as nonpoint source insecticide runoff control techniques was very effective at the TRT Site at reducing surface water risks and impacts to organisms in adjacent estuarine tidal creeks. Ecotoxicological biomonitoring at the TRT Site indicated recovery of the macropelagic fauna at this site to levels comparable at the CTL Site. The integrated field and laboratory toxicological methods employed in this study were not only effective in quantifying insecticides impacts, but were equally as sensitive in documenting ecological recovery as contaminant risks were reduced. These results imply that the methods employed in this study would be effective not only in pesticide risk characterizations, but also in risk reduction evaluations. **This is extremely important!**

Highly correlated field and laboratory results for pesticide risk assessment imply a mechanism for simultaneously quantifying toxicological risk and evaluating risk management options. In practice, very few studies have been able to document both. The present study has clearly indicated the utility of this approach for a variety of insecticides (organophosphates, organochlorines, and pyrethroids), in a variety of species (fish and shellfish), using a variety of risk management options.

Future studies should be better focused at evaluating the implications of sublethal effects or biomarkers in terms of population level impacts. Sublethal effects studies should distinguish between biomarkers which are indicators of specific contaminant exposure (i.e., AChE) and those which are nonspecific indicators of significant sublethal effects (i.e. bioenergetic metabolism). Linking cause and effect with generalized physiological indicators is tenuous at best. Utilization of both specific (i.e. AChE) and nonspecific (bioenergetic metabolism) sublethal indicators provides a holistic method of determining if significant contaminant exposure has occurred and if that exposure is translatable into ecophysiologically significant effects. The approach used in this study provides a template for evaluating this question and has partially answered this question for estuarine fish and

shellfish exposed to azinphosmethyl. Further study of this issue is needed for other insecticides to adequately address this question.

Future laboratory studies which utilize specific and nonspecific biomarkers should be conducted to evaluate the persistence of any sublethal effect beyond just the initial exposure phase. Such studies would distinguish between labile effects and permanent impairment. Obviously, any organism when exposed to a pesticide may exhibit altered physiological responses. Once insecticide exposure is terminated, if the effect disappears rapidly, then the observed biomarker was not ecophysiologically significant. If the effect persists, then the potential for permanent impairment exists. The application of this approach should begin with better designed laboratory studies so that those biomarkers of prolonged ecophysiological impairment may be identified and distinguished from indicators of exposure per se. Application of laboratory results to field studies in populations would be made easier and more directly translatable to environmental risk assessment for insecticides.

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INTRODUCTION

Throughout the U.S., continued public scrutiny has been placed on the use of pesticides in the environment (i.e., Med-fly spraying in southern California). While the use of pesticides in agriculture and vector control has been justified due to world shortages of food and public health concerns, respectively, their impacts on the environment are being more widely studied, both in the U.S. and other countries of the world. A total of nearly one billion pounds of active ingredient (PAI) pesticide was produced in the U.S. in 1983. Agriculture was the dominant use (77%) followed by industrial and government use (16%) and home and garden use (7%).

There are over 50 companies which produce over 960 pesticides, in the U.S., sold in more than 25,000 formulations. Ten percent of all U.S. pesticide production consists of unregistered (in the U.S.) or banned products (i.e., DDT[1]) which are sold in overseas market (Revelle and Revelle, 1988). For many developing third world nations, pesticide usage is indispensable to prevent starvation and disease (Atuma, 1985). Risk assessments of pesticide use must be balanced differently in economically developed and underdeveloped nations of the world. Risk assessments for pesticides must be far ranging, protective of human-consumers, farm workers, and avian, terrestrial and aquatic resources. Particular emphasis must be placed on coastal and estuarine ecosystems, given their ecological importance and as a commercial and recreational source of food.

Pait et al (1989) summarized agricultural pesticide usage for 28 pesticides in estuarine drainage areas of the U.S. Nationally some 800 million PAI pesticides were applied to agriculture in the contiguous U.S. The 28 pesticides which were evaluated, accounted for 50% of all pesticide applications nationwide. Over 34 million PAI of these 28 pesticides were applied in coastal areas, representing 8% of their total use in the U.S. The greatest amount of pesticide was applied to corn (>10,000,000 PAI/yr), followed by soybeans (>8,000,000 PAI/yr), rice (>2,000,000 PAI/yr), peanuts (>1,200,000 PAI/yr) and pasture/range land (>1,000,000 PAI/yr) in coastal habitats of the U.S. The coastal estuarine drainage areas with the highest pesticide use

[1]Trade names are provided for information only and do not imply endorsement by the National Oceanic and Atmospheric Administration.

were Chesapeake Bay (5,290,000 PAI/yr), Winyah Bay (3,240,000 PAI/yr), Albemarle Sound (2,132,000 PAI/yr), Pamlico Sound (1,963,000 PAI/yr) and Laguna Madre (1,902,00 PAI/yr). Pesticide usage was greatest in the Gulf and Southeast coast, followed by Northeast coast, and West coast. The dominant pesticides applied to crops in the coastal zone of the U.S. were Alachlor (herbicide - >6,000,000 PAI/yr), Atrazine (herbicide - 5,000,000 PAI/yr), Metolachlor (herbicide - >2,500,000 PAI/yr), 2,4D (herbicide - >2,000,000 PAI/yr, and Carbaryl (insecticide - 1,500,000 PAI/yr). While the 28 pesticides evaluated in this study represented the majority of pesticides applied to agricultural crops in the coastal areas of the U.S., several important and highly toxic pesticides were not considered, including azinphosmethyl, fenvalerate and many of the other pyrethrins. Additionally, the size of the drainage basin, the proportion of the drainage basin cultivated in agriculture, and the pesticide use per unit of cropland were dominant factors affecting the overall ranking of potentially high risk areas. As a result, some site specific effects may have been overlooked. For example, soybeans (and the pesticide applied to this crop) were the dominant crop in most estuarine drainage areas. As a result, the pesticide applied to soybeans would dominate summary statistics for pesticide usage. Yet throughout several estuarine drainage areas, other crops (i.e., tomatoes) may have much higher pesticide application rates and consequently pose a greater toxicological risk.

Estuarine habitats adjacent to these agricultural areas would be at greatest risk from pesticide effects and impacts. More than 70% of commercial and recreational fisheries landings are taken from estuaries (Department of Commerce, 1988). Additionally, these estuarine and coastal habitats provide significant recreational and aesthetic pleasures to the public. More than $7 billion of public funds are spent annually on outdoor marine and estuarine recreation in the 22 coastal states of the U.S. (NOAA, 1988). It is imperative that effective methodologies for pesticide risk be developed which adequately protect these fragile estuarine habitats.

In many of the estuarine drainage areas of the U.S., agricultural lands comprise a substantial portion of the land use. This is particularly true in the Southeast and Gulf coast regions of the U.S. Pait et al, (1989) reported that 8 of the top 10 estuarine drainage basins, with the highest proportion of land use as agriculture were located in the Southeast and Gulf coast regions of the U.S., with agriculture comprising anywhere from 36%-75% of the land surface area in given estuarine drainage areas. It is not surprising that nonpoint source (NPS) runoff from agriculture is thus a major concern in terms of water quality contraventions in estuarine habitat impacts.

Recent reports (NOAA, 1988; Humenik, 1987; EPA, 1984; and EPA, 1983) have indicated that in most regions of the U.S., NPS runoff remains one of the more pervasive, least understood, and poorly managed sources of water pollution. NOAA (1988) reported in a survey of 145 marine pollution experts that NPS pollution ranked fourth in terms of severity out of 83 marine pollution problems evaluated. The Association of State and Interstate Water Pollution Control Administrators (ASIWPCA) evaluated the effects of NPS runoff in 49 states in the U.S. and reported that water quality was threatened and/or degraded due to NPS runoff in 42% of the estuarine habitats surveyed (ASIWPCA, 1985). On a national basis the major sources of NPS runoff are agriculture, urbanization, mining, silviculture, and construction/building activities (EPA, 1984). These findings clearly indicate the importance of estuarine habitats and their potential vulnerability to chemical contaminants present in NPS runoff.

Although the effects of NPS runoff on estuarine habitats have not been fully studied, efforts have been made to identify estuarine impacts associated with this type of pollution. Trim and Marcus (1990) reported that in South Carolina from 1978-88, a total of 805 fish kills occurred, of which 354 (44%) occurred in estuarine waters. In evaluating these estuarine fish kills, it was found that 43% were from natural causes (i.e., depleted DO_2), 35% were from anthropogenic causes and 22% were from undetermined causes. Nearly 54% of the anthropogenic induced fish kills were from

coastal pesticide usage including weed control around resorts (21%), agriculture (20%) and vector control (13%). Almost all of these pesticide related fish kills were from NPS runoff rather than spray drift or misapplication. Of additional interest was the fact that point source discharges from the 123 permitted estuarine discharges accounted for only 12% of all anthropogenic related fish kill (Trim and Marcus, 1990). Moreover, a recent assessment has also indicated that 65% of all closed shellfish harvesting waters in South Carolina are due to bacterial pollution from nonpoint sources of pollution (SCDHEC, 1988). These results clearly indicate the significance of NPS pollution in the State of South Carolina. In other coastal states similar problems with NPS pollution abound.

Fish kills and shellfish closure clearly represent environmental episodes and/or conditions where environmental management, permitting procedures and regulatory policies have failed. These episodic events must be considered over a long time frame and when integrated with other available data bases (i.e., ambient monitoring data) so that management alternatives can be formulated, tested and evaluated.

Such is often the case with pesticides registration processes within the federal statutory authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) used to register pesticides within the U.S. Environmental hazard evaluation is a critical and significant part of the pesticide registration process. Scott et al (1990) in an integrated laboratory and field study of pesticide impacts to estuarine organisms clearly found significant statistical correlations between 96 hour laboratory toxicity tests and *in situ* field toxicity tests and in stream ecotoxicological biomonitoring approaches. These three approaches - laboratory toxicity tests, *in situ* toxicity tests, and ecotoxicological biomonitoring provide the toxicological cornerstones found in pesticide hazard evaluations and environmental risk assessments. Additionally (Fulton, 1989), also reported in Scott et al (1990) found significant statistical correlations between sublethal physiological biomarkers (brain acetylcholinesterase) in comparisons between fish exposed in the laboratory and to NPS pesticide runoff in the field. While these studies were significant in defining and relating the integration and interrelationships between field toxicity tests and ecotoxicological biomonitoring with laboratory toxicity test methodologies, additional studies are needed to better define these associations.

The objective of this present study was to continue and expand the approaches used by Scott et al (1990) in pesticide hazard evaluation processes by:

1) Continued study of *in situ* field toxicity testing and ecotoxicological biomonitoring of sites impacted by agricultural NPS runoff;
2) Comparing NPS runoff *in situ* effects at field sites with [retention ponds, Best Management Practices (BMP) and Integrated Pest Management (IPM)] and without (Calendar Spray - a spray application every three - five days, no IPM/BMP) significant NPS runoff controls measures;
3) Evaluating and comparing the significance of biomarkers (brain -AChE) as measures of both exposure and sublethal physiological effects;
4) Evaluating the utility of bioenergetic metabolism approaches (i.e. scope for growth) in assessing NPS runoff effects in the American oyster, Crassostrea virginica (Gmelin); and
5) Evaluating and comparing the use of more rapid assessment (i.e., push netting) ecotoxicological sampling approaches with more traditional biomonitoring methods (i.e., block seining) in assessing pesticide NPS runoff effects.

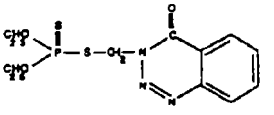
These additional studies were undertaken as a Cooperative Research Agreement between the University of South Carolina, School of Public Health and the U.S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory. The purpose of this present study was to better define the relationship between conventional 96 hour Laboratory toxicity tests and *in situ* field effects for three major classes of insecticides - organochlorines (endosulfan), pyrethroids (fenvalerate) and organophosphates (azinphosmethyl). By better understanding the toxicological interrelationships between laboratory and field toxicity tests and ecotoxicological studies, greater insight into effective pesticide risk assessment may be gained.

MATERIALS AND METHODS

Insecticides Studied

Azinphosmethyl

Azinphosmethyl, also known as Guthion or Gusathion, is an organophosphate having the chemical designation: O,O-dimethyl S-[(4-oxo-1,2,3benzotriazin-3(4H)-yl) methyl] phosphorodithioate (Turner, 1977). Azinphosmethyl was developed by Bayer A.G. in 1953 and is used as a nonsystemic insecticide and acaricide. The structure of azinphosmethyl, along with selected physicochemical factors are listed below:



Azinphosmethyl

Molecular weight: 317.3
Octanol/water partition coefficient: 360 @ 20°C
Solubility in water: 29mg/L @ 25°C
Melting Point: 73-74°C (Verttorazi, 1976; Morifusca, 1977)
Persistence: 14 days (water); 12-28 days (soils)
(Shultz et. al 1972; Staiff et. al., 1975:
Gunther et. al., 1977; and Engelhart et. al., 1984).

Like most organophosphate insecticides, azinphosmethyl acts by blocking synaptic transmission. The disruption of the nerve impulse is caused by excessive amounts of the neurotransmitter acetylcholine (ACh) at the synapses which is normally broken down by acetylcholinesterase (AChE). In order for azinphosmethyl to exert its cholinergic effect it must first be metabolized by replacing the sulfur of the thiophosphate linkage with an oxygen. This oxygen analogue then binds to the active site of the AChE to prevent breakdown of ACh. Once

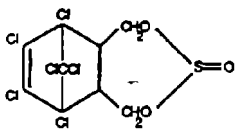
neurotransmission in the respiratory center of the brain or the neuromuscular junction of the respiratory apparatus has been blocked, death rapidly ensues. Depressed AChE activity may persist for weeks and it is possible with repeated exposure to see an additive effect (Dubois et al., 1957; Coppage and Matthews, 1974). Of the more than 16 metabolites that have been identified only the oxygen analogue of azinphosmethyl has been shown to exert toxicity (Yaron et al., 1974).

Despite the fact that azinphosmethyl has been registered for agricultural uses for some time, there are relatively few studies concerning its effects on nontarget species. Loosanoff et al. (1957) examined the effects on certain forms of plankton and found azinphosmethyl to be nontoxic to two species of freshwater algae, *Chlorella sp.* and *Chlamydomonas sp.* at a concentration of 1 ug/L. Further, he found that it exerted no toxic effects on oyster larvae and at a concentration of 0.05 ug/L actually' enhanced growth (Loosanoff et al., 1957). A study by Benke and Murphy (1974) compared toxicity of azinphosmethyl and methyl parathion, another organophosphate, in mice and fish. They found azinphosmethyl to be approximately 400X more toxic than methyl parathion in the sunfish *Lepomis gibbosus.* This difference was explained in part by the greater sensitivity of brain and muscle acetylcholinesterase to azinphosmethyl as indicated by *in vitro* I_{50} (Concentration causing 50% AChE Inhibition) values: $4.8x10^{-10}$M for brain and $2.4x10^{-10}$M for muscle tissue with azinphosmethyl as compared to $2x10^{-8}$M for brain and $4x10^{-8}$M for muscle with methyl parathion. Additionally the authors found additive effects with repeated exposure (Benke et al., 1973; Benke and Murphy, 1974). In a study examining the toxicity of azinphosmethyl in salmonids, Katz (1961) found 96h LC_{50}s ranging from 3.2 to 4.3 ug/L for three freshwater species (*Oncorhynchus tshawytscha, Oncorhynchus kisutch*, and *Salmo gairdneri*) while for a marine species, *Gasterosteus aeuleatus,* the 96h LC_{50} ranged from 4.8 ug/L (salinity-25 ppt) to 12.1 ug/L (salinity-5 ppt). In a study by Adelman *et al.* (1976) on the toxicity of azinphosmethyl to the Fathead minnow, *Pimephales promelas,* a concentration of 0.51 ug/L was found to drastically reduce fecundity. Meyer (1965) determined 48h LC_{50}s for four freshwater species ranging from 25 ug/L in green sunfish, *Lepomis macrochirus,* and Largemouth bass, *Micropterus salmonids,* to 9,000 ug/L in channel catfish, *Ictalurus punctatus.* Other studies examining the toxicity of azinphosmethyl in various American freshwater fish have reported 96h LC_{50}s ranging from 0.4 ug/L to 4300 ug/L with most values less than

50 ug/L (Lewallen and Brydon, 1958; Weiss, 1961; Pickering *et al.*, 1962; Macek and McCallister, 1970; and Murty, 1986). A limited number of studies have examined the toxicity of azinphosmethyl in saltwater species with toxicity values ranging from a 48h LC_{50} in Brown shrimp, *Penaeus aztecus*, of 2.4 ug/L to a 96h LC_{50} of more than a 1000 ug/L for the Eastern oyster, *Crassostrea virginica*, again with most values less than 50 ug/L (Coppage and Matthews, 1974; Miura and Takahashi, 1976; and Mayer, 1987).

Endosulfan

Endosulfan also known as Thiodan, Thiosulfan, Cyclodan, and several other trade names is a chlorinated cyclodiene having the chemical designation: 6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a- hexahydro-6,9-methane-2,4,3-benzodioxathiepin-3-oxide (Berg, 1985). Endosulfan is a nonsystemic contact insecticide and acaricide. It was developed in Germany by Hoechst AG in 1965 and is distributed in the U.S. by the FMC Corporation (Thomson, 1985). The structure of endosulfan, along with selected physicochemical properties are listed below:



Endosulfan

Molecular weight: 407.0
Solubility in hexane: 24g/L @ 20°C
Solubility in water: alpha isomer 0.32mg/L @25°C
beta isomer 0.33mg/L @ 25°C
Melting Point: alpha isomer 109°C beta isomer 213.3°C
(Rao and Murty, 1980; BCPC, 1983)
Persistence: 14 days (water); 60-160 days (soil) metabolite (endosulfan cyclic sulfate) is highly persistent. (Eichelberger and Lichtenberg. 1979; McEwen and Stephenson, 1979; Rao and Murty, 1980).

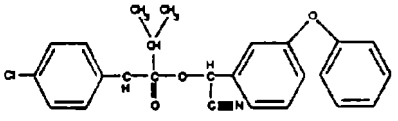
The exact mode and site of action of endosulfan is not completely known. Like most insecticides, the cyclodienes are neurotoxins and are generally considered to be central nervous system stimulants. Biochemical studies with dieldrin, another cyclodiene, showed an alteration of amino acid ratios and an increase in ammonia levels in the brain (Murphy, 1980). A study by Truhaut et al., (1974) found endosulfan to cause inhibition of hamster serum and rat hepatic cholinesterase. In a study by Gupta, (1976), it was found that acetylcholinesterase activity in rat brain was decreased by 23-33% following an intraperitoneal injection of 30-60 mg/Kg of endosulfan.

There is a considerable volume of data concerning the toxicity of endosulfan to nontarget aquatic species. A study by Mathiessen and Logan (1984) examined the toxicity of endosulfan to tropical cichlids *(Tilapia rendalii and Sarotherodon mossambicus)* following aerial spraying of endosulfan for control of the tse tse fly. They found 75 fewer cichlids nests in treated areas and a 25% reduction in juvenile recruitment. In this same study the 24h LC_{50} for *Sarotherodon mossambicus* was found to be 10.4 ug/L with a concentration of 0.6 ug/L affecting breeding behavior (manifested as a delay in spawning). In a study by Roberts (1975), mussels and scallops were exposed to 450 ug/L technical grade endosulfan for 24 hours. Results indicated a 50% reduction in byssal attachment. Another study by Netrawali *et al.*, (1986) examined the effects of endosulfan on the sexual life cycle of *Chlamydomonas reinhardtii* and found a delay in the onset of meiosis in the zygote at a concentration of 0.25 x 10^{-4}m (10.18 ug/L). One study which compared sediment versus superficial water exposures using the shrimp, *Crangon septemspinosa,* reported that exposure through water was the primary factor controlling toxicity in this species with a 96h LC_{50} of 0.2 ug/L in water and 3.5-49 ug/Kg in sediment exposures (McLease and Metcalfe, 1980). Haider and Inbaraj (1986) compared the toxicity of the technical material and commercial formulations of endosulfan in adult *Channa punctatus* and found the 96h LC_{50} for the emulsifiable concentrate (3.0 ug/L) to be 1.88 times less than that for the technical material (5.78 ug/L). Additionally, exposed animals in both formulations exhibited definable behavioral changes such as: 1) erratic swimming; 2) convulsions; 3) increased or difficulty in respiration; 4) loss of equilibrium; 5) pale color; and 6) excessive mucous about the gill epithelium. In a study comparing the toxicity of the two isomers of the parent compound, the alpha isomer was found to be more toxic than the beta isomer in the fish, *Channa punctatus,* with 96h LC_{50} values of 0.16 p,g/L versus 6.6 ug/L respectively (Devi *et al.*, 1981). Other studies examining the toxicity of endosulfan in various species of freshwater fish reported

96h LC_{50}s ranging from 0:.9 to 8.1 ug/L depending on the organism tested (Macek *et al.*, 1969; Johnson, 1980). In general, results from the toxicity tests on freshwater species indicate that endosulfan is generally more toxic to fish than invertebrates. Fewer tests have been conducted with saltwater species but available results indicated marine species are at least equally if not more sensitive to endosulfan than freshwater organisms. Results from toxicity tests on various saltwater species indicated 96h LC_{50} ranging from 0.04 ug/L for the pink shrimp, *Penaeus duorarum,* to 1.31 ug/L for the grass shrimp, *Palaemonetes pugio* (Schimmel et al., 1977).

Fenvalerate

Fenvalerate also known as Pydrin, Belmark, Ectrin, Sumicidin, and other trade names is a synthetic pyrethroid with the chemical formula Cyano(3-phenoxyphenyl)- methyl 4-chloro alpha (l-methylethyl) benzeneacetate. Fenvalerate is used as a selective contact and stomach poison insecticide. It was developed by Sumitomo Chemical Co. of Japan in 1974 was originally distributed in the US by Shell, and is currently distributed in the U.S. by DuPont (Thomson, 1985). The structure of fenvalerate, along with selected physicochemical properties are listed below:



Fenvalerate

Molecular weight: 419.9
Octanol/water partition coefficient: 1.58×10^6 @ 20°C
Solubility in water: 0.002mg/L @ 23°C
Melting point: viscous liquid at room temperature
Persistence: reported half-life 2-7 weeks (Mulla *et. al.*, 1978; Ware, 1980; Schimmel *et. al.*, 1983; Caplan *et. al.*, 1984; and Smith and Stratten, 1986).

Fenvalerate is a type II pyrethroid which acts on the central nervous system (Bradbury *et al.*, 1986). Fenvalerate causes a depolarization of the nerve membrane by effecting its permeability to Na^+ and K^+ ions resulting in rapid and repetitive firing of the nerve impulses, leading to disorientation and death. It is thought that fenvalerate wedges in the open Na^+ channels so they cannot close. As a result, the membrane potential is unable to return to resting state and remains partially depolarized. When the level of depolarization is near the threshold voltage, repetitive firing of the nerve cell may occur. The repetitive discharge is manifested as hyperexcitability and convulsions in the affected animal. In the continued presence of the insecticide, the nerve cell becomes increasingly depolarized until impulse conduction is blocked and death results (Shell, 1977).

Numerous studies have considered the effects of fenvalerate on nontarget aquatic organisms. A study by Coats and Jeffery (1987) compared the toxicity of the technical and emulsifiable formulations of fenvalerate on Rainbow trout, *Salmo gairdneri,* in static tests. They found the emulsifiable formulation to be 3.2 times more toxic than the technical grade material with 24h LC_{50}s of 21 ug/L and 76 ug/L, respectively. However, a study by Bradbury et al., (1986) comparing the toxicities of the technical and emulsifiable formulations of fenvalerate on the Fathead minnow, *Pimephales promelas,* found no significant difference in toxicity between formulations. Behavioral changes observed during acute toxicity studies included: 1) rapid gill movement; 2) erratic swimming; 3) altered schooling activity; and 4) swimming at the surface (Holcombe et al., 1982; Bradbury et al., 1986). In a study by Dyer et al., (1986) increasing water hardness was found to enhance the toxicity of fenvalerate to the Bluegill, *Lepomis macrochirus.* Symonik et al. (1986) exposed bluegills *Lepomis macrochirus,* to the technical material and the individual isomers (2S, aS; 2S, aR; 2R, aS; 2R, aR) of fenvalerate and found that the 2S, aS isomer was 100 times more toxic than the next most toxic isomer 2S, aR. All the R-acid isomers were found to be essentially nontoxic.

A study by McKenney and Hamaker (1984) examined the effects of fenvalerate on the larval development and metabolism of grass shrimp, *Palaemonetes pugio,* during osmotic stress. Flow-through exposures to a nominal concentration of 3.2 ng/L significantly reduced the number of larvae completing metamorphosis. Further, larvae reared continuously in a sublethal concentrations of

0.1 and 0.2 ng/L showed significant increased metabolic rates when subjected to acute fluctuations in salinity as compared to controls at salinities of 10 ppt and 30 ppt. Fenvalerate has also been shown to exhibit a negative temperature coefficient *(i.e.* greater toxicity with decreasing temperature).

Other studies (Shell, 1977) examining the toxicity of fenvalerate in various species of freshwater fish found 96h LC_{50}s ranging from 0.64 ug/L in bluegill to 6.2 ug/L in rainbow trout. Toxicity values in marine organisms have been found to range from a 96h LC_{50} value of 0.003 ug/L for larval grass shrimp, *Palaemonetes pugio,* to 1600 ug/L for Amphioxus (Schimmel et al., 1983; Clark et al., 1985).

Study Sites

The study sites for field research were located south of Charleston, South Carolina at Leadenwah Creek (Coordinates - Latitude N32°36'12" Longitude W80°07') on Wadmalaw Island and an unnamed tidal creek (Coordinates-Latitude N32°36'7", Longitude W80°07') on Johns Island. (Figure 5). The eastern branch of Leadenwah Creek is surrounded by extensive agricultural fields used for vegetable (tomatoes, snap beans, cucumbers, and squash) farming (Plate 1). Fields here are drained by ditches which discharge into the eastern branch of Leadenwah Creek. The eastern branch of Leadenwah Creek has been the site of numerous fish kills over the past 10 years and was designated the Treatment Site (TRT).

A reference or Control Site (CTL) was selected on the western branch of Leadenwah Creek, which lies within the drainage basin of rural, single family dwellings bordered by upland forests and saltmarsh (Plate 2). There are approximately 40 acres of tomato fields under cultivation which drain into a pond at this site. Both Leadenwah Creek sites are remarkably similar in terms of hydrographic regime, salinity, dissolved oxygen, pH, temperature, and sediment substrates.

An additional agricultural site was selected for study on an unnamed tidal creek, near Kiawah Island (Plate 3). This site was designated the Kiawah Site (KWA) which lies in the drainage basin of several large agricultural fields used for

vegetable crop cultivation. Salinities and pH were slightly higher at this site and the sediments were finer grain-sized (muds versus coarse-fine sand) than at the Leadenwah Creek study sites. Additional differences were that agricultural fields in this area have an extensive vegetative border when compared to the TRT site and farmers do not use Integrated Pest Management (IPM) or Best Management Practices (BMP) nor is runoff controlled by retention ponds.

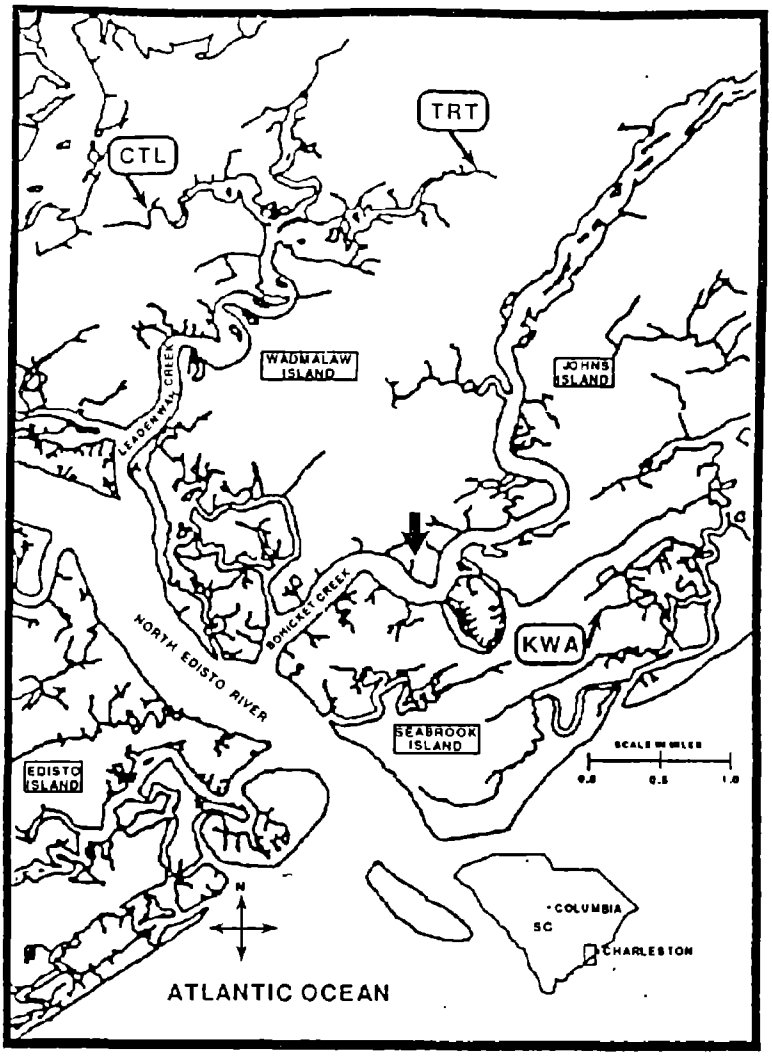


Figure 1. Map of study sites used in 1989-90 field study. Sites include the Reference (REF) or Control (CTL) Site on the west branch of Leadenwah Creek; the Exposure 1 (EXP-1) or TRT Site on the eastern branch of Leadenwah Creek; and the Exposure 2 (EXP-2) or Kiawah Site on an unnamed tidal tributary of Haulover Creek. Arrow (↑) denotes the Cherry Point (CP) Collection Site for mummichogs deployed in field toxicity tests.

Plate 1 Aerial photograph of the CTL Site located on the west branch of Leadenwah Creek. Note the presence of scattered rural single family dwellings and deciduous forests in the area.

Plate 2A Aerial photograph of the TRT Site located on the eastern branch of Leadenwah Creek. Note the presence of extensive agricultural fields at this site.

Plate 2B Retention pond constructed at the TRT Site in 1988.

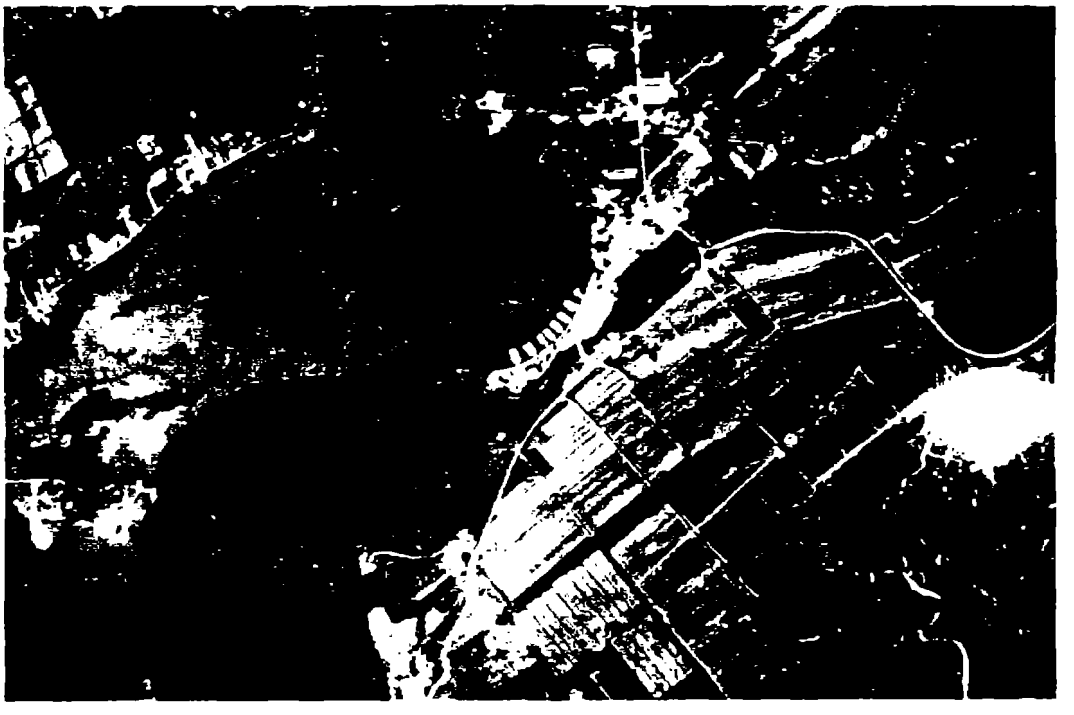


Plate 3 Aerial photograph of the KWA Site located on an unnamed title tributary of Haulover Creek. Note the extensive agricultural fields surrounding this site.

These three study sites offer a diverse approach for dealing with nonpoint source runoff. Drainage ditches are prevalent in agricultural fields at all three sites. The CTL Site, has less agricultural acreage (relative to the other sites) and nonpoint source runoff has been controlled by channeling all runoff into a small retention pond. The TRT Site has extensive agricultural fields (>1,000 acres), without extensive vegetative buffer strips. During 1985-87, agricultural runoff at the TRT Site flowed directly into ditches that extensively drained agricultural fields in this area, which then discharged directly into the headwaters of eastern branch of Leadenwah Creek. Following significant droughts during 1986 and 1987, an extensive retention pond system was constructed in agricultural fields at the TRT Site. During 1988, this retention pond system effectively drained and retained approximately 50% of the agricultural runoff at the TRT Site. The water in the retention ponds was used for drip fertigation (drip irrigation of sand filtered, fertilized water) in tomato fields. During 1988, 1989 and 1990, certain portions of agricultural fields were planted with a rye grass, vegetative strip buffer. The farmer at the TRT Site has adhered rigorously since 1987 to suggested BMP and utilized recommended IPM strategies. The KWA Site has extensive agricultural fields with a natural vegetative buffer strip surrounding each field. Runoff is channeled into an extensive ditch network which discharges directly into the headwaters of a small unnamed tidal tributary. The farmers at the KWA Site do not utilize BMP or IPM practices such as those employed at the TRT Site.

Field Toxicity Tests

Field toxicity tests were conducted during May-June, 1989 and May-June, 1990.

During 1989, field toxicity tests were conducted from 25 May - 27 June, 1989 at the CTL, TRT and KWA Sites. In tests conducted from 25 May - 15 June, 1989, a total of five test species were utilized including: 1) adult grass shrimp (15-35 mm *P. pugio*); 2) adult mummichogs (35-100 mm *F. heteroclitus*); 3) juvenile penaied shrimp (35 - >100 mm *P. aztecus* and *P. setiferus*); 4)adult mysid shrimp (*M. bahia*); and 5) juvenile sheepshead minnow (<20 mm - *C. variegatus*). In tests conducted after June 15, 1989 only four species - sheepshead minnow, grass shrimp, mummichogs, and juvenile penaied shrimp were used. All toxicity tests with grass shrimp, mummichogs, juvenile penaied shrimp and mysid shrimp were of 96 hour duration. Field toxicity tests with juvenile sheepshead minnow were either 14 [Group 2 (1-15 June 1989)] or 16 day [Groups 1 (24 May-9 June, 1989) and 3 (11-27 June 1989)].

During 1990, field toxicity tests were conducted from 24 May - 23 June, 1990 at the CTL, TRT and KWA sites. In tests conducted from 24 May - June 1990, a total of six species were utilized including: 1) Adult grass shrimp (15-35 mm *P. pugio*); 2) adult mummichogs (35-100 mm *F. heteroclitus*); 3) juvenile penaied shrimp (35-100 mm *P. aztecus* and *p. setiferus*); 4) adult mysid shrimp (*M. bahia*); 5) juvenile sheepshead minnow (<23 mm *C. variegatus*) and 6) juvenile tide water silversides (13-22 mm - *Menidia beryllina*). Mysid shrimp tests were only conducted through 13 June and silversides through June 17, 1990. In tests conducted after June 17, 1990 only four species - grass shrimp, penaied shrimp, mummichogs, and sheepshead minnow were used. All field tests with grass shrimp, mummichogs, juvenile penaied shrimp, and mysid shrimp were of 96 hour duration. Field toxicity tests with juvenile sheepshead minnow (6-9 days) and juvenile menidia (4-9 days) were of variable duration due to additional growth experiments (*C. variegatus*) and problems in field survival (*M. berylina*).

All grass shrimp and penaied shrimp were collected by seine at the CTL Site on Leadenwah Creek. Mummichogs were collected by minnow trap from an unnamed tidal tributary of Bohicket Creek, near Cherry Point Landing. Mysid shrimp, juvenile sheepshead minnows and silversides were taken from existing laboratory stocks at the Gulf Breeze Environmental Research Laboratory, Pensacola, Florida.

Each test species was deployed in different types of cages during field toxicity tests as follows: 1) grass shrimp (rectangular, plexiglass cages - 25 x 5.3 x 5.3 cm with 2 mm nytex screen with styrofoam floats); 2) mummichogs (rectangular, plexiglass cages - 51.5 x 12.5 x 11.7 cm with nytex 2 mm screen with and without styrofoam floats); 3) penaied shrimp (rectangular plexiglass cage - 51.5 x 12.5 x 11.7 cm with 2 mm nytex screen); 4) Mysid shrimp (circular, nalgene plastic cages - 8.25 cm diameter x 13.97 cm height with 0.45 μ nytex screen); 5) sheepshead minnow (circular, nalgene plastic cages - 8.25 cm diameter x 13.97 cm height with 1.000 μ nytex screen) and 6) silversides (circular, nalgene cage - 11 cm diameter x 7 cm height with 1 μ nytex screen). All caged organisms were placed in a larger wire cage to exclude predators. During each toxicity test, a total of three replicate cages/species, 10 organism per replicate (n=30/species/site) were deployed at each field site.

During the 1989-90 field toxicity testing period, new animals were deployed every 96 hours with the exception of juvenile sheepshead minnows, which were deployed every seven days.

In each field toxicity test, the following parameters were recorded daily: 1) percent mortality and survival; 2) water temperature (YSI Model 64 oxygen meter and Taylor Min-Max thermometers - °C); 3) Salinity (A.O. refractometer-ppt); 4) dissolved oxygen (YSI Model 64 oxygen meter - mg 0_2/L); 5) pH (Hanna Model 0064 pH meter; 6) Rainfall (cm/day); and 7) surface water samples (4.25 L) were collected for pesticide residue analysis. In addition, surface sediments and adult oysters (*Crassostrea virginica*) were collected weekly for pesticide residue analysis. During rain events, additional grab and composite water samples were collected for residue analysis at the CTL, TRT and KWA Sites. Composite water samples (250ml/20 minutes) were normally collected over a 12 hour period, using a Sigma water sampler. Water samples were solvent (dichloromethane) extracted in the field and refrigerated until analyzed. Oyster samples were cleaned, shucked, placed in solvent-cleaned glass jars and frozen until analysis. Sediment samples were also placed in solvent-cleaned glass jars and frozen until analyzed. All samples were analyzed by capillary column gas chromatography using methods outlined by EPA (1980).

During the May - June 1989 sampling period, continuous (every fifteen minutes) measurements of water depth (m), salinity (ppt), conductivity (mmhos), water temperature (°C), pH, and dissolved oxygen (mg 0_2/L) were recorded at the two Leadenwah Creek Sites using a Hydrolab in-stream water monitor. Similarly during May - June 1990, Hydrolabs were deployed at the CTL, TRT, and KWA Sites. Continuous (every fifteen minutes) measurements of water depth (m), salinity (ppt), conductivity (mmhos), water temperature (°C), pH, and dissolved oxygen (mg 0_2/L) were recorded.

To ensure uniformity among all test animals used in field toxicity tests, quality control, static 96 hour Quality Assurance (QA) toxicity tests were conducted weekly on each test species (grass shrimp, penaied shrimp, mummichogs, and sheepshead minnow - 1989; and grass shrimp, penaied shrimp, mummichogs, sheepshead minnow and silversides - 1990). The Emulsifiable Concentrate (EC) of endosulfan (24% AI) was used as the reference toxicant. Exposure concentration varied among test species (grass shrimp and penaied shrimp - nominal 0.01, 1.00, 1.15 and 2.50 μg/L; mummichogs - nominal 0.01, 1.15, 2,50 and 5.00

μg/L; and silversides and sheepshead minnow - 0.01, 1.15 and 2.50 μg/L). Test species were also exposed to the carrier (0.1% acetone) in seawater. During 1989, tests were conducted at salinities ranging from 25 to 34 ppt, water temperature of 21.2 - 28.3 °C, dissolved oxygen levels ranging from 2.00 - 8.80 mg/L, and pH ranging from 7.20 - 8.10. During 1990, tests were conducted at salinities ranging from 30-34 ppt, dissolved oxygen levels ranging from 1.40 - 7.60 mg/L, water temperatures ranging from 19.8 - 26.2°C, and pH ranging from 7.3 - 8.2 Water changes were made daily. Pesticide concentrations were based on nominal dilutions of a measured stock. All tests were conducted at ambient light: dark cycles (~14:10 L:D cycle). Quality control tests were not conducted on mysid shrimp, since test groups were taken from existing laboratory stocks at GBERL.

Chemical Analysis of Environmental Samples

Seawater Samples

During field toxicity testing, seawater samples (4.25 L) were collected daily and at prescribed sampling intervals, following significant rainfall (>1.27 cm/24 hr) events, for pesticide residue analysis at all study sites. Samples were placed on ice, transported immediately back to the lab and processed in the following manner:

(1) Initially, all samples were thoroughly shaken by hand and 750 ml of sample was decanted.

(2) Two 20 ml aliquots of samples were taken from this 750 ml portion and filtered through a preweighed filter (pore size 0.70um). Each filter was placed in aluminum foil and frozen. Later each filter was dried in a drying oven at 65°C for 24 hours and reweighed. Contents on each filter represented total filterable solids residue (TFR-g/L).

(3) Three hundred ml of dichloromethane was added to the remaining 3.50L of sample. Each sample was then shaken thoroughly by hand and placed on a jar mill for a minimum of two h. The solvent layer was then placed into a 2000 ml separatory funnel and decanted into a solvent-cleaned, 500 ml, Teflon-capped amber glass bottle. Each sample bottle was sealed and stored in a refrigerator for subsequent analysis.

(4) Each extracted water sample was placed into a glass round bottom flask and flash evaporated at 30°C to a final volume of 5-10 ml.

(5) Florisil columns were used to remove interfering, biogenic compounds from each sample. During the 1989-90 studies, microflorisil columns were prepared by placing 0.5 cm (0.70 g) of florisil and 0.50 cm of sodium sulfate into a solvent cleaned pasteur pipette (14.40 cm). The concentrated sample was then added to the microcolumn and eluted with 7 ml of a 20% (by volume) ethyl acetate in hexane solution. Each sample was then evaporated under a stream of dry nitrogen gas (ultra high purity 99.999%) to near dryness and diluted up to 1 ml with isooctane.

(6) All samples were analyzed by capillary column gas chromatography (CC-GC) in accordance with procedures outlined by EPA, (1980), using a Hewlett-Packard GC (Model 5890A) with an electron capture detector and a BP-1 bonded phase silica based capillary column (25 m). Samples were injected in the splitless mode. Helium was used as the carrier gas at a flow rate of 1 ml/min. Peak heights areas were determined by a Hewlett-Packard (Model 3393 A) integrator. The injector temperature was 220°C and the detector temperature was 300°C for the analysis of each insecticide. A temperature program with a 20°C/min ramp from 90-200°C and then 10°C/min ramp from 200-290°C with a 15 min hold at 290°C was used.

(7) Peak heights and retention times from each sample were compared with analytical standards (fenvalerate, endosulfan I, endosulfan II and endosulfan cyclic sulfate; ethyl parathion; methyl parathion, and azinphosmethyl) obtained from the U.S. EPA Pesticides-Industrial Chemicals Repository, Research Triangle Park, Raleigh, NC for compound identification and quantification. Insecticide concentrations are reported in ng/L for summary tables and μg/L for figures depicting measured insecticide concentrations.

Water samples from all laboratory toxicity tests were also analyzed in a similar manner as that described for field toxicity tests.

Sediment Samples

During 1989 field toxicity testing, sediment samples were collected at discrete sampling periods (weekly) for pesticide residue analysis. Sediment samples were collected at each field site by scraping soils from the top 20-30 mm of surface and placing sediments into a solvent cleaned, glass jar (0.5L)> Following collection, all samples were sealed and frozen until analyzed.

At the lab, each sample was thawed and three, 20 mg aliquots of wet sediment were each placed in an aluminum tin and dried at 60°C for 24 hour in a dry air incubator. At the end of 24 hour samples were reweighed to determine % water content of each sample as described by EPA (1980). The % water content estimate for each sample was used in the final calculation of sediment pesticide concentration/g sediment.

Next, 50 g of wet sediment from each sample was placed in solvent-cleaned freeze drying flask. Each sample was then freeze dried at -50°C for 24 h at 0.001 mm Hg pressure. Following freeze drying, each sample was broken into a powder with a mortar and pestle and then placed in a solvent-cleaned cellulose thimble (Whatman-43 x 123 mm). Each sample was then soxhlet extracted for 24 h with dichloromethane (250 ml volume) at a rate of 1 cycle volume/h. Next, samples were flash evaporated to 5-10 ml volume and then blown to dryness under a dry stream of nitrogen and 1 ml hexane (nanograde) was added. Then 0.5 ml of Hg (triple distilled) was added to each sample. Samples were vortexed for 30 sec, allowed to settle and vortexed again for another 30 sec. The precipitate in each sample was allowed to settle and the clean solvent (hexane) layer was decanted.

Samples were then placed in a florisil column and eluted into three separate fractions with 15 ml of 6, 15, and 50% ethyl ether in hexane, respectively. Each of the three fractions was evaporated under a dry stream of nitrogen (ultra high purity - 99.999%) to one ml volume and were then diluted up to 5 ml with isooctane.

Sediment samples were then analyzed by capillary column chromatography in accordance with EPA (1980) methodologies using the same procedure used in the analysis of water samples. Results are reported in μg/kg (dry weight) for each insecticide detected.

Oyster, Shrimp and Fish Tissue Samples

Adult oysters (*Crassostrea virginia*) were collected at discrete sampling periods (weekly) for pesticide residue analysis during field toxicity tests. During 1989, all oysters were initially collected from a reference site located at the mouth of Leadenwah Creek on 24 May, 1989. Oysters were transported back to the lab, an initial tissue sample was taken, and the remaining animals were deployed at each site (CTL, TRT, and KWA Sites) in plastic trays (92 x 61 x 15 cm).

During 1990, oysters were initially collected on 24 May 1990 from a reference site located at the mouth of Leadenwah Creek. Oysters were transported back to the lab, an initial tissue sample was taken, and the remaining animals were deployed at the CTL, TRT and KWA Sites in plastic trays (92 x 61 15 cm) on 25 May 1990.

Trays were affixed to the creek bottom at each site in the mid-lower intertidal zone with reinforcing rods (rebar for concrete) and subsequent samples were collected weekly throughout the study. Each collected sample was immediately cleaned, shucked into a solvent-cleaned, glass jar using a solvent-cleaned, oyster knife. Samples were sealed and frozen until further analysis. In addition to oyster tissues, penaied shrimp, grass shrimp, blue crab, mullet and mummichogs were collected during field toxicity tests, when significant mortality occurred. These samples were placed in solvent-cleaned glass jars, transported back to the lab on ice, and frozen until further analysis.

Quality Control

During field toxicity tests, weekly water, sediment and oyster tissue samples from the CTL Site were spiked with endosulfan, fenvalerate, azinphosmethyl and methyl parathion to determine "spiked" recovery efficiencies for the various extraction and clean up methods used with each sample procedure. Results were reported in μg/L (water) and μg/kg (sediment and tissue) and as % recovery efficiency (water, sediments and tissues).

Oyster Field Studies, 1989-90

The estuarine habitat is a very complex, dynamic and sensitive ecosystem. These tidal creeks support many indigenous species of ecological, commercial and/or recreational importance. They also serve as nursery grounds and refuges for many ecologically, recreationally and commercially important species of fish and shellfish (Bearden 1982; Cain and Dean 1976; Hampton 1987; Patterson 1986; Scott *et al.* 1986). One of the most important indigenous species of commercial, recreational and overall ecological importance is the American oyster, *Crassostrea virginica* (Gmelin), which may be sensitive to inputs of toxic chemicals due to its ability to bioconcentrate and bioaccumulate pollutants. A dominant secondary producer in the *Spartina* marshes of South Carolina and the southeastern United States, as well as a prime candidate for aquaculture, is the intertidal filter-feeding bivalve, *C. virginica*. *C. virginica* is also a very important species in the healthy ecological functioning of estuarine creeks as it provides hard substrate habitat for many other estuarine species.

Oysters filter and ingest a mixture of inorganic particles, phytoplankton and detrital complexes. The energy gained through digestion of algae and detrital complexes may be directed to any of a suite of physiological processes within the oyster. Both exogenous (i.e., food quality and quantity, temperature, etc) and endogenous (i.e., age, size, reproductive state, nutritional status, disease, etc.) factors will also affect the energy partitioning of the organism. The degree of fecundity and growth within an oyster population, then, results from a complex balance between gametic production, somatic production, and metabolism (respiration and excretion) for the energy absorbed from the oysters' food ration. This relationship has been described by Winberg (1960) as : $C - F = A = R + U + P$ or $P = A - (R + U)$. Where: C = food energy consumed, F = energy lost as feces, A = energy absorbed from the food, R = energy respired, U = energy excreted, P = energy into somatic and gametic production. Energy available for growth and reproduction has also been referred to as "scope for growth" (Warren and Davis, 1967). Positive scope for growth values indicate energy is available for production of gametes and somatic growth, while negative values are indicative of stressful conditions requiring utilization of body reserves. Since direct measurement of growth and fecundity (production and viability of gametes) can be difficult in bivalve species, scope for growth has become a useful index for these physiological parameters (for a review, see Bayne *et al.*, 1985). The use of this index for the measurement of the physiological performance of mussels subjected to environmental stress has been discussed by Bayne *et al.* (1979, 1982) and Widdows *et al.* (1981) a, b).

Bivalves with reduced scope for growth due, for example, to decreased food availability, may produce fewer eggs which have low nutrient reserves (Sastry, 1975; Bayne *et al.*, 1978). The result is a reduced probability of successful larval metamorphosis and setting of spat (juvenile oysters), resulting in population instability. Lipid content in bivalve eggs and larvae seems to be related to parental scope for growth. Bayne *et al.* (1978) reported eggs produced by *Mytilus edulis* having a negative scope for growth were smaller and had less organic matter per egg than those produced by mussels with a positive scope for growth. Adult *Ostrea edulis* maintained on low food rations released larvae having slower rates of growth and lower lipid content than larvae released from adults maintained on high rations (Helm *et al.*, 1973). The resulting extension of the larval period may well increase mortality, thus reducing recruitment potential. *Crassostrea virginica* having a positive scope for growth need not deplete its glycogen reserves, which are used, in part, as a source of lipid in eggs (Gabbott, 1976; Holland, 1978) and in part as a source of energy for adults during winter for routine maintenance and disease fighting metabolism. The oysters would then be more likely to produce gametes with adequate nutrient reserves which, by extension, leads to viable larvae, greater over-wintering survival of adults, and a replenishing or increase in the population as a whole. Scope for growth, as a measure of the energy status of an oyster, measures not only level of stress within oysters but the potential for growth and fecundity as well.

Two additional indices of stress, condition index and O:N ratios, are also useful measurements. Scope for growth may be viewed as a measure of energy status and fundamental adaptive responses, while condition index is a measure of alterations in the nutritive status of an organism, and O:N ratios measure alterations in the balance between catabolic processes. Organisms under stressful conditions (i.e., parasitic infection), which cause their metabolic requirements to increase above normal levels, tend to utilize nutrient reserves in order to meet the elevated metabolic demands. The ratio of oxygen utilized to the amount of ammonia excreted is an index of the relative use of protein in an organism's metabolism, with lower ratios indicative of greater use of protein relative to carbohydrate and lipid. As an example, Widdows *et al.* (1981) found that mussels transplanted along a pollution gradient in Narragansett Bay demonstrated a decline in O:N from 75 to 30 with increasing contamination.

During 1989 an assessment was made using a modification of these various bioenergic metabolic approaches to evaluate the effects of nonpoint source agricultural pesticide runoff

on the American oyster at the CTL and TRT Sites. During 1990, this assessment was repeated at the CTL and KWA Sites. The pesticides of concern during this 1989-90 assessment were those used during the growing season in the area and included: methyl parathion, azinphosmethyl, endosulfan and fenvalerate. This assessment consisted of measuring pesticide uptake rates and resulting lethal and sublethal, physiological effects in adult oysters (by use of whole animal respiration, nitrogen excretion rates, O:N ratios, condition and gonadal index) using methods described by Scott *et al.*, 1990 and Crosby (1988). In addition, larval settlement, size-frequency distributions (1989-90) and *Perkinsus marinus* infections were also measured. This integrated approach generally follows the concepts of Sastry and Miller (1981) who suggest the use of multi-media sampling and linkage to biological species for definition of effects or impacts.

Oyster Collection and Transplantation

During the 1989 study adult American oysters, *Crassostrea virginica* (Gmelin), of legally harvestable size ($\geq$7.5 cm in height) were collected on 24 May 1989 by hand from the mid-intertidal zone of a well-established, healthy reef near the mouth of Leadenwah Creek. The total number of oysters harvested were split into three groups and placed into cages for re-laying. Plastic trays were used as cages (dimensions: 92 cm length x 61 cm width x 15 cm height). The interior walls of the trays were lined with nylon screening (1.00 mm) to preclude easy access to the cages by predators.

Each group consisted of three cages in which approximately 100 oysters were placed in two of the three. One cage held only 30 oysters, each marked with an individual identification code. All cages were anchored to the mid-intertidal zone by reinforcing rods and supported above the mud by concrete blocks. The two cages of 100 oysters each were used as the sample pool for physiological and chemical measurements. The cage of 30 oysters served for *in situ* mortality determinations.

During 1990, three groups of oysters were again deployed at the CTL, TRT and KWA field sites. Each group consisted of three cages in which approximately 100 oysters were placed. These three cages were used as a sample pool for physiological and chemical analysis. All cages at each site were deployed in the mid-low intertidal zone by placing each cage on small cement blocks (to provide support above the mud) and were then anchored in place by reinforcing rods.

Physicochemical Measurements

A Fisher minimum-maximum thermometer was affixed to one transplant cage per site to record overall temperature extremes (in degrees Celsius °C) between each sampling visit. Discrete salinity measurements were made at each site during each sampling period using an American Optical refractometer and reported in parts per thousand (ppt). Water temperature at the time of sampling was recorded from each site using a standard stick thermometer in °C. Rainfall in cm per day was recorded daily at each site using a standard rainfall collection gauge.

Chemical Analyses

During 1989, analyses for methyl parathion, endosulfan, azinphosmethyl and fenvalerate were conducted on a composite sample of 15 oysters per site at exposure days 0, 6, 13, 23, 32 and 63.

Following collection, all oysters were returned to the laboratory and washed with tap water using a spray nozzle and brush to remove all external dirt and debris. They were then shucked into glass containers with tinfoil lined caps that had been washed with laboratory detergent, rinsed four times with deionized water and then three times with pesticide-grade solvents (acetone, petroleum ether). The tin foil-lined caps were prepared in the same way. All shucked oysters were stored at -15°C until laboratory analyses began. Analyses were conducted by gas chromatography with all analytical procedures following USEPA Methods (1980).

Physiological Analyses

During 1989, 10 to 15 oysters per site were sampled for respiration, nitrogen excretion, O:N ratios, condition and gonadal index measurements at exposure days 0 (17 May 1989), 28, 54 and 72 (28 July 1989). During 1990, 10 to 15 oysters per site were similarly sampled for respiration, nitrogen excretion, O:N ratios, condition and gonadal index measurements at exposure days 0 (15 May 1990), 30, and 60 (14 July 1990). After washing as described for the chemical analyses, all fouling and commensal organisms were removed, oysters were immersed in a chlorine bleach-tap water solution for 5-10 minutes, thoroughly rinsed and

placed in a flow-through respirometer chamber. Following an appropriate period of acclimation, chambers were sealed off and measurements of whole animal respiration determined. Following respiration determinations, each oyster was placed in an acid clean container for one hour at room temperature (20°C). Water samples were then collected, fixed with phenol, and stored in a refrigerator for subsequent nitrogen ammonia determinations (Solarzano, 1979). Each oyster was then sacrificed for condition index following the method of Lawrence and Scott (1982). This technique measures the cavity volume of shell by subtraction of the dry shell weight (without soft tissues) from the total weight (shell and soft tissues). The resultant cavity volume is then used to calculate the condition index (CI) by utilizing the following formula:

CI = [total dry body weight (g) / cavity volume ml] x 100

The gonadal tissues were removed by cutting just above the dark area of the digestive diverticulum and then along the adductor muscle. These tissues were dried at 60°C for 48 hours, as were the remaining soft tissues for the CI analysis. The gonadal index (GI) was then calculated by using the following formula:

GI = [dry gonad weight (g) / total dry body weight (g)] x 100

Field Mortality Analyses

During 1989 and 1990, the level of *in situ* mortality was monitored using 30 oysters maintained in one cage. After harvest from the endemic area, 30 oysters per site were cleaned by scrubbing with a bristle brush to remove mud and debris. After allowing to air dry at room temperature for 30 minutes, a unique identifying code from K1 to K30, (KWA Site), T1 to T30 (TRT Site), C1 to C30 (CTL Site) was assigned to each oyster and painted onto the cleaned shell using fingernail polish.

Prior to each field visit, three replicates of 10 coded oysters per replicate were formed by random number selection from the pool of 1 to 30 at each site. Using these three replicate groups at each station, mortality trays were then checked for dead oysters and any such mortality or otherwise missing oysters were recorded. The identifying codes of missing or dead oysters were not carried into subsequent replicate formulations. Thus, by the end of the

study, there were less than 10 oysters per replicate. The level of mortality was reported as the mean of the percent from each replicate.

Perkinsus Marinus Analyses

During 1989-90, rectum and labial palps were dissected from the oysters used in the physiological measurements and infection incidences and intensities of *Perkinsus marinus* were estimated by culturing those tissues in thioglycolate medium fortified with dextrose (Ray, 1966). Mycostatin and chloramphenicol were added to the medium to inhibit bacterial growth and tissue putrefication. After incubation at room temperature (up to six months), tissues were stained with Lugol's Iodine Solution and hypnospores were counted using a microscope at 45X magnification.

Densities were determined by averaging the counts observed for three non-overlapping areas (4.71 mm^2) in each tissue. A number code (NC) was assigned to these averages to facilitate interpretation (Scott *et al.*, 1983) as follows:

No. of hypnospores	Intensity Class	Number Code
0	Negative	0
1-5	Very Light	1
6-10	Light	2
11-30	Lightly Moderate	3
31-300	Moderate	4
301-1000	Moderately Heavy	5
1001-3000	Heavy	6
> 3000	Very Heavy	7

Spat Settlement

Recruitment of oyster larvae to the existing reefs at each site was investigated by assessing larval settlement.

During 1989, spat plates, consisting of corrugated PVC pipe (1.90 cm diameter x 183 cm height) were deployed on 3 June, 1989 in three transects, each 30 cm apart, throughout the upper to lower intertidal zone at the CTL, TRT, and KWA Sites. Along each transect, collectors were spaced at 183 cm intervals from the upper to lower intertidal zone. A total of nine spat collectors were deployed at each site. Initially steel reinforcing rods were driven into the sediment substrate and then the larger corrugated PVC pipe was placed over each reinforcing rod. Spat collectors were collected 365 days later and analyzed for settlement of both oysters and barnacles by enumerating the number settled (#/cm^2) throughout the entire spat collector at respective vertical elevations (0-15, 15-30, 30-45, 45-60 and 60-75 cm above the sediment surface). In addition, oysters were measured for shell height (cm) and weight (g). Barnacles were identified to species, divided into two distinct size classes (1-5 mm and 6-18 mm) and enumerated.

Statistical Analyses - Oyster Studies

Tests for significant differences in means between sampling sites for selected parameters were made using both parametric and nonparametric procedures. The Wilcoxon Rank Sum Test and the Kruskal-Wallis one way Analysis of Variance Test (SAS 1985; Wilcoxon and Wilcox 1964) were used. These nonparametric techniques were selected because of the usual non-normal distribution of environmental data and the poor transformation response of the data to a normal distribution (Gertz 1978; Wright *et al.* 1985). Additional parametric procedures (T-Test and ANOVA) were used when possible.

Laboratory Toxicity Tests

Earlier studies by Scott *et al.*, (1990) have established 96 hour LC50 and 6 hour pulsed dosed LC50 values for grass shrimp (*P. pugio*) and mummichogs (*F. heteroclitus*) exposed to azinphosmethyl, acephate, endosulfan, fenvalerate, and various insecticide mixtures (i.e., azinphosmethyl-endosulfan, endosulfan-fenvalerate, azinphosmethyl-fenvalerate). Additionally, extrinsic (salinity) and intrinsic (life stage) factors were evaluated. The results of these studies indicated that these factors enhanced the traditional 96 hour LC50 toxicity at 20 ppt salinity by no more than a factor of 2.86. Given the extensive data base reported by Scott *et al.* (1990) additional 96 hour laboratory toxicity tests were not conducted during this

study. Emphasis in the laboratory was rather placed on sublethal effects of azinphosmethyl on the mummichog, *Fundulus heteroclitus*.

Effects of Azinphosmethyl on Brain AChE Activity in Mummichogs

Laboratory Phase

Azinphosmethyl was selected for laboratory study because of its known occurrence in runoff at field sites (TRT - 1986, 1987 and KWA - 1988) and its mode of toxicity to the mummichog (Inhibition of AChE). Laboratory experiments were conducted to determine the effects of short-term azinphosmethyl exposure on brain AChE activity in the mummichog.

Two groups of 12 adult (> 35mm) mummichogs (*F. heteroclitus*), collected by minnow trap from the Cherry Point (CP) collection site were exposed to 2.4 μg/L of EC azinphosmethyl for 24 hours. This exposure concentration of azinphosmethyl was selected as the result of range finding studies which suggested this concentration would produce 80% brain AChE inhibition after 24 hours of exposure. Two additional groups of 12 fish were exposed only to the carrier (acetone) and served as controls.

Exposure duration was 24h in 5L glass aquaria at 20 ppt salinity. Temperature in the aquaria was ambient and ranged from 20 - 21°C. Azinphosmethyl exposure concentrations ranged from 0.24 to 3.90 μg/L. Fish utilized in the bioassays ranged in length from 45 - 80 mm. A total of six fish were exposed per concentration. Total exposure volume was 4.8L. An additional group of six fish was maintained as a control. All test concentrations and the control contained the same carrier (acetone) concentration. Following 24h of exposure, all animals were removed from the exposure media and sacrificed. The brains were removed, wrapped in aluminum foil and stored at -20°C until analyzed for AChE activity as previously described. The level of AChE inhibition produced in each of the azinphosmethyl concentrations was then determined based on a comparison to the level in control animals. The results of these tests were then used to calculate a 24h EC_{50} for azinphosmethyl-induced AChE inhibition.

Following the 24 hour exposure whole animal respiration rates ($\mu g O_2/g/h$) were determined for fish in one of the treatment groups and one of the control groups. In

addition, samples were collected for ammonia determinations. Both of these determinations were made using methods described by Scott *et al.*, 1987.

Respiration rates (μgO_2 consumed/g of tissue/h) were determined by measuring oxygen consumption with a dissolved oxygen (DO) meter (YSI Model 58). BOD bottles were filled with high salinity (20 ppt), filtered (0.45μm) seawater and immersed in a water bath kept constant at 20°C. Each BOD bottle was allowed to acclimate for one hour, then initial DO levels and time of measurement were recorded prior to the introduction of test animals into each BOD bottle. Additional BOD bottles containing 300 ml of filtered seawater without test animals were examined to determine the effects of aerobic activity other than that of test animals. Final DO determinations were made after 1 hour of immersion in the water bath.

At the end of each final respiration determination, a 20 ml sample of seawater was collected from each BOD bottle, preserved with two (2) ml of phenol (80%) and promptly refrigerated until later analysis for ammonia. After ammonia samples were collected, fish were removed from the BOD bottle, measured for standard length (mm), sexed, placed in preweighed aluminum pans, and dried for at least 72 h at 90°C before final dry weight determinations (g) were taken. Results were expressed as $\mu g0_2/g/hr$).

All water samples collected for ammonia analysis were analyzed within two weeks of collection using the procedure described by Solarzano (1969). An Orion Scientific Auto Analyzer (Model 140) was used for ammonia determinations of samples and standard curves. Results were expressed as μg of ammonia excreted per gram of dry weight per hour ($\mu gNH_4/g/hr$).

Bayne (1975) and McKenney (1982) have suggested that alterations in balance between the catabolism of carbohydrate, protein, and lipid substrates may be useful as a measure of stress in aquatic organisms. Alterations in the ratio between oxygen consumption and ammonia excreted has been used to assess stress in aquatic organisms. Oxygen/nitrogen (O/N) ratios were calculated for all mummichogs exposed to azinphosmethyl and in controls by dividing the oxygen consumption (μg atoms) by the nitrogen excretion rates (in μg atoms) for each fish.

Fish were also sacrificed and the brains removed and stored at -20°C for subsequent determination of brain AChE activity using a modification of the method described by Ellman *et al.*, 1961.

The remaining control and treatment fish were transferred to clean water and held for 8 days. Water was changed daily and fish were fed during their depuration period.

At the end of 8 days, respiration measurements were made, samples collected for ammonia determinations and the fish sacrificed and the brains removed as previously described.

From these determinations, mean fish respiration rate (nitrogen excretion rate), and O/N ratios were determined and statistically compared using ANOVA. An alpha ≤ 0.05 was the minimum level of significance used. Both temporal (24 hour exposure versus 192 hour depuration: azinphosmethyl and control groups) and between group (control versus azinphosmethyl groups) comparisons were made.

BIOMARKER STUDIES

Toxicity studies with pesticides in fish and other aquatic organisms generally assess only the acute toxicity of individual insecticides. In order to accurately predict the ecological impact of insecticide exposure in the environment, additional information concerning the sublethal effects of insecticides in aquatic organisms is needed. One goal of this project was to evaluate specific sublethal toxic responses in aquatic organisms exposed to agricultural insecticides.

Organophosphorus (OP) insecticides are believed to produce toxicity by severely inhibiting the enzyme, acetylcholinesterase (AChE). This inhibition causes an accumulation of acetylcholine at the post-synaptic membrane which leads to excessive activity at the synapses followed by a blockade of nervous impulses (O'Brien, 1967).

Earlier studies by Fulton 1989 also reported in Scott *et al.*, (1990), have indicated the effect of azinphosmethyl on brain AChE enzyme. The 24 hour EC50 was 0.81 μg/L.

Additional statistical analyses of various intrinsic (sex) and extrinsic (salinity and the presence of more than one insecticide) were conducted to evaluate their importance. Results indicated:

1) ANOVA analysis indicated that salinity and brain AChE were significantly correlated. Additional, multiple mean comparisons of high and low salinity indicated there were no differences in brain AChE levels at high (20 ppt) and low (5 ppt) salinities.
2) ANOVA analysis also indicated there was no significant interaction between azinphosmethyl and other insecticides (endosulfan); and
3) ANOVA analysis further indicated no significant interactions between azinphosmethyl exposure and sex associated with brain AChE levels.

These extensive laboratory studies in fish were then compared with measured levels of brain AChE inhibition in field exposures to azinphosmethyl. Results indicated excellent agreement between field and laboratory results.

This present study was designed to further examine the effects of sublethal insecticide exposure on the level of brain AChE activity under field conditions. Additional comparisons of whole animal AChE levels in oysters were made to compare different species. The goal of this research was to enhance our knowledge of the utility of AChE inhibition as a biomarker of nonpersistent pesticide exposure in the field.

Field Exposure Phase

Study sites for the field exposure tests were those previously described in the Materials and Methods section. Field exposure tests were conducted during May - June 1989 - 90. During 1989 and 1990, 96h field exposure tests were conducted during the vegetable growing season at the TRT, KWA and CTL Sites.

F. heteroclitus were collected using a minnow trap at the Cherry Point (CP) collection site (Figure 1). All animals utilized in the field exposure tests were between 45-100 mm. Animals were deployed by placing them in rectangular plexiglass cages - 25 (L) x 5.3 (W) x 5.3 (H) cm with 2.0 mm nytex screen. Ten animals were deployed per cage. A total of 30 animals were deployed in three cages at each site. All plexiglass cages were placed in larger wire cages to exclude predators.

At the end of the 96 hour field exposure, the animals deployed at each test site were removed from the field and transported back to the laboratory in large, insulated coolers. At the laboratory, animals from each exposure site were sorted by sex. A total of 15 - 20 fish from each site were sacrificed. All animals were sacrificed within 12 hours of their removal from the field. The brains from these animals were removed, wrapped in aluminum foil (5 brains per sample) and stored at -20°C until analyzed for AChE activity.

During field exposure tests, seawater samples were collected daily and at prescribed sampling intervals following significant rainfall. These samples were collected and analyzed using procedures described in the Materials and Methods Section.

Assay of AChE Activity

Brain AChE activity was determined using a continuous assay procedure modified from Ellman *et al.*, (1961). Each brain tissue sample was homogenized on ice with a TenBroeck® ground glass homogenizer in 50 mm Tris-HCl buffer (pH = 8.1) at 20 mg/ml. Next, 250 μl of this homogenate were added to a test tube containing 4.75 ml of the buffer. After incubation for 15 minutes in a shaking water bath at 30 °C, 2.9 ml of the dilute homogenate were added to a cuvet containing 100 μl of 0.87% 5, 5-dithiobis-(2-nitrobenzoic acid), the color reagent. Finally, 15 μl of 75 μM acetylthiocholine, the substrate, were then added to the cuvet which was covered by parafilm and inverted to mix. The absorbance was then read continuously for 1 minute at 412 nm using a Bausch and Lomb Spectronic® 1001 spectrophotometer. Enzyme velocities were linear during the assay period. A minimum of three subsamples were assayed for each brain tissue sample. In addition, a subsample incubated with 10.0 μM eserine was used to account for non-enzymatic, non-AChE hydrolysis of the substrate. The protein content of the homogenate was determined using the Sigma^R assay procedure, a modification of the original Lowry method (Lowry *et al.*, 1951). Enzyme activity was calculated as nmol product formed min^{-1} mg protein $^{-1}$.

Whole Body Insecticide Residue Analysis

Following significant runoff events, fish deployed in field bioassays were sacrificed for whole animal analysis of pesticide levels.

Tissue preparation, sample cleanup and insecticide quantification were performed using the methods described by Bush *et al.*, (1977) and Bush *et al.*, (1978).

After thawing, approximately-5g of fish tissue were placed in a high speed blender jar. About 50 g of Na_2SO_4 and 300 ml of ethyl acetate were added, and the sample was blended for 5 - 10 minutes. This homogenate was then filtered with suction through a 9-cm diameter Buckner funnel fitted with a Reeves Angel glass filter paper into a 500 ml suction flask. The filtrate was then transferred to a boiling flask and taken to dryness using a rotary evaporator at 50°C. This extract was then made to 10 ml with ethyl acetate-toluene (75:25). Fat was removed by gel permeation chromatography (GPC) using an automated GPC AutoPrep® 1001.

Gas Chromatography (GC) analysis was conducted with a Tracor gas chromatograph (Model 550) equipped with 6 ft x 0.25 inch coiled glass columns and Ni^{63} electron capture detectors. Identification and quantification of insecticide residues was based on their retention time and peak height relative to those of reference insecticide-standard solutions.

Bioconcentration factors for the insecticides in *F. heteroclitus* exposed in field exposures were calculated by dividing the insecticide concentration measured in the fish by the insecticide concentration measured in stream at each field site. Detection limits were 50 μg/kg for azinphosmethyl and 10 μg/kg for endosulfan I, endosulfan II and endosulfan sulfate.

Ecotoxicological Studies of Macropelagic Organisms: 1989 - 1990

Block Seining

The east (TRT) and west (CTL) branches of Leadenwah Creek were sampled for macropelagic fauna, using a block seining technique during 1989-90 (Figure 2). At each site, three consecutive 50 m stretches of stream were permanently marked with metal stakes. During each sampling period (monthly, February - May, 1989; bimonthly (every 14 days), June - August, 1990; monthly, September 1989 - May 1990; bimonthly, June - August, 1990; and monthly, September 1990 - March, 1991), a total of four seine nets (12 m x 1.5 m x 4 mm mesh) with 2 m long poles were anchored into the sediments for each 50 m interval. At each site, lead lines for each net were pushed into the sediments and held in place by bricks.

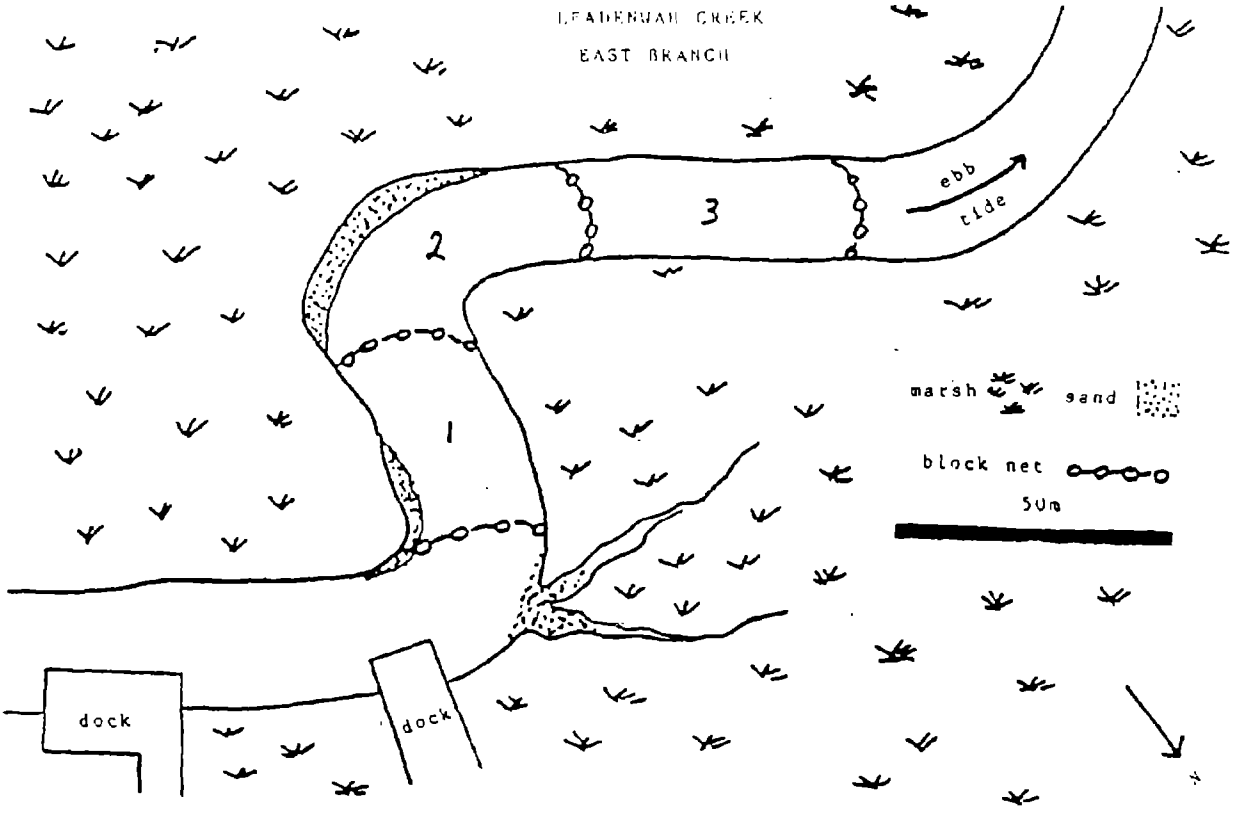


Figure 2. Sketch of net deployments during block seining at the TRT Site.

Another net was then pulled between each set of block nets and the contents of each seine placed into a plastic bucket, preserved in a 10% buffered formalin, and stored for subsequent taxonomic identification.

At the laboratory, each bucket was opened and the contents poured onto a large diameter (10 mm) screen, washed and all detritus, vegetation and algae was removed from each sample. Each sample was then placed back into the bucket and weighed wet to determine total sample biomass (g/50m of stream).

Following biomass measurements, all large (> 10 cm) organisms (fish, shrimp and blue crabs) were removed from each sample, identified to genus or species, counted (density/50 m of stream), and the wet weight (g/species) of each species noted. The remainder of the sample (organisms < 10 cm) was then identified to genus or species, enumerated (density/50 m of stream) and the wet weight (g/species) of each species noted. For small biomass samples (<2000 g/samples) this procedure was followed for each sample, but for larger biomass samples (>2000 g/sample) a subsampling procedure for small organisms (< 10 cm) was used for each sample. Three randomly selected subsamples (500 g each) were taken from each sample. Each subsample was sorted and identified to genus/species, enumerated (density/species) and weighed wet (g/species). Each subsample was then multiplied by a sample weight (g)/subsample weight (g) conversion factor to estimate the number of organisms in each sample. Each of the three subsamples were then averaged and a mean density (±95% CL) and biomass (±95%CL) calculated for each species. This subsampling procedure was used primarily for grass shrimp (*P. pugio*), mummichogs (*F. heteroclitus*), juvenile spot (*L. xanthurus*), and bay anchovies (*A. mitchilli*). Following subsampling, the remainder of each sample was poured back out onto the screen to identify any rare species such as sheepshead minnow (*Cyprinodon variegatus*) which had been excluded by the subsampling procedure.

From this procedure the following ecological parameters were calculated for each sample:

(1) Total sample biomass (g/50 m of stream);
(2) Total grass shrimp (*P. pugio*) densities (#/50 m of stream);
(3) Total mummichogs (*Fundulus heteroclitus*) densities (#/50m of stream);
(4) Total penaied shrimp (*Penaeus aztecus, Penaeus setiferus,* and *Penaeus duorarum*) densities (#/50m of stream);

(5) Total blue crab (*Callinectes sapidus*) densities (#/50m of stream); and
(6) Total fin fish densities (#/50m of stream).

Ecotoxicological Sampling Statistical Procedures

Ecological parameters at each sampling site (CTL and TRT) were statistically compared using:

(1) The Mann-Whitney or Wilcoxon Rank Sums Nonparametric Method for unpaired samples (Wilcoxon, 1964).
(2) The Wilcoxon Rank Sum Nonparametric Method for paired samples (Wilcoxon, 1964). This procedure was found to be more appropriate since statistical analysis generally indicated a slight statistical bias in our sampling method, as higher densities and biomass were found in our most seaward net (#1 at the CTL and #3 at the TRT Sites). Therefore the paired procedure was found to be more appropriate for our data analysis.

In all samples, statistical comparisons were based upon a sample size of n = 6 (three replicates at the CTL and three replicates at the TRT Site); however, in samples which were subsampled, statistical comparisons of paired samples were possible with an n = 8 - 18. In samples which were subsampled, additional statistical comparisons were made using the Wilcoxon Rank Sums Tests for paired data as previously described. An alpha level of $\leq$ 0.05 was chosen as a minimum significant level in comparisons of samples.

An additional method of statistical data analysis was used in which the ecological parameter of interest at the TRT Site was subtracted from the same paired parameter at the CTL Site. If the two sites were similar, the numerical difference between the matched replicate pairs should approach a theoretical zero difference. Large deviations from this zero difference may occur if the TRT Site was impacted by pesticide runoff (I.e. toxic effects, behavioral avoidance, or both). During periods of significant pesticide runoff, increased densities at the CTL Site may occur relative to the TRT Site, resulting in significant deviations from the zero difference line. Statistical difference between CTL and TRT Sites were based upon the Wilcoxon Rank Sum Nonparametric Method. An alpha level of $\leq$ 0.05 was the minimum significance level used.

Water Quality Parameters

During each sampling event during 1989-90, temperature (°C), dissolved oxygen (mg O_2/L) and salinity (ppt) were measured by a YSI oxygen meter (Model 64) and an American Optics Salinity Refractometer, using methods described in Standard Methods (1982) for calibration and sample determination. The pH was also measured using either an Orion (Model 250) or a Hanna (Model 0064) pH meter.

Push Netting

While block seining has been shown to be an effective method for assessing population level effects in the macropelagic community, it is extremely time consuming, labor intensive, and produces large amounts of solid (animal carcass) and hazardous wastes (i.e. formalin waste). Additionally, certain habitats may be extremely difficult to sample in this manner. Alternative methods which are less labor intensive, time consuming and waste generating are needed.

Results of block seining studies have clearly indicated the importance of *P. pugio* in tidal creek habitats and their known sensitivity to various agricultural pesticides. Welch (1975) in earlier studies has demonstrated the use of push netting to characterize field populations of *P. pugio* in estuarine habitats of Texas, with densities varying from 20-300 animals/m^2 being reported. Welch's method was modified and evaluated as a rapid census method for grass shrimp in estuarine tidal creeks at the CTL, TRT, and KWA Sites from March - December, 1990. At each site, three consecutive, 50m stretches of stream were permanently marked with metal stakes and sampled monthly with a push net. (31 cm length x 30 cm width x 5 mm mesh). Two tows (by hand), one along each bank, were made in each stream reach at or near dead low tide. Each tow was made going against the tide. The contents of the two tows were pooled, placed in 10% formalin and stored for subsequent taxonomic identification.

At the laboratory, each sample was opened and poured onto a large diameter screen (10 mm mesh), washed and all detritus, vegetation, and algae were removed. The remainder of the sample was blotted dry and weighed wet to determine total biomass (g/m^2). Following biomass measurements, all crabs (primarily juvenile *Callinectes sapidus*), penaied shrimp, (primarily *Penaeus aztecus* or *Penaeus setiferus*), and small fish (primarily *F. heteroclitus*)

were identified to genus and/or species, enumerated (density/50m) and weighed wet (g/50m). The remainder of each sample containing grass shrimp (*P.pugio*) was enumerated (density/50m) and weighed wet (g/50m). From this procedure the following ecological parameters were calculated for each sample:

1) Total sample biomass (g/50m) and total sample density for all species (density/50 m);
2) Total grass shrimp (*P.pugio*) density (density/50m)and biomass (g/50m); and
3) Total non grass shrimp biomass (g/50 m) and density (density/50m).

These ecological parameters at each sampling site were statistically compared using nonparametric procedures (Mann-Whitney, Wilcoxon Rank Sums and Kruskal-Wallis)(Zar, 1974; Armor, 1973; and Wilcoxon, 1964). An alpha level of ≤ 0.05 was chosen as a minimum for significance levels when comparing samples between sites.

RESULTS

I. Field Toxicity Tests

A. Daily Physicochemical Parameters

1. 1989, Daily Water Quality Parameters

Results of physicochemical water quality parameters measured daily at each site during the 1989 field study are listed in Table 1. Mean daily seawater temperature ranged from 24.3 - 31.0°C, averaging 27.64°C at the CTL Site. Similarly, temperatures at the TRT Site ranged from 23.5 - 33.4°C, averaging 27.34°C. At the KWA Site, temperatures ranged from 22.0 - 37.0°C, averaging 28.10°C. Statistical analysis indicates that seawater temperatures at the three field sites were not significantly different during May - June, 1989.

Mean salinities ranged from 16 - 33.2 ppt, averaging 28.59 ppt at CTL Site. Salinities were lower at the TRT Site, ranging from 6.0 - 32.0, averaging 22.04 ppt. Statistical analysis indicated that salinities were significantly ($p \leq 0.05$) lower at the TRT when compared to the CTL Site. The lower salinities at the TRT Site were the result of freshwater inputs of agricultural runoff following major rain events. The low salinities at the TRT Site occurred despite the fact that most of agricultural drainage area's runoff was channeled into retention ponds.

Salinities at the KWA Site were even lower, ranging from 2 - 35 ppt, averaging 15.79 ppt. Statistical analysis indicated that salinities at the KWA Site were significantly ($p \leq 0.05$) different from both the CTL and TRT Sites. The much lower salinities at the KWA Site demonstrates the significance that freshwater discharge from agriculture may have on salinity. Of particular interest is the fact that salinity comparisons between the TRT Site, an agriculture site with BMP, IPM, and retention ponds, were significantly different from the KWA Site, an agricultural site without BMP, IPM, and retention ponds. Although salinities at the TRT Site were significantly lower than the CTL Site, the retention ponds there appeared to provide some degree of protection by moderating fresh water inputs.

TABLE 1. Summary of physicochemical water quality parameters measured at field sites during the 1989 field study
Pooled means with different letters (A ,B, C) were significantly ($p < 0.05$) different from one another.

1989			Water Temperature (°C)			Salinity (ppt)			DO_2 (mg 0_2/L)			pH		
SITE	GRP #	DATE	X	SE	Range	X	SE	Range	X	SE	Range	X	SE	Range
CTL	1	5/24/89 through 5/29/89	26.51	0.72	24.5 - 29.7	32.08	0.28	31.1 - 33.1	4.48	0.74	3.31 - 8.12	7.29	0.09	7.11 - 7.60
TRT			26.17	0.77	24.2 - 29.7	28.23	0.71	26.1 - 30.82	3.95	0.49	2.63 - 6.02	7.38	0.05	7.24 - 7.59
KWA			28.00	1.04	25.0 - 32.5	29.83	1.45	25.0 - 35.0	4.92	0.70	1.40 - 9.30	7.83	0.10	7.70 - 8.30
CTL	2	5/29/89 through 6/2/89	25.90	0.67	24.3 - 27.9	32.82	0.18	32.2 - 33.2	3.52	0.30	3.10 - 4.41	7.17	0.02	7.11 - 7.21
TRT			26.30	0.57	25.1 - 28.2	31.24	0.43	30.3 - 32.8	3.68	0.25	3.18 - 4.60	7.24	0.04	7.10 - 7.29
KWA			30.30	2.08	25.0 - 36.0	34.00	0.63	32.0 - 35.0	6.93	1.19	4.10 - 11.2	7.82	0.10	7.50 - 8.10
CTL	3	6/2/89 through 6/7/89	28.52	0.74	25.8 - 30.7	30.38	1.27	25.8 - 32.7	4.62	0.69	2.70 - 5.20	7.20	0.05	7.00 - 7.30
TRT			28.93	1.77	23.5 - 33.4	24.83	4.40	9.0 - 32.9	5.50	0.62	3.70 - 7.20	7.22	0.08	7.00 - 7.50
KWA			31.75	2.79	22.0 - 37.0	23.33	6.15	2.0 - 35.0	9.53	1.50	4.20 - 13.5	8.00	0.11	7.50 - 8.30
CTL	4	6/7/89 through 6/11/89	25.94	0.22	25.5 - 26.5	25.60	0.46	24.1 - 27.0	2.94	0.39	1.80 - 4.20	7.02	0.04	6.90 - 7.10
TRT			24.62	0.39	23.5 - 25.6	10.88	1.38	6.0 - 13.7	4.06	0.56	2.60 - 5.50	7.17	0.06	7.04 - 7.40
KWA			24.30	0.58	24.0 - 26.0	2.40	0.40	2.0 - 4.0	4.96	1.14	3.50 - 9.50	7.92	0.10	7.60 - 8.10
CTL	5	6/11/89 through 6/15/89	28.02	0.64	26.4 - 30.3	27.34	0.45	25.7 - 28.0	3.00	0.53	1.80 - 4.70	7.16	0.09	7.00 - 7.50
TRT			27.64	1.05	25.3 - 31.4	19.22	2.28	11.5 - 23.7	3.73	1.29	0.89 - 9.90	7.36	0.11	7.10 - 7.70
KWA			26.24	0.69	24.0 - 28.2	5.60	1.17	4.0 - 10.0	6.40	0.84	5.00 - 9.50	7.74	0.13	7.30 - 8.10

1989			Water Temperature (°C)			Salinity (ppt)			DO_2 (mg 0_2/L)			pH		
SITE	GRP #	DATE	X	SE	Range	X	SE	Range	X	SE	Range	X	SE	Range
CTL	6	6/15/89 through 6/19/89	29.12	0.67	27.7 - 31.0	26.40	0.62	24.5 - 28.0	4.28	0.51	2.70 - 5.50	7.70	0.12	7.40 - 8.10
TRT			29.66	0.94	26.5 - 31.6	21.40	3.93	7.0 - 27.5	5.94	1.33	2.10 - 9.90	7.68	0.13	7.40 - 8.10
KWA			27.50	0.82	25.1 - 30.6	6.17	1.30	3.0 - 10.0	5.17	0.65	2.70 - 7.40	7.53	0.16	7.20 - 8.30
CTL	7	6/19/89 through 6/23/89	27.76	0.26	27.7 - 28.5	25.20	2.33	16.0 - 28.0	3.25	0.23	2.70 - 3.83	7.36	0.04	7.30 - 7.50
TRT			27.36	1.02	25.6 - 31.3	18.20	2.11	14.0 - 26.0	3.55	0.66	2.10 - 5.20	7.48	0.13	7.30 - 8.00
KWA			27.52	0.55	25.7 - 28.6	15.30	3.27	6.0 - 24.0	4.69	0.65	2.40 - 5.90	7.58	0.10	7.30 - 7.90
CTL	8	6/23/89 through 6/27/89	28.70	0.77	27.1 - 30.9	27.20	1.83	20.0 - 30.0	3.52	0.61	2.26 - 5.20	7.32	0.04	7.20 - 7.40
TRT			27.16	0.36	25.8 - 27.9	19.90	2.21	15.0 - 27.5	2.94	0.18	2.50 - 3.37	7.28	0.07	7.10 - 7.50
KWA			28.08	0.49	26.4 - 29.5	5.00	1.41	2.0 - 10.0	9.88	1.38	5.14 - 12.65	7.70	0.18	7.30 - 8.30
CTL	Grp 1 through Grp 8	5/24/89 through 6/27/89	27.64^{A}	0.30	24.3 - 31.0	28.59^{A}	0.65	16.0 - 33.2	3.84^{A}	0.22	1.80 - 8.12	7.29^{A}	0.04	6.90 - 8.10
TRT			27.34^{A}	0.45	23.5 - 33.4	22.04^{B}	1.38	6.0 - 32.9	4.19^{A}	0.31	0.89 - 9.90	7.36^{A}	0.04	7.00 - 8.10
KWA			28.10^{A}	0.62	22.0 - 37.0	15.79^{C}	2.21	2.0 - 35.0	6.53^{B}	0.50	1.40 - 13.50	7.76^{B}	0.05	7.30 - 8.30

Mean dissolved oxygen concentrations ranged from 1.80 - 8.12, averaging 3.84 mg/L at the CTL Site compared to levels ranging from 0.89 - 9.90, averaging 4.19 mg/L at the TRT Site. Statistical analysis indicated the mean dissolved oxygen concentrations were not significantly different in comparisons between the CTL and TRT Sites. Additionally dissolved oxygen levels were at concentrations sufficient to support crustacean and fish populations observed at these sites.

At the KWA Site, dissolved oxygen concentrations ranged from 1.40 - 13.50, averaging 6.53 mg/L. Statistical analysis indicated that mean dissolved oxygen concentrations were significantly ($p \leq 0.05$) higher at the KWA Site when compared to the CTL and TRT Sites. Although dissolved oxygen levels were somewhat similar at the sites, it is interesting to note that dissolved oxygen concentrations had a much greater range, than was observed at the CTL Site (1.80 - 8.12 mg/L). In some instances, (i.e. afternoon ebb tides) oxygen levels were supersaturated ($> 100\%$) suggesting possible nutrient enrichment at the KWA Site. At the TRT Site, similar supersaturated conditions were observed, similarly suggesting nutrient enrichment. Additional morphometric features, such as the broad, shallow habitats conducive for benthic diatoms and other phytoplankton growth, must also be considered.

Mean daily pH ranged from 6.90 - 8.10, averaging 7.29 at CTL Site compared to values ranging from 7.00 - 8.10, averaging 7.36 at the TRT Site. Statistical analysis indicated that pH was not significantly different in comparisons between the two sites.

At the KWA Site, pH ranged from 7.20 - 8.30, averaging 7.76. Statistical analysis indicated that pH at the KWA Site was significantly ($p \leq 0.05$) higher than at the TRT and CTL Sites. Generally pH declines with reduction in salinity due to decreased carbonate/bicarbonate buffering capacities in the water. This trend was not observed at the KWA Site, as pH increased with generally decreased salinities. This suggests possible agricultural influences (i.e, nutrients, fertilizer) which may have affected pH values there. Although, there were statistically significant differences in pH between sites, these differences were not biologically significant (i.e., pH values were within the zone of compatibility for most organisms) in terms of survival of estuarine organisms residing there. The CO_2 - Carbonate buffering system in seawater maintains pH at a range of 7.50 - 8.50 (Valiela, 1984). EPA (1976) reported in the Red Book that a pH range of 6.5 - 9.0 provides adequate protection for fresh water and marine organisms. These slight differences however, may reflect possible eutrophic conditions due to nutrient enrichment (i.e, increase of plankton production) as evidenced by the increased dissolved oxygen levels, observed at the KWA Site. This may result in increased plankton densities and possible plankton species differences which may cause increased pH.

2. 1990, Daily Water Quality Parameters

Results of physicochemical water quality parameters measured daily at each site during the 1990 field studies are listed in Table 2. Mean daily seawater temperatures ranged from 21.3 - 32.1°C at the CTL Site, averaging 26.83°C for the May - June, 1989 study period. At the TRT Site, seawater temperatures ranged from 21.6 - 34.4°C, averaging 27.08°C compared to temperatures ranging from 22.5 to 34.8°C, averaging 28.59°C at the KWA Site. Statistical analysis indicated that seawater temperatures were significantly ($p \leq 0.05$) higher at the KWA Site than at the CTL and TRT Sites; however, these differences were more the result of the generally later daily sampling time at the KWA Site rather than actual physical between site differences.

Mean daily salinities ranged from 24.0 - 34.0, averaging 30.4 ppt at CTL Site compared to salinities ranging from 14.2 - 35.0, averaging 27.78 at the TRT Site. Similarly salinities were slightly lower at the KWA Site compared to the CTL Site, ranging from 21.6 - 35.5, averaging 30.84 ppt. Statistical analysis indicated that salinities at the TRT Site were significantly ($P \leq 0.05$) lower than values measured at both the CTL and KWA Sites.

Mean daily dissolved oxygen levels at the CTL Site ranged from 2.10 - 7.20, averaging 4.72mg/L. Similar levels at the TRT Site ranged from 2.40 - 9.10, averaging 4.89 mg/L. At the KWA Site, daily dissolved oxygen levels were slightly higher, ranging from 1.60 - 11.70, averaging 6.26 mg/L. Statistical analysis indicated that dissolved oxygen levels were significantly ($p \leq 0.05$) higher at the KWA Site, when compared to the CTL and TRT Sites. Generally dissolved oxygen levels were somewhat similar at all sites, but it is interesting to note that at the KWA Site the range of daily dissolved oxygen levels was again much greater than the CTL or TRT Sites during 1990, suggesting possible nutrient enrichment. Measurements of Chlorophyll A at the KWA, TRT, and CTL Sites during 1990 made by EPA (John Macauley, USEPA, Personal Communications) directly supported this observation, as Chlorophyll A levels were higher at the KWA Site than the TRT or CTL Sites. Higher Chlorophyll A levels would result from high phytoplankton biomass and resulting hyperproduction of oxygen during periods of high photic activity. While minimum dissolved levels were low relative to EPA water quality criteria, the organisms residing in these tidal creeks and used in toxicity tests are well adapted to the rigors of this environment. None of the low dissolved oxygen levels observed were detrimental to the fish and crustaceans tested. Recent findings (Dr. L. Burdette, University of Charleston, personal communication) report that at dissolved oxygen levels of <2mg/L, grass shrimp become respiro-conformers rather than respiro-regulators. This suggests that these species are quite capable of adapting to low dissolved oxygen levels.

TABLE 2. Summary of physicochemical water quality parameters measured at field sites during the 1990 field study
Pooled means with different letters (A ,B, C) were significantly ($p < 0.05$) different from one another.

1990			Water Temperature (°C)			Salinity (ppt)			DO_2 (mg 0_2/L)			pH		
SITE	GRP #	DATE	X	SE	Range	X	SE	Range	X	SE	Range	X	SE	Range
CTL	1	5/24/90 through 5/28/90	25.98	1.32	22.3 - 29.9	29.38	0.87	26.0 - 31.0	5.98	0.65	4.30 - 7.20	7.50	0.11	7.20 - 7.80
TRT			25.58	1.32	22.5 - 30.1	30.78	0.49	30.0 - 32.3	4.39	0.52	3.08 - 5.69	7.31	0.07	7.10 - 7.49
KWA			29.68	0.58	27.6 - 30.8	26.74	0.93	25.0 - 30.0	7.68	0.83	4.60 - 9.40	7.56	0.04	7.40 - 7.60
CTL	2	5/28/90 through 6/1/90	24.26	1.54	21.3 - 29.9	27.00	1.00	24.0 - 30.0	4.63	0.62	3.45 - 6.90	7.40	0.08	7.10 - 7.60
TRT			23.98	1.62	21.6 - 30.1	20.84	2.90	14.2 - 30.0	4.21	0.42	3.07 - 5.69	7.39	0.05	7.27 - 7.50
KWA			25.32	1.32	22.5 - 30.2	25.50	1.64	21.6 - 30.0	4.56	0.77	2.90 - 7.40	7.38	0.06	7.20 - 7.50
CTL	3	6/1/90 through 6/5/90	25.10	0.83	22.9 - 27.9	29.50	0.50	28.0 - 31.0	4.26	0.39	3.70 - 5.00	7.30	0.07	7.10 - 7.50
TRT			25.12	0.92	22.0 - 27.4	24.20	1.11	22.0 - 28.0	4.92	0.42	4.20 - 6.50	7.36	0.06	7.20 - 7.50
KWA			28.96	1.33	25.3 - 32.8	30.10	0.72	28.0 - 32.5	8.42	0.92	6.20 - 11.70	7.52	0.06	7.40 - 7.70
CTL	4	6/5/90 through 6/9/90	27.72	0.88	25.3 - 29.6	30.90	0.40	30.0 - 31.5	4.80	0.43	3.70 - 6.10	7.28	0.02	7.10 - 7.50
TRT			29.40	0.60	28.0 - 31.0	29.40	0.60	28.0 - 31.0	5.22	0.47	4.22 - 6.70	7.40	0.06	7.30 - 7.60
KWA			32.46	0.92	30.0 - 34.8	33.10	0.60	32.0 - 35.0	8.88	0.45	7.60 - 10.10	7.58	0.08	7.40 - 7.80

CTL	5	6/9/90	29.46	0.37	28.7 - 30.5	31.60	0.37	31.0 - 33.0	5.68	0.31	5.10 - 6.80	7.36	0.04	7.30 - 7.50
TRT		through	29.46	1.14	26.8 - 31.9	29.40	1.08	26.0 - 32.0	7.36	0.60	5.80 - 9.10	7.64	0.10	7.40 - 8.00
KWA		6/13/90	30.30	1.59	26.5 - 33.5	34.20	0.58	32.0 - 35.0	8.24	1.06	5.50 - 10.30	7.78	0.10	7.40 - 8.00
CTL	6	6/13/89	26.90	1.01	24.6 - 29.3	31.00	0.63	29.0 - 33.0	4.38	0.90	2.10 - 5.90	7.24	0.04	7.10 - 7.30
TRT		through	25.98	0.75	24.5 - 28.5	28.30	2.13	24.0 - 32.5	4.38	0.93	2.40 - 6.80	7.28	0.04	7.20 - 7.40
KWA		6/17/90	26.16	0.88	22.8 - 27.8	32.20	1.49	27.5 - 35.5	4.54	0.45	3.20 - 5.80	7.34	0.02	7.30 - 7.40
CTL	7	6/17/90	28.38	1.33	25.2 - 31.2	32.4	0.40	31.0 - 33.0	3.80	0.27	3.20 - 4.70	7.20	0.05	7.10 - 7.40
TRT		through	29.00	1.63	24.5 - 34.4	28.10	0.68	26.0 - 30.0	3.98	0.49	2.70 - 5.40	7.44	0.09	7.20 - 7.70
KWA		6/21/90	28.04	1.72	25.1 - 34.6	32.50	0.77	30.0 - 34.0	3.08	0.50	1.60 - 4.60	7.28	0.05	7.10 - 7.40
CTL	8	6/21/90	30.33	1.34	27.7 - 32.1	33.33	0.33	33.0 - 34.0	4.33	0.19	4.1 - 4.70	7.20	0.12	7.00 - 7.40
TRT		through	32.60	1.14	30.5 - 34.4	32.67	1.45	30.0 - 35.0	5.10	0.85	3.50 - 6.40	7.50	0.12	7.30 - 7.70
KWA		6/23/90	29.87	2.83	24.8 - 34.6	34.83	0.44	34.0 - 35.5	3.54	0.70	2.30 - 4.73	7.17	0.07	7.10 - 7.30
CTL	Grp 1	5/24/90	26.83^{A}	0.51	21.3 - 32.1	30.40^{A}	0.41	24.0 - 34.0	4.72^{A}	0.24	2.10 - 7.20	7.31^{A}	0.03	7.00 - 7.80
TRT	through	through	27.08^{A}	0.61	21.6 - 34.4	27.68^{B}	0.89	14.2 - 35.0	4.89^{A}	0.29	2.40 - 9.10	7.41^{B}	0.03	7.10 - 8.00
KWA	Grp 8	6/23/90	28.59^{B}	0.63	22.5 - 34.8	30.84^{A}	0.73	21.6 - 35.5	6.26^{B}	0.51	1.60 - 11.70	7.47^{B}	0.04	7.10 - 8.00

Mean daily pH ranged from 7.00 - 7.80, averaging 7.31 at the CTL Site. Values at the TRT Site were slightly higher ranging form 7.10 - 8.00, averaging 7.41. Similarly, values at the KWA Sites were slightly higher, ranging from 7.10 -8.00, averaging 7.47. Statistical analysis indicated that mean pH values were significantly ($p < 0.05$) higher at the TRT and KWA Sites when compared to the CTL Site. The slight differences in pH between sites were not biologically significant (i.e. would not affect survival of most estuarine organisms) but may be indicative of agricultural influences. During 1989, pH was also significantly higher at the KWA Site despite very low salinities (< 5 ppt). Generally pH declines with reductions in salinity. This trend was not observed at the TRT and KWA Sites suggesting possible agricultural influences.

B. Rainfall Measurements

1. 1989 Study Period

Cumulative rainfall totals throughout the 1989 study period (May 24 - June 27, 1989) were 16.36 cm (± 0.25) at the CTL Site, 17.04 cm (± 0.36) at the TRT Site, and 25.40 cm (± 0.30) at the KWA Site (Table 3). During the study period rainfall occurred on a total of 14 days at the CTL Site, 15 days at the TRT Site, and 10 days at the KWA Site (Table 3). The largest daily (within 24 hours) rainfall amounts were 4.75 cm (± 0.05 at the CTL Site, 4.90 cm (± 0.05) at the TRT Site and 8.46 cm (± 0.08) at the KWA Site (Table 3).

Also during the study period, the number of significant (>1.27cm/day) rainfall days was 4 days at the CTL Site, 5 days at the TRT Site, and 5 days at the KWA Site (Table 4). At the CTL Site the greatest rainfall amounts were observed on June 5 (4.75 ± 0.05cm) and June 6 (3.43 ± 0.08 cm). Similarly at the TRT Site, the greatest rainfall amounts occurred on June 5 (4.90 ± 0.05 cm) and June 6 (3.43 ± 0.08 cm). At the KWA Site the greatest rainfall amounts occurred at June 5 (7.54 ± 0.08 cm), June 6 (8.46 ± 0.08 cm) and June 24 (4.57 ± 0.00 cm).

2. 1990 Study Period

Cumulative rainfall totals throughout the 1990 Study Period (May 24 - June 23, 1990) were 4.88 cm (± 0.08) at the CTL Site, 5.31 cm (± 0.15) at the TRT Site, and 4.32 cm (± 0.03) at the KWA Site (Table 5). During the study period, rainfall occurred on a total of 4 days at the CTL Site, 4 days at the TRT Site, and 5 days at the KWA Site (Table 5). The largest daily (within a 24 hour period) rainfall amounts were 3.02 cm (± 0.03) at the CTL Site, 2.90 cm (± 0.13) at the TRT Site, and 2.24 cm (± 0.03) at the KWA Site (Table 5).

Table 3. Summary of rainfall observed during the 1989 field study.

1989			Cumulative Rainfall		# Days of Rain	Greatest Rainfall Amount/Day (cm/day)	
			(cm)	Range[1]			
SITE	Group #	Date	X (± SE)	(cm)	(Days/Grp)	X	SE
CTL	1	5/24/89 through 5/29/89	0	NC	0	0	NC
TRT			0	NC	0	0	NC
KWA			0	NC	0	0	NC
CTL	2	5/29/89 through 6/2/89	0	NC	0	0	NC
TRT			0	NC	0	0	NC
KWA			0	NC	0	0	NC
CTL	3	6/2/89 through 6/7/89	8.26 (±0.08)	8.10 - 8.36	4	4.75	0.05
TRT			3.37 (±0.10)	8.36 - 8.71	4	4.90	0.05
KWA			16.20 (±0.15)	15.95-16.46	4	8.46	0.08
CTL	4	6/7/89 through 6/11/89	1.60 (±0.08)	1.52 - 1.78	3	1.35	0.08
TRT			1.80 (±0.05)	1.75 - 1.91	3	1.58	0.05
KWA			2.21 (±0.08)	2.13 - 2.39	2	2.11	0.08
CTL	5	6/11/89 through 6/15/89	0	NC	0	0	NC
TRT			0	NC	0	0	NC
KWA			0	NC	0	0	NC

1989			Cumulative Rainfall		# Days of Rain	Greatest Rainfall Amount/Day (cm/day)	
			(cm)	Range			
SITE	Grp #	Date	X (± SE)	(Inches)	(Days/Grp)	X	SE
CTL	6	6/15/89	5.13 (±0.05)	5.05 - 5.21	4	3.02	0.03
TRT		through	1.61 (±0.01)	4.03 - 4.13	4	2.06	0.03
KWA		6/19/89	2.21 (±0.05)	2.10 - 2.29	2	1.47	0.03
CTL	7	6/19/89	3.51 (±0.02)	3.47 - 3.53	4	3.02	0.03
TRT		through	2.67 (0.08)	2.57 - 2.82	5	1.22	0.03
KWA		6/23/89	0.03 (0.03)	0.00 - 0.13	1	0.05	0.05
CTL	8	6/23/89	1.02 (±0.00)	1.02 - 1.02	2	0.76	0.00
TRT		through	1.42 (±0.02)	1.40 - 1.46	3	1.14	0.00
KWA		6/27/89	4.83 (±0.00)	4.83 - 4.83	2	4.57	0.00
CTL	Grp 1	5/24/89	16.36 (±0.25)	15.95-16.84	14	4.75	0.05
TRT	through	through	17.04 (±0.36)	16.46-17.65	15	4.90	0.05
KWA	Grp 8	6/27/89	25.40 (±0.30)	24.92-25.98	10	8.46	0.08

1 = Between 3 Rain Gauges
X = Mean
SE = Standard Error
NC = Not Calculated
GRP = Group

Table 4. Dates of signifcant rainfall (>1.27 cm/day) during the 1989 field study

1989		Rainfall Amount (cm/day)		
SITE	Date	Range[A]	X	(± SE)
CTL	6/5/89	4.70 - 4.83	4.75	(0.05)
	6/6/89	3.30 - 3.56	3.43	(0.08)
	6/9/89	1.27 - 1.52	1.35	(0.08)
	6/16/89	0.89 - 0.89[B]	0.89	(0.00)
	6/19/89	2.97 - 3.05	3.02	(0.03)
	6/24/89	0.25 - 0.25[B]	0.25	(0.00)
TRT	6/5/89	4.83 - 4.95	4.90	(0.05)
	6/6/89	3.30 - 3.53	3.43	(0.08)
	6/9/89	1.52 - 1.65	1.57	(0.05)
	6/16/89	2.03 - 2.10	2.03	(0.02)
	6/19/89	1.21 - 1.27[B]	1.22	(0.03)
	6/24/89	0.19 - 0.32[B]	0.25	(0.03)
KWA	6/5/89	7.37 - 7.62	7.54	(0.08)
	6/6/89	8.38 - 8.64	8.46	(0.08)
	6/9/89	2.03 - 2.29	2.11	(0.08)
	6/16/89	1.40 - 1.52	1.47	(0.03)
	6/19/89	0.00 - 0.00[B]	0.00	(0.00)
	6/24/89	4.57 - 4.57	4.57	(0.00)

A = Range between three rain gauges
B = Rainfall < 1.27 cm/day but included for comparative purposes
X = Mean
SE = Standard Error

Table 5. Summary of rainfall observed during the 1990 field study

1990			Cumulative Rainfall (cm)	Range[1]	# Days of Rain	Greatest Rainfall Amount cm/day	
SITE	Grp #	Date	X (± SE)	(cm)	(Days/Grp)	X	SE
CTL	1	5/24/90 through 5/28/90	3.15 (±0.03)	3.10 - 3.18	2	3.02	0.03
TRT			3.02 (±0.13)	2.77 - 3.18	2	2.90	0.13
KWA			2.34 (±0.03)	2.31 - 2.41	2	3.02	0.03
CTL	2	5/28/90 through 6/1/90	3.02 (±0.03)	3.00 - 3.05	1	3.02	0.03
TRT			2.90 (±0.13)	2.67 - 3.05	1	2.90	0.13
KWA			2.24 (±0.03)	2.21 - 2.31	1	2.24	0.03
CTL	3	6/1/90 through 6/5/90	0	NC	0	0	NC
TRT			0	NC	0	0	NC
KWA			0	NC	0	0	NC
CTL	4	6/5/90 through 6/9/90	0	NC	0	0	NC
TRT			0	NC	0	0	NC
KWA			0	NC	0	0	NC
CTL	5	6/9/90 through 6/13/90	0.25 (±0.00)	0.25 - 0.25	1	0.25	0.00
TRT			0.25 (±0.03)	0.25 - 0.25	1	0.25	0.00
KWA			<0.13 (±0.00)	<0.13-<0.13	1	<0.13	0.00

1990			Cumulative Rainfall (cm)	Range[1] (cm)	# Days of Rain (Days/Grp)	Greatest Rainfall Amount cm/day X	Greatest Rainfall Amount cm/day SE
SITE	**Grp #**	**Date**	**X (± SE)**			**X**	**SE**
CTL	6	6/13/90 through 6/17/90	1.45 (±0.05)	1.45 - 1.52	1	1.45	0.05
TRT			2.01 (±0.03)	1.98 - 2.03	1	2.01	0.03
KWA			1.78 (±0.00)	1.78 - 1.78	1	1.78	0.00 I
CTL	7	6/17/90 through 6/21/90	0	NC	0	0	NC
TRT			0	NC	0	0	NC
KWA			0	NC	0	0	NC
CTL	8	6/21/90 through 6/23/90	0	NC	0	0	NC
TRT			0	NC	0	0	NC
KWA			<0.13	<0.13 - <0.13	1	<0.13	0.00
CTL	Grp 1 through Grp 8	5/24/90 through 6/23/90	4.88 (±0.08)	4.78 - 5.00	4	3.02	0.03
TRT			5.31 (±0.15)	5.00 - 5.51	4	2.90	0.13
KWA			1.70 (±0.01)	4.29 - 4.39	5	2.24	0.03

I = Range between 3 rain gauges
X = Mean
SE = Standard Error
NC = Not calculated
GRP = Group

A total of two days of significant (> 1.27 cm/day) rainfall occurred at each site (Table 6). At the CTL Site, significant rainfall occurred on May 28 (3.02 ± 0.13 cm) and June 15 (1.45 ± 0.05 cm) (Table 6). At the TRT Site, significant rainfall days were also May 28 (2.90 ± 0.13 cm) and June 15 (2.01 ± 0.03 cm) (Table 6). On May 28 (2.24 ± 0.03 cm) and June 15 (1.78 ± 0.00 cm) significant rainfall was observed at the KWA Site (Table 6).

C. Measured Insecticide Concentrations in Water Samples

1. Results for the 1989 Study Period

a. **Water Samples** - Results for analysis of selected seawater samples collected during the 1989 field study are listed in Table 7 (CTL Site - grab samples); Table 8 (CTL Site - Composite Samples); Table 9 (TRT Site - grab samples), Table 10 (TRT Site - Composite Samples); Table 11 (KWA Site - grab samples); Table 12 (Tomato field discharges, KWA Site -grab samples); Table 13 (Pesticide Transport - Haulover Creek adjacent to the KWA Site) and Table 14 (spiked recovery efficiencies). Figures 3 (CTL), 4, (TRT) and 5 (KWA) depict measured insecticide levels in grab seawater samples from each site throughout the 1989 study.

Analysis of spiked water samples indicated generally good recovery efficiencies ranging from 78.0 - 112.6%, averaging 93.1% (± 6.6%) for azinphosmethyl; from 60.0 -92.2%, averaging 75.9% (± 5.1%) for endosulfan I; from 61.7 - 99.7%, averaging 80.5% (± 5.8%) for endosulfan II; from 65.3 - 99.3%, averaging 82.0% (± 5%) for endosulfan sulfate; from 51.8 -96.5%, averaging 76.9% (± 6.9%) for fenvalerate; and from 69.1 - 99.6%, averaging 84.3% (± 4.6) for methyl parathion (Table 14). Pooled spiked recovery efficiencies for all pesticides was 82.1% (± 2.5%). This compares favorably with spiked recoveries for 1986 - 88, which ranged from 77.5 - 84.0% (Scott et al, 1990).

At the CTL Site, only background levels of endosulfan (≤ 10 ng/L) were observed in most (73%) of the grab water samples analyzed, with concentrations ranging from 2 - 10 ng/L (Table 7 and Figure 3). In the 27% of samples where levels exceeded background, endosulfan concentrations ranged from 11 - 14 ng/L and were observed during ebb tides following significant (> 1.27 cm/day) rain events. The average endosulfan concentrations for the CTL Site during 1989 was 8.0 ng/L (± 0.60 ng/L). None of the measured concentrations at the CTL Site during 1989 exceeded the 96h LC50 values for any test species deployed in field toxicity tests.

Table 6. Dates of significant rainfall (> 1.27 cm/day) during 1990 field study

1990_		Rainfall Amount (cm/day)		
SITE	DATE	RANGE[A]	X	(± SE)
CTL	5/28/90	3.00 - 3.05	3.02	(0.03)
	6/15/90	1.40 - 1.52	1.45	(0.05)
TRT	5/28/90	2.67 - 3.05	2.90	(0.13)
	6/15/90	1.98 - 2.03	2.01	(0.03)
KWA	5/28/90	2.21 - 2.31	2.24	(0.03)
	6/15/90	1.78 - 1.78	1.78	(0.00)

A = Range between three rain gauges
X = Mean
SE = Standard Error

Table 7. Summary of measured insecticide concentrations (ng/L) in water samples collected at the CTL Site during the 1989 field study

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
1	51	6/5/89	1330	CTL	32	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 4.0 (± 0.0) < DL < DL
2	55	6/6/89	0015 Initial Post Rain	CTL	26	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 7.0 (± 3.4) < DL < DL
3	57	6/6/89	0202 2h Post Rain	CTL	26	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 7.0 (± 3.8) < DL < DL
4	59	6/6/89	0445 4h Post Rain Dead Low	CTL	20	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 2.0 (±0.06) < DL < DL
5	73	6/6/89	1615	CTL	6	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 11.0 (±1.3) < DL < DL
6	75	6/6/89 through 6/16/90	1842 Post Rain ⅓ Flood	CTL	5	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 8.0 (± 5.1) < DL < DL
7	84	6/7/89	0530	CTL	26	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 11.0 (± 1.5) < DL < DL

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
8	106	6/8/89 –	0910 1 Day Post Rain	CTL	NM	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 14.0 (±3.0) < DL < DL
9	121	6/9/89	0915 Initial Post Rain Dead Low	CTL	25	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 9.0 (± 4.5) < DL < DL
10	140	6/11/89	0930 2 days Post Rain	CTL	26	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 12.0 (±0.6) < DL < DL
11	144	6/12/89	0930	CTL	27	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 11.0 (±4.0) < DL < DL
12	148	6/13/89	0920	CTL	28	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 5.0 (± 0.5) < DL < DL
13	155	6/15/89	1240	CTL	28	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 8.0 (± 3.1) < DL < DL
14	168	6/16/89	0030 Initial Post Rain Dead Low	CTL	25	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 10.0 (± 1.0) < DL < DL
15	174	6/16/89	1155 11.5h Post Rain ⅔ Ebb	CTL	23	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 9.0 (± 2.2) < DL < DL

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
16	159	6/16/89	1400 13.5h Post Rain ⅓ Flood	CTL	24	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 10.0 (± 0.6) < DL < DL
17	180	6/17/89	0100 24h Post Rain Dead Low	CTL	25	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 4.0 (± 0.6) < DL < DL
18	189	6/17/89	1315 37h Post Rain Dead Low	CTL	28	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 4.0 (± 1.0) < DL < DL
19	200	6/18/89	1620 54h Post Rain ⅓ Ebb	CTL	NM	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 12.0 (± 1.0) < DL < DL
20	209	6/20/89	1000	CTL	28	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 9.0 (± 0.8) < DL < DL
21	216	6/22/89	1100	CTL	16	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 8.0 (± 4.0) < DL < DL
22	221	6/23/89	0830	CTL	28	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 10.0 (± 6.0) < DL < DL
23	230	6/24/89	1532 Post Rain	CTL	30	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 8.0 (± 1.5) < DL < DL

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
24	242	6/25/89	1532 Post Rain ¼ Flood	CTL	29	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 4.0 (± 0.5) < DL < DL
25	250	6/26/89	0915 Dead Low	CTL	20	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 4.0 (± 1.0) < DL < DL
26	260	6/27/89	1015	CTL	29	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 8.0 (± 1.0) < DL < DL

NM = Not measure
<DL = Less than lower limits of detection

Limits of detection:

Azinphosmethyl	<5ng/L
Endosulfan	<3ng/L
Fenvalerate	<2ng/L
Methyl Parathion	<1ng/L

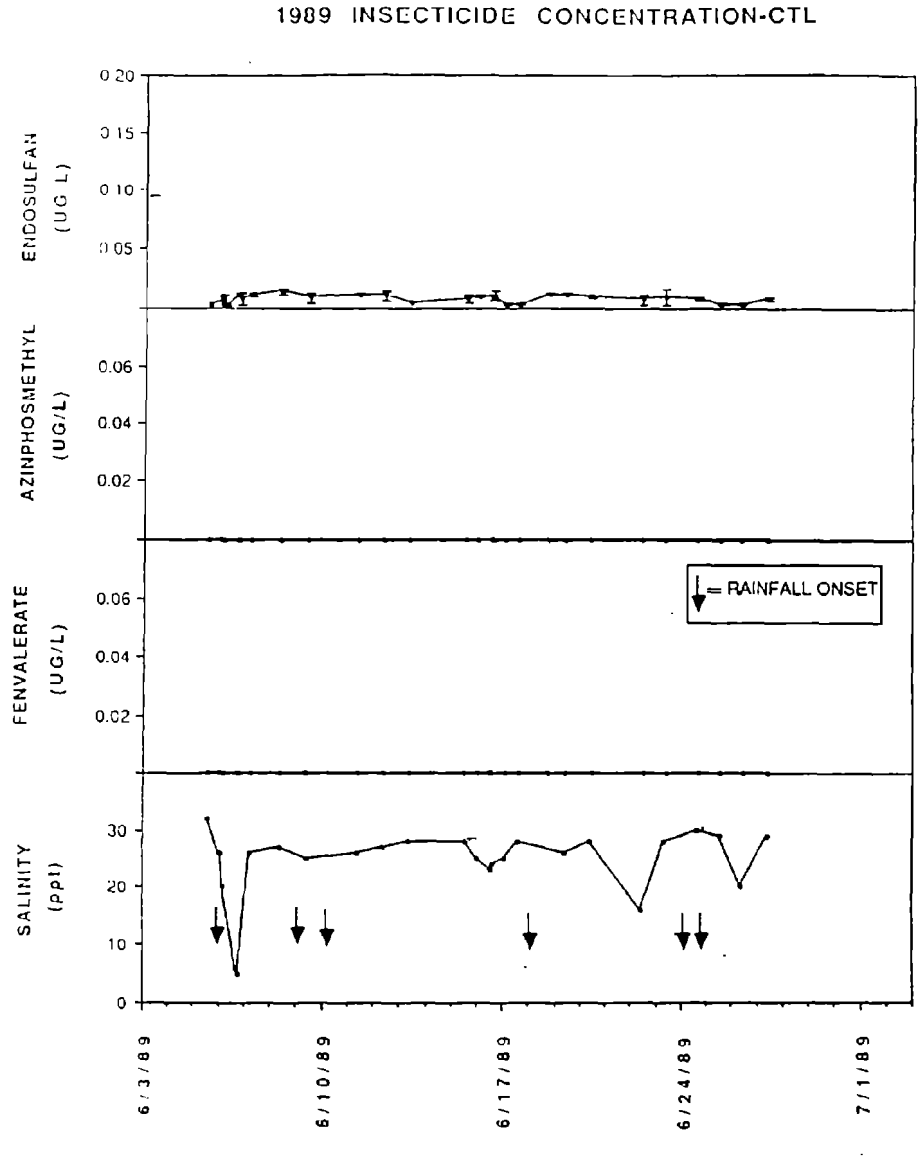


Figure 3. Measured insecticide concentrations (ug/L) and salinities (ppt) observed at the CTL Site during the 1989 field study. Values reported are maximum daily concentrations observed at the CTL Site on the dates sampled (•) at ebb tide. While concentrations are shown continuously for dates sampled, it should be noted that actual insecticide concentrations may fluctuate temporally at each site with tidal flushing. Measured insecticide levels are in ug/L, while results for tables are reported in ng/L. To convert ug/L to ng/L, multiply by 1000.

Analysis of composite water samples collected at the CTL Site during periods of significant (> 1.27 cm/day) rainfall (Table 8) indicated only detectable levels of endosulfan (Endosulfan I and Endosulfan Sulfate) with concentrations ranging from 2 - 9 ng/L, averaging 5.5 (± 1.18 ng/L). The peak/composite ratio (peak concentration in grab samples/average concentration in a composite sample) for endosulfan concentrations measured during significant rain events at the CTL Site ranged from 1.11 - 3.50, averaging 2.12 (± 0.44). These findings suggest that composite sampling at the CTL Site would underestimate peak endosulfan concentrations by a factor of 2 (i.e. peak concentrations would be double average concentrations measured in composite samples).

At the TRT Site (Tables 9-10, Figure 4), only detectable levels of endosulfan were observed during periods of fair weather. Following major rain events detectable levels of azinphosmethyl, endosulfan, and fenvalerate were observed. For endosulfan, concentrations ranged from 2 - 10 ng/L, averaging 5.2 ng/L (± 1.35 ng/L) during periods of fair weather (21% of all samples). During rain events, (79% of all samples), endosulfan concentrations ranged from 2 - 20 ng/L, averaging 8.2 ng/L (± 0.99 ng/L). Analysis of composite sampling during these same rain events indicated endosulfan concentrations ranging from 3 - 9 ng/L, averaging 5.4 ng/L (± 0.95 ng/L). Peak/composite ratios ranged from 1.25 - 2.80, averaging 2.32 (± 0.58 ng/L). For the entire 1989 study period, endosulfan concentrations ranged from 2-20 ng/L, averaging 7.54 ng/L (± 0.86 ng/L). These levels were quite comparable to the average endosulfan concentrations of 8.0 ng/L for 1989. These 1989 results also compare favorably with TRT Site runoff sampling results for 1987 (endosulfan concentrations of 2 - 59 ng/L and peak/composite ratios of 2.46) and 1988 (endosulfan concentrations of 2.2 ng/L and peak/composite ratio of 2:46) (Scott et al, 1990).

During fair weather periods azinphosmethyl was not detected at the TRT Site. Detectable levels of azinphosmethyl of 16 ng/L were observed in one sample, 4h post rain at dead low tide on June 6, 1989.

Similarly, fenvalerate was not detected at the TRT Site during periods of fair weather. Following periods of significant rainfall (> 1.27 cm/day) detectable levels of fenvalerate were observed, ranging from < DL - 93 ng/L, averaging 11.4 ng/L (± 4.91 ng/L). Analysis of composite sampling during these same rain events indicated fenvalerate concentrations ranging from < DL - 65 ng/L, averaging 15.6 ng/L. The peak/composite ratio for fenvalerate ranged from 0.95 - > 11, averaging 5.23 (± 2.44).

Table 8. Summary of measured insecticide concentratrations (ng/L) observed in composite water samples collected at the CTL Site during the 1989 field study.

1989				Sample Description	Measured Concentration (ng/L)	
#	Code #	Date - Time	Site		Insecticide	X (± SD)
1	69	6/5 - 2320 through 6/6 - 1120	CTL	Composite (F→E→F)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL < DL < DL 2.0 (± 0.5) < DL < DL
2	92	6/7 - 0000 through 6/7 - 1300	CTL	Composite (⅓F→F→E→⅓F)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL 5.0 (± 0.5) < DL 4.0 (± 0.5) < DL < DL
3	81	6/7 - 1600 through 6/7 - 2400	CTL	Composite (F→E→⅓F)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL 2.0 (± 1.0) < DL 3.0 (± 1.0) < DL < DL
4	131	6/9 - 0300 through 6/9 - 1515	CTL	Composite (E→F→E)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL 2.0 (± 0.0) < DL 2.0 (± 0.6) < DL < DL
5	175	6/16 - 0030 through 6/16 - 1400	CTL	Composite (E→F→E)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL 2.0 (± 0.6) < DL 2.0 (± 0.6) < DL < DL
6	190	6/16 - 1400 through 6/17 - 0030	CTL	Composite (E→F→E)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL 4.0 (± 2.0) < DL 5.0 (± 0.5) < DL < DL

E = Ebb Tide; F = Flood Tide; Samples composited every 20 minutes for each time period.

Limits of Detection: Azinphosmethyl < 5 ng/L; Endosulfan I < 1 ng/L; Endosulfan II < 1 ng/L; Endosulfan Sulfate < 1 ng/L; Fenvalerate < 2 ng/L; Methyl Parathion < 1 ng/L

< DL = Less than lower limits of detection

Table 9. Summary of measured insecticide concentrations (ng/L) in water samples collected at the TRT Site during the 1989 field study

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
1	52	6/5/89	1400	TRT	33	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 2.0 (± 1.5) < DL < DL
2	54	6/5/89	2350 Initial Post Rain	TRT	24	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 7.0 (± 2.8) < DL < DL
3	56	6/6/89	0137 2h Post Rain ⅔ Ebb	TRT	26	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 6.0 (± 2.6) < DL < DL
4	58	6/6/89	0415 4h Post Rain Dead Low	TRT	5	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	16.0 (± 6.0) 13.0 (± 4.1) 93.0 (± 17.0) < DL
5	60	6/6/89	0725 7.5h Post Rain ½ Flood	TRT	9	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 9.0 (± 1.9) 50.0 (± 12.0) < DL
6	66	6/6/89	1055 11h Post Rain Flood Tide	TRT	28	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 4.0 (± 1.0) < DL < DL
7	70	6/6/89	1320 Initial Post Rain ⅓ Ebb	TRT	21	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 7.0 (± 3.8) < DL < DL
8	72	6/6/89	1614 3h Post Rain ⅔ Ebb	TRT	14	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 16.0 (± 3.2) < DL < DL

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
9	74	6/6/89	1820 5h Post Rain Dead Low	TRT	5	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 20.0 (± 1.7) 40.0 (± 47.0) < DL
10	85	6/7/89	1100 21.5h Post Rain Dead Low	TRT	8	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 13.0 (± 0.5) 22.0 (± 0.0) < DL
11	122	6/9/89	0830 Initial Post Rain Dead Low	TRT	6	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 5.0 (± 0.5) 21.0 (± 2.0) < DL
12	139	6/11/89	0830 48h Post Rain	TRT	12	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 5.0 (± 0.5) < DL < DL
13	143	6/12/89	0845 72h Post Rain	TRT	18	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 6.0 (± 0.5) < DL < DL
14	147	6/13/89	0830	TRT	24	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 7.0 (± 1.0) < DL < DL
15	154	6/15/89	1130	TRT	19	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 10.0 (± 2.4) < DL < DL
16	167	6/16/89	0000 Initial Post Rain Dead Low	TRT	15	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 5.0 (± 0.5) < DL < DL
17	173	6/16/89	1145 12h Post Rain Dead Low	TRT	7	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 14.0 (± 1.4) 15.0 (± 6.0) < DL

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
18	161	6/16/89	1320 13.25h Post Rain – ⅓ Flood	TRT	7	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 6.0 (± 2.4) < DL < DL
19	181	6/17/89	0030 24.5h Post Rain Dead Low	TRT	NM	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 11.0 (± 1.5) < DL < DL
20	188	6/17/89	1625 40.5h Post Rain Dead Low	TRT	28	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 2.0 (± 0.5) < DL < DL
21	201	6/18/89	1800 66h Post Rain	TRT	28	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 11.0 (± 1.0) 10.0 (± 12.0) < DL
22	204	6/19/89	0600 77.5h Post Rain	TRT	26	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 7.0 (± 3.4) < DL < DL
23	210	6/20/89	1430	TRT	18	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 6.0 (± 1.7) < DL < DL
24	219	6/23/89	0800	TRT	18	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 5.0 (± 2.6) < DL < DL
25	222	6/24/89	0930 Initial Post Rain ⅓ Flood	TRT	28	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 5.0 (± 1.0) < DL < DL

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
26	241	6/25/89	0930 Initial Post Rain ½ Flood	TRT	22	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 6.0 (± 1.2) < DL < DL
27	249	6/26/89	0800 22.5h Post Rain	TRT	15	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 2.0 (± 1.7) < DL < DL
28	258	6/27/89	0830 47h Post Rain	TRT	17	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 1.0 (± 1.0) < DL < DL

< DL = Less than lower limits of detection

NM = Not Measured

Limits of Detection:

Azinphosmethyl	< 5 ng/L
Endosulfan	< 3 ng/L
Fenvalerate	< 2 ng/L
Methyl Parathion	< 1 ng/L

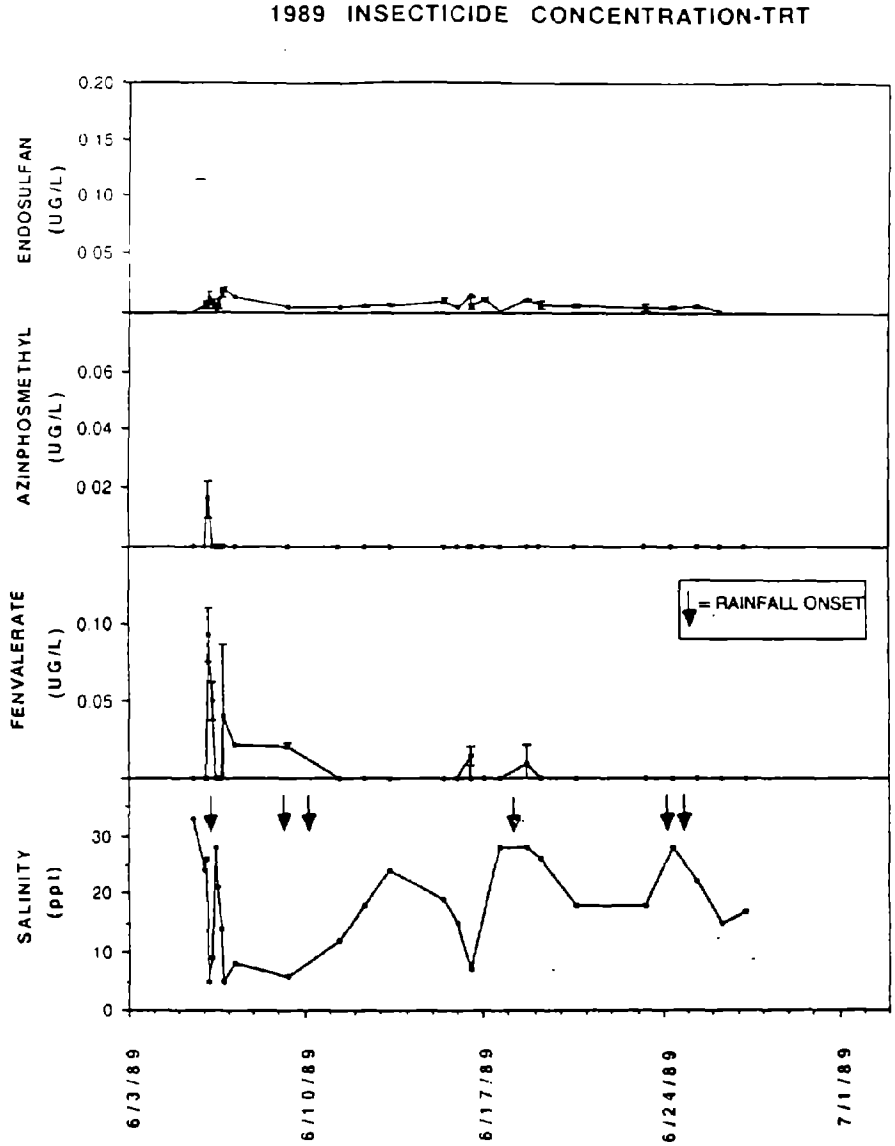


Figure 4. Measured insecticide concentrations (ug/L) and salinities observed at the TRT Site during the 1989 field study. Values reported are maximum daily concentrations observed at the TRT Site on the dates (•) sampled at ebb tide. While concentrations depicted are generally representative for the dates sampled, actual pesticide concentrations may fluctuate temporally with tidal flushing. Measured insecticide levels reported are in ug/L rather than the ng/L levels reported in tables. To convert ug/L to ng/L, multiply by 1000.

Table 10. Summary of measured insecticide concentrations (ng/L) observed in composite water samples collected at the TRT Site during the 1989 field study.

1989				Sample Description	Measured Concentration (ng/L)	
#	Code #	Date - Time	Site		Insecticide	X (± SD)
1	68	6/5 - 2320 through 6/6 - 1120	TRT	Composite (F→E→F)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL 3.0 (± 2.0) < DL 6.0 (± 2.O) 65.0 (± 3.0) < DL
2	95	6/7 - 0000 through 6/7 - 1300	TRT	Composite (F→E→F)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL 5.0 (± 2.0) < DL 4.0 (± 1.0) < DL < DL
3	83	6/7 - 1600 through 6/8 - 0030	TRT	Composite (⅔E→E→F)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL < DL < DL 4.0 (± 0.6) 22.0 (± 8.8) < DL
4	129	6/9 - 0300 through 6/9 - 1515	TRT	Composite (½E→E→F→½E)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL < DL < DL 4.0 (± 2.0) 22.0 (± 11.0) < DL
5	191	6/16 - 1230 through 6/17 - 0000	TRT	Composite (E→F→E)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL 2.0 (± 2.0) < DL 2.0 (± 1.0) < DL < DL
6	247	6/25 - 1000 through 6/25 - 1930	TRT	Composite (½F→F→E)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL < DL < DL 5.0 (± 1.7) < DL < DL
7	257	6/25 - 2000 through 6/26 - 0830	TRT	Composite (⅔E→E→F→⅓E)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL < DL < DL 3.0 (± 0.5) < DL < DL

E = Ebb Tide; F = Flood Tide; Samples composited every 20 minutes for each time period.

Limits of Detection:				
	Azinphosmethyl	< 5 ng/L	Endosulfan Sulfate	< 1 ng/L
	Endosulfan I	< 1 ng/L	Fenvalerate	< 2 ng/L
	Endosulfan II	< 1 ng/L	Methyl Parathion	< 1 ng/L

<DL = Less than lower limits of detection

These results agree favorably with 1988 results when post rain fenvalerate levels of < DL - 68 ng/L were observed at the TRT Site, with peak grab ratios of 2.06 (Scott et al, 1990). For the entire study period, fenvalerate concentrations ranged from < DL - 93 ng/L, averaging 9.0 ng/L. At the TRT Site, no measured endosulfan or azinphosmethyl concentrations exceeded the 96h LC50 values for any of the test species deployed. Measured fenvalerate concentrations during rain events exceeded the 96H LC50 values for mysid shrimp and *P. pugio* and may have exceeded the no observable effect concentration (NOEC) for penaied shrimp. Measured fenvalerate concentrations did not exceed 96h LC50 values for mummichogs and sheepshead minnow during rain events monitored during 1989.

These data suggest that during 1989, 3 days of rainfall (June 6, 1989; June 9, 1989; and June 16, 1989) occurred which resulted in significant pesticide runoff at concentrations high enough to pose acute toxicity risk to crustaceans (*M. bahia*, *P. pugio*, and *Penaeus sp.*) deployed in field toxicity tests (Figure 16). Peak/composite sample comparisons during runoff events suggest that peak (i.e. pulsed) insecticide concentrations were 2.32 - 5.23 times greater than time weighted average concentrations obtained from composite water samples.

At the KWA Site (Tables 11 - 13; Figure 5), significant runoff of endosulfan and azinphosmethyl following rain events on June 5, June 6, June 9, June 16, and June 24, 1989, resulted in elevated levels of both pesticides for most of the 1989 study period. During periods of fair weather insecticide concentrations ranged from < DL - 211 ng/L, averaging 68.7 ng/L (± 31.05 ng/L) for azinphosmethyl; from 5 - 64 ng/L, averaging 33.0 ng/L for endosulfan; and only non detectable levels of fenvalerate. At the KWA Site, measured azinphosmethyl and fenvalerate concentrations following major rainfall events exceeded the 96h LC50 values for Penaeus species, *P. pugio*, *M. bahia*, juvenile *C. variegatus* and the NOEC for *F. heteroclitus*. Similarly, concentrations of endosulfan exceeded the NOEC and Lowest Observable Effect Concentration (LOEC) for some species (*P. pugio, M. bahia,* Penaeas species, juvenile *C. variegatus* and juvenile *F. heteroclitus*). In fact, the average in stream concentration of azinphosmethyl (1,078 ng.L) for the entire study period (May 23 - June 24,1989) exceeded reported 96h LC50 value (970 - 1050 ng/L for P. pugio). Similarly the average in stream concentrations of endosulfan (48.42 ng/L) and fenvalerate (4.48 ng/L) represented 21% and 64% respectively of the 96h LC50 value for the most sensitive species (juvenile *F. heteroclitus* and zoeal *P. pugio*, respectively).

Table 11. Summary of measured insecticide concentrations (ng/L) in water samples collected at the KWA Site during the 1989 field study

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
1	50	6/4/89	1415	KWA	33	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 5.0 (±1.0) < DL < DL
2	53	6/5/89	1500 Initial Post Rain ⅔ Ebb	KWA	35	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 4.0 (± 1.0) < DL < DL
3	63	6/6/89	0345 13h Post Rain ⅔ Ebb	KWA	2	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	817.0 (± 69.0) 18.0 (± 4.5) 54.0 (± 3.8) < DL
4	64	6/6/89	0545 15h Post Rain Dead Low	KWA	4	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	480.0 (± 47.0) 16.0 (± 4.3) < DL < DL
5	65	6/6/89	0745 17h Post Rain ⅓ Flood	KWA	6	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	1,730.0 (± 137.0) 69.0 (± 5.1) < DL < DL
6	99	6/6/89	1200 21h Post Rain Flood Tide	KWA	9	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	809.0 (± 51.0) 144.0 (± 7.1) < DL < DL
7	100	6/6/89	1400 23h Post Rain ⅓ Ebb	KWA	9.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	306.0 (±28.0) 50.0 (± 4.8) < DL < DL

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
8	101	6/6/89	1630 26.5h Post Rain ⅔ Ebb	KWA	NM	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	508.0 (± 44.0) 122.0 (± 8.1) < DL < DL
9	102	6/6/89	1830 28.5 Post Rain Dead Low	KWA	NM	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	350.0 (± 41.0) 46.0 (± 5.4) 39.0 (± 5.0) < DL
10	103	6/7/89	0400 Initial Post Rain ⅔ Ebb	KWA	2	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	1,078.0 (± 87.0) 71.0 (± 5.6) < DL < DL
11	104	6/7/89	0600 2h Post Rain Dead Low	KWA	0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	151.0 (± 26.0) 96.0 (± 14.0) 64.0 (± 7.0) < DL
12	105	6/7/89	0800 4h Post Rain ⅓ Flood	KWA	3	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	1,222.0 (± 83.0) 163.0 (± 11.9) < DL < DL
13	88	6/7/89	1100 7h Post Rain ⅔ Flood	KWA	2	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	28.0 (± 13.0) 50.0 (± 1.0) < DL < DL
14	115	6/7/89	1200 8h Post Rain Flood Tide	KWA	2	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	1,155.0 (± 192.0) 122.0 (± 12.6) < DL < DL

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
15	109	6/8/89	1030 30.5h Post Rain ½ Flood	KWA	2	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	382.0 (± 55.0) 64.0 (± 5.8) < DL < DL
16	124	6/9/89	0800 Initial Post Rain ⅓ Flood	KWA	2	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	76.0 (± 4.0) 41.0 (± 4.5) < DL < DL
17	135	6/9/89	1245 4h Post Rain High Tide	KWA	NM	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	50.0 (± 10.0) 43.0 (± 1.7) < DL < DL
18	134	6/10/89	0800 24h Post Rain Dead Low	KWA	2	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	31.0 (± 4.0) 43.0 (± 5.4) < DL < DL
19	141	6/11/89	1130	KWA	4	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	368.0 (± 90.0) 54.0 (± 6.2) < DL < DL
20	145	6/12/89	0800	KWA	4	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	211.0 (± 74.0) 50.0 (± 8.7) 31.0 (± 36.0) < DL
21	149	6/13/89	1030	KWA	6	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	53.0 (± 62.0) 64.0 (± 8.5) < DL < DL

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
22	152	6/14/89	0830	KWA	4	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	89.0 (± 13.0) 32.0 (± 4.1) < DL < DL
23	153	6/15/89	0900	KWA	10	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	40.0 (± 6.0) 32.0 (± 3.3) < DL < DL
24	169	6/16/89	0130 Initial Post Rain Dead Low	KWA	6	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	168.0 (± 19.0) 28.0 (± 4.0) < DL < DL
25	160	6/16/89	1020 9h Post Rain ⅓ Ebb Fish Kill Observed	KWA	10	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	2457.0 (± 256.0) 25.0 (± 1.2) < DL < DL
26	172	6/16/89	1250 11.5h Post Rain ¾ Ebb	KWA	3	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	1100.0 (± 158.0) 30.0 (± 1.3) < DL < DL
27	179	6/16/89	1515 14h Post Rain ½ Flood	KWA	4	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	2222.0 (± 520.0) 38.0 (± 5.1) < DL < DL
28	178	6/16/89	1800 17h Post Rain Flood Tide	KWA	6	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	1528.0 (± 366.0) 24.0 (± 2.1) < DL < DL

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
29	182	6/17/89	0100 23h Post Rain Dead Low	KWA	7	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	578.0 (± 122.0) 27.0 (± 2.4) < DL < DL
30	186	6/17/89	1100 33.5h Post Rain ⅓ Ebb	KWA	3	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	716.0 (± 49.0) 27.0 (± 2.4) < DL < DL
31	192	6/18/89	0645 53.25h Post Rain ⅓ Flood	KWA	5	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	426.0 (± 53.0) 23.0 (± 1.5) < DL < DL
32	202	6/18/89	1830 55h Post Rain Dead Low	KWA	NM	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	1710.0 (± 766.0) 32.0 (± 3.3) < DL < DL
33	205	6/19/89	1100 76.5h Post Rain ⅓ Flood	KWA	6	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	1351.0 (± 267.0) 27.0 (± 30.) < DL < DL
34	211	6/20/89	0930 99h Post Rain Dead Low	KWA	17	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	214.0 (± 26.0) 12.0 (± 2.2) < DL < DL
35	220	6/23/89	1000	KWA	10	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	19.0 (± 4.0) 15.0 (± 1.0) < DL < DL

1989					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
36	224	6/24/89	1040 Initial Post Rain ⅓ Flood Fish Kill Observed	KWA	4 Fish Kill	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	3111.0 (± 377.0) 64.0 (± 6.6) < DL < DL
37	237	6/24/89	1930 9h Post Rain Dead Low	KWA	2	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	1612.0 (± 150.0) 34.0 (± 1.9) < DL < DL
38	239	6/24/89	2200 11.5h Post Rain ½ Flood	KWA	NM	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	2383.0 (± 352.0) 42.0 (± 2.4) < DL < DL
39	243	6/25/89	1100 Initial Post Rain ⅔ Flood	KWA	6	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	5288.0 (± 742.0) 65.0 (± 9.6) < DL < DL
40	248	6/25/89	2011 9h Post Rain ⅓ Ebb	KWA	3	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	1956.0 (± 439.0) 50.0 (± 2.2) < DL < DL
41	251	6/29/89	2011 24H Post Rain Dead Low	KWA	2	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	1604.0 (± 353.0) 38.0 (± 2.7) < DL < DL
42	261	6/27/89	1115 48h Post Rain	KWA	2	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	7002.0 (± 759.0) 32.0 (± 2.4) < DL < DL

NM = Not Measured

< DL = Less than lower limits of detection

Limits of detection: Azinphosmethyl < 5 ng/L
Endosulfan < 2 ng/L
Fenvalerate < 3 ng/L
Methyl Parthion < 1 ng/L

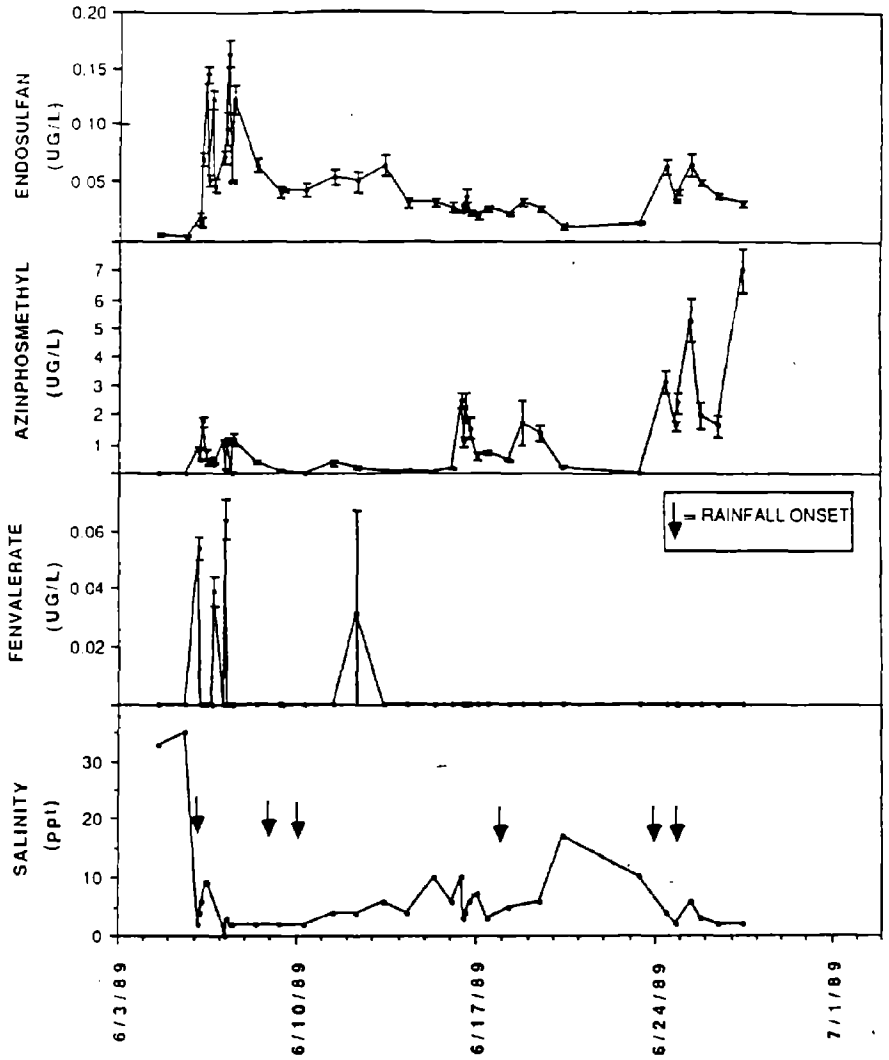


Figure 5. Measured insecticide concentrations (ug/L) and salinities (ppt) observed at the KWA Site during the 1989 field study. Values depicted are maximum daily concentrations at the KWA Site on the dates (●) sampled at ebb tide. While concentrations depicted are generally representative for the dates sampled, actual pesticide levels may fluctuate temporally with tidal flushing. Measured insecticide concentrations depicted are in ug/L rather than the ng/L levels reported in tables. To convert ug/L to ng/L, multiply by 1000.

At the KWA Siteduring rain events, azinphosmethyl concentrations ranged from < DL - 7,002 ng/L, averaging 1,246.31 ng/L (± 243.79 ng/L). Endosulfan concentrations ranged from 4 - 163 ng/L, averaging 50.92 ng/L (± 5.95 ng/L). Fenvalerate concentrations ranged from < DL - 64 ng/L, averaging 5.2 ng/L (± 2.60 ng/L).

At the KWA Site during the entire 1989 study period, azinphosmethyl concentrations ranged from < DL - 7002 ng/L, averaging 1078.07 ng/L (± 218.28 ng/L). Endosulfan concentrations ranged from 4 - 163 ng/L, averaging 48.42 ng/L (± 5.33 ng/L). Fenvalerate concentrations ranged from < DL - 64 ng/L, averaging 4.48 ng/L (± 2.24 ng/L).

b. Pesticide Loadings

Table 12 lists results of water samples collected from drainage ditches from tomato fields at the KWA Site approximately 20m upstream of the KWA Site. These samples were taken to address pesticide loading, potential at the KWA Site. During the rain event of June 16, 1989, 1.47 cm of rain at the KWA Site caused significant azinphosmethyl runoff with concentrations of 15, 497 ng/L (± 1796 ng/L) and slight runoff of endosulfan (concentrations of 119 ng/L). In stream water samples 20m downstream from this tomato field site contain much lower levels of azinphosmethyl (2457 ng/L) and endosulfan. The field/stream ratio (insecticide concentration measured exiting the tomato field/tidal stream concentration) was 6.31 for azinphosmethyl and 4.76 for endosulfan. A fish kill was observed at the KWA Site during this runoff event.

On June 18, 1989, 44h post rain significant azinphosmethyl (7,567 ng/L) and endosulfan (117 ng/L) concentrations were still observed in ditches exiting the tomato field. In stream concentrations of azinphosmethyl (426 ng/L) and endosulfan (23 ng/L) at the KWA Site 20m downstream were much lower than levels observed in the tomato field ditch. The field stream ratio was 17.76 for azinphosmethyl and 5.09 for endosulfan.

On June 24, 1989, 4.57 cm of rainfall occurred at the KWA Site resulting in significant runoff of azinphosmethyl (1574 ng/L) and endosulfan (100 ng/L) from tomato fields adjacent to the site which caused a fish kill (Plates 4-7). At the KWA Site, in stream concentrations of azinphosmethyl (3, 111 ng/L) were nearly double concentrations measured in the ditches exiting the tomato field. This suggests that the majority of the azinphosmethyl runoff had exited the field and entered the tidal creek. For endosulfan,

Table 12. Summary of measured insecticide concentratrations (ng/L) observed in composite water samples collected from a tomato field drainage ditch as it enters an estuarine tidal creek at the KWA Site during the 1989 field study.

1989				Sample Description	Measured Concentration (ng/L)	
#	Code #	Date - Time	Site		Insecticide	X (± SD)
1	171	6/16/89 - 1100	KWA	Tomato field drainage ditch as it enters tidal creek	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	15497.0 (± 1796.0) 3.0 (± 0.5) 24.0 (± 3.0) 92.0 (± 12.0) < DL < DL
2	193	6/18/89 - 0645	KWA	Tomato field drainage ditch as it enters tidal creek	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	7567.0 (± 802.0) < DL 24.0 (± 3.0) 93.0 (± 39.0) < DL < DL
3	223	6/24/89 - 1100	KWA	Tomato field drainage ditch as it enters tidal creek	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	1574.0 (± 247.0) < DL 19.0 (± 1.0) 81.0 (± 6.0) < DL < DL

Limits of Detection:

Azinphosmethyl	< 5 ng/L	Endosulfan Sulfate	< 1 ng/L
Endosulfan I	< 1 ng/L	Fenvalerate	< 2 ng/L
Endosulfan II	< 1 ng/L	Methyl Parathion	< 1 ng/L

< DL = Less than lower limits of detection

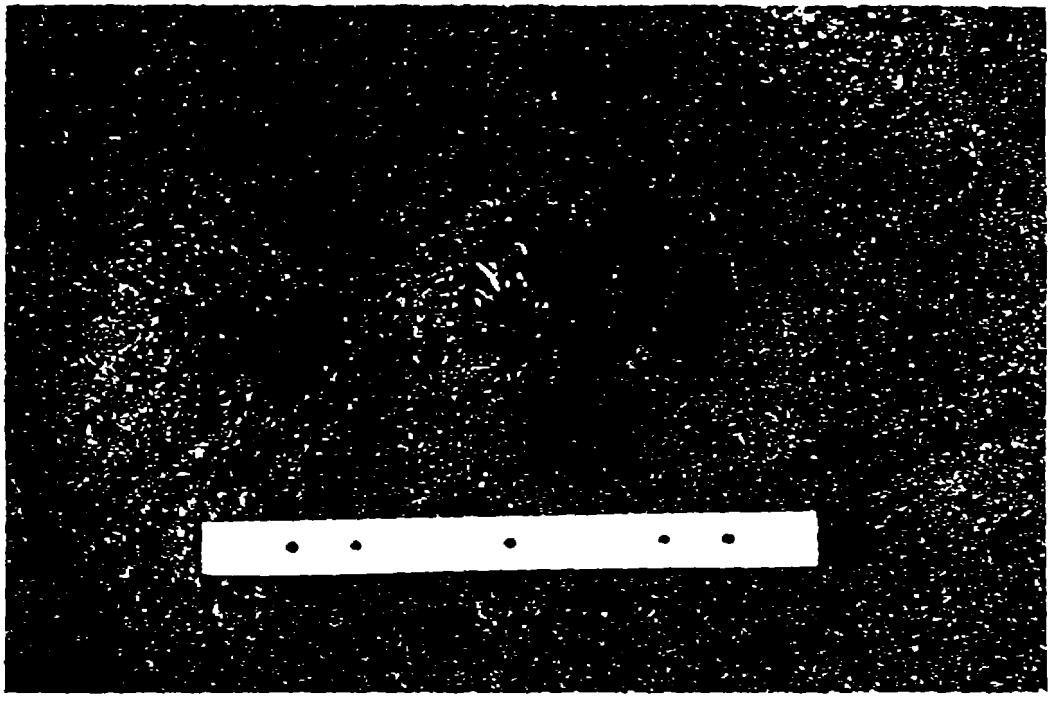


Plate 4 Photograph of dead *F. heteroclitus* at the KWA Site following significant rainfall and resulting fish kill. Note how all dead mummichogs were juvenile - young adult size classes.

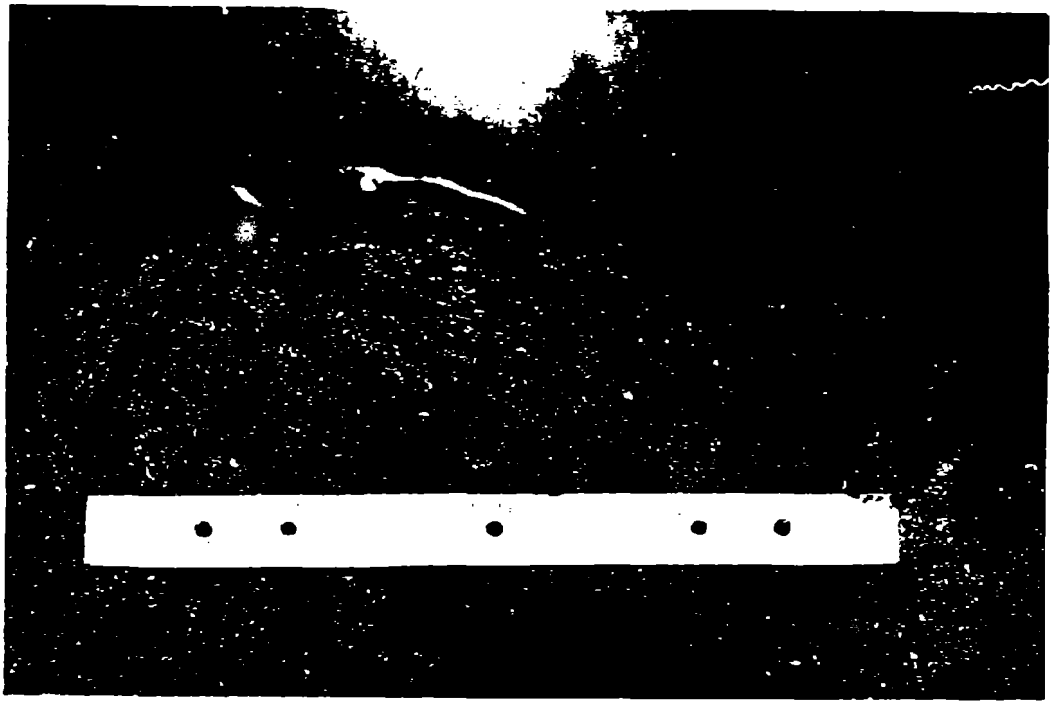


Plate 5 Photograph of dead *P. pugio* at the KWA Site following significant rainfall and resulting fish kill. Dead grass shrimp were found along the bank of the tidal creek throughout this area.

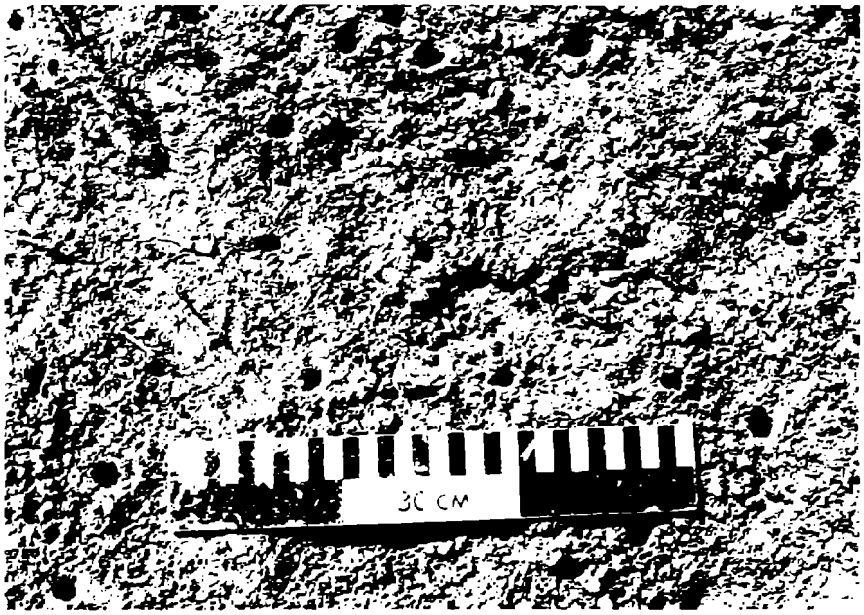


Plate 6A. Photograph of dead *Uca pugilator* at the KWA Site following significant rainfall and resulting fish kill. There was significant mortality in fiddler crabs at this site.

Plate 6B. Photograph of dead polychaetes at the KWA Site following significant rainfall and resulting fish kill. This was the first time that dead polychaetes were observed at a fish kill during the 6 years of these studies.

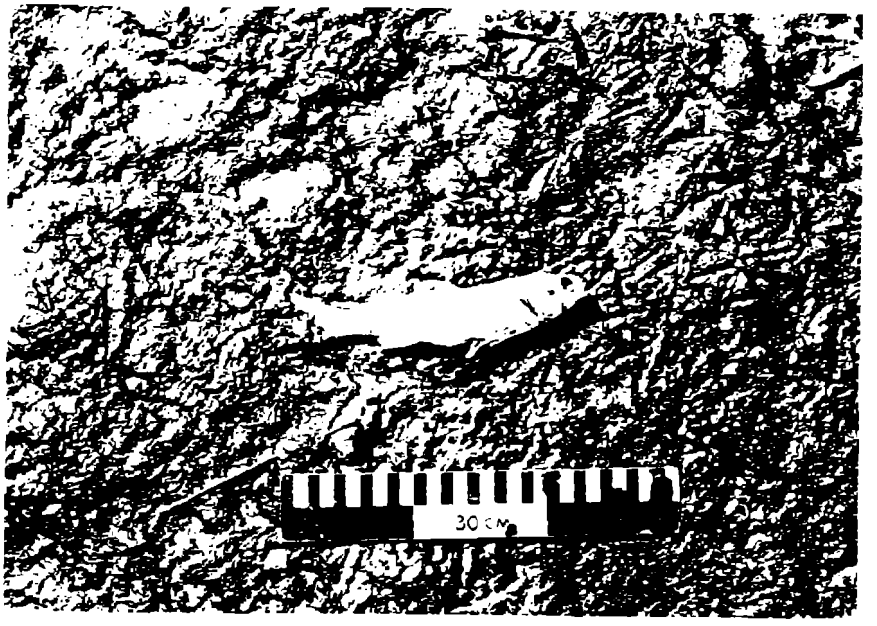


Plate 7A. Photograph of dead *Mugil cephalus* at the KWA Site following significant rainfall and resulting fish kill.

Plate 7B. Photograph of shorebirds (gulls, wading shorebirds and egrets) consuming dead fish, crustaceans and invertebrates at the KWA Site during the fish kill.

a less water soluble, organochlorine insecticide, the reverse was seen. Endosulfan concentrations in the tomato field runoff (100 ng/L) were higher than in stream endosulfan concentrations (64 ng/L) suggesting that less water soluble insecticides may runoff from the field at slightly different rates than more water soluble pesticides such as azinphosmethyl. Azinphosmethyl is nearly 100 times more soluble in water than endosulfan (29 ng/L at 25°C versus 0.32 - 0.33 ng/L at 25°C in distilled water), which explains its greater mobility and transport. The field/stream ratio for this rain event was 0.51 for azinphosmethyl, resulting from the rapid runoff from the field, and 1.56 for endosulfan.

c. Pesticide Transport Studies

To further evaluate pesticide transport, samples were analyzed from Haulover Creek, a small tidal creek 2 river miles (4.5 km) northwest of the KWA Site during periods of significant rainfall (Table 13). On June 24, 1989, 4.57 cm of rain fell at the KWA Site resulting in significant runoff of azinphosmethyl (3111 ng/L) and endosulfan (64 ng/L) at the KWA Site. A resulting fish kill occurred at the KWA Site at 1040 on June 24, 1989. At Haulover Creek at 1440 on June 24, 1989 only slight concentrations of azinphosmethyl (116 ng/L) and endosulfan (4 ng/L) were observed. Concentrations of endosulfan and azinphosmethyl were 16 and 26.8 time respectively higher at the KWA Site. By 1726 on June 24, 1989, azinphosmethyl concentration at the Haulover Creek Site had increased to 1631 ng/L and were identical to concentrations at the KWA Site (1612 ng/L). Similarly endosulfan concentrations increased to 18 ng/L at the Haulover Site. A fish kill then began to occur at the Haulover Site. By 1945, azinphosmethyl concentrations (1700 ng/L) were still similar to levels measured at the KWA Site. Similarly endosulfan concentrations at the Haulover Site (23 ng/L) were nearly identical to concentrations at the KWA Site (34 ng/L).

These results clearly indicate the rapid transport and mobility of azinphosmethyl and endosulfan in agricultural runoff. In a 12 - 14h time period, significant levels of these insecticides were discharged from a tomato field into a small tidal creek causing a fish kill. On the initial ebb tide these insecticides wee discharged out of this small tidal tributary into a larger portion of Haulover Creek. As the flood tide occurred, this runoff was transported > 2.0 km upstream in Haulover Creek, causing a second fish kill. These findings clearly indicate that in small drainage basins with very slow flushing rates, runoff of insecticides may cause significant spatial impacts which are not restricted to the stream site adjacent to the point of discharge into the stream.

Table 13. Summary of measured insecticide concentrations (ng/L) at fish kills at Haulover Creek, adjacent to the KWA Site and at a tidal canal adjacent to the golf links at Seabrook Island.

1989				Sample Description	Measured Concentration (ng/L)	
#	Code #	Date - Time	Site		Insecticide	X (± SD)
1	142	6/11/89 1330	Seabrook Island Golf Course	Fish Kill at tidal canal adjacent to golf course	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	< DL < DL < DL < DL < DL < DL
2	233	6/24/89 1440	Haulover Creek	Fish Kill in creek adjacent to KWA Site (Flood Tide)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	116.0 (± 27.0) < DL < DL 4.0 (± 1.0) < DL < DL
3	235	6/24/89 1726	Haulover Creek	Fish Kill in creek adjacent to KWA Site (½ Ebb)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	1631.0 (± 290.0) 2.0 (± 1.0) 4.0 (± 1.0) 10.0 (± 1.0) < DL < DL
4	238	6/24/89 1945	Haulover Creek	Fish Kill in creek adjacent to KWA Site (Dead Low)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	1700.0 (± 792.0) 2.0 (± 0.5) 4.0 (± 0.6) 17.0 (± 2.0) < DL < DL
5	240	6/24/89 2210	Haulover Creek	Fish Kill in creek adjacent to KWA Site (½ Flood)	Azinphosmethyl Endosulfan I Endosulfan II Endosulfan Sulfate Fenvalerate Methyl Parathion	2670.0 (± 262.0) < DL 4.0 (± 0.6) 12.0 (± 1.0) < DL < DL

Limits of Detection:

Azinphosmethyl	< 5 ng/L	Endosulfan Sulfate	< 1 ng/L
Endosulfan I	< 1 ng/L	Fenvalerate	< 2 ng/L
Endosulfan II	< 1 ng/L	Methyl Parathion	< 1 ng/L

< DL = Less than lower limits of detection

Table 14. Spiked recovery efficiencies (% recovery) for water samples during the 1989 field study

Pesticide	Spike Concentration (ug)	Percent Recovery (%)		
		X	SE	Range
Azinphosmethyl	0.944	93.1	6.6	78.0 - 112.6%
Endosulfan I	0.295	75.9	5.1	60.0 - 92.2%
Endosulfan II	0.322	80.5	5.8	61.7 - 99.7%
Endosulfan Sulfate	0.311	82.0	5.0	65.3 - 99.3%
Fenvalerate	0.224	76.9	6.9	51.8 - 96.5%
Methyl Parathion	0.250	84.3	4.6	69.1 - 99.6%
Pooled All Insecticides		82.1	2.5	51.8 - 112.6

2. Results for the 1990 Field Study

Results of analysis of selected seawater samples collected during the 1990 field study are listed in Tables 15 (CTL Site - Grab and Composite samples), Table 16 (TRT Site - grab and composite samples), Table 17 (KWA Site - grab and composite samples) and Table 18 (Spiked recovery efficiencies). Figure 6 (CTL Site), Figure 7 (TRT Site) and Figure 8 (KWA Site) depict measured insecticide levels in grab samples from each site during the 1990 study. Analysis of spiked water samples indicated generally good recovery efficiencies ranging from 55.0 -80.0%, averaging 69.2% (± 4.5%) for azinphosmethyl, from 62.0 - 81.0%, averaging 70.8% (± 3.6%) for endosulfan I. From 68.0 - 85.0%, averaging 78.2% (± 3.3%) for endosulfan II, from 79.0 - 102.0%, averaging 90.0% (± 5.3%) for endosulfan sulfate, from 66.0 - 94.0%, averaging 83.4% (± 4.7%) for fenvalerate, and 71.0 - 92.0%, averaging 82.2% (± 3.8%) for methyl parathion (Table 18). Pooled spiked recovery efficiencies for all pesticides was 79.0% (± 3.2%). This compares favorably with spiked recovery efficiencies for 1989 (82.1%) and for results from 1986-88, which ranged from 77.5 - 84.0% (Scott et al, 1990).

At the CTL Site, only background levels of endosulfan (≤10 ng/L) were observed in water samples analyzed during the 1990 field study (Table 15 and Figure 6). Endosulfan was the only pesticide detected, with concentrations ranging from < DL - 9 ng/L, averaging 2.3 ng/L (± 1.20 ng). Detectable endosulfan concentrations were observed in only 33% of the samples analyzed. The water samples analyzed were those associated with the two major rain events (May 28, 1990 and June 15, 1990) during the 1990 studies. These findings clearly indicate that at the CTL Site, pesticides were below concentrations which cause toxicity in acute exposures to those species deployed in field toxicity tests.

At the TRT Site (Table 16 and Figure 7), detectable concentrations of endosulfan and fenvalerate were observed. Endosulfan concentrations ranged from <DL to 14 ng/L, averaging 3.8 ng/L (± 2.23 ng/L). Detectable levels of endosulfan were found in 50% of all samples analyzed and only 17% of the samples exceeded background levels of < 10 ng/L. Fenvalerate concentrations ranged from <DL - 123 ng/L, averaging 20.5 ng/L (± 20.50 ng/L). Detectable levels of fenvalerate were found in only 17% of the samples, primarily those grab samples taken during the rain event of May 20, 1990. During this rain event, potentially toxic levels of fenvalerate (123 ng/L ± 5.1 ng/L) were detected only in one grab sample at dead low tide. The composite sample collected during the same time period (initial post rain - 13h post rain) contained <DL of fenvalerate. Earlier studies at the TRT Site have

Table 15. Summary of measured insecticide concentrations (ng/L) observed in water samples from the CTL Site during 1990.

Values are for grab samples unless otherwise denoted (composite samples).

1990					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
1	302	5/24/90	1445	CTL	29.7	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
2	344	5/28/90 through 5/29/90	2200-1030 Initial 12.5h Post Rain	CTL	Composite F→ E →F	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
3	342	5/30/90	0900	CTL	24.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
4	382	6/7/90	1333	CTL	31.5	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 6.0 (±0.60) < DL < DL
5	413	6/15/90	0808	CTL	31.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 6.0 (±0.05) < DL < DL
6	428	6/16/90 through 6/16/90	0015-1300 (7-20h) Post Rain	CTL	Composite ⅔F→F→E→F	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
7	424	6/16/90	1100 18h Post Rain ⅓ Flood	CTL	31.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 9.0 (±1.5) < DL < DL

1990					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
8	431	6/17/90	1030 41.5h Post Rain Dead Low	CTL	31.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
9	456	6/22/90	1400	CTL	34.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL

E = Ebb
F = Flood Tide

Lower Limits of Detection:

Azinphosmethyl	< 5ng/L
Endosulfan	< 3ng/L
Fenvalerate	< 2ng/L
Methyl Parathion	< 1ng/L

<DL = Less than lower limit of detection

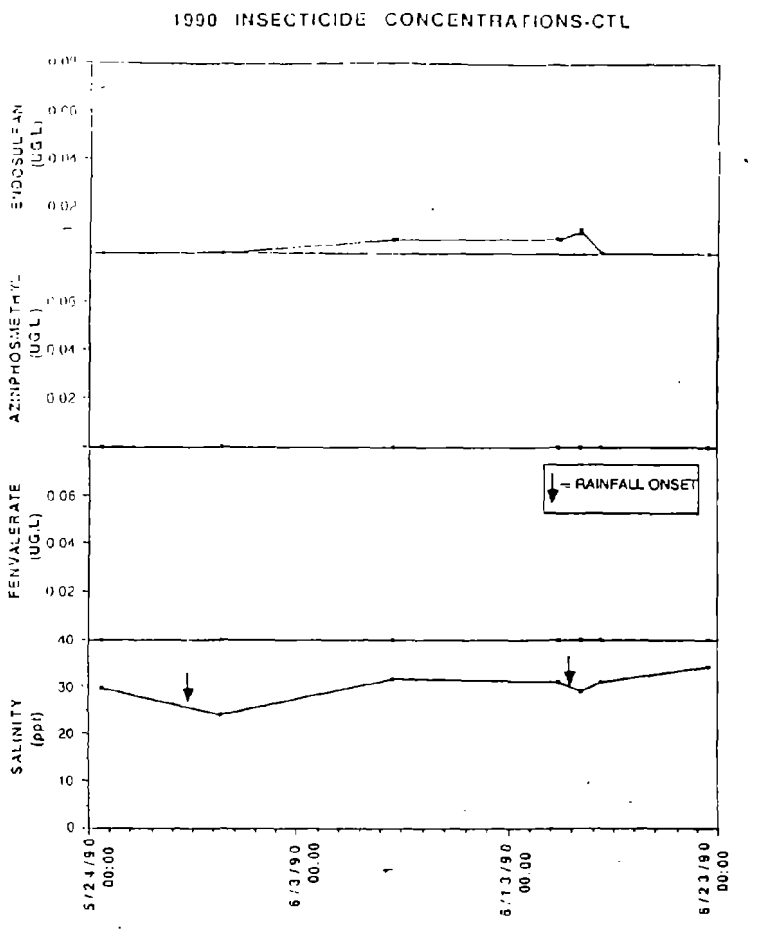


Figure 6. Measured insecticide concentrations (ug/L) and salinities (ppt) observed at the CTL Site during the 1990 field study. Values depicted are maximum daily concentrations at the CTL Site on the dates (•) sampled at ebb tide. While concentrations depicted are generally representative for the dates sampled, actual pesticide levels may fluctuate temporally with tidal flushing. Measured insecticide concentrations depicted are in ug/L rather than ng/L levels reported in tables. To convert ug/L to ng/L, multiply by 1000.

Table 16. Summary of measured insecticide concentrations (ng/L) observed in water samples form the TRT Site during the 1990 field study.

Values are for grab samples unless otherwise denoted (i.e. composite).

1990					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
1	332	5/29/90	0620 8h Post Rain Dead Low	TRT	6.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 14.0 (± 1.7) 123.0 (±5.1) < DL
2	343	5/28/90 through 5/29/90	2200-1100 Initial 13h Post Rain	TRT	Composite F→E→F	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
3	419	6/16/90	0044 8h Post Rain ⅔ Flood	TRT	30.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 4.0 (± 0.8) < DL < DL
4	425	6/16/90	0945 17h Post Rain Dead Low	TRT	31.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
5	429	6/16/90 through 6/16/90	0045-1400 8 - 21h Post Rain	TRT	Composite ⅔F→F→E→F	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL 5.0 (±3.7) < DL < DL
6	431	6/17/90	0900 40h Post Rain ⅓ Flood	TRT	26.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL

E = Ebb Tide; F=Flood Tide;

Lower Limits of Detection:

Azinphosmethyl	< 5 ng/L
Endosulfan	< 3 ng/L
Fenvalerate	< 2 ng/L
Methyl Parathion	< 1 ng/L

< DL = Less than lower limits of detection

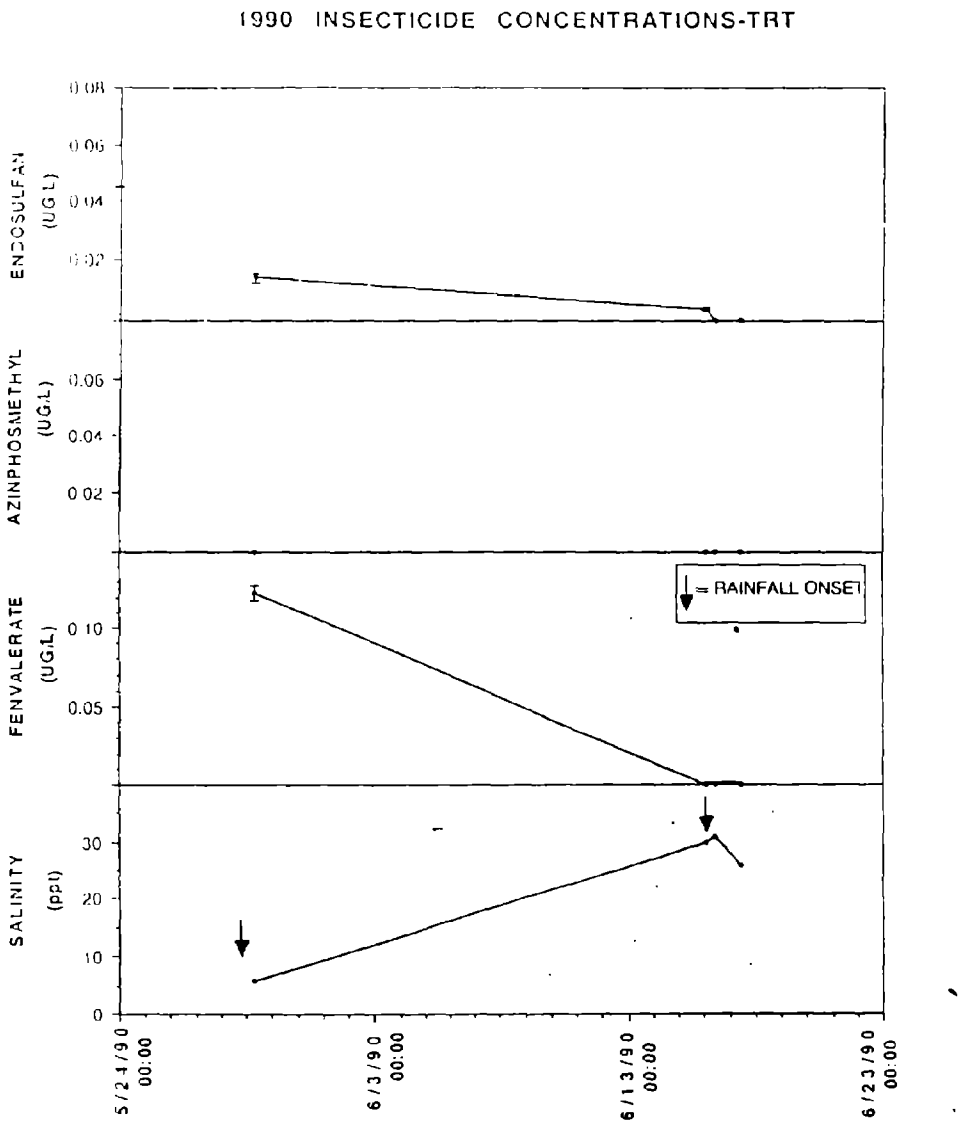


Figure 7. Measured insecticide concentrations (ug/L) and salinities (ppt) observed at the TRT Site during the 1990 field study. Values depicted are representative for the dates sampled (•). Actual pesticide levels may fluctuate temporally with tidal flushing. Measured insecticide concentrations depicted are in ug/L rather than ng/L levels reported in tables. To convert ug/L to ng/L, multiply by 1000.

noted peak/composite ratios ranging as high as 10.91 (Scott et al, 1990) - > 11.01 (this study). Using those extrapolations, one would have estimated fenvalerate concentrations of approximately 11 ng/L, just above DL. Non detectable levels of fenvalerate were noted in composite samples suggesting that only a small "slug" of fenvalerate was discharged into the environment during this rain event. During the rain event of June 15, 1990, only background levels of endosulfan were observed.

During 1990, at the TRT Site, no measured endosulfan concentrations exceeded the 96h LC50 values for any of the species deployed. Measured fenvalerate concentrations during the rain event of May 28, 1990, exceeded the 96h LC50 values for several crustacean species (M. bahia and *P. pugio*) and the LOEC for Penaeus species. Measured fenvalerate concentrations did not exceed 96h LC50 values for mummichogs, sheepshead minnow and silversides.

At the KWA Site (Table 17 and Figure 8), only detectable levels of azinphosmethyl were observed. Azinphosmethyl concentrations ranged from < DL - 62 ng/L, averaging 13.4 ng/L (± 6.51 ng/L). Detectable levels of azinphosmethyl were noted in 40% of the samples, mainly in those samples associated with June 15, 1990 rain event. During this rain event, azinphosmethyl concentrations in grab samples ranged from < DL - 62 ng/L, averaging 22.20 ng/L (± 11.39 ng/L). Analysis of composite samples indicated an azinphosmethyl concentrations of 24 ng/L (± 3.7 ng/L). The peak/composite ratio was 1.13. These results for the KWA Site, suggest that during 1990 only detectable levels of azinphosmethyl were observed which were below levels considered acutely toxic to any of the species deployed in field toxicity studies.

The 1990 study period (May - June) was an extremely dry period compared with results for 1989. Generally, dry weather results in decreased numbers of crop pests which reduces the amounts and types of insecticides applied. The 1990 study provided a period of stark contrast to the 1989 study characterized by:

1) Relatively low rainfall
2) Relatively little, if any significant insecticide runoff; and
3) Generally very high survival in species deployed in acute toxicity tests at each site.

Table 17. Summary of measured insecticide concentrations (ng/L) observed in water samples from the KWA Site during the 1990 field study. Values are for grab samples unless otherwise denoted (composite samples).

1990					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	$\bar{X}$ (±SD)
1	321	5/28/90	1830 Dead Low	KWA	27.6	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
2	324	5/28/89	2000 Initial Post Rain ⅓ Flood	KWA	12.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
3	331	5/29/90	0600 10h Post Rain Dead Low	KWA	30.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
4	339	5/28/90 through 5/29/90	2000-0820 Initial 12h Post Rain	KWA	Composite ⅓F→F→E→⅓F	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
5	417	6/15/90	2010 Initial Post Rain Dead Low	KWA	34.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	62.0 (±9.2) < DL < DL < DL
6	420	6/16/90	0135 5.5h Post Rain ⅔ Flood	KWA	NM	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL

1990					Salinity	Measured Concentration (ng/L)	
#	Code #	Date	Time	Site	(ppt)	Insecticide	X (±SD)
7	423	6/16/90	0615 10h Post Rain ⅔ Ebb	KWA	31.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	22.0 (±1.3) < DL < DL < DL
8	426	6/16/90	0845 13h Post Rain Dead Low	KWA	28.0	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	< DL < DL < DL < DL
9	427	6/16/90	1215 16h Post Rain ⅔ Flood	KWA	27.5	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	27.0 (±2.0) < DL < DL < DL
10	430	6/16/90 through 6/16/90	0135 through 1235 5.5h - 16h Post Rain	KWA	Composite ⅔F→F→E→⅔F	Azinphosmethyl Endosulfan Fenvalerate Methyl Parathion	24.0 (±3.7) < DL < DL < DL

NM = Not Measured
E = Ebb
F = Flood Tide

Lower Limits of Detection:

Azinphosmethyl	< 5ng/L
Endosulfan	< 3ng/L
Fenvalerate	< 2ng/L
Methyl Parathion	< 1ng/L

< DL = Less than lower limit of detection

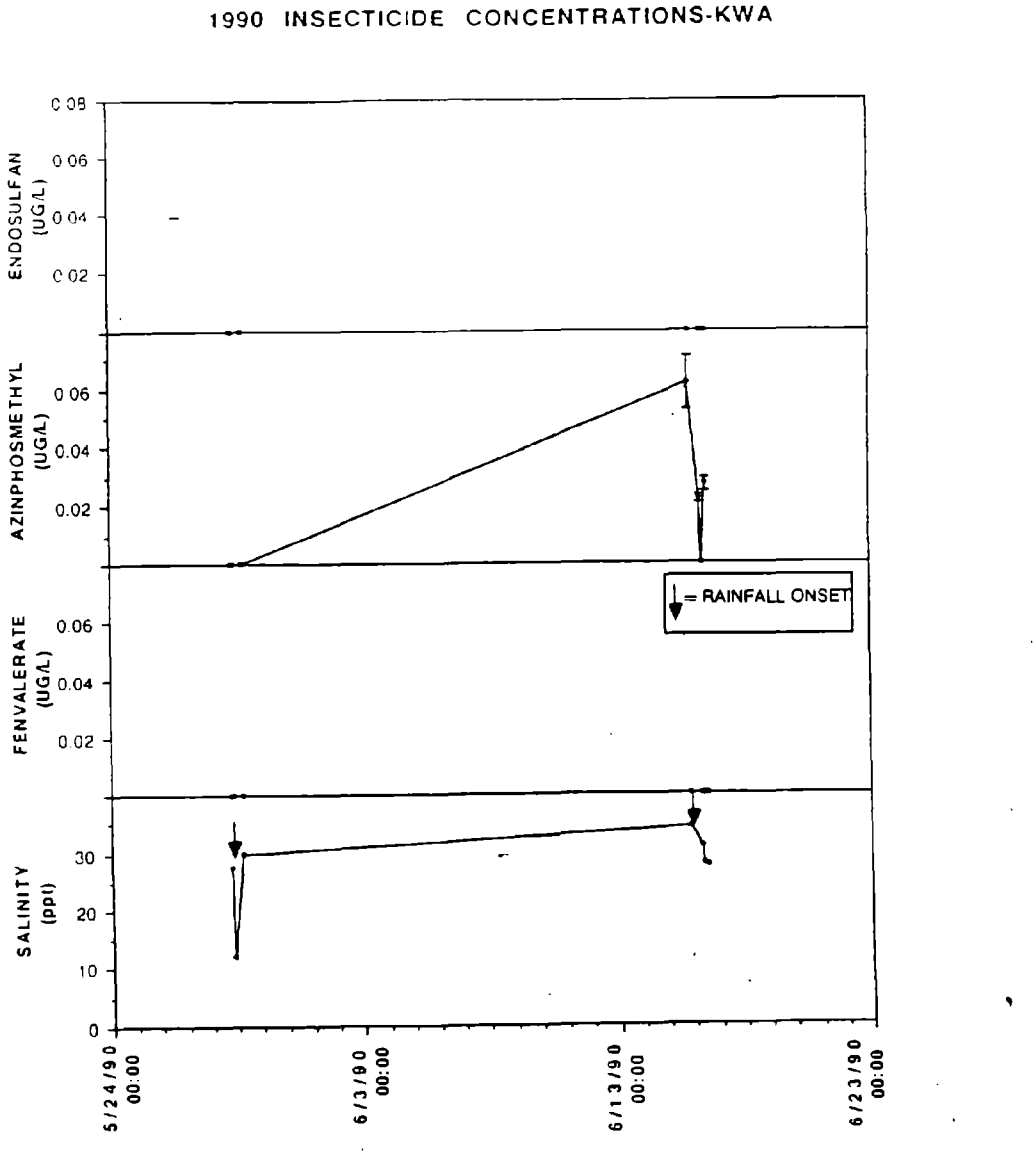


Figure 8. Measured insecticide concentrations (ug/L) and salinities (ppt) observed at the KWA Site during the 1990 field study. Values depicted are representative of maximum daily concentrations at the KWA Site on the dates (•) sampled at ebb tide. While concentrations depicted are generally representative for the dates sampled, actual pesticide levels may fluctuate temporally with tidal flushing. Measured insecticide concentrations depicted are in ug/L rather than the ng/L levels reported in tables and the text. To convert ug/L to ng/L, multiply by 1000.

Table 18. Spiked recovery efficiencies (% recovery) for water samples during the 1990 field study.

Pesticide	Spike Concentration (ug)	Percent Recovery (%)		
		X	SE	Range
Azinphosmethyl	1.260	69.2	4.5	55.0 - 80.0%
Endosulfan I	0.321	70.8	3.6	62.0 - 81.0%
Endosulfan II	0.331	78.2	3.3	68.0 - 85.0%
Endosulfan Sulfate	0.271	90.0	5.3	79.0 - 102.0
Fenvalerate	0.292	83.4	4.7	66.0 - 94.0%
Methyl Parathion	0.532	82.2	3.8	71.0 - 92.0%
Pooled All Insecticides		79.0	3.2	55.0 - 102.0

D. Hydrolab Results for the 1989 Study Period

1. Hydrolab Results for the 1989 Study Period

During the 1989 study, hydrolabs were deployed at the CTL and TRT Sites only. Results of hydrolab studies (May 24 - June 13, 1989) are depicted in Figures 9 -20. Figures 9 - 14 depict hydrographic conditions during fair weather periods at both sites. There were three periods of significant (> 1.27 cm/day) rainfall which occurred during the time of hydrolab deployment. These occurred on June 5, June 6, and June 15, 1989. The effects of each rain event on physicochemical water quality are depicted in Figure 15 (CTL Site - June 5, 6), Figure 10 (TRT Site - June 5, 6), Figure 16 (CTL Site - June 9) and Figure 8 (TRT Site - June 9). Figures 9 (CTL) and 20 (TRT) depict recovery in physicochemical water quality following these three major rain events. No hydrolab data were available for the KWA Site; hence no results for the KWA Site are presented.

During fair weather periods (May 24 - June 4) note the normal tidal (depth, salinity) and diurnal (water temperature, dissolved oxygen, and pH) fluctuations at the CTL (Figures 9, 11, and 13) and TRT (Figures 10, 12, and 14) Sites. During fair weather periods, note the small range in salinity at each site (TRT - ≈26-32 ppt and CTL - 30-33 ppt). Note that the highest dissolved oxygen levels were observed during periods of maximum daily water temperatures concomitant with maximum daily pH values. Also note the supersaturated dissolved concentrations (> 10 mg/L) at the TRT Site on June 3 - 5, 1989 (Figures 12, 14, 16). Also note the concomitant higher pH values observed at the TRT Site during the same time period. These hydrolab results indicate the dynamic nature of the environment at both sites.

During the first significant rain event of June 5th, a total of 4.75 and 4.90 cm of rain fell at the CTL and TRT Sites, respectively. At the CTL Site, this resulted in significant NPS runoff which caused significant decline in salinity from > 31 ppt to 20 ppt on the first post rain, ebb tide and a further decline to 7 ppt on the second post rain, ebb tide (Figure 15). Concomitant with the declines in ebb tide salinity were declines in dissolved oxygen and water temperature. Note the slight increase in water depth on the second post rain ebb tide. The only insecticide detected in water samples was endosulfan (2-11 ng/L) generally at or below background levels (< 10 ng/L) (Table 7).

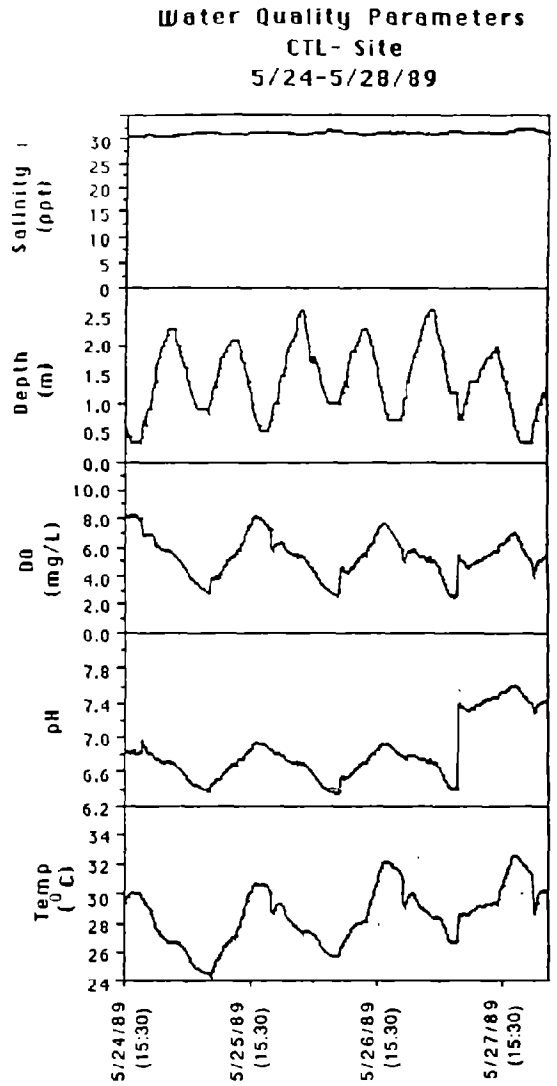


Figure 9. Hydrolab results for the CTL Site, 24-27 May, 1989.

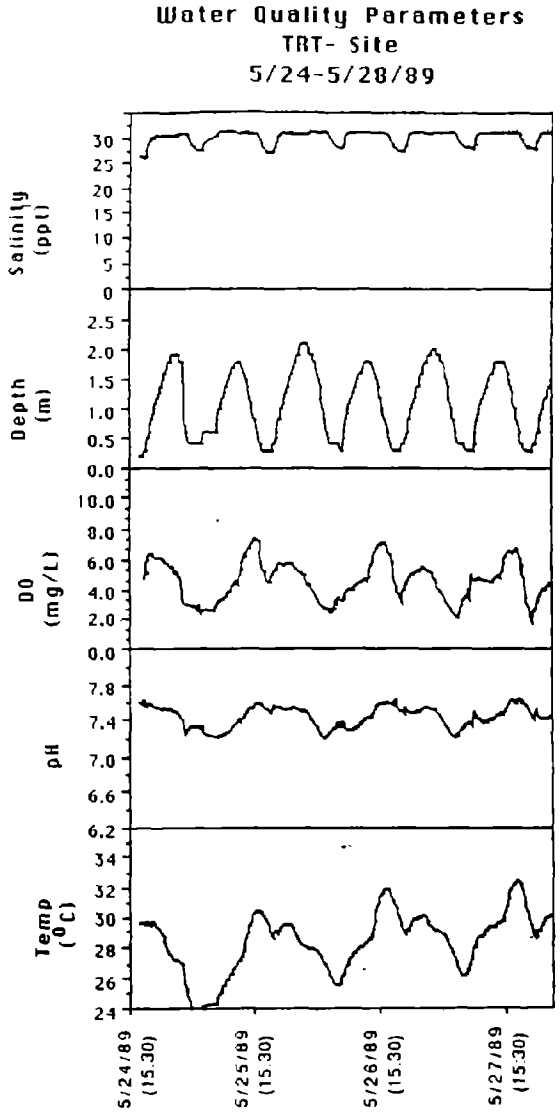


Figure 10. Hydrolab results for the TRT Site, 24-27 May, 1989.

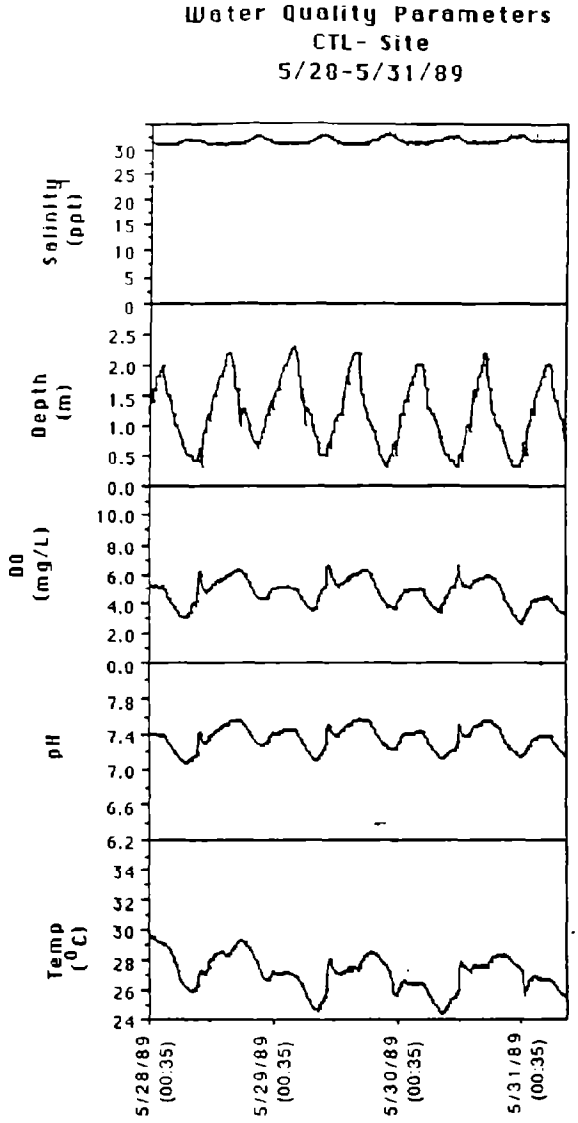


Figure 11. Hydrolab results for the CTL Site, 28-31 May, 1989.

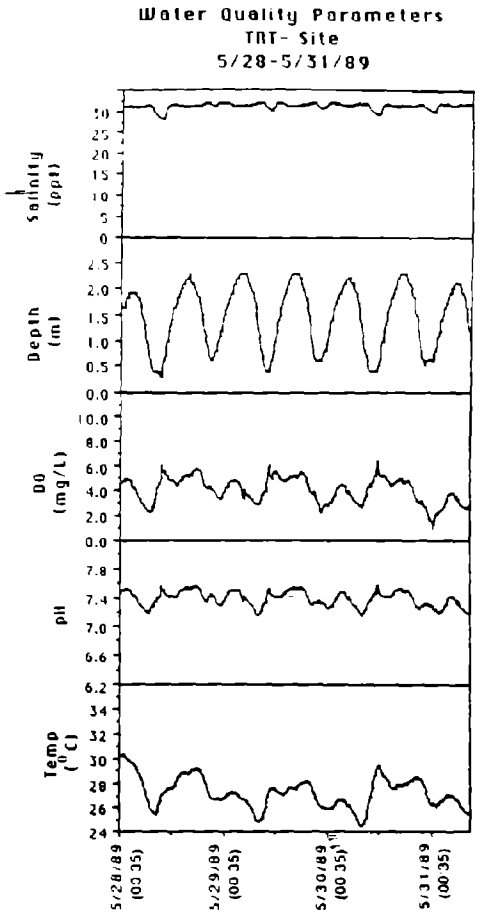


Figure 12. Hydrolab results for the TRT Site, 28-31 May, 1989.

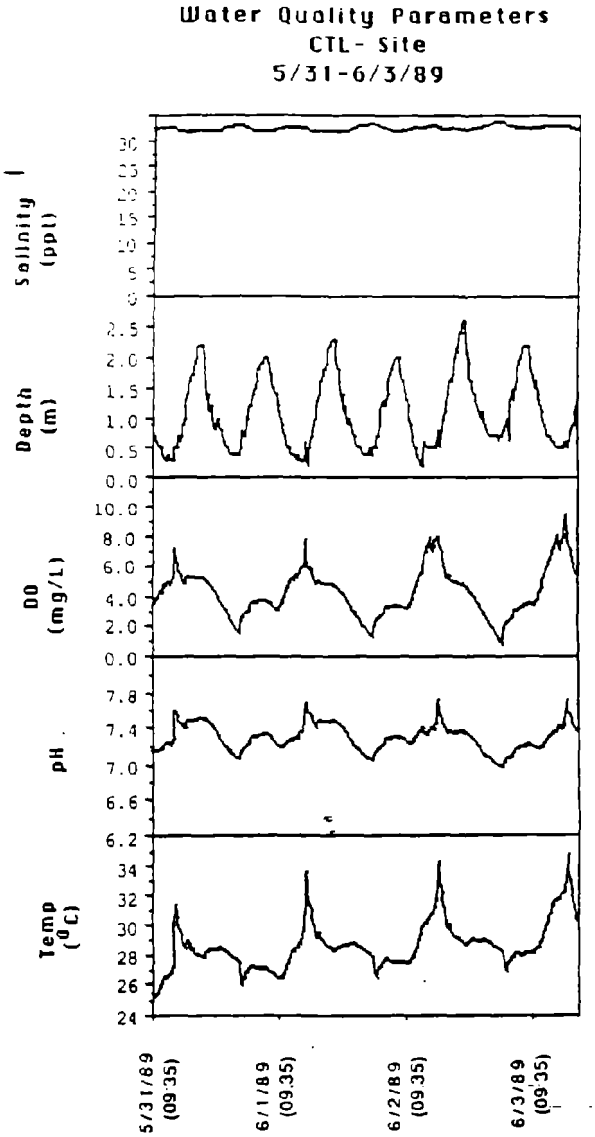


Figure 13. Hydrolab results for the CTL Site, 31 May-3 June, 1989.

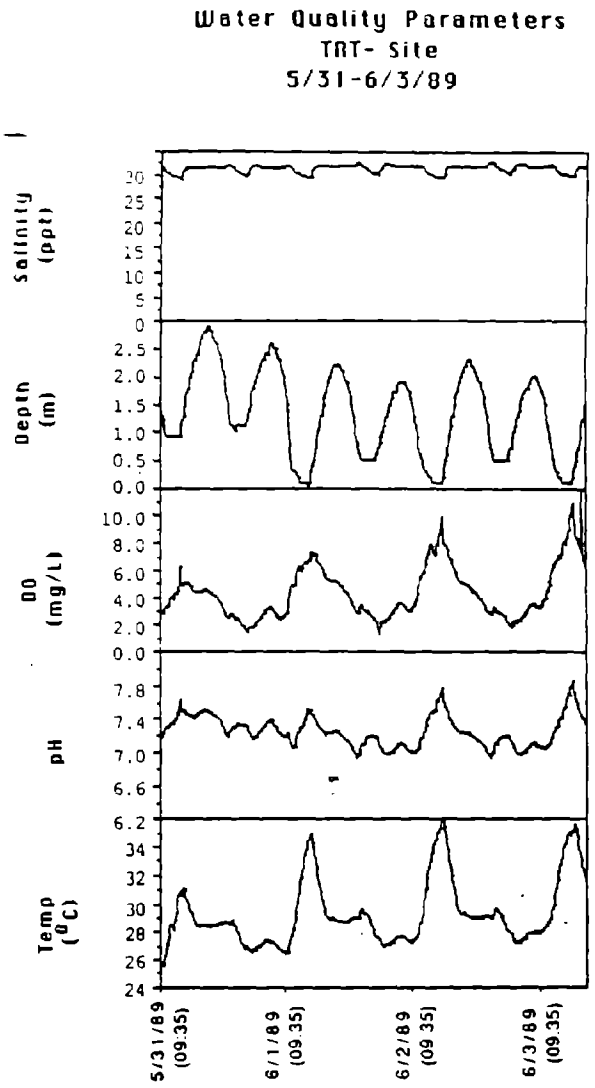


Figure 14. Hydrolab results for the TRT Site, 31 May-3 June, 1989.

At the TRT Site (Figure 6) note the much steeper drop in post rain, ebb tide salinities from 30 to < 5 ppt. Also there were concomitant declines in dissolved oxygen, pH and water temperature during the post rain ebb tides. Additionally, water depth was 0.3 - 0.4 m greater during post rain ebb tides when lowest salinities were observed. This represented the discharge of significant volumes of NPS agricultural runoff as evidenced by the significant concentrations of endosulfan (6-20 ng/L), fenvalerate (< DL - 93 ng/L), and azinphosmethyl (<DL - 16 ng/L) observed (Table 9). Highest pesticide concentrations were observed at the initial post rain ebb tide, 4 hour post rain which represented the "first flush."

The second significant (> 1.27 cm/day) rain event occurred during the afternoon of June 6, 1989 when 3.43 cm of rain was observed at both the CTL and TRT Sites. Note the continued decrease in salinities at both sites, during post rain ebb tides (Figures 15 - 18). At the CTL Site, low tide salinities generally recovered from 7 ppt to 25 ppt by June 8th (≈ 48h post rain) (Figure 17). At the TRT Site, however, ebb tide salinities only recovered slightly from < 5 ppt to 11 ppt (Figure 18). Dissolved oxygen, water temperature and pH during this time period remained at levels lower than those found during fair weather periods. Only slight concentrations of endosulfan (8 -14 ng/L) were observed at CTL Site during this second rain event (Table 7). At the TRT Site slightly elevated (above background) concentrations of endosulfan (7-20 ng/L) were observed, although significant (> 96h LC50 values for most sensitive crustaceans) levels of fenvalerate (< DL - 40 ng/L) were again found (Table 9). Highest pesticide concentrations were observed at the initial post rain ebb tide (i.e. first flush).

The third significant (> 1.27 cm/day) rain event occurred on June 9, 1989 when 1.35 cm of rain fell at the CTL Site. A total of 1.57 cm of rainfall was recorded at the TRT Site. At the CTL Site, this rainfall appeared to have only minimal effects, as post rain low rain salinities remained at or about 25 ppt, similar to levels measured on June 8 (Figure 19). Endosulfan concentrations remained at levels at or below background (9 ng/L; background = < 10 ng/L) (Table 7). The data suggests only minimal runoff occurred at the CTL Site during this rain event. At the TRT Site, as low tide salinities fell from 11 ppt to 6 ppt and generally remained suppressed (< 10 ppt) at subsequent low tides until June 11, 1989 (1235) (Figures 18 and 20). In fact, low tide salinities were very slow to recover throughout the post rain period (June 10 - 13, 1989) (Figure 20). This suggests continued runoff possibly due to regulated discharge from the retention

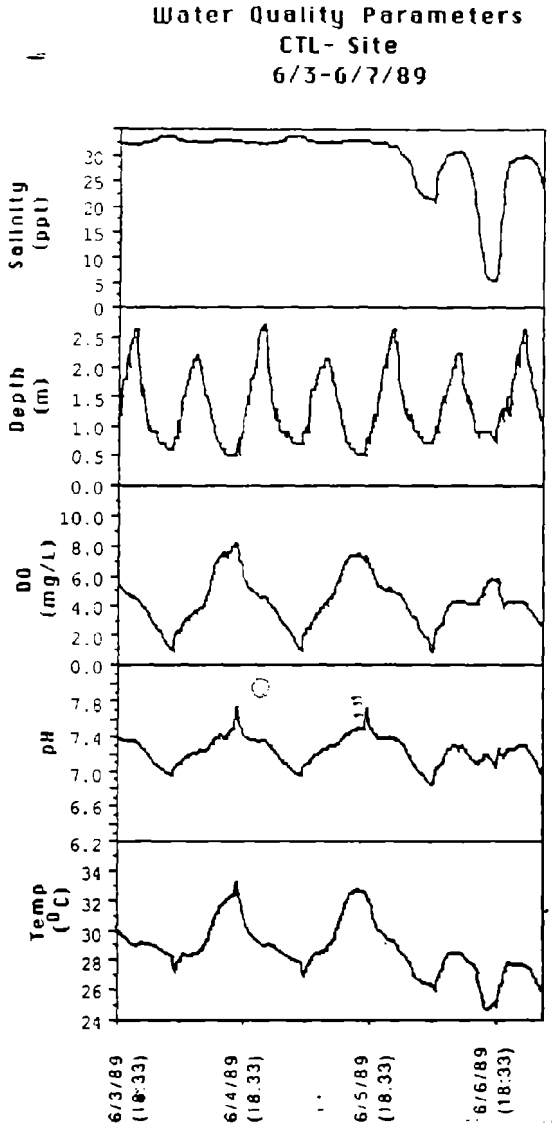


Figure 15. Hydrolab results for the CTL Site, 3 -7 June, 1989. Note the effects of rain events on the 5 - 6 June on salinity and other water quality parameters.

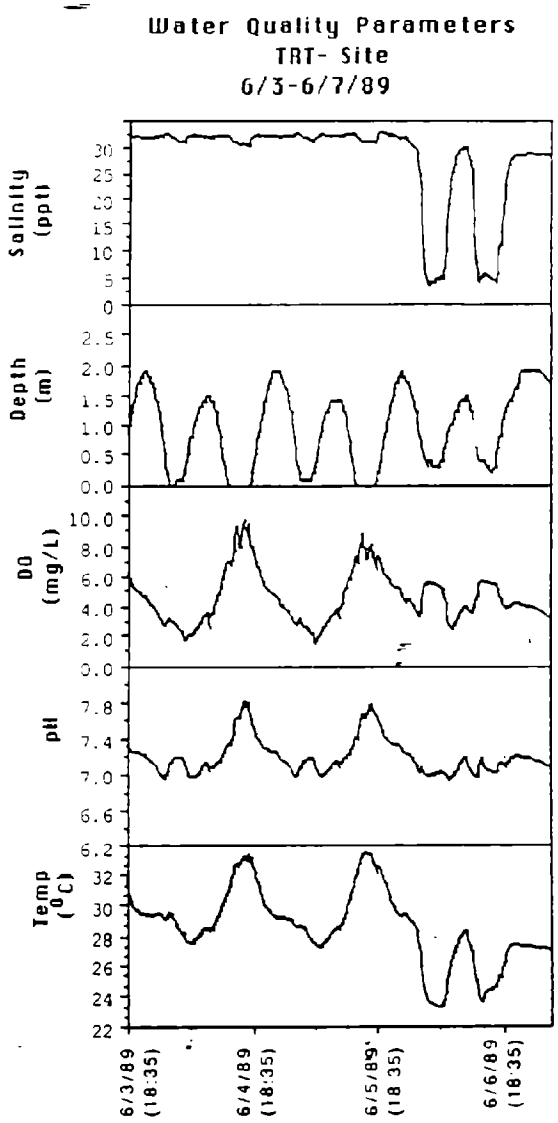


Figure 16. Hydrolab results for the TRT Site, 3 - 7 June, 1989. Note the effects of rain events on the 5 - 6 June on salinity and other water quality parameters.

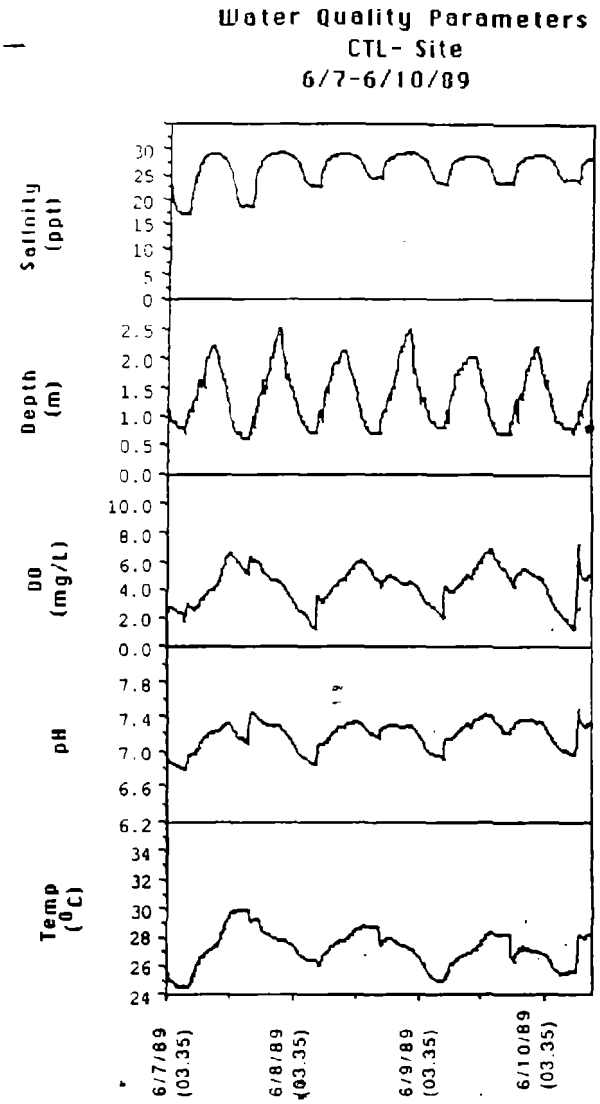


Figure 17. Hydrolab results for the CTL Site, 7 - 10 June, 1989. Note the slightly reduced salinities at this site following rain events on the 5 - 6 June and 9 June, 1989.

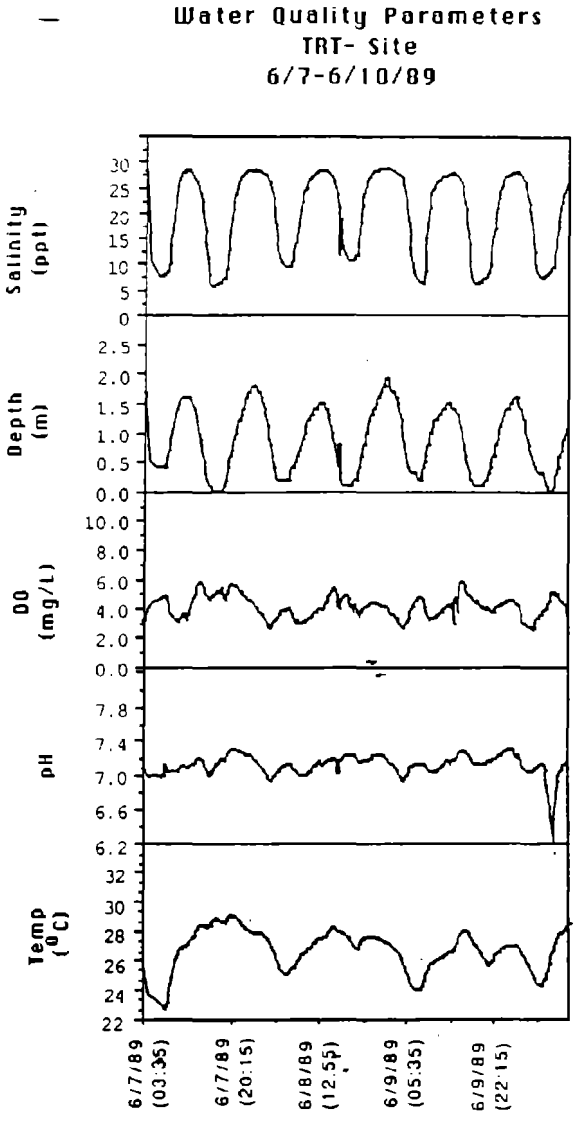


Figure 18. Hydrolab results for the TRT Site, 7 - 10 June, 1989. Note the significant reductions in salinity following rain events on the 5 - 6 June and 9 June, 1989.

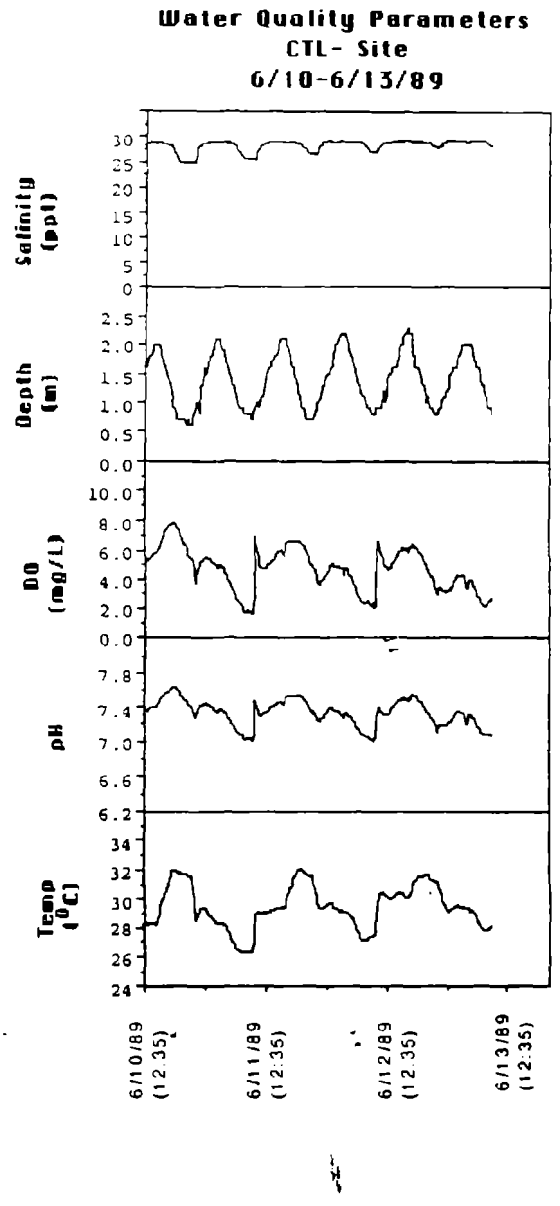


Figure 19. Hydrolab results for the CTL Site, 10-13 June, 1989. Salinity at this site has recovered back to levels comparable to pre-rain conditions.

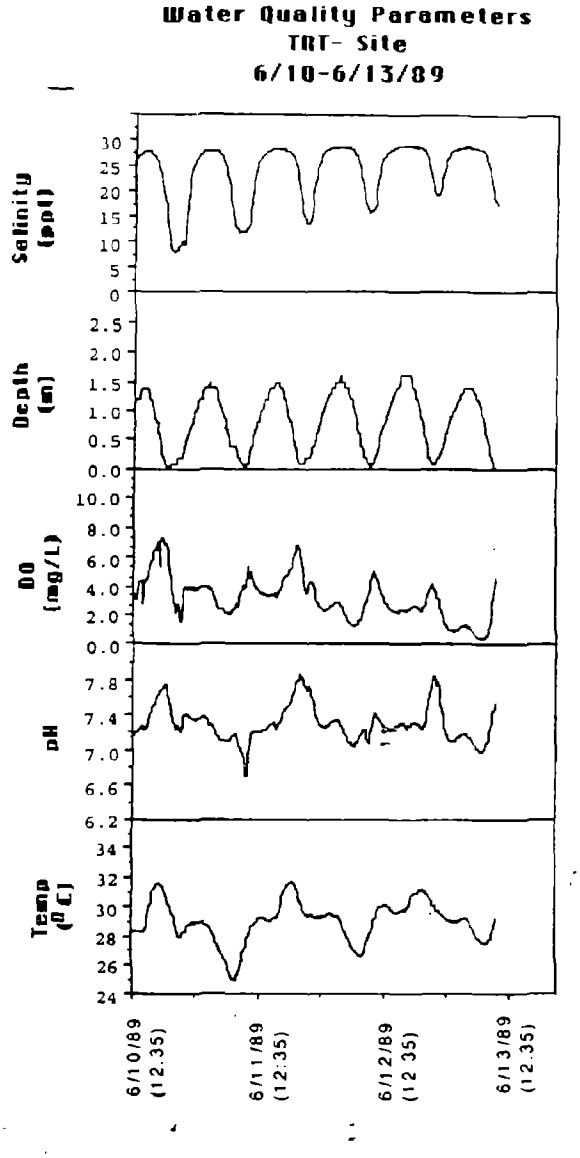


Figure 20. Hydrolab results for the TRT Site, 10-13 June, 1989. Note the continued reduced salinities at this site.

ponds at this site. Also note that immediately, post rain on June 9, low tide water depths were 0.2 m higher than previous ebb tides suggesting discharge of fresh water. Chemical analysis of water samples indicated only background levels of endosulfan (5 - 7 ng/L) but significant levels of fenvalerate (<DL - 21 ng/L) (Table 9). Detectable levels of fenvalerate were only observed during the initial post rain sample (first flush).

2. Hydrolab Results for the 1990 Study Period

During the 1990 Study period hydrolabs were deployed at the CTL, TRT and KWA Sites. Only two significant rainfall (> 1.27 cm/day) event occurred with only minimal levels of pesticides detected at each Site. As a result, hydrolab results for 1990 were not included in this report.

E. Survival Data for Field Toxicity Test

1. 1989 Field Toxicity Test

Results of *in situ* toxicity tests conducted May 25 - June 27, 1989 are listed in Tables 19 - 23 and depicted in Figures 21 - 25. The dates for caged animal deployment do not always directly overlap or correspond with the dates of analytical chemical analysis, due to the different bioassay deployment and water sample collection schedules. When evaluating *in situ* toxicity test results (Tables 19 - 23) and pesticide analytical results (Tables 7-17), note dates of deployment and sample collection in interpreting results.

Results of grass shrimp (Table 19 and Figure 21) *in situ* toxicity tests indicated that: 1) Survival at the CTL Site ranged from 90 - 100%, averaging 96.5% (± 1.46%); 2) Survival at the TRT Site ranged from 28.5 - 96.7%, averaging 81.8% (±8.53%); and 3) Survival at the KWA Site ranged from 0.0 - 86.7%, averaging 26.1% (± 9.27%). Statistical analysis indicated that survival was significantly ($p \leq 0.05 - 0.01$) reduced at the TRT and KWA Sites compared to the CTL Site due to exposure to fenvalerate (TRT Site - June 2 - 7, 1989) and combined fenvalerate, endosulfan, and azinphosmethyl (KWA Site - June 6 - 27, 1989) exposures, respectively. . Additionally, survival at the KWA Site was significantly ($p \leq 0.05$) lower than at the TRT Site.

Table 19. Summary of survial in *P. pugio* at all sites during the 1989 field study. Pooled means with different letters (A,B,C) were significantly ($p \leq 0.05$) different.

Group #	1989		% Survival	
	Date	Site	X	SE
1	5/25 - 29/89	CTL	100.0	0.00
		TRT	93.3	3.33
		KWA	72.3	11.83
2	5/29 - 6/2/89	CTL	96.7	3.33
		TRT	93.3	3.33
		KWA	86.7	8.82
3	6/2 - 7/89	CTL	93.0	3.53
		TRT	28.5	13.82
		KWA	20.0	20.00
4	6/6 - 11/89	CTL	90.0	5.77
		TRT	63.3	3.33
		KWA[A]	00.0	0.00
		KWA[B]	00.0	0.00
5	6/11 - 15/89	CTL	100.0	0.00
		TRT	96.7	3.33
		KWA	36.7	3.33
6	6/15 - 19/89	CTL	100.0	0.00
		TRT	93.3	6.67
		KWA[C]	0.0	0.00
		KWA[D]	37.4	8.12
7	6/19 - 23/89	CTL	100.0	0.00
		TRT	89.3	6.43
		KWA	60.0	15.28
8	6/23 - 27/89	CTL	92.6	7.41
		TRT	96.7	3.33
		KWA[E]	0.0	0.00
		KWA[F]	0.0	0.00
		KWA[G]	0.0	0.00
1 - 8	5/25 - 6/27/89	CTL	96.5[A]	1.46 Range = 90.0 - 100.0
		TRT	81.8[B]	8.53 Range = 28.5 - 96.7
		KWA	26.1[C]	9.27 Range = 0.0 - 86.7

A = Group deployed from 6/6 - 8/89.
B = Group deployed from 6/8 - 11/89.
C = Group deployed from 6/15 - 19/89.
D = Group deployed from 6/18 - 23/89.
E = Group deployed from 6/23 - 24/89.
F = Group deployed from 6/24 - 26/89.
G = Group deployed from 6/25 - 27/89.

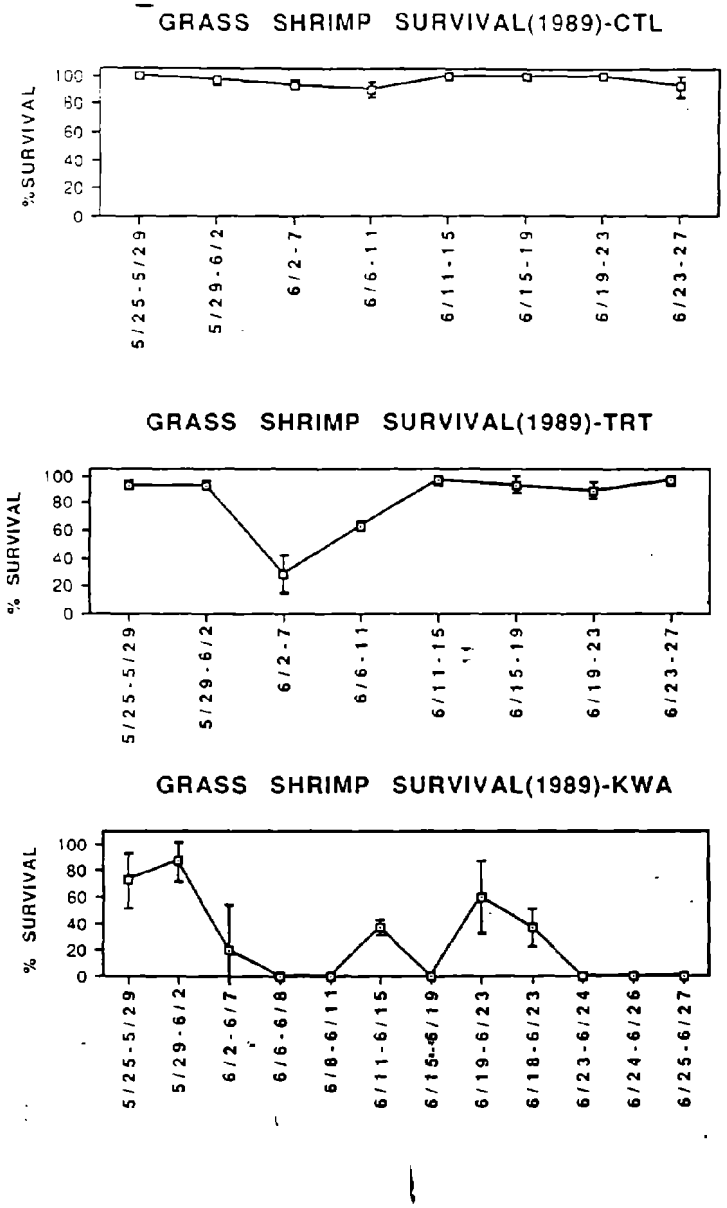


Figure 21. Survival of *P. pugio* in field toxicity tests during the 1989 field study. Note the significant mortality observed at the KWA Site. Also note one period of reduced survival(6/2-7/89) at the TRT Site.

Results of penaied shrimp (Table 20 and Figure 22) *in situ* toxicity test indicated that: 1) Survival at the CTL Site ranged from 58.3 - 100%, averaging 90.9% (± 4.9%); 2) Survival at the TRT Site ranged from 51.9 - 100%, averaging 92.3% (± 6.01%); and 3) Survival at the KWA Site ranged from 0.0 - 35.8%, averaging 5.7% (± 4.47%). Statistical analysis indicated that survival at the KWA Site was significantly ($p \leq 0.001$) lower than at the CTL and TRT Sites. This resulted from significant runoff of azinphosmethyl, endosulfan, and fenvalerate at this site. Significant mortality (48%) was also observed at the TRT Site following the rain events of June 5 - 6, 1989, when high concentrations of fenvalerate were measured in runoff at the site.

Results of mysid shrimp (Table 21 and Figure 23) *in situ* toxicity tests indicated that: 1) Survival at the CTL Site ranged from 0 -100%, averaging 40.0% (± 24.5%); Survival at the TRT Site ranged from 1 - 100%, averaging 60.0% (±24.5); and 3) Survival at the KWA Site ranging from 0 - 100%, averaging 37.2% (± 22.87). Poor survival was observed at all sites due to: 1) the inability of mysids to survive low salinity (< 10 ppt) exposure following significant rainfall (i.e. Group 3 - June 2 - 7, 1989); and 2) problem with cage fouling on the tether line, (i.e. CTL Site - Groups 1 - 2). Statistical analysis indicated no significant between site differences in survival, despite cage deployment problems, described above.

Results of mummichogs *in situ* toxicity tests (Table 22 and Figure 24) indicated that: 1) Survival at the CTL Site ranged form 96.3 - 100%, averaging 98.7% (± 0.63%); 2) Survival at the TRT Site ranging from 96.7 - 100.00%, averaging 99.6% (± 0.41%); and 3) Survival at the KWA Site ranging from 83.3 - 100.0%, averaging 97.1% (± 2.04%). Statistical analysis generally indicated no significant between site difference in survival. Survival at the KWA Site, Group 8 (83.3 ± 6.67%) was significantly ($p < 0.05$) reduced, however, compared to the CTL and TRT Sites due to significant azinphosmethyl runoff.

Results of juvenile sheepshead minnow *in situ* toxicity tests (Table 23 and Figure 25) indicated that: 1) Survival at the CTL Site ranged from 36.1 - 86.7%, averaging 56.1% (± 15.53%); 2) Survival at the TRT Site ranged form 80 - 100%, averaging 92.1% (± 6.14%); and 3) Survival at the KWA Site ranged from 0 -44%, averaging 24.9% (± 13.04%). Statistical analysis indicated that survival was significantly

Table 20. Summary of survival in Penaeus Species at all sites during the 1989 field study. Pooled means with the different letters (A,B) were not significantly ($p \leq 0.05$) different.

Group #	1989		% Survival	
	Date	Site	X	SE
1	5/25 - 29/89	CTL	93.3	6.67
		TRT	100.0	0.00
		KWA	10.0	5.77
2	5/29 - 6/2/89	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	0.0	0.00
3	6/2 - 7/89	CTL	89.6	0.37
		TRT	51.9	6.06
		KWA	0.0	0.00
4	6/7 - 11/89	CTL	100.0	0.00
		TRT	86.7	8.82
		KWA	0.0	0.00
5	6/11 - 15/89	CTL	100.0	0.0
		TRT	100.0	0.0
		KWA	0.0	0.0
6	6/15 - 19/89	CTL	58.3[1]	23.95
		TRT	100.0	0.00
		KWA	0.0	0.00
7	6/19 - 23/89	CTL	95.8	4.17
		TRT	100.0	0.00
		KWA	35.8	8.70
8	6/23 - 27/89	CTL	90.5	4.76
		TRT	100.0	0.00
		KWA	0.0	0.00
1 - 8	5/25 - 6/27/89	CTL	90.9[A]	4.90 Range = 58.3 - 100.0
		TRT	92.3[A]	6.01 Range = 51.9 - 100.0
		KWA	5.7[B]	4.47 Range = 0.0 - 35.8

[1] = Mortality caused by extremely heavy siltation in cages following heavy rains at dead low tide which eroded large quantities of sediment into Leadenwah Creek.

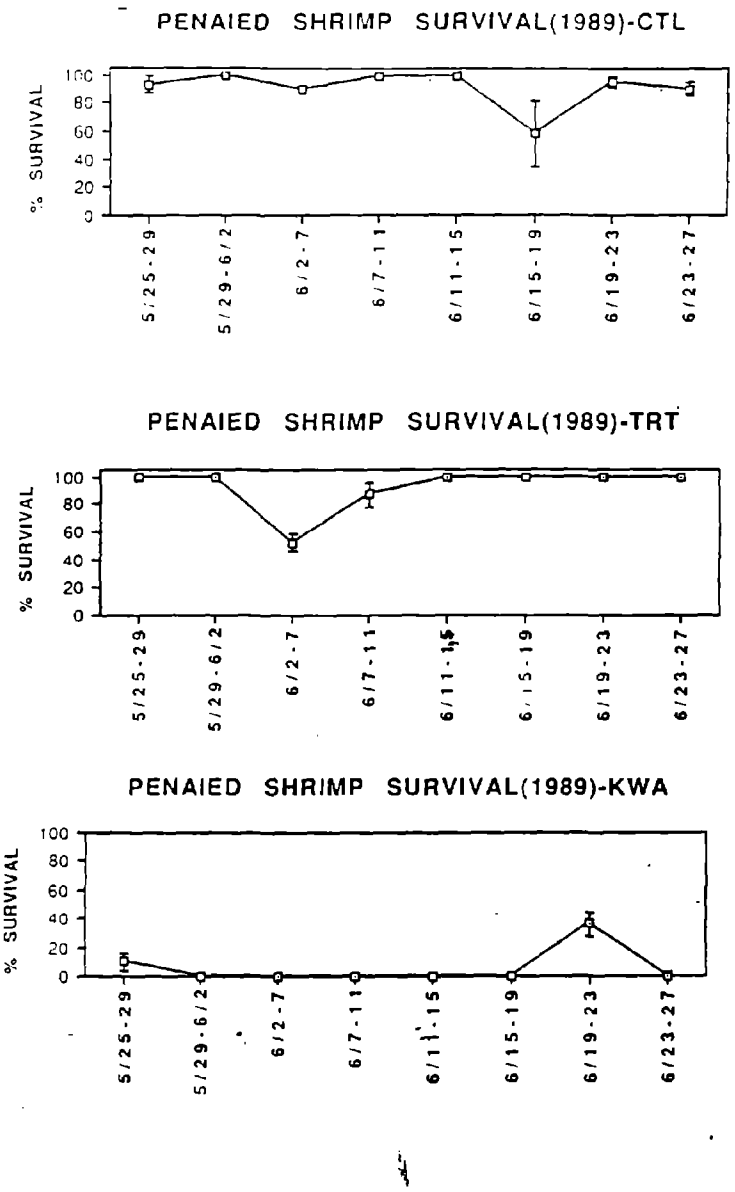


Figure 22. Survival of *Penaeus* species in field toxicity tests during the 1989 field study. Note the significant mortality in penaied shrimp at the KWA Site. Also note one period of reduced survival(6/2-7/89) at the TRT Site.

Table 21. Summary of survival in *Mysidopsis bahia* at all sites during the 1989 field study. Pooled means with the same letter (A) were not significantly ($p > 0.05$) different.

Group #	1989		% Survival	
	Date	Site	X	SE
1	5/25 - 29/89	CTL	0.0[1]	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
2	5/29 - 6/2/89	CTL	0.0[2]	0.00
		TRT	100.0	0.00
		KWA	85.8	14.20
3	6/2 - 7/89	CTL	0.0[3]	0.00
		TRT	0.0[3]	0.00
		KWA	0.0[3]	0.00
4	6/7 - 11/89	CTL	100.0	0.00
		TRT	0.0	0.00
		KWA	0.0	0.00
5	6/11 - 15/89	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	0.0	0.00
1 - 5	5/25 - 6/15/89	CTL	40.0[A]	24.50 Range = 0 - 100
		TRT	60.0[A]	24.50 Range = 0 - 100
		KWA	37.2[A]	22.87 Range = 0 - 14.2

[1] = Control Group mortality due to cage becoming aerially exposed resulting in dessication.

[2] = Control Group mortality die to cage becoming hung up due to extremely high tides.

[3] = Mortality at all sites probably due to effects of low salinity (CTL) and low salinity -pesticide exposures (TRT and KWA).

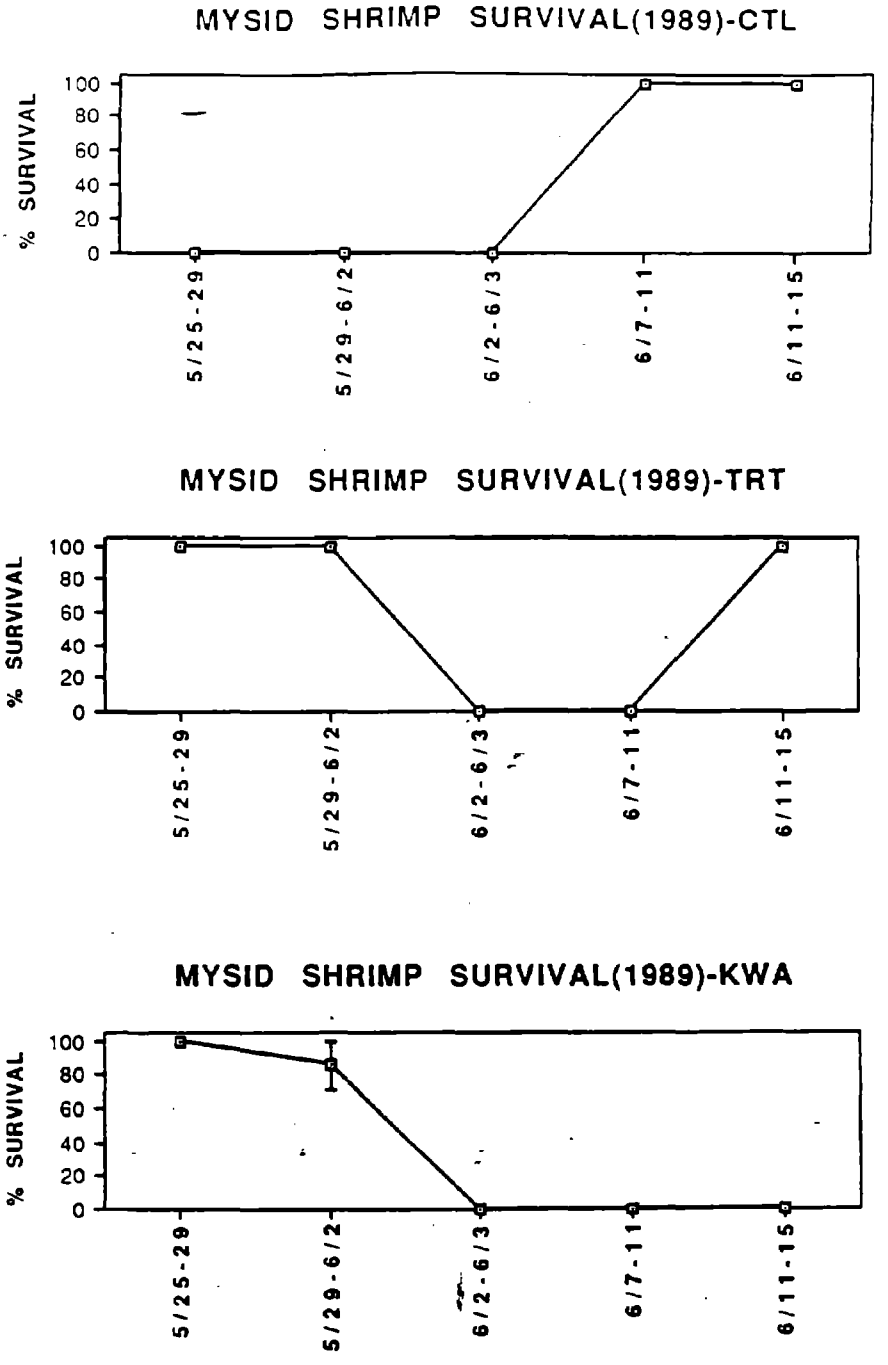


Figure 23. Survival of *Mysidopsis bahia* in field toxicity tests during the 1989 field study. Note the generally poor survival at all field sites.

Table 22. Summary of survival in *F. heteroclitus* at all sites during the 1989 field study. Pooled means with the same letter (A) were not significantly ($p > 0.05$) different.

Group #	1989		% Survival		
	Date	Site	X	SE	
1	5/25 - 29/89	CTL	100.0	0.00	
		TRT	96.7	3.33	
		KWA	100.0	0.00	
2	5/29 - 6/2/89	CTL	96.7	3.33	
		TRT	100.0	0.00	
		KWA	96.7	3.33	
3	6/2 - 6/7/89	CTL	96.7	3.33	
		TRT	100.0	0.00	
		KWA	100.0	0.00	
4	6/7 - 11/89	CTL	100.0	0.00	
		TRT	100.0	0.00	
		KWA	100.0	0.00	
5	6/11 - 15/89	CTL	96.3	3.70	
		TRT	100.0	0.00	
		KWA	100.0	0.00	
6	6/15 - 19/89	CTL	100.0	0.00	
		TRT	100.0	0.00	
		KWA	100.0	0.00	
7	6/19 - 23/89	CTL	100.0	0.00	
		TRT	100.0	0.00	
		KWA	96.7	3.33	
8	6/23 - 27/89	CTL	100.0	0.00	
		TRT	100.0	0.00	
		KWA	83.3	6.67	
1 - 8	5/25 - 6/27/89	CTL	98.7[A]	0.63	Range = 96.3 - 100.0
		TRT	99.6[A]	0.41	Range = 96.7 - 100.0
		KWA	97.1[A]	2.04	Range = 83.3 - 100.0

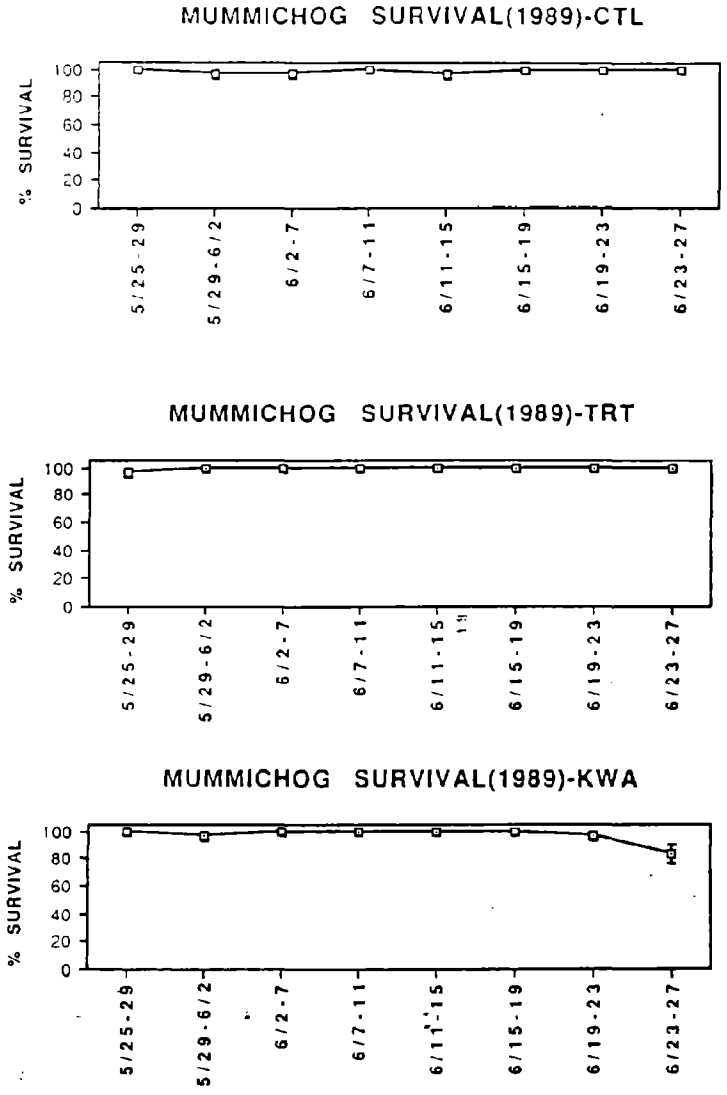


Figure 24. Survival of *F. heteroclitus* in field toxicity tests during the 1989 field study. Note the one period of reduced survival at the KWA Site (6/23-27/89).

Table 23. Summary of survival in juvenile *Cyprinodon variegatus* at all sites during the 1989 field study. Pooled means with different letters (A,B,C) were significantly ($p \leq 0.05$) different.

Group #	1989		% Survival		
	Date	Site	X	SE	
1	5/24 - 28/90	CTL	96.7	3.33	
		TRT	36.7	20.28	
		KWA	100.0	0.00	
2	5/28 - 6/1/89	CTL	93.0	3.53	
		TRT	96.7	3.33	
		KWA	93.3	3.33	
3	6/11 - 27/89	CTL	36.1	7.35	
		TRT	100.0	7.35	
		KWA	0.0	0.00	
1 - 3	5/25 - 6/27/89	CTL	56.1^{A}	15.53	Range = 36.1 - 86.7
		TRT	92.1^{B}	6.14	Range = 80.0 - 100.0
		KWA	24.9^{C}	13.04	Range = 0.0 - 44.0

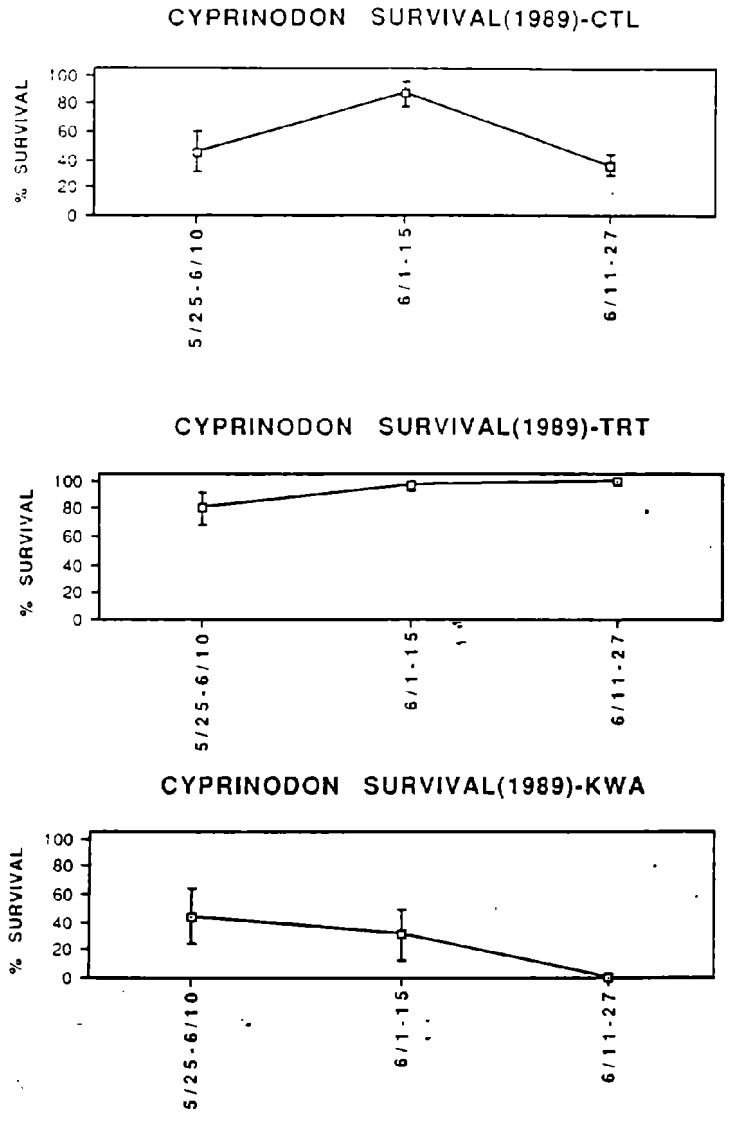


Figure 25. Survival of *Cyprinodon variegatus* in field toxicity tests during the 1989 field study. Note the reduced survival at the KWA Site and variable survival at the CTL Site.

($p < 0.05$) higher at TRT Site when compared to the CTL and KWA Sites. Additionally, survival at the CTL Site was significantly ($p < 0.05$) higher than at the KWA Site. Survival at the TRT Site was high despite the significant runoff of fenvalerate observed following significant rainfall on June 5, June 6 and June 9. Reduced survival at the CTL Site was most likely related to prolonged exposure duration (14 - 16 days), as most mortality was incurred beyond 10 days post deployment. This may have resulted due to starvation. Although fish at both sites were deployed for the same time using the same cage type, food availability was dependent upon what passed through the mesh on each container. At the TRT Site, cages were deployed in a shallower stream stretch than at the CTL Site, which may have resulted in greater food availability. Differences in survival between the TRT and CTL Sites may have resulted from differences in food availability among animals deployed at each site. This generally was not the case at the KWA Site as mortality occurred concomitant with periods of significant runoff and pesticide exposure (azinphosmethyl and endosulfan), irrespective of exposure (i.e. food availability) duration. Most mortalities at the KWA Site were attributed to azinphosmethyl exposure rather than endosulfan or fenvalerate exposure. The basis for this conclusion were two fold. First significant AChE enzyme inhibition was observed in organisms at the KWA Site indicating significant azinphosmethyl exposure. Secondly, levels of endosulfan and fenvalerate were generally below 96h LC_{50} values reported for the species tested.

2. Quality Assurance and Quality Control for Bioassay Organisms Used in Field Toxicity Test during the 1989 Field Study

Results of quality assurance and control bioassays conducted during 1989 using endosulfan as a reference toxicant are listed in Table 24.

Results for adult *P. pugio* exposed to endosulfan indicated mortalities ranging from 0 - 40%, averaging 8% (± 8%) at 0.01 ug/L; from 60 - 100%, averaging 72% (± 8%) at 1.15 ug/L; and from 100 - 100%, averaging 100% (± 0%) at 2.50 ug/L. Control mortality ranged from 0 - 0%, averaging 0% (± 0%) in high salinity (> 25 ppt) controls and was also 0% (N = 1) in low salinity controls (2ppt). The 96h LC50 for endosulfan *P. pugio* was 0.18 ug/L (CL = 0.10 - 0.33 ug/L), which was comparable to previous reported LC50 values (0.25 - 1.01 ug/L) for grass shrimp (Scott et al, 1990).

Results for adult *F. heteroclitus* exposed to endosulfan indicated mortalities ranging from 0 - 0%, averaging 0% (± 0%) at 0.01 ug/L; from 0 - 40%, averaging 8% (± 8%) at 1.15 ug/L; 60% at 2.50 ug/L (n = 1); and from 100 - 100%, averaging 100%

Table 24. Summary of Quality Control/Quality Assurance Bioassays for 1989 using endosulfan as the reference toxicant, for the five test periods of the study.

Nominal Endosulfan Concentration	Species (*)	% Mortality[4]					Pooled Results	
		#1	#2	#3	#4	#5	X	(± SE)
0.00[1]	*P. pugio* (A)	0	0	0	0	ND	0.0	(± 0.00)
0.00[2]		ND	0	ND	ND	ND	0.0	(NC)
0.01		0	40	0	0	0	8.0	(± 8.00)
1.15		60	60	60	100	80	72.0	(± 8.00)
2.50		100	100	100	100	ND	100.0	(± 0.00)
							96h LC_{50} = 0.18 ug/L	
							(59%CL = 0.10 - 0.33 ug/L)	
0.00	*F. heteroclitus* (A)	0	0	0	0	0	0.0	(± 0.00)
0.01		0	0	0	0	0	0.0	(± 0.00)
1.15		0	40	0	0	0	8.0	(± 8.00)
2.50		60	ND	ND	ND	ND	60.0	(NC)
5.00		ND	100	100	100	100	100.0	(± 0.00)
							96h LC_{50} = 1.82 ug/L	
							(95%CL = 1.18 - 2.83 ug/L)	
0.00[1]	*Penaeus sp.* (J)	20	0	0	0	ND	5.0	(± 5.00)
0.00[2]		ND	40	ND	ND	ND	40.0	(NC)
0.01		20	0	0	0	0	4.0	(± 4.0)
1.15		80	80	100	33	60	70.6	(± 11.33)
2.50		80	100	100	100	ND	95.0	(± 5.00)
							48h LC_{50} = 0.18ug/L	

Nominal Endosulfan Concentration	Species (*)	% Mortality[4]					Pooled Results	
		#1	#2	#3	#4	#5	X	(± SE)
						(95%CL = 0.13 - 0.75 ug/L)		
0.00[3]	*C. variegatus* (J)	0	0	40	0	ND	10.0	(± 10.0)
0.01		0	0	40	0	ND	10.0	(± 10.0)
1.15		0	100	100	25	ND	56.3	(± 25.8)
2.50		100	100	100	ND	ND	100.0	(± 0.0)
							96h LC_{50} = 0.31 ug/L	
						(95% CL = 0.13 - 0.75 ug/L)		

* = Lifestage: A = Adult; J = Juvenile

ND = Not Determined
NC = Not Calculated

1 = High Salinity Control = 20 ppt salinity
2 = Low Salinity Control = 2 ppt salinity
3 = High Control Mortality due to handling stress
4 = Exposure periods were 96 hours for all species except *Penaeus sp.* which was 48 hours.

(± 0%) at 5.00 ug/L. Control mortality ranged from 0 - 0%, averaging 0% (± 0%). The 96h LC50 for endosulfan *F. heteroclitus* was 1.82 ug/L (CL = 1.18 - 2.83 ug/L), which was comparable to previously reported 96h LC50 valves (1.29 - 1.45 ug/L) for mummichogs (Scott et al. 1990).

Results for juvenile penaied shrimp (*Penaeus aztecus* and *Penaeus setiferus*) exposed to endosulfan indicated 48h mortalities ranging from 0-20%, averaging 4% (± 4%) at 0.01 ug/L; from 33 - 100%, averaging 70.6% (± 11.3%) at 1.15 ug/L; and from 80 - 100%, averaging 95.% (± 5%) at 2.50 ug/L. Control mortality ranged from 0 - 20% averaging 5% (± 5%) at high salinity (> 20 ppt). At low salinities (2 ppt - used to simulate KWA Site) mortality was 40% (N = 1). The 48h LC50 for endosulfan in juvenile penaied shrimp was 0.18 ug/L (CL = 0.09 - 0.38 ug/L).

Results for juvenile *C. variegatus* exposed to endosulfan indicated mortalities ranging from 0 - 40%, averaging 10% (± 10%) at 0.01 ug/L; from 0 - 100%, averaging 56.3% (± 25.8%), and from 100 - 100%, averaging 100% (± 0%). Control survival ranged from 0 - 40%, averaging 10% (± 10%). All control mortality occurred during QA Test #3, suggesting possible handling stress. The 96h LC50 for endosulfan in juvenile *C. variegatus* was 0.31 ug/L (CL = 0.13 - 0.75 ug/L).

These results generally indicated that each group of organism were comparable to previously reported (Scott et al, 1990) acute toxicity results in terms of their response to endosulfan exposure. Although some variation was noted between groups, much of this may have resulted from differences in ambient temperature, given the inverse or negative temperature coefficient for endosulfan (i.e. less toxic at higher temperatures).

3. 1990 Field Toxicity Tests

Results of *in situ* toxicity tests conducted May 24 - June 23, 1990 are listed in Tables 25 - 30 and depicted in Figures 26 - 31.

Results of grass shrimp (Table 25 and Figure 26) *in situ* toxicity tests indicated that: 1) Survival at the CTL Site ranged from 93 - 100%, averaging 98.3% (± 0.92%), 2) Survival at the TRT Site ranged from 36.7 - 100.0%, averaging 90.4% (± 7.69%); and

Table 25. Summary of survial in *P. pugio* at all sites during the 1990 field study. Pooled means with the same letters (A) were not significantly ($p > 0.05$) different.

Group #	Date	Site	% Survival	
			X	SE
1	5/24 - 28/90	CTL	96.7	3.33
		TRT	36.7[1]	20.28
		KWA	100.0	0.00
2	5/28 - 6/1/90	CTL	93.0	3.53
		TRT	93.7	3.33
		KWA	93.3	3.53
3	6/1 - 5/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	86.7	3.33
4	6/5 - 9/90	CTL	96.7	3.33
		TRT	96.7	3.33
		KWA	96.7	3.33
5	6/9 - 13/90	CTL	100.0	0.00
		TRT	96.7	3.33
		KWA	97.0	3.03
6	6/13 - 17/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	96.7	3.33
7	6/17 - 21/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	96.7	3.33
8	6/21 - 23/90	CTL	100.0	0.00
		TRT	96.7	3.33
		KWA	100.0	0.00
1 - 8	5/24 - 6/23/90	CTL	98.3[A]	0.92 Range = 93.0 - 100.0
		TRT	90.4[A]	7.69 Range = 36.7 - 100.0
		KWA	95.9[A]	1.52 Range = 86.7 - 100.0

[1] = Cage at TRT Site turned on side resulting in aerial exposure and desiccation.

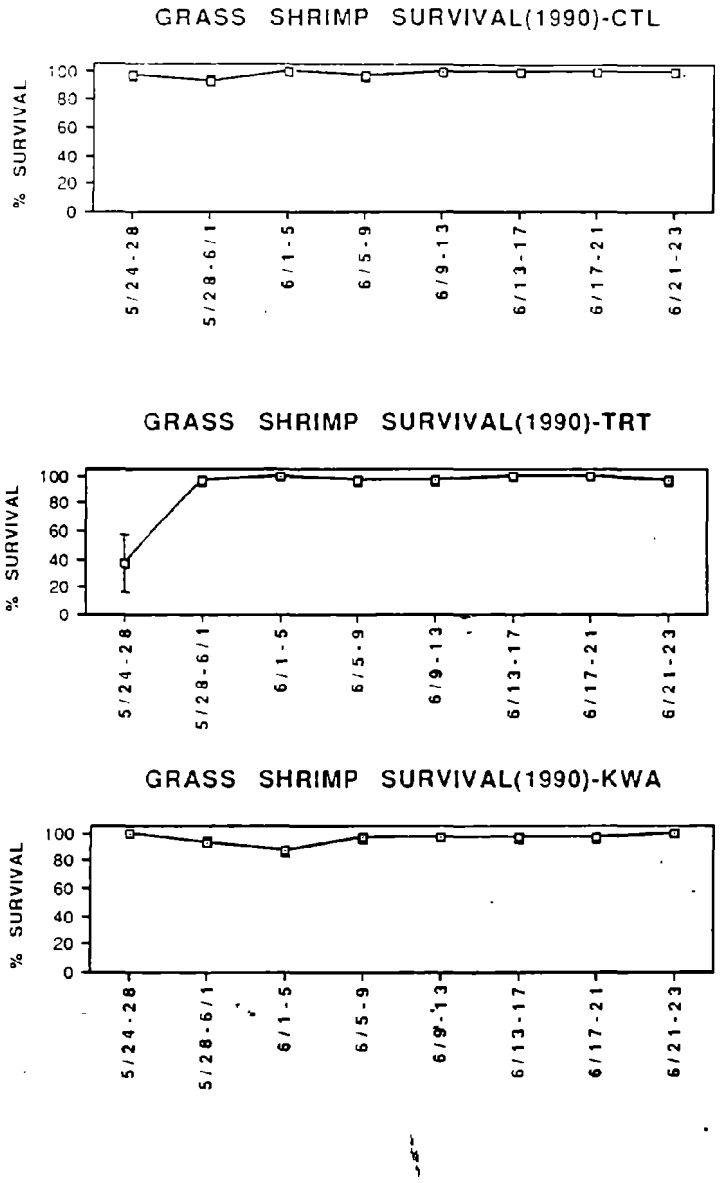


Figure 26. Survival of *P. pugio* in field toxicity tests during the 1990 field. Note the high survival at all sites.

3) Survival at the KWA Site ranged from 86.7 - 95.9%, averaging 95.9% (± 1.52%). Statistical analysis indicated no between site differences in survival during the 1990 study period, despite significant fenvalerate runoff (concentration > 96h LC50 value for crustaceans) at the TRT Site (Table 16 and Figure 7). It is noteworthy that while one grab sample exceeded the 96h LC50 values, the composite sample (time- weighted average exposure) was < DL. This suggests that only a small volume discharge of fenvalerate occurred at the TRT Site, such that even though peak concentrations at ebb tide exceeded 96h LC50 values, rapid dilution by incoming tides, reduced in-stream concentrations to below toxic thresholds.

Results of juvenile penaied shrimp (Table 26 and Figure 27) *in situ* toxicity tests indicated that: 1) Survival at the CTL Site ranged from 96.3 - 100%, averaging 99.5% (± 0.46%); 2) Survival at the TRT Site ranging from 96.7 - 100.0%, averaging 99.2% (± 0.54%); and 3) Survival at the KWA Site ranging from 96.3 - 100.0%, averaging 98.7% (± 0.65%). Statistical analysis indicated no between site differences in survival during the 1990 field study.

Results of mysid shrimp (Table 27 and Figure 28) *in situ* toxicity tests indicated that: 1) Survival at the CTL Site ranged from 22.2 - 100.0%, averaging 80.9% (± 12.88%); 2) Survival at the TRT Site ranged from 8.3 - 100%, averaging 76.3% (± 22.66%); 3) Survival at the KWA Site ranged 77.8 - 100.0%, averaging 94.5% (± 5.55%). Statistical analysis indicated no significant between site differences in survival during the 1990 Study. Low survival was observed at the CTL (Groups 2 and 4) and TRT (Group 1) when cages were hung up on the tether line, resulting in aerial exposure and desiccation. Significant runoff of fenvalerate at the TRT Site on May 28 was not assessed due to the cage deployment problems just described.

Results of mummichog (Table 28 and Figure 29) *in situ* toxicity tests indicated that: 1) Survival at the CTL Site ranged from 93.3 - 100.0%, averaging 98.3% (± 0.91%); 2) Survival at the TRT Site ranged from 93.3 - 100%, averaging 98.3% (± 0.89%); and 3) Survival at the KWA Site ranged from 100 - 100%, averaging 100.0% (± 0%). Statistical analysis indicated no significant between site differences during the 1990 field study.

Table 26. Summary of survival in *penaeus species* at all sites during the 1990 field study. Pooled means with the same letter (A) were not significantly ($p > 0.05$) different.

Group #	Date	Site	% Survival	
			X	SE
1	5/24 - 28/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
2	5/28 - 6/1/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
3	6/1 - 5/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	96.7	3.33
4	6/5 - 9/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
5	6/9 - 13/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
6	6/13 - 17/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
7	6/17 - 21/90	CTL	100.0	0.00
		TRT	96.7	3.33
		KWA	96.3	3.70
8	6/21 - 23/90	CTL	96.3	3.70
		TRT	96.7	3.33
		KWA	96.3	3.33
1 - 8	5/24 - 6/23/90	CTL	99.5^	0.46 Range = 96.3 - 100.0
		TRT	99.2^	0.54 Range = 96.7 - 100.0
		KWA	98.7^	0.65 Range = 96.3 - 100.0

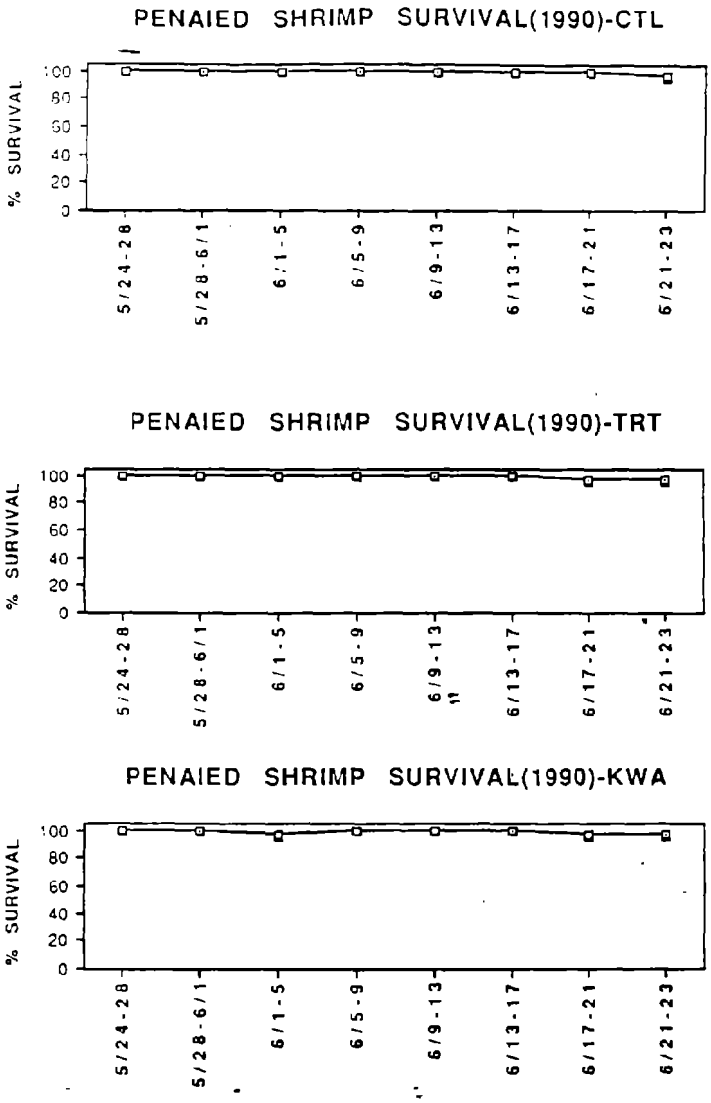


Figure 27. Survival of *Penaeus* species in field toxicity tests during the 1990 field study. Note the high survival at all sites.

Table 27. Summary of survival in *Mysidopsis bahia* at all sites during the 1990 field study. Posted means with the same letter (A) were not significantly ($p > 0.05$) different.

Group #	Date	Site	% Survival	
			X	SE
1	5/28 - 6/1/90	CTL	96.3	3.70
		TRT	8.3*	8.33
		KWA	100.0	0.00
2	6/1 - 5/90	CTL[1]	66.7	33.33
		CTL[2]	100.0	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
3	6/5 - 9/90	CTL	100.0	0.00
		TRT	96.7	3.33
		KWA	77.8	11.11
4	6/9 - 13/90	CTL[3]	22.20	22.20
		CTL[4]	100.0	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
1 - 4	5/28 - 6/13/90	CTL	80.9[A]	12.88 Range = 22.2 - 100.0
		TRT	76.3[A]	22.66 Range = 8.3 - 100.0
		KWA	94.5[A]	5.55 Range = 77.8 - 100.0

* = Low survival due to cage hanging up on tether resulting in aerial exposure and dessication.

[1] = Group 2[1] deployed 6/1 - 3/90 and resulting mortality occurred due to problems with cage deployment.

[2] = Group 2[2] deployed 6/3 - 5/90 and problem was corrected.

[3] = Group 4[3] deployed 6/9 - 13/90 and resulting mortality was due to problems with cage deployment.

[4] = Group 4[4] deployed 6/11 - 13/90 and deployment problem was corrected.

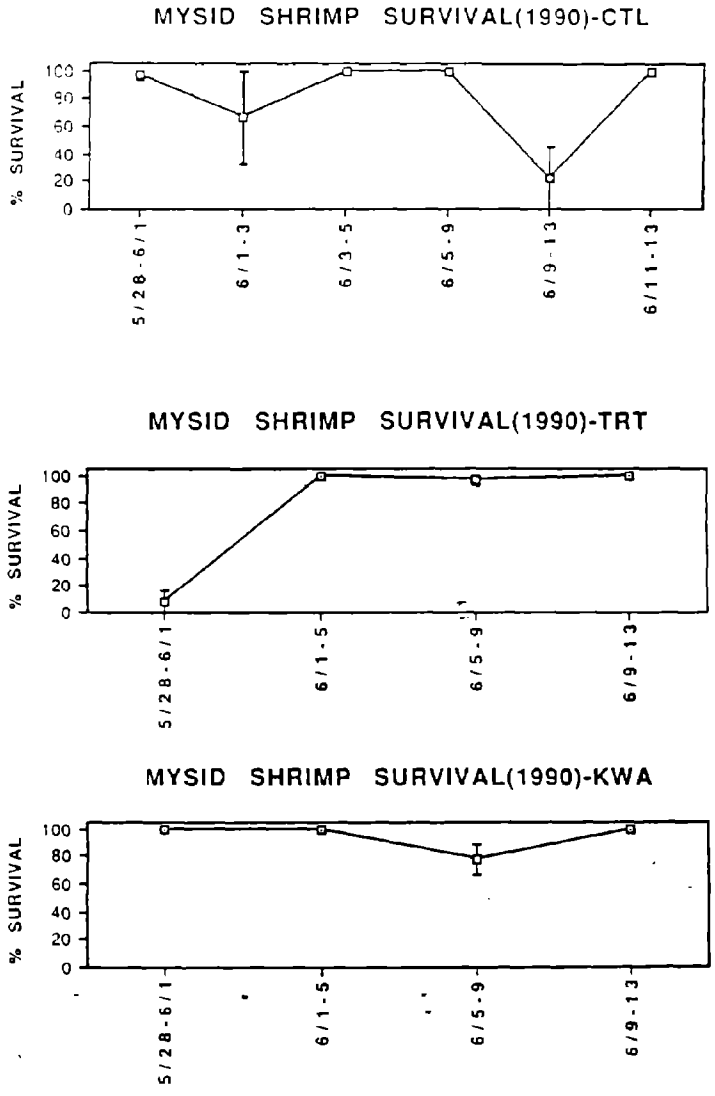


Figure 28. Survival of *Mysidopsis bahia* in field toxicity tests during the 1990 field study. Note the generally good survival at all sites.

Table 28. Summary of survival in *F. heteroclitus* at all sites during the 1990 field study. Pooled means with the same letter (A) were not significantly ($p > 0.05$) different.

Group #	Date	Site	% Survival	
			X	SE
1	5/24 - 28/90	CTL	100.0	0.00
		TRT	93.3	6.67
		KWA	100.0	0.00
2	5/28 - 6/1/90	CTL	96.3	3.70
		TRT	96.7	3.33
		KWA	100.0	0.00
3	6/1 - 5/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
4	6/5 - 9/90	CTL	93.3	6.67
		TRT	100.0	0.00
		KWA	100.0	0.00
5	6/9 - 13/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
6	6/13 - 17/90	CTL	96.7	3.33
		TRT	100.0	0.00
		KWA	100.0	0.00
7	6/17 - 21/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
8	6/21 - 23/90	CTL	100.0	0.00
		TRT	96.7	3.33
		KWA	100.0	0.00
1 - 8	5/24 - 6/23/90	CTL	98.3[A]	0.91 Range = 93.3 - 100.0
		TRT	98.3[A]	0.89 Range = 93.3 - 100.0
		KWA	100.0[A]	0.00 Range = 100.0 - 100.0

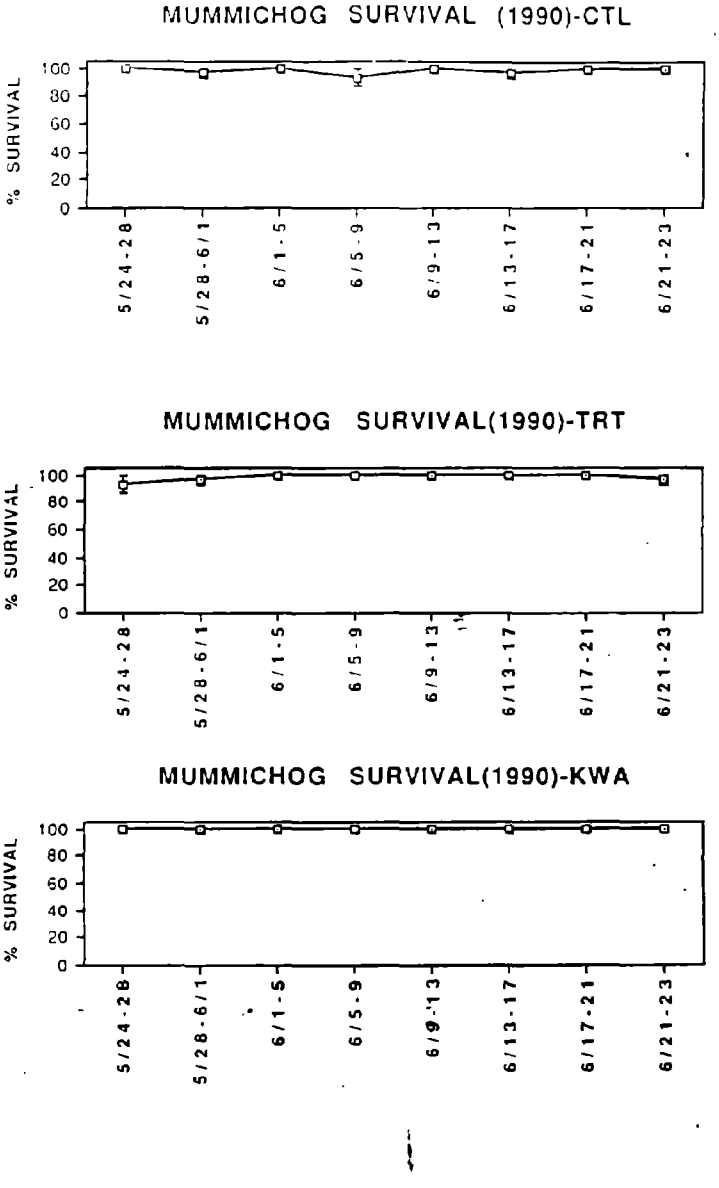


Figure 29. Survival of *Fundulus heteroclitus* in field toxicity tests during the 1990 field study. Note the high survival at all sites.

Results of juvenile sheepshead minnow (Table 29 and Figure 30) *in situ* toxicity test indicated that: 1) Survival at the CTL Site ranged from 60.9 - 100.0%, averaging 89.4% (± 9.53%); 2) Survival at the TRT Site ranged form 64.1 - 100.0%, averaging 91% (± 8.98%) and 3) Survival at the KWA Site ranged from 83.0 - 100%, averaging 95.8% (± 4.18%). Statistical analysis indicated no significant between site differences in survival. The reduced deployment time (7 - 8 days) used in 1990 (versus 14 - 15 days in 1989) appeared to greatly enhance survival. A seven - eight day deployment time appears optimal.

Results of juvenile *Menidia berylina* (Table 30 and Figure 31) survival in field toxicity tests indicated: 1) Survival at the CTL Site ranged from 0 - 40%, averaging 17.2% (± 5.20); 2) Survival at the TRT Site ranged from 0 - 100%, averaging 66.7% (± 22.61%); and 3) Survival at the KWA Site ranged from 0 - 65.7%, averaging 23.4% (± 12.91%) Statistical analysis indicated no significant between site differences in survival. Juvenile *M. berylina* appeared to have poor survival at all sites. Deployment in different cages and in different exclusion cage positions (surface and bottom) appeared to have little effect on survival (see Group 4 - CTL Site - Table 30). Other factors, such as extreme current flow or low dissolved oxygen levels in the tidal creek, may have been stressful enough to cause mortality.

4. Quality Assurance and Quality Control for Bioassay Organisms Used in Field Toxicity Tests during the 1990 Field Study

Results of quality assurance and quality control bioassays conducted during 1990, using endosulfan as a reference toxicant are listed in Table 31.

Results for adult *P. pugio* exposed to endosulfan indicated mortalities ranging from 0 - 20%, averaging 10% (± 5.78%) at 0.01 ug/L; from 60 - 100%, averaging 75% (± 9.58%) at 1.15 ug/L; and from 80 - 100%, averaging 90% (± 5.78%) at 2.50 ug/L. Control mortality ranged from 0 - 20%, averaging 5% (± 5.0%). The 96h LC50 for endosulfan in *P. pugio* during 1990 was 0.18 ug/L (CL = 0.08 - 0.39 ug/L) which compared favorable with previously reported 96h LC50 values of 0.25 - 1.01 ug/L (Scott et al, 1990) and with 1989 Quality Control bioassay results (LC50 = 0.18 ug/L with CL = 0.10 - 0.33 ug/L).

Table 29. Summary of survival in juvenile *Cyprinodon vareigatus* at all sites during the 1990 field study. Pooled mans with different letters (A) were not significantly ($p > 0.05$) different.

Group #	Date	Site	% Survival	
			X	SE
1	5/24 - 6/2/90	CTL	60.9*	15.48
		TRT[1]	64.1*	21.12
		KWA	83.3	16.70
2	6/2 - 10/90	CTL	100.0	0.00
		TRT[2]	100.0	0.00
		KWA	100.0	0.00
3	6/10 - 17/90	CTL	100.0	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
4	6/17 - 24/90	CTL	96.7	0.00
		TRT	100.0	0.00
		KWA	100.0	0.00
1 - 4	5/24 - 6/24/90	CTL	89.4^	9.53 Range = 60.9 - 100.0
		TRT	91.0^	8.98 Range = 64.1 - 100.0
		KWA	95.8^	4.18 Range = 83.0 - 100.0

[1] = TRT Group 1 deployed from 5/24 - 6/1/90.

[2] = TRT Group 2 deployed from 6/1 - 6/10/90.

* = Mortality was caused by extremely heavy situations in cages following heavy rains at ebb tide which eroded large quantities of sediment into Leadenwah Creek.

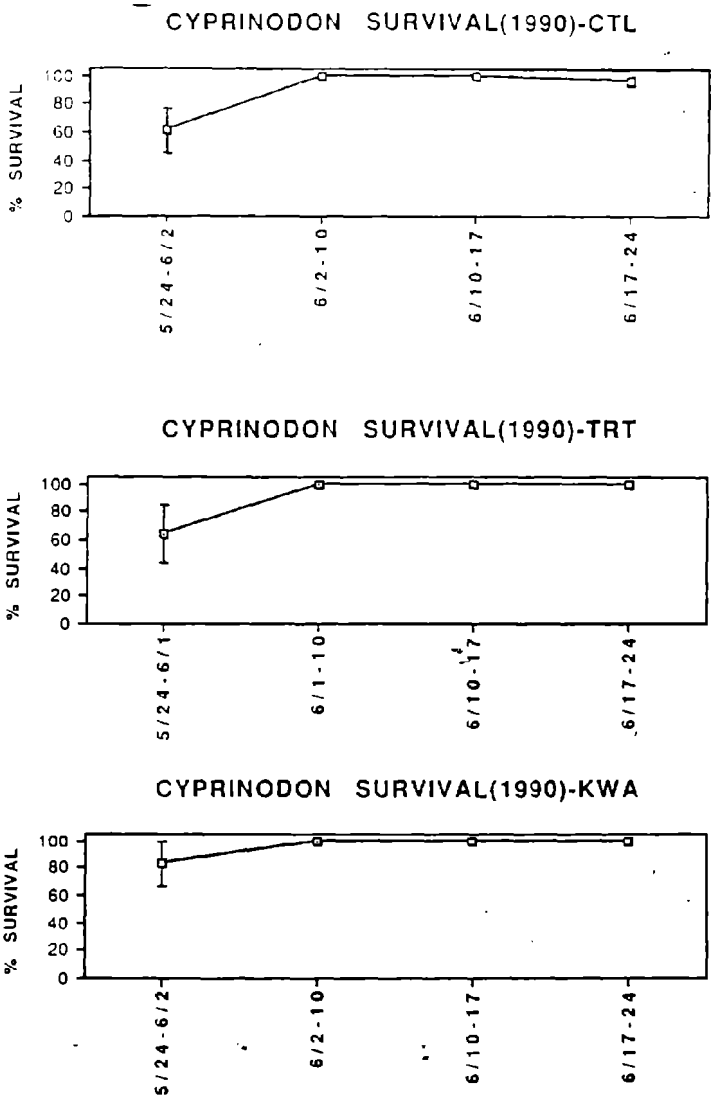


Figure 30. Survival of *Cyprinodon variegatus* in field toxicity tests during the 1990 field study. Note the generally high survival at all sites.

Table 30. Summary of survial in *M. berylina* at all sites during the 1990 field study. Pooled means with same letter (A) were not significantly ($p > 0.05$) different. All deployments were in *Menidia* cage unless other noted.

Group #	Date	Site	% Survival	
			X	SE
1	5/24 - 28/90	CTL	0.0	0.00
		TRT	0.0	0.00
		KWA	6.7	6.67
2	5/28 - 6/2/90	CTL	40.0	30.55
		TRT	100.0	0.00
		KWA	65.7	8.69
3	6/2 - 10/90	CTL[1]	36.7	12.02
		CTL[2]	14.0	4.0
		CTL[3]	6.7	6.67
		TRT	80.0	5.77
		KWA[4]	3.3	3.33
		KWA[5]	0.0	0.00
4	6/10 - 18/90	CTL[6]	10.0	0.00
		CTL[7]	23.3	23.33
		CTL[8]	6.7	3.33
		TRT	86.7	13.33
		KWA[9]	41.1	24.05
1 - 8	5/24 - 6/18/90	CTL	17.2[A]	5.20 Range = 0.0 - 40.0
		TRT	66.7[A]	22.60 Range = 0.0 - 100.0
		KWA	23.4[A]	12.91 Range = 0.0 - 65.7

[1] = Group 3[1] deployed 6/2 - 4/90 at CTL Site.

[2] = Group 3[2] deployed 6/4 - 7/90 at CTL Site.

[3] = Group 3[3] deployed 6/7 - 10/90 at CTL Site.

[4] = Group 3[4] deployed 6/4 - 8/90 at KWA Site.

[5] = Group 3[5] deployed 6/7 - 8/90 at KWA Site.

[6] = Group 4[6] deployed 6/10 - 13/90 in Cyprinodon cage at CTL Site on the surface.

[7] = Group 4[7] deployed 6/10 - 13/90 in Menidia cage at CTL Site on the bottom.

[8] = Group 4[8] deployed 6/10 - 13/90 in Cyprinodon cage at the CTL Site on the bottom.

[9] = Group 4[9] deployed 6/10 - 13/90 at KWA Site.

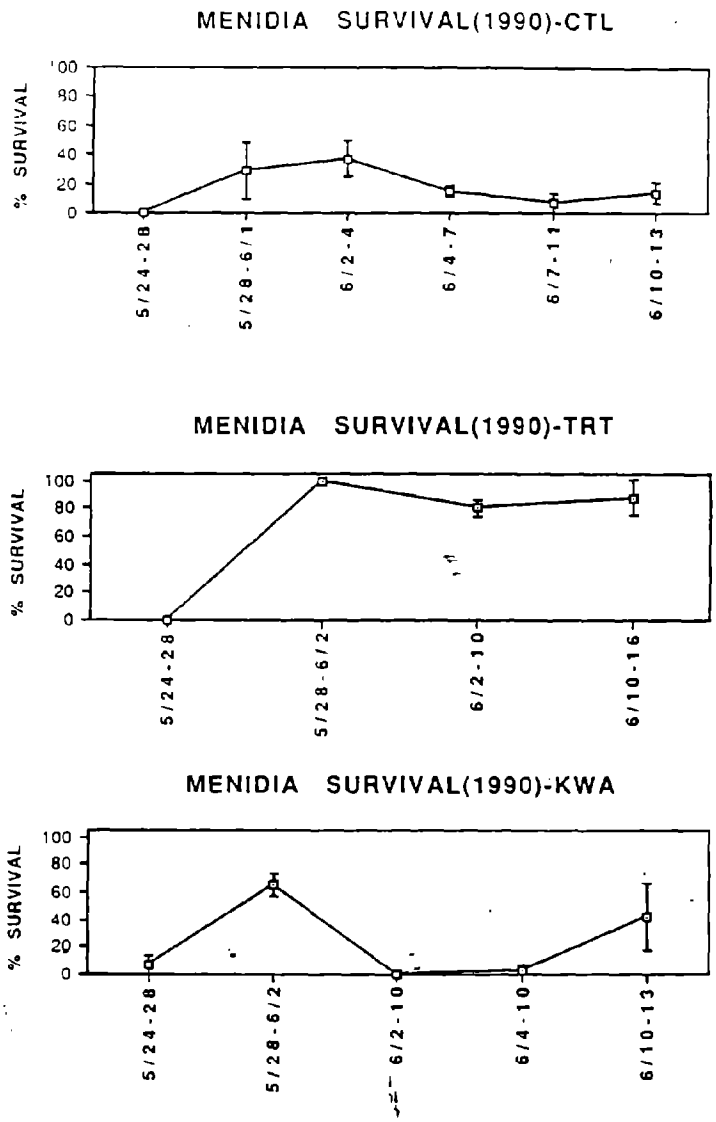


Figure 31. Survival of *Menidia berylina* in field toxicity tests during the 1990 field studies. Note the generally poor survival at all sites.

Table 31. Summary of Quality Control/Quality Assurance Bioassays using endosulfan as the reference toxicant for the five toxicity tests conducted over the course of the 1990 study.

Nominal Endosulfan Concentration	Species (*)	% Mortality[1]				Pooled Results	
		#1	#2	#3	#5	X	(± SE)
0.00	*P. pugio* (A)	0	20	0	0	5.0	(± 5.00)
0.01		0	20	20	0	10.0	(± 5.78)
1.15		60	100	80	60	75.0	(± 9.58)
2.50		100	80	100	50	90.0	(± 5.78)
						96h LC_{50} = 0.18 ug/L	
						(95% CL = 0.08 - 0.39 ug/L)	
0.00	*F. heteroclitus* (A)	0	0	0	0	0.0	(± 0.00)
1.15		0	0	0	0	0.0	(± 0.00)
2.50		60	20	25	0	26.3	(± 12.48)
5.00		100	80	100	80	90.0	(± 5.78)
						96h LC_{50} = 0.31 ug/L	
						(95% CL = 2.55 - 3.86 ug/L)	
0.00	*Penaeus sp.* (J)	0	0	0	0	0.0	(± 0.00)
0.01		20	0	0	0	5.0	(± 0.00)
1.15		40	80	100	40	65.0	(± 15.00)
2.50		80	80	100	60	80.0	(± 8.17)
						48h LC_{50} = 0.31 ug/L	
						(95% CL = 0.13 - 0.77 ug/L)	

Nominal Endosulfan Concentration	Species (*)	% Mortality[1]				Pooled Results	
		#1	#2	#3	#5	X	(± SE)
0.00	*C. variegatus* (J)	0	0	0	0	0.0	(± 0.00)
0.01		0	0	0	0	0.0	(± 0.00)
1.15		0	0	0	20	5.0	(± 5.00)
2.50		100	100	60	20	70.0	(± 19.15)
						96h LC_{50} = 1.97 ug/L	
						(95% CL = 1.66 - 2.34 ug/L)	
0.00	*M. berylina* (J)	0	25	20[2]	ND	15.0	(± 7.64)
0.01		20	0	20	ND	13.3	(± 6.67)
1.15		100	100	100	ND	100.0	(± 0.00)
2.50		100	100	100	ND	100.0	(± 0.00)
						96h LC_{50} = 0.07 ug/L	
						(95% CL = NC)	

* = Life Stage: A = Adult; J = Juvenile

1 = Exposure periods were 96h for all species expect *Penaeus species*, which was 48 hours.

2 = Handling stress caused mortality

NC = Confidence Limits not calculatedTable 31

Results for adult *F. heteroclitus* exposed to endosulfan indicated mortalities ranging from 0 - 0%, averaging 0% (± 0%) at 1.15 ug/L; from 0 - 60%, averaging 26.3% (± 12.48%) at 2.50 ug/L; and 80 - 100%, averaging 90% (± 5.78) at 5.00 ug/L. Control mortality ranged from 0 - 0%, averaging (± 0%). The 96h LC50 for endosulfan in *F. heteroclitus* during 1990 was 3.14 ug/L (CL = 2.55 - 3.86 ug/L) which compared favorably with previously reported LC_{50} values of 1.29 -1.45 ug/L (CL = 1.29 - 1.59 ug/L) by Scott et al (1990) and with results for 1989 QA/QC results (LC50 = 1.82 ug/L with CL = 1.18 - 2.83 ug/L). The slightly higher LC_{50} value in *F. heteroclitus* obtained during 1990 was largely the result of higher exposure temperature, particularly during the second and fourth tests. Given the inverse temperature coefficient for endosulfan, acute toxicity was reduced with these higher temperature. Results for 1989 and 1990 were not significantly different in statistical comparisons (upper and lower 95% CL overlap).

Results for juvenile penaied shrimp (*Penaeus aztecus* and *Penaeus setiferus*) exposed to endosulfan indicated 48h mortalities ranging form 0 -20 %, averaging 5% (± 5%) at 0.01 ug/L; from 40 - 100%, averaging 65% (± 15%) at 1.15 ug/L; and from 60 - 100%, averaging 80% (± 8.17%). Control mortality ranged from 0 -0%, averaging 0% (± 0%). The 48h LC_{50} for endosulfan in juvenile penaied shrimp was 0.31 ug/L (CL = 0.13 - 0.77 ug/L) which compared favorably with 1989 QA/QC results for penaied shrimp (LC_{50} = 0.18 ug/L with CL = 0.09 - 038 ug/L).

Results for juvenile *Cyprinodon variegatus* exposed to endosulfan indicated mortalities ranging from 0 - 0%, averaging 0% (± 0%) at 0.01 ug/L; from 0 - 20%, averaging 5% (± 5%) at 1.15 ug/L; and from 20 - 100%, averaging 70% (± 19.15%) at 2.50 ug/L. Control mortality ranged from 0 -0%, averaging 0% (± 0%). The 96h LC_{50} for endosulfan in juvenile *Cyprinodon variegatus* was 1.97 ug/L (CL = 1.66 - 2.34 ug/L) which was much higher than the QA/QC results for 1989 (96h LC_{50} = 0.31 ug/L with CL = 0.13 - 0.75 ug/L). The statistically higher LC_{50} obtained during 1990 were the result of the higher exposure temperatures during 1990 (inverse temperature coefficient previously discussed in *F. heteroclitus*) and the fact that U.S. EPA laboratory stocks of juvenile sheepshead minnow during 1990 were generally larger size juveniles (late stage) compared to 1989 stocks (early stage). The larger size juveniles would be more resistant than earlier staged juveniles. Similar results have been reported with adult and juvenile *F. heteroclitus* exposed to endosulfan (Scott et al, 1990).

Results for juvenile *Menidia berylina* exposed to endosulfan indicated mortalities ranging from 0 - 20%, averaging 13.3% (± 6.67%) at 0.01 ug/L; from 100 - 100%, averaging 100% (± 0%) at 1.15 ug/L; and from 100 - 100%, averaging 100% (± 0%) at 2.50 ug/L). Control mortality ranged 0 - 25%, averaging 15% (± 7.64%). The high control mortality was the result of handling stress in some instances, as this was the first time *Menidia berylina* had been used in toxicity tests. The 96h LC_{50} for endosulfan in juvenile *Menidia berylina* was 0.07 ug/L (CL = not calculated).

These results generally indicated that each group of organisms used in each field deployment were comparable. Although some variations were observed between groups much of these differences were related to extrinsic (exposure temperature) and intrinsic (different life history stage) factors encountered during the bioassay, which would account for these differences.

II. OYSTER ECOPHYSIOLOGY STUDIES, 1989-90

A. 1989 Studies

Results of oyster ecophysiology studies conducted at the CTL and TRT Sites during 1989 are listed in Tables 32-38 and Figures 32-38.

During May, 1989 at the mouth of Leadenwah Creek, where oysters were initially collected prior to transplantation, salinities ranged from 24 - 27 ppt, seawater temperatures ranged from 21.5 - 27°C, and dissolved oxygen from 6.45 - 7.05 mg/L (Table 32). During June, 1989 at the TRT Site salinities ranged from 20 - 25 ppt, water temperatures from 29.1 - 30.4°C, and dissolved oxygen levels from 6.00 - 7.20 mg/L. Physicochemical parameters were quite similar at the CTL Site, with salinities ranging from 21.0 - 28.5 ppt, seawater temperatures from 29.0-29.7°C, and dissolved oxygen levels from 6.30 - 6.70 mg/L. During July, 1989 salinities ranged from 21.5 - 29.0 ppt, seawater temperatures from 30.3-32.4°C, and dissolved oxygen levels from 5.80 - 7.00 mg/L at the CTL Site versus salinities ranging from 16.0 - 25.0 ppt, seawater temperatures from 27.9 - 33.6°C, and dissolved oxygen levels from 5.90 - 6.80 mg/L at the TRT Site. The lower salinities at the TRT Site during July were the result of heavy rainfall and runoff, which as a result, lowered salinities.

Table 32. Summary of physicochemical water quality parameters measured in oyster studies at the CTL, TRT and KWA Sites during 1989 - 90. Note the lower salinities at the TRT Site during June 1989 following periods of significant rainfall.

Date	Site	Salinity (ppt)	Dissolved Oxygen (mg/L)	Water Temperature (°C)
17-18 May, 1989	Mouth of Leadenwah Creek	24.0 - 27.0	6.74 - 7.05	21.5 - 27.0
12-13 June, 1989	CTL	21.0 - 28.5	6.30 - 6.70	29.0 - 29.7
14-15 June, 1989	TRT	20.0 - 25.0	6.00 - 7.20	29.1 - 30.4
10-11 July, 1989	CTL	21.5 - 29.0	5.80 - 7.00	30.3 - 32.4
12-13 July, 1989	TRT	16.0 - 25.0	5.90 - 6.80	27.9 - 33.6
16-17 May, 1990	Mouth of Leadenwah Creek	28.0 - 30.0	6.28 - 7.70	26.5 - 29.4
13 June, 1990	CTL	31.0 - 31.0	6.40 - 7.60	23.0 - 25.7
14 June, 1990	KWA	35.0 - 36.0	6.10 - 6.75	23.2 - 26.9
6 July, 1990	CTL	34.0 - 35.0	5.20 - 5.95	30.2 - 30.9
7 July, 1990	KWA	35.0 - 36.0	5.40 - 5.85	26.7 - 31.0

During 1989, significant runoff of fenvalerate was observed at the TRT Site on 6/5 - 6/89 (0.065 - 0.093 ug/L fenvalerate), 6/9/89 (0.021-0.022 ug/L fenvalerate) and on 6/15/89 (0.015 ug/L fenvalerate) and possible exposure to oysters may have occurred.

Results of condition index measured in oysters from both sites (Table 33; Figure 32) indicated there were no significant differences in between site comparisons during June - July, 1989. The initial condition index in May prior to transplantation, was 93.10 (± 2.57). Mean condition indices measured during June-July ranged from 64.60 (± 3.48) in June to 71.49 (± 4.48) in July at the TRT Site and ranged from 84.38 (±4.31) in June to 72.72 (± 3.53) in July at the CTL Site. These data indicate that immediately following transplantation condition indices at both sites declined nearly 30%. This was probably the result of spawning activities in oysters as noted earlier by Scott (1979) and Scott *et al.* (1990).

Results of *Perkinsus marinus* infection intensities analysis (Table 34; Figure 33) indicated low-moderate infection intensities of this oyster parasite at both sites. During June, infection intensities ranged from 2.08 (±0.34) at the TRT Site to 2.33 (±0.28) at the CTL Site. During July, infection intensities increased in oysters at both sites, ranging from 3.50 (±0.20) at the TRT Site to 3.17 (±0.39) at the CTL Site. Statistical analysis indicated that there were no significant between site differences observed. These results generally agree with earlier studies by Scott *et al.* (1990).

Table 33. Summary of condition indices measured in oysters at the CTL, TRT, and KWA sites 1989-90. Statistical analysis indicated no significant differences in temporal comparisons between paired sites.

Parameter	Date	Site	X	SE
Condition Index	May, 1989	CTL	93.10	2.57
	June, 1989	CTL	84.38	4.31
		TRT	64.60	3.48
	July 1989	CTL	72.72	3.53
		TRT	71.49	4.48
	May, 1990	CTL	61.11	3.91
	June, 1990	CTL	84.49	8.57
		KWA	79.15	9.68
	July, 1990	CTL	79.77	6.62
		KWA	69.35	4.12

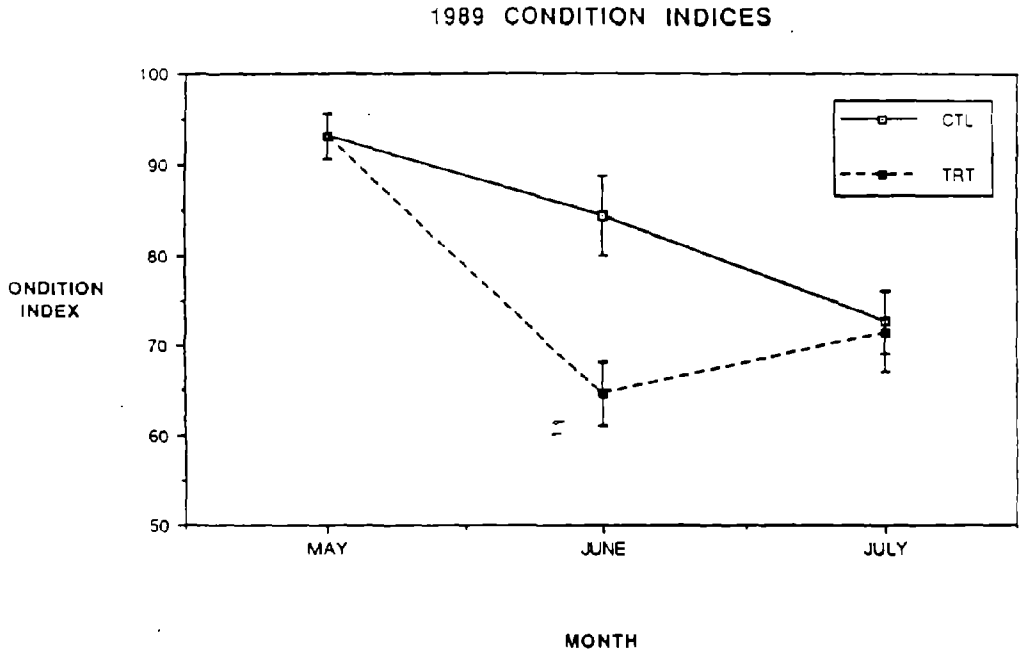


Figure 32. Condition index in oysters deployed at the CTL and TRT Sites during the 1989 field study. Note the similarities in condition indices for oysters from both sites.

Table 34. Summary of *Perkinsus marinus* infection intensities measured at the CTL, TRT and KWA Sites during 1989-90. Statistical analysis indicated no significant differences in temporally paired comparisons between the CTL and TRT Sites (1989) and the CTL and KWA Sites (1990).

Parameter	Date	Site	X	SE
Perkinsus marinus Infection Intensity	June, 1989	CTL	2.33	0.28
		TRT	2.08	0.34
	July, 1989	CTL	3.17	0.39
		TRT	3.50	0.20
	May, 1990	CTL	2.17	0.27
	June, 1990	CTL	3.33	0.33
		KWA	2.94	0.29
	July, 1991	CTL	2.77	0.40
		KWA	3.77	0.51

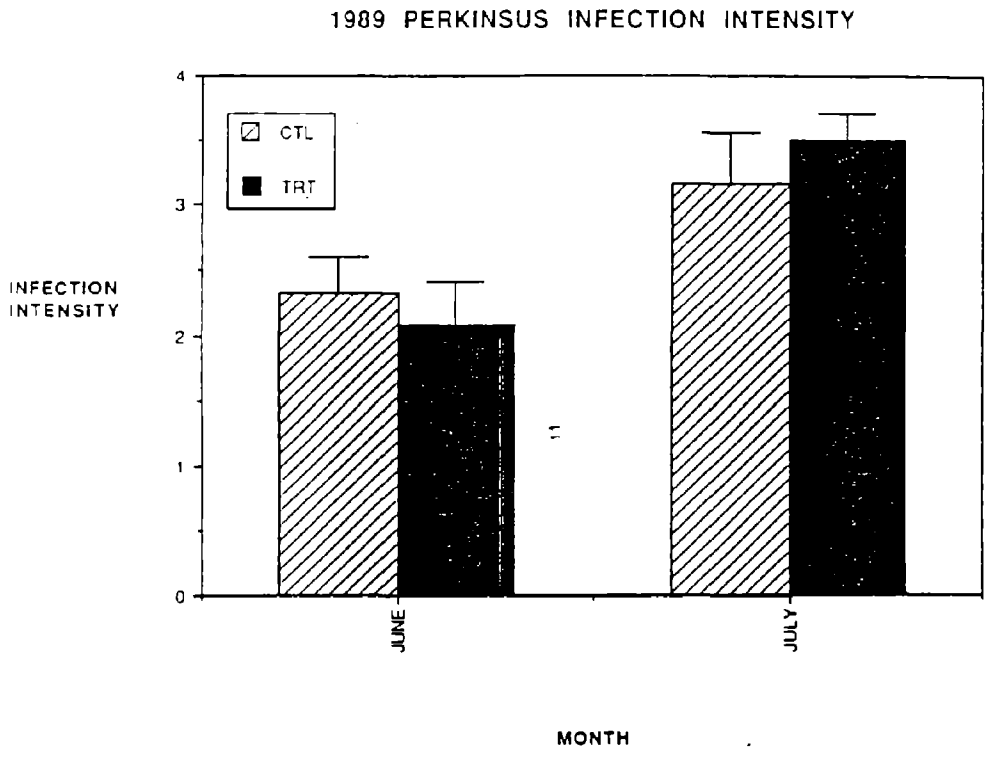


Figure 33. *Perkinsus marinus* infection intensities in oysters from the CTL and TRT Sites during the 1989 field study. Note the similarities in infection intensities for oysters from both sites.

Results of *in situ*, whole animal respiration rate determinations are listed in Table 35. Initially, a mean respiration rate (23°C) of 1.020 ml/0.685 g/h (±0.070) was measured in oysters during May, prior to transplantation. Following transplantation, June respiration rates (25°C) ranged from 1.600 ml/0.685 g/h (±0.080) at the TRT Site to 1.290 ml/0.685 g/h (±0.100) at the CTL Site. During July, (30°C) respiration rates ranged from 2.470 ml/0.685 g/h (±0.100) at the TRT Site to 2.250 ml/0.685 g/h (±0.110) at the CTL Site. Statistical analysis indicated there were significant ($p \leq 0.002$) between site differences in respiration rates observed during June following periods of low salinity associated with fenvalerate runoff at the TRT Site. An earlier study by Scott (1979) reported similar gill and mantle respiration rates as were observed in whole animals at the CTL Site during May - June, in oysters from Leadenwah Creek.

Respiration rates in oysters from both sites increased during May - July, primarily due to increased ambient seawater temperatures (May-21.5 - 27.5°C to July- 27.9 -33.6°C). Oysters are poikilothermic organisms, whose metabolic rates conform directly with ambient temperatures. As a result, respiration rates in oysters from each site varied temporally due to changes in exposure temperature. To compensate for this effect, respiration rates for each sampling period were Q_{10} adjusted at 23°C (May), 25°C (June), and 30°C (July), so that temporal comparisons between groups, could be made (Table 35; Figure 34). Q_{10} respiration adjustments allow the physiological effects of pesticide runoff to be better elucidated by normalizing the effects of temperature on respiration, so that pesticide effects can be discerned.

At 23°C, the initial Q_{10} adjusted respiration rate for oysters during May, prior to transplantation was 1.020 ml/0.685 g/h (±0.070). During June, Q_{10} adjusted respiration rates ranged from 1.250 ml/0.685 g/h (±0.060) at the TRT Site to 0.950 ml/0.685 g/h (±0.080) at the CTL Site.

Table 35. Summary of Q_{10} Adjusted Respiration Rates ($m/0_2/0.685g/h$) in oysters deployed at the CTL, TRT and KWA Sites during 1989-90. Statistical analysis indicated significant (*) differences ($p \leq 0.05$) in paired comparisons between the CTL and TRT Sites (1989) and the CTL and KWA Sites (1990) as indicated in the table.

Parameter	Temp (°C)	Date	Site	X	SE
Respiration Q_{10} Adjusted ($ml/0_2/0.685g/h$)	23[1]	May, 1989	CTL	1.020	0.070
	25			1.220	0.090
	30			1.720	0.120
	23	June, 1989	CTL	0.950	0.080
	25[1]			1.290	0.100
	30			2.140	0.170
	23		TRT	1.250 *(0.005)	0.060
	25[1]			1.600 *(0.002)	0.080
	30			2.460	0.130
	23	July, 1989	CTL	1.000	0.050
	25			1.350	0.060
	30[1]			2.250	0.110
	23		TRT	1.260 *(0.001)	0.050
	25			1.600 *(0.001)	0.060
	30[1]			2.470	0.100
	23[1]	May, 1990	CTL	1.080	0.060
	25			1.290	0.070
	30			1.820	0.100
	23	June, 1990	CTL	1.070	0.020
	25[1]			1.260	0.030
	30			1.750	0.040
	23		KWA	0.900 *(0.01)	0.040
	25[1]			1.180	0.050
	30			1.880	0.080
	23	July, 1990	CTL	1.120	0.080
	25			1.320	0.090
	30[1]			1.840	0.130
	23		KWA	1.000	0.040
	25			1.310	0.050
	30[1]			2.100	0.080

* = Significantly different in paired comparisons between CTL and TRT (1990) and CTL and KWA (1990) at each respective date and temperature. Values in parentheses ( ) are P values.

[1] = Actual time periods and temperatures respiration measurements were taken. Values for other time periods and temperatures combinations are Q_{10} derived values.

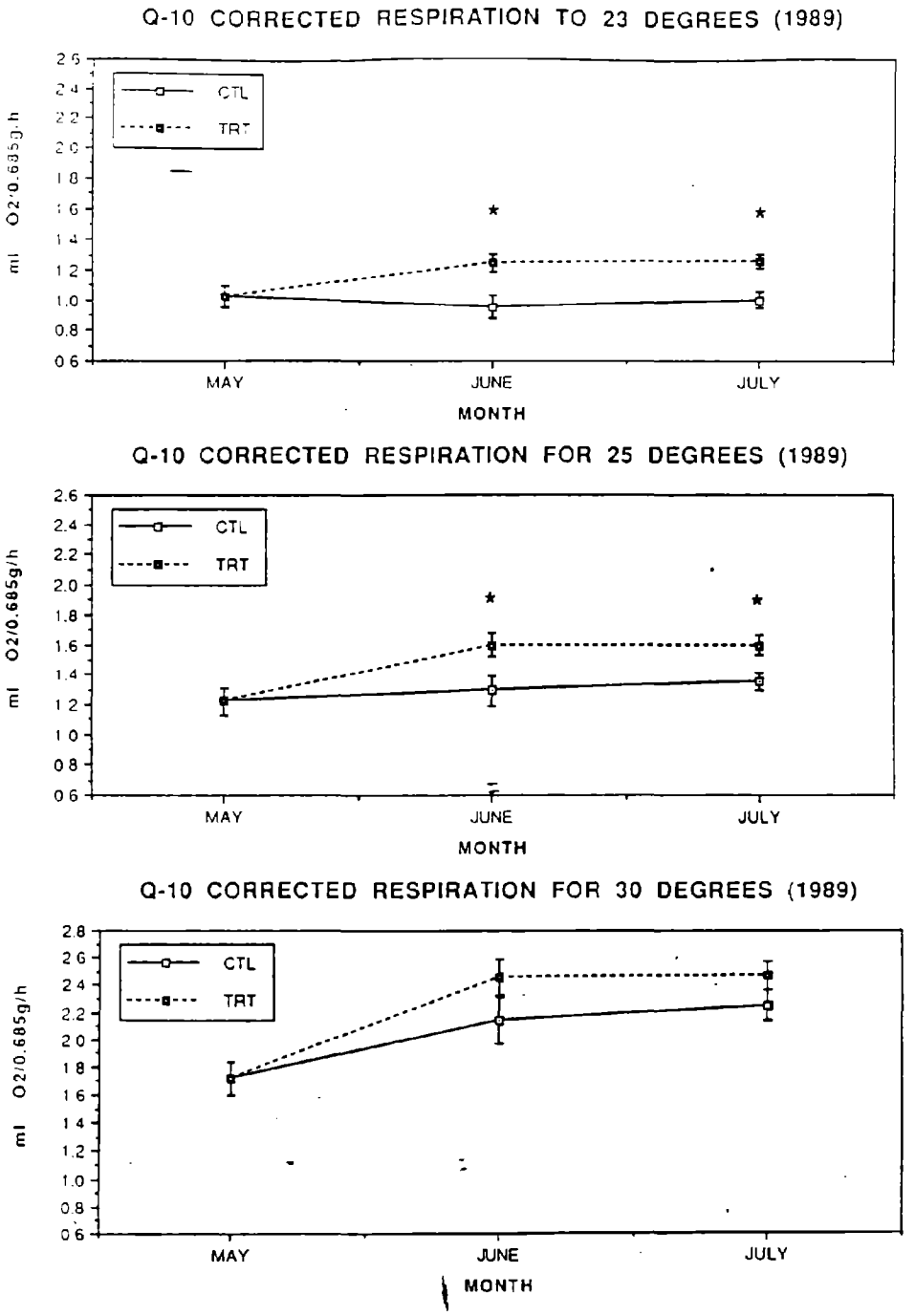


Figure 34. Q10 adjusted respiration rates (ml/0.685g/h) for oysters at three exposure temperatures (23°, 25°, and 30° C) during the 1989 field study. Note the increased respiration rates in TRT Site oysters at 23° and 25°C.

During July, Q_{10} adjusted respiration rates ranged from 1.260 ml/0.685 g/h (± 0.050) at the TRT Site to 1.000 ml/0.685 g/h (± 0.050) at the CTL Site. Statistical analysis clearly indicated that there were significant ($p \leq 0.001$-0.005) between site differences in Q_{10} adjusted respiration rates during June and July at 23°C. At 23°C, respiration rates in oysters at the TRT Site were much higher than at the CTL Site. Also note that Q_{10} adjusted respiration rates at the CTL Site were virtually unchanged from May through July.

At 25°C, the initial Q_{10} adjusted respiration rate was 1.220 ml/0.685 g/h (± 0.090) during May, prior to transplantation. During June, Q_{10} adjusted respiration rates at 25°C, ranged from 1.600 ml/0.685 g/h (± 0.080) at the TRT Site to 1.290 ml/0.685 g/h (± 0.100) at the CTL Site. During July, Q_{10} adjusted respiration rates at 25°C, ranged from 1.600 ml/0.685 g/h (± 0.060) at the TRT Site to 1.35 ml/0.685 g/h (± 0.060) at the CTL Site. Statistical analysis indicated that there were significant ($p \leq 0.001$-0.002) between site differences in Q_{10} adjusted respiration rates observed during June and July at 25°C. Respiration rates were higher in TRT Site oysters while remaining relatively constant at the CTL Site.

At 30°C, the initial Q_{10} adjusted respiration rate for oysters collected in May, prior to transplantation was 1.720 ml/0.685 g/h (± 0.120). During June, Q_{10} adjusted respiration rates at 30°C, ranged from 2.460 ml/0.685 g/h (± 0.130) at the TRT Site to 2.140 ml/0.685 g/h (± 0.170) at the CTL Site. During July, Q_{10} adjusted respiration rates at 30°C, ranged from 2.470 ml/0.685 g/h (± 0.100) at the TRT Site to 2.250 ml/0.685 g/h (± 0.110) at the CTL Site. Statistical analysis indicated that there were no significant between site differences observed in Q_{10} adjusted respiration rates at 30°C during June and July.

Figure 35 depicts the mean Q_{10} standardized respiration rates for oysters at the CTL and TRT Sites from May - July for the 23-30°C temperature range observed. Note the significantly ($p \leq 0.0001$-0.0365) higher respiration rates at all temperatures (23-30°C), in oysters from the TRT Site when compared to CTL Site organisms. These results suggest that metabolic rates in oysters from the TRT Site were significantly higher at temperatures ranging from 23-30°C. This would be at the upper limits of their zone of compatibility or capacity adaptations for temperature exposure. These differences in respiration rates may, in part, be the result of fenvalerate exposure in oysters at the TRT Site, although other factors such as low salinity must also be considered. Low salinity (< 5ppt) and resulting reduced particulate levels (including phytoplankton) may also be significant factors, which may adversely affect oysters. For example, low salinity (< 5ppt) exposure per se, would cause oysters to close their valves

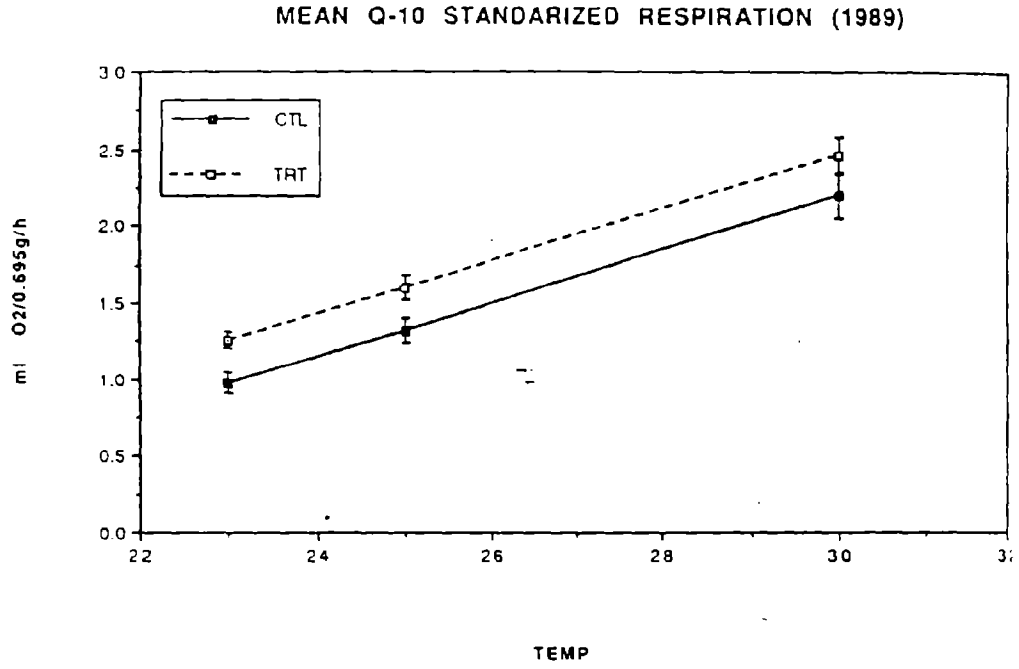


Figure 35. Mean Q10 standardized respiration rates (ml/0.685g/h) measured during the 1989 field study. Note the significantly increased respiration rates at all temperatures tested (23-30°C) for TRT Site oysters.

and utilize reverse glycolysis to maintain metabolic activity but with resulting increased respiration rates due to the oxygen debt incurred. Respiration rates during hypoxia would vary directly with temperature (Scott, 1979); however, respiration rates during low salinity exposures ranging from 7-10 ppt, would not affect gill respiration rates in oysters (Scott *et al.*, 1985). It is extremely difficult to differentiate effects from low salinity and fenvalerate exposure in the field, since both factors may co-occur during runoff events. Results from this study suggest that exposure to agricultural nonpoint source insecticide runoff and resulting low salinity conditions increased the cost of maintenance metabolism in oysters adjacent to agricultural sites.

Results of nitrogen excretion rate measurements are listed in Table 36 and Figure 36. During May, the initial nitrogen excretion rate was 2.30 ug atoms N/g/h (± 0.29 ug atoms N/g/h). During June, nitrogen excretion rates increased at the TRT Site to 9.70 ug atoms N/g/h (± 1.54 ug atoms N/g/h) compared to only 3.84 ug atoms N/g/h (± 0.60 ug atoms N/g/h) in CTL Site oysters. During July, nitrogen excretion rates decreased at the TRT Site to 4.44 ug atoms N/g/h (± 0.33 ug atoms N/g/h) which was comparable to levels of 3.00 ug atoms N/g/h (± 0.74 ug atoms N/g/h) at the CTL Site. Statistical analysis indicated that nitrogen excretion rates during June (peak of the tomato growing season and four days post fenvalerate exposure) were significantly ($p \leq 0.01$) higher in TRT Site oysters. On 5, 6 and 9 June, significant (> 1.25 cm/day) rainfall occurred at the TRT Site, which resulted in substantial runoff of fenvalerate (0.065-0.093 ug/L on 6/5 - 6/89 and 0.021 - 0.022 ug/L on 6/9/89) and concomitant periods of extended low salinity (< 10 ppt) exposure. Oysters exposed to low salinity will catabolize amino acids (ninhydrin positive - glycine, alanine and taurine) in order to osmoregulate. An earlier study by Scott *et al.* (1985) reported that low salinity does not significantly affect respiration rate; however, other studies have shown that low salinity may significantly increase nitrogen excretion rates in mussels (Widdows *et al.*, 1981) and fish (Scott *et al.*, 1987). Fenvalerate exposure caused significantly increased respiration rates in juvenile crustaceans which was enhanced by low salinity osmotic stress (Mckenny and Hamaker, 1984). Exposure of fish to fenvalerate appeared to have no effect on nitrogen excretion rates (Scott *et al.*, 1990). Results from this study indicated significantly increased nitrogen excretion rates in oysters at the TRT Site exposed to low salinity, fenvalerate agricultural runoff during June. By July, nitrogen excretion rates in oysters at the TRT Site decreased to levels comparable to CTL Site oysters.

Table 36. Summary of Nitrogen Excretion Rates (ug atoms N/g/h) in oysters deployed at the CTL, TRT and KWA Sites during 1989 - 90. Asterisks (*) indicate where paired comparisons were significantly ($p \leq 0.05$) different.

Parameter	Date	Site	X	SE
Nitrogen Excretion (ug atoms N/g/h)	May, 1989	CTL	2.30	0.29
	June, 1989	CTL	3.84	0.60
		TRT	9.70 *($p \leq 0.01$)	1.54
	July, 1989	CTL	3.00	0.76
		TRT	4.44	0.33
	May, 1990	CTL	10.06	1.46
	June, 1990	CTL	11.70	1.63
		KWA	9.34	1.17
	July, 1990	CTL	5.36	0.97
		KWA	9.51	2.87

* = Signifcantly different from paired control value.
Values in ( ) were p values.

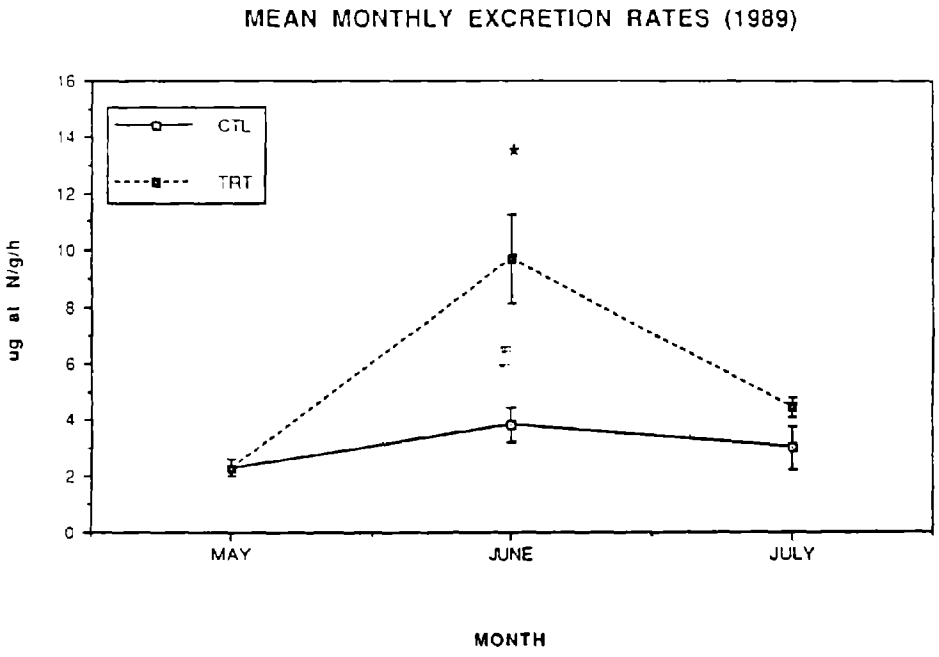


Figure 36. Ammonia nitrogen excretion rates (ug atoms N/g/h) in oysters deployed during the 1989 field study. Note the increased nitrogen excretion rate in TRT Site oysters during June, following periods of significant fenvalerate-low salinity runoff conditions.

Results of O/N Ratios are listed in Table 37 and Figure 37. The initial O/N ratio measured in May was 65.04 (± 9.66). During June, O/N ratios ranged from 50.61 (± 10.12) at the TRT Site to 74.74 (± 11.38) at the CTL Site. During July, O/N ratios ranged from 84.39 (± 10.99) at the TRT Site to 151.91 (± 25.96) at the CTL Site. Statistical analysis indicated there were significant ($p \leq 0.009$) differences in between site comparisons during July as higher O/N ratios were measured at the CTL Site.

Results of Q_{10} adjusted O/N ratios are listed in Tables 38 and Figure 38. The initial Q_{10} adjusted O/N ratio in May was 74.96 (± 10.13). During June, Q_{10} adjusted O/N ratios ranged from 31.43 (± 6.48) to 46.79 (± 6.83) at the CTL Site. During July, Q_{10} adjusted O/N ratios ranged from 50.54 (± 5.22) at the TRT Site to 82.20 (± 13.91) at the CTL Site. Statistical analysis indicated there were no significant differences in between site comparisons of Q_{10} adjusted O/N ratios during June. During July, Q_{10} adjusted O/N ratios were significantly ($p \leq 0.03$) lower at the TRT Site.

O/N ratios and Q_{10} adjusted O/N ratios were generally above 40 throughout the 1989 study, suggesting that oysters were healthy with a dominance of lipid and carbohydrate metabolism, with minor protein catabolism (NAS, 1980). The generally lower values measured at the TRT Site were indicative of higher nitrogen production by oysters there. This higher nitrogen out put by oysters at the TRT Site was in all likelihood, a metabolic adaptation (i.e. osmoregulation) to lower salinity conditions there, resulting from agricultural runoff. To compensate oysters catabolize protein to maintain homeosmocity; as a result increased nitrogen excretion will occur, with resulting decreased O/N ratios. Fenvalerate exposure must also be considered since fenvalerate may inhibit ATPase. This enzyme is important in the maintenance of osmotic balance (i.e. $Na^{+}K^{+}Mg^{++}$ ATPase).

Table 37. **Summary of O/N Ratios measured in oysters deployed at the CTL, TRT and KWA Sites during 1989 - 90. Asterisks (*) indicate where paired statistical comparisons were significantly ($p \leq 0.05$) different.**

Parameter	Date	Site	X	SE
O/N Ratio	May, 1989	CTL	65.04	9.66
	June, 1989	CTL	74.72	11.38
		TRT	50.61	10.12
	July, 1989	CTL	151.91	25.96
		TRT	84.39 *($p \leq 0.009$)	10.99
	May, 1990	CTL	29.16	7.13
	June, 1990	CTL	16.78	1.79
		KWA	26.86	6.70
	July, 1990	CTL	63.11	7.82
		KWA	65.12	17.48

* = Significantly different in comparisons with paired control values.
Values in ( ) were p values.

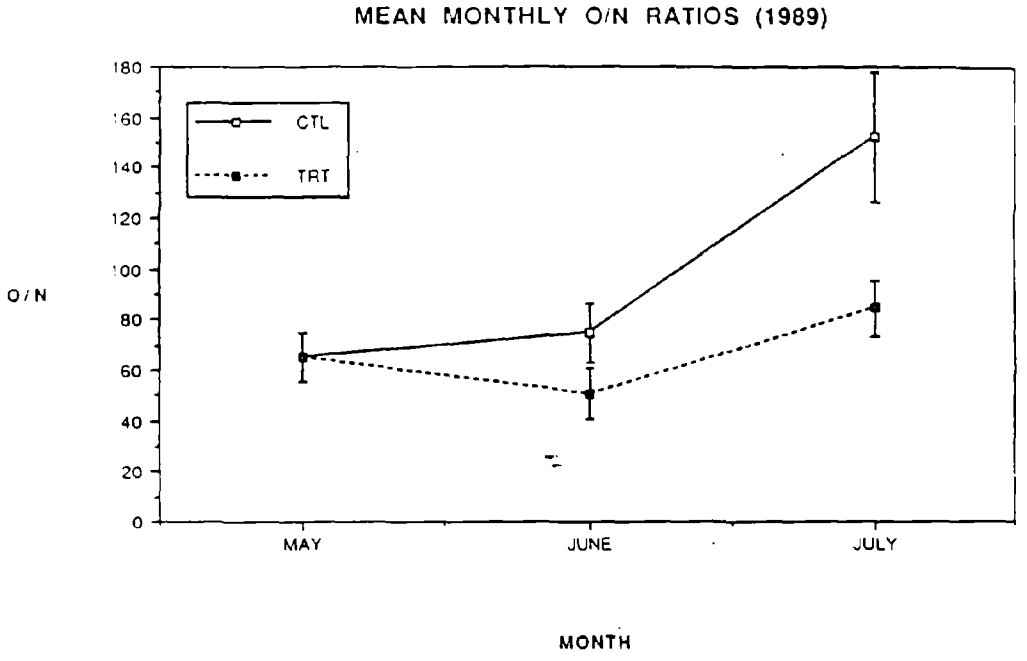


Figure 37. Mean O/N Ratios in oysters deployed during the 1989 field study. Note how O/N ratios were significantly lower at the TRT Site during July.

Table 38. Summary of Q_{10} Corrected O/N Ratios in oysters deployed at the CTL, TRT and KWA Sites during 1989 - 90. Asterisks (*) indicate where paired statistical comparisons were significantly ($P \leq 0.05$) different.

Parameter	Date	Site	X	SE
Q/10 Corrected O/N Ratios	May, 1989	CTL	74.96	10.13
	June, 1989	CTL	46.79	6.83
		TRT	31.43	6.48
	July, 1989	CTL	82.20	13.91
		TRT	50.54 *($p \leq 0.03$)	5.22
	May, 1990	CTL	23.40	5.85
	June, 1990	CTL	16.35	1.90
		KWA	23.28	5.56
	July, 1990	CTL	40.91	5.51
		KWA	40.39	12.57

* = Significantly different in comparison with paired control value.
Values in ( ) were p values.

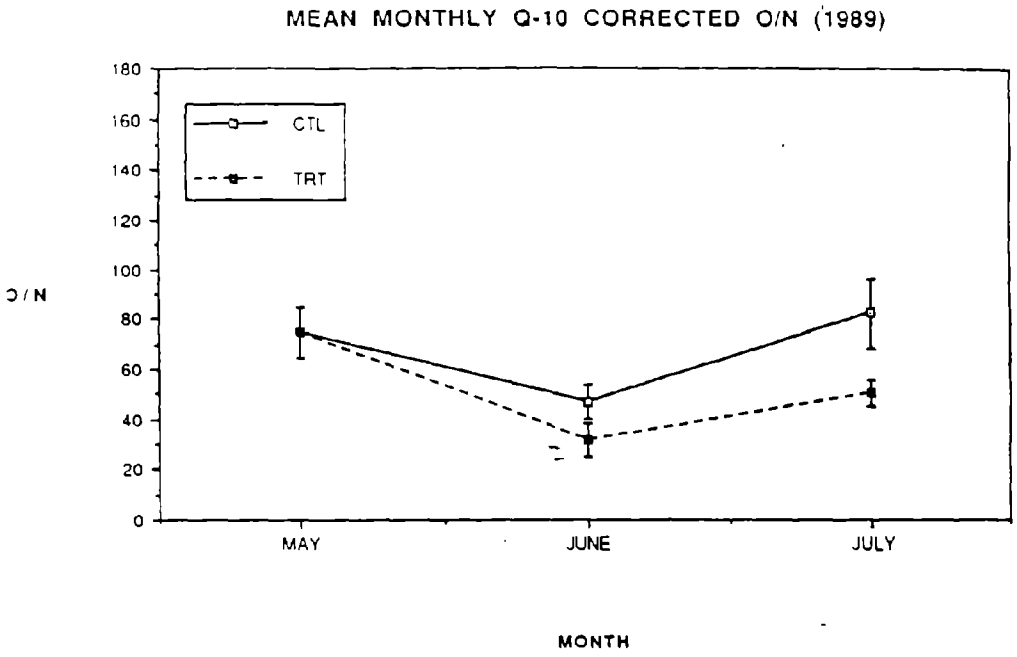


Figure 38. Mean Q10 adjusted O/N Ratios in oysters deployed during the 1989 field study. Note how O/N ratios were significantly lower in July at the TRT Site.

B. 1990 Results

Results of oyster ecophysiology studies conducted at the CTL and KWA Sites during 1990 are listed in Tables 32-38 and Figures 39-45.

During May, 1990 at the mouth of Leadenwah Creek, where oysters were initially collected prior to transplantation at the CTL and KWA Sites, salinities ranged from 28-30 ppt, seawater temperatures from 26.5-29.4°C, and dissolved oxygen from 6.28-7.70 mg/L (Table 32). During June, 1989 at the CTL Site, salinities ranged from 31.0-31.0 ppt, water temperatures from 23.0-25.7°C, and dissolved oxygen from 6.40-7.60 mg/L. At the KWA Site, salinities (35.0-36.0 ppt), water temperatures (23.2-26.9°C), and dissolved oxygen levels (6.40-7.60 mg/L) were quite similar to the CTL Site during June, 1990. During July, 1990 salinities ranged from 35.0-36.0 ppt, water temperatures from 26.7-31.0°C, and dissolved oxygen levels from 5.40-5.85 mg/L at the KWA Site versus salinities ranging from 34.0-35.0 ppt, water temperatures from 30.2-30.9°C, and dissolved oxygen levels from 5.20-5.95 mg/L at the CTL Site. Generally physicochemical parameters were similar at both sites during June and July, 1990. The small amount of rainfall during this study resulted in very little agricultural runoff. As a result salinities remained high throughout the study.

During 1990, only slight runoff (concentrations < 96h LC_{50} values for most sensitive estuarine species) of azinphosmethyl was observed at the KWA Site on 6/15/90 (0.024-0.062 μg/L). Oysters at the KWA Site may have been potentially exposed to azinphosmethyl as a result. No significant levels of pesticides were observed at the CTL Site during 1990.

Results of condition index measured in oysters from both sites (Table 33; Figure 39) indicated there were no significant differences in condition index in between site comparisons during June and July, 1990. The initial condition index in May 1990 prior to transplantation averaged 61.11 (± 3.91). Condition indices measured in June and July ranged from 69.35 (± 4.12) - 79.15 (± 9.68) at the KWA Site and from 79.77 (± 6.62) - 84.49 (± 8.57) at the CTL Site. These data indicated that immediately following transplantation, condition indices at both sites increased nearly 30%. This was the result of glycogen accumulation in oysters at these sites prior to spawning. Earlier studies by Scott (1979), Scott *et al.*, (1990) and 1989 results reported in this study indicated a slightly earlier period (May) of glycogen accumulation and spawn at Leadenwah Creek. Results for 1990, indicated that glycogen accumulation were delayed until June when resulting spawning activity occurred in July at both sites.

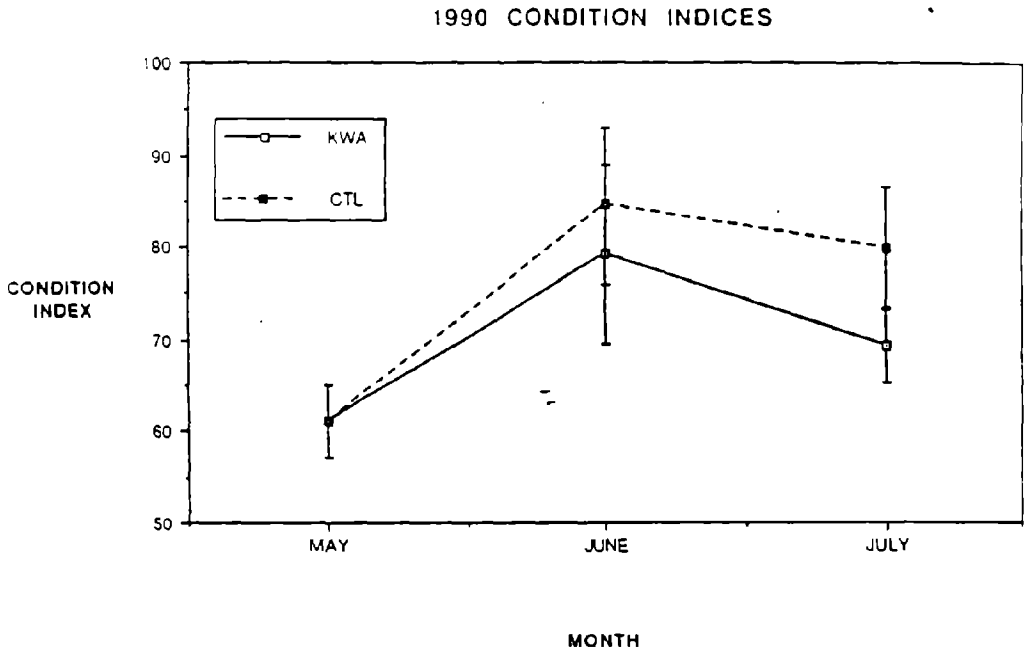


Figure 39. Condition index in oysters deployed at the CTL and KWA Sites during the 1990 field study. Note the similarities in condition indices in oysters at both sites.

Results of *Perkinsus marinus* intensity analysis (Table 34; Figure 40) indicated low - moderate infection intensities of this oyster parasite at both sites. The initial infection intensity was 2.17 (± 0.27) in oysters collected from Leadenwah Creek during May, prior to transplantation. During June, infection intensities ranged from 2.94 (± 0.29) at the KWA Site to 3.33 (± 0.33) at the CTL Site. During July infection intensity increased, in oysters at the TRT Site with intensities averaging 3.77 (± 0.51). At the CTL Site, intensities decreased slightly, averaging 2.77 (± 0.40). Statistical analyses indicated that there were no significant between site differences observed. These results generally agreed with earlier studies by Scott *et al.*, (1990) and results for 1989 in this study.

Results of *in situ* whole animal respiration rate determinations are listed in Table 35 and Figure 41. Initially, respiration rates averaged 1.080 ml/0.685 g/h (± 0.060 ml/0.685 g/h) in oysters collected during May, 1989 prior to transplantation. During June respiration rates ranged from 1.180 ml/0.685 g/h (± 0.050 ml/0.684 g/h) at the KWA Site to 1.260 ml/0.685 g/h (± 0.030 ml /0.685 g/h) at the CTL Site. During July, respiration rates ranged from 2.100 ml/0.685 g/h (± 0.080 ml/0.685 g/h) at the KWA Site to 1.840 ml/0.685 g/hr (± 0.130 ml/0.685 g/h) at the CTL Site. Statistical analysis indicated there were no significant between site differences in oyster respiration rates during June and July. An earlier study by Scott (1979) indicated similar gill and mantle tissue respiration rates during May-July in oysters at Leadenwah Creek.

Respiration rates in oysters from both sites increased from May-July, primarily due to increased ambient seawater temperatures (May - 26.5 - 29.4°C to 26.7 - 31.0°C during July). Oysters are poikilothermic organisms, whose metabolic rates will conform directly with ambient temperatures as a result. Respiration rates in oysters from each site varied temporally, due to changes in exposure temperature. To compensate for this effect, the Q-10s for each respiration determinations from each sampling were calculated at 23°C (May), 25°C (June) and 30°C (July) so that temporal comparisons between groups could be made (Table 35; Figure 41). At 23°C, the initial Q_{10} adjusted respirations for oysters during May prior to transplantation was 1.080 ml/0.685 g/h (± 0.060 ml/0.685 g/h). During June, Q_{10} adjusted respiration rates at 23°C ranged from 0.900 ml/0.685 g/h (± 0.040 ml/0.685 g/h) at the KWA Site to 1.070 ml/0.685 g/h (± 0.020 ml/0.685 g/h) at the CTL Site. Statistical analysis indicated significant ($p \leq 0.01$) between site differences, as respiration rates were decreased during June at the KWA Site. During July, Q_{10} adjusted respiration rates at 23°C ranged from 1.000 ml/0.685 g/H (± 0.040 ml/0.685 g/h) at the KWA Site to 1.120 ml/0.685 g/h (± 0.080 ml/0.685 g/h) at the CTL Site. Statistical analysis indicated, there were no significant between site differences observed in Q_{10} adjusted respiration rates during July at 23°C.

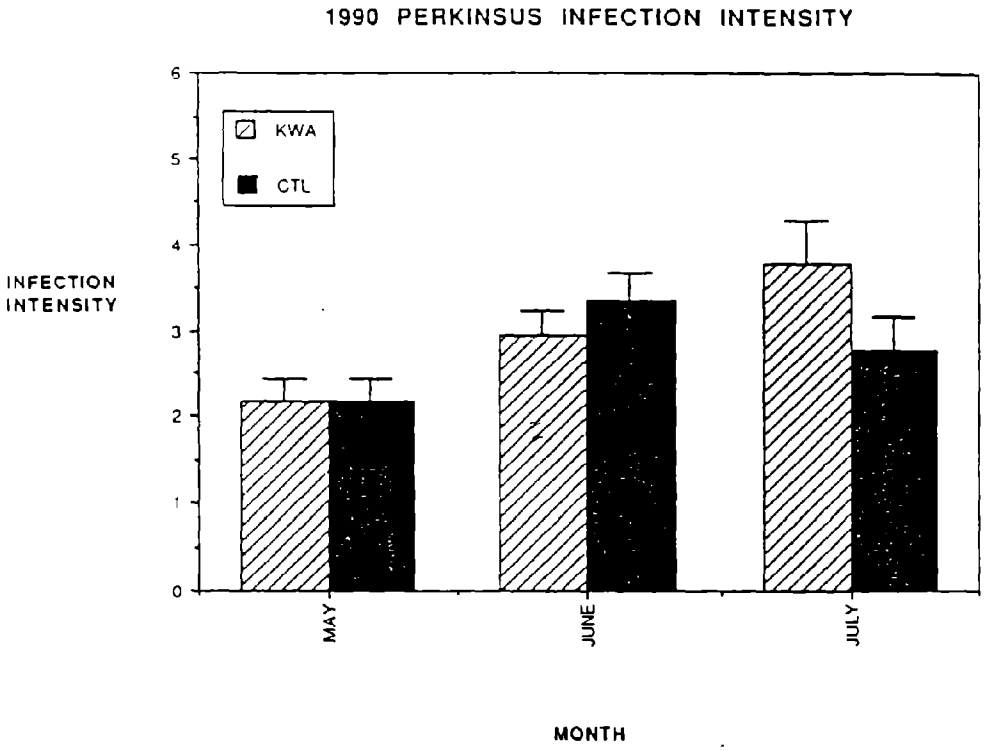


Figure 40. *Perkinsus marinus* infection intensities in oysters deployed during the 1990 field study. Note the similarities in infection intensities for oysters at the CTL and KWA Sites.

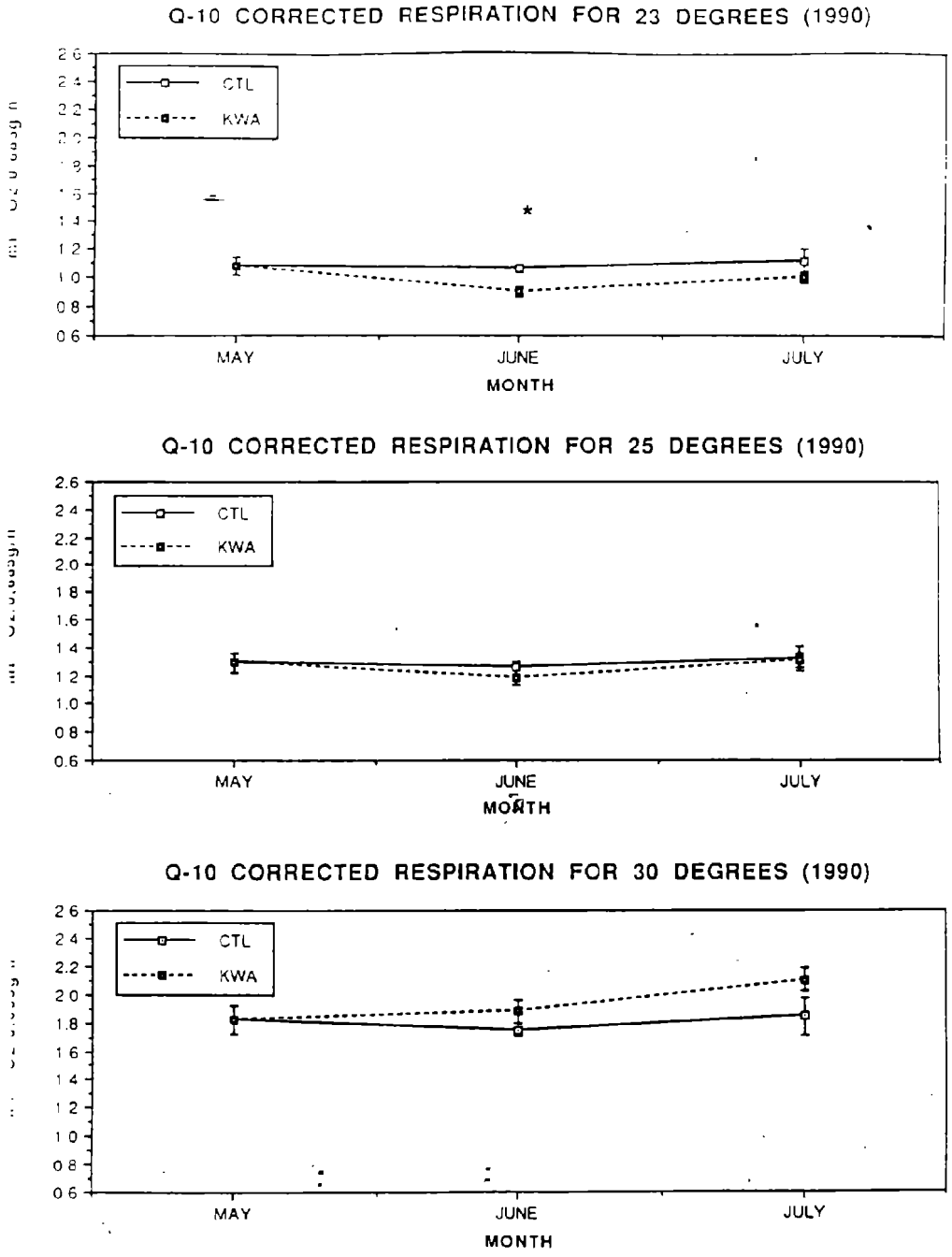


Figure 41. Q10 adjusted respiration rates (ml/0.685g/h) in oysters deployed during the 1990 field study. Generally there were no significant differences in respiration rates observed between oysters deployed at both sites for all temperatures tested (23-30°C).

At 25°C, the initial Q_{10} adjusted respiration rate during May, prior to transplantation was 1.290 ml/0.685 g/h (± 0.070 ml/0.685 g/h). During June, Q_{10} adjusted respiration rates at 25°C ranged from 1.180 ml/0.685 g/h (± 0.050 ml/0.685 g/h) at the KWA Site to 1.260 ml/0.685 g/h (± 0.030 ml/0.685 g/h) at the CTL Site. During July, Q_{10} adjusted respiration rates at 25°C ranged from 1.310 ml/0.685 g/h (± 0.050 ml/0.685 g/h) at the KWA Site to 1.320 ml/0.685 g/h (± 0.090 ml/0.685 g/h) at the CTL Site. Statistical analysis indicated there were no significant between site differences observed during June and July at 25°C.

At 30°C the initial Q_{10} adjusted respiration rate in May for oysters prior to transplantation, was 1.820 ml/0.685 g/h (± 0.100 ml/0.685 g/h). During June, Q_{10} adjusted respiration rates at 30°C ranged from 1.880 ml/0.685 g/h (± 0.080 ml/0.685 g/h) at the KWA Site to 1.750 ml/ 0.685 g/h (± 0.040 ml/0.685 g/h) at the CTL Site. During July, Q_{10} adjusted respiration rates at 30°C ranged from 2.100 ml/0.685 g/h (± 0.080 ml/0.685 g/h) at the KWA Site to 1.840 ml/0.685 g/h (± 0.130 ml/0.685 g/h) at the CTL Sites. Statistical analysis indicated there were no significant between site differences observed in Q_{10} adjusted respiration rate comparisons during June and July at 30°C.

Figure 42 depicts the mean Q_{10} Standardized respiration rates for oysters at the CTL and KWA Sites from May-June, for the 23-30°C temperature ranged observed. At 23°C, there were no significant ($p \leq 0.61$) differences in Q_{10} standardized respiration rates for comparisons between the KWA (X = 0.900 ml/0.685 g/h ± 0.040 ml/0.685 g/h) and CTL (X = 1.070 ml 0.685 g/h ± 0.020 ml/0.685 g/h) Sites. At 25°C, there were no significant differences in Q_{10} standardized respiration rates in comparisons of oysters at each site (X = 1.180 ml/0.685 g/h ± 0.050 ml/0.685 g/h at the KWA Site versus X = 1.260 ml/0.685 g/h ± 0.030 ml/0.685 g/h at the CTL Site). Also at 30°C, there were no significant differences in Q_{10} standardized respiration rates in comparisons of oysters at the KWA (X = 1.880 ml/0.685 g/h I 0.080 ml/0.685 g/h ± 0.080 ml/0.685 g/h) and CTL (X = 1.750 ml/0.685 g/h) Sites. These results suggest that metabolic rates in oysters from both sites were similar at all test temperatures (23-30°C), which would be at upper thermal limits of their zone of capacity adaptations for temperature exposure. The lack of significant differences in respiration rates in oysters at the CTL and KWA Sites during 1990 was not surprising given the small amounts of rainfall, resulting similarities in the physicochemical environmental at both sites, and the resulting low levels of insecticide exposure (azinphosmethyl) observed during 1990. These results are in sharp contrast in results for 1989, when marked differences in respiration rates were observed in TRT Site Oysters following significant fenvalerate and low salinity exposure. During 1990 low salinities (< 5 ppt) were not observed at the KWA Site due to the small amount of rainfall.

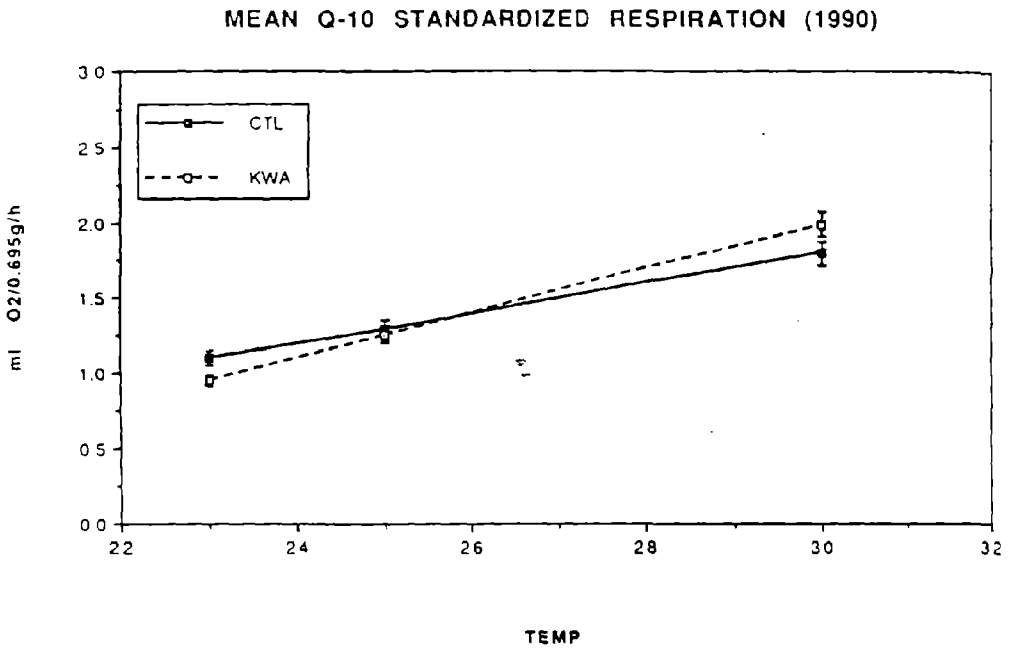


Figure 42. Mean Q10 standardized respiration rates (ml/0.685g/h) in oysters deployed during the 1990 field study. Note the similarities in respiration rates in oysters from each site at all temperatures tested (23-30°C).

Results of nitrogen excretion rates are listed in Table 36 and Figure 43. During May, the initial nitrogen excretion rate was 10.06 ug atoms N/h/h (± 1.46 ug atoms N/g/h). During June, nitrogen excretion rates ranged from 9.34 ug atom N/g/h (± 1.17 ug atom N/g/h) at the KWA Site to 11.70 ug atom N/g/h (± 1.63 ug atoms N/g/h) at the CTL Site. During July, nitrogen excretion rates ranged from 9.51 ug atoms N/g/h (± 2.87 ug atoms N/g/h) at the KWA Site to 5.36 ug atom N/g/h (± 0.97 ug atoms N/g/h) at the CTL Site. Statistical analysis indicated nitrogen excretion rates during June and July were not significantly different in between site comparisons.

Results of O/N ratios are listed in Table 37 and Figure 44. The initial O/N ratio measured in May was 29.16 (± 7.13). During June O/N ratios ranged from 26.86 (± 6.70) at the KWA Site to 16.78 (± 1.79) at the CTL Site. During July, O/N ratios ranged from 65.12 (± 17.48) at the KWA Site to 63.11 (± 7.82) at the CTL Site. Statistical analysis indicated these were no significant differences in between site comparisons during June and July.

Results of Q_{10} adjusted O/N ratios are listed in Table 38 and Figure 45. The initial Q_{10} adjusted O/N ratio in May was 23.40 (± 5.85). During June, Q_{10} adjusted O/N ratios ranged from 23.28 (± 5.56) at the KWA Site to 16.35 (± 1.90) at the CTL Site. During July, Q_{10} adjusted O/N ratios ranged from 40.39 (± 12.57) at the KWA Site to 40.91 (± 5.51) at the CTL Site. Statistical analysis indicated these were no significant differences in between site comparisons of Q_{10} adjusted O/N ratios.

The lower O/N ratio values measured during May and June (<30) are indicative of healthy oysters, but signify some possible protein catabolism. During July the higher O/N ratios (> 40) measured were indicative of a predominance of lipid and carbohydrate metabolism rather than significant protein catabolism. As noted by the changes in condition index, oysters were accumulating gametes for spawning from May - July. As this maturation process occurred, O/N ratios increased as nitrogen output and protein catabolism decreased.

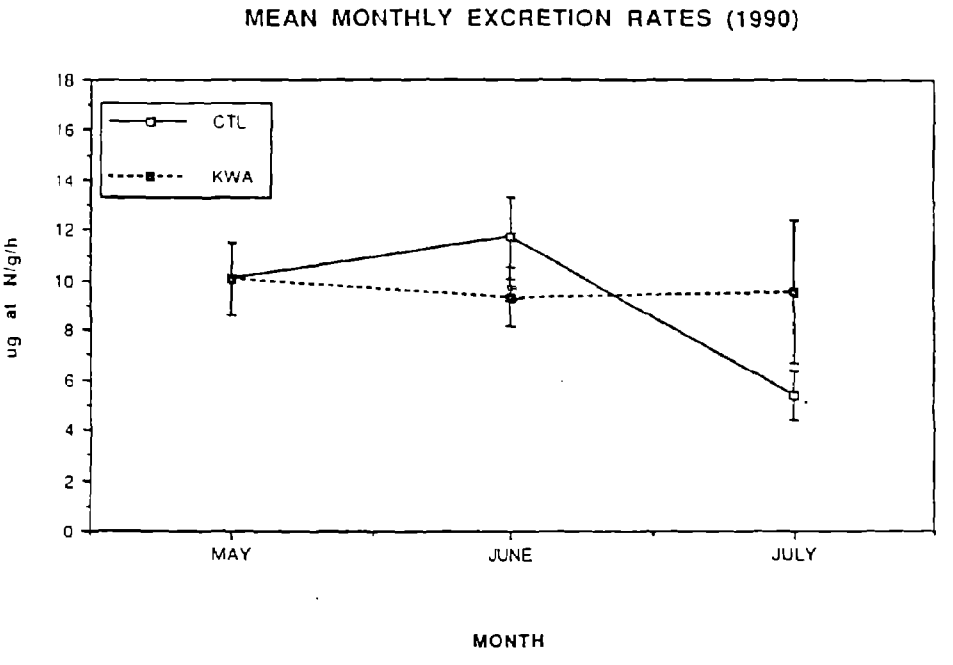


Figure 43. Ammonia nitrogen excretion rates (ug atoms N/g/h) in oysters deployed during the 1990 field study. There were no differences in nitrogen excretion rates observed in comparison of CTL and KWA Site oysters.

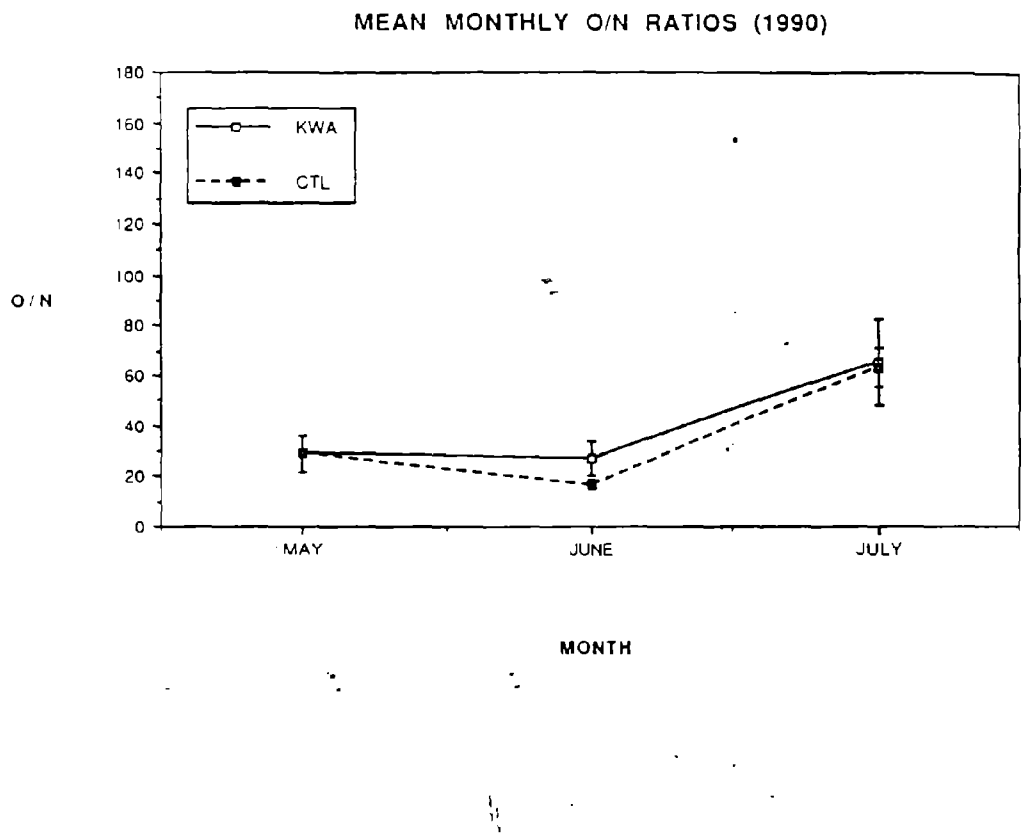


Figure 44. Mean O/N Ratios in oysters deployed during the 1990 field study. Note the similarities in O/N ratios in oysters deployed at the CTL and KWA Sites during 1990.

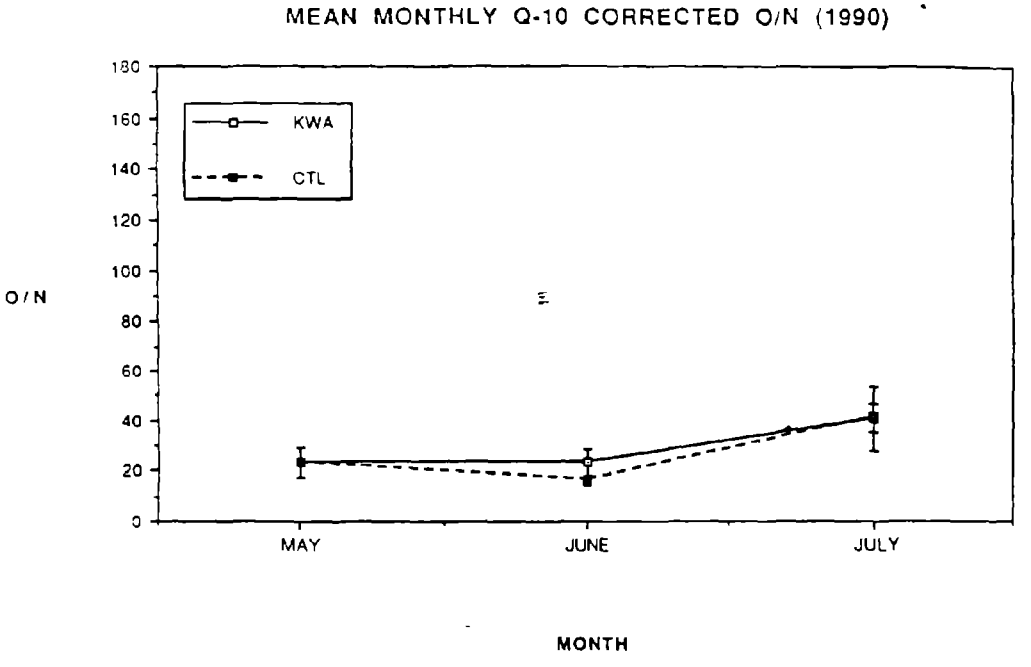


Figure 45. Mean Q10 adjusted O/N Ratios in oysters deployed during the 1990 field study. Note the similarities in O/N ratios in oysters deployed at the CTL and KWA Sites during 1990.

C. Discussion and Conclusions: Oyster Ecophysiology Studies 1989-90.

Results of ecophysiology studies clearly indicated the utility and sensitivity for an integrated battery of physiological parameters to assess agricultural NPS pesticide runoff effects in oysters. During 1989, significant runoff of fenvalerate may have, in part, caused significant sublethal stress as measured by Q_{10} adjusted respiration rates and nitrogen excretion rates in oysters at the TRT Site. Alterations of these physiological parameters did not cause resulting effects in oyster condition index or parasite infection intensity. These results suggest that while significant fenvalerate exposure occurred, with measurable physiological differences in respiration and nitrogen excretion rates resulting, no gross changes in body component indices (i.e., condition index) occurred. This is suggestive that while specific physiological differences were measured, effects were not severe enough to cause large gross scale physiological effects.

Results from 1990, indicated a slightly different seasonal patterns of physiological change, as condition indices were suggestive of a delayed period of glycogen accumulation (June) and spawning (July) in 1990 compared to 1989 (May -glycogen accumulations and June - spawning activity). An absence of significant pesticide runoff, other than the small azinphosmethyl concentrations observed at the KWA Site, was observed during 1990. As a result, physiological parameters were not significantly different in comparisons of oysters between both sites during 1990.

While the results for 1989-90, demonstrated the usefulness of oyster ecophysiology measurements to assess nonpoint source pesticide runoff effects, it is important to note the significance of confounding factors such as low salinity exposure, which may co-occur with pesticide exposure. Only by careful study and appropriate study design may the effects of confounding factors such as salinity be differentiated from pesticide effects per se. Thus it is extremely important that study design incorporate an appropriate number of controls to address physiological responses to non-contaminant environmental fluctuations, so that contaminant effects per se may be elucidated and statistically discerned. Low salinity is a particularly important factor, since it will occur concomitant with NPS runoff of chemical contaminants.

III. Laboratory Toxicity Tests

A. Effects on Brain AChE Activity

1. Laboratory Phase - EC_{50} Determination

The results of the 24h laboratory exposure experiments to determine the level of brain AChE inhibition produced in mummichogs exposed to a series of azinphosmethyl concentrations are shown in Figure 46. The predicted 24h EC_{50} (concentration necessary to produce a 50% reduction in AChE activity following 24h of exposure) was 0.90 μg/L.

2. Relationship Between Specific Levels of Azinphosmethyl - Induced Brain AChE Inhibition and Sublethal Effects on Respiration, Nitrogen Excretion and O/N Ratios

Figure 47 shows the effects on brain AChE observed in mummichogs exposed to azinphosmethyl at 2.4μg/L for 24h, both initially and following eight days of depuration. Brain AChE activity was reduced by 81% in the mummichogs immediately following 24 hours of exposure. Following eight days of depuration, brain AChE activity had recovered to about 70% of normal but was still significantly ($p \leq 0.05$) lower than controls.

Figure 48 shows the oxygen consumption rates observed in mummichogs exposed to azinphosmethyl at 2.4 μg/L, both immediately following 24h of exposure and after eight days of depuration in clean water. Oxygen consumption rates observed in control animals at 24h and at eight days are also shown. There was no significant ($p > 0.05$) difference in oxygen consumption between the 24 h azinphosmethyl exposed animals and the corresponding control group. Neither was there a significant ($p > 0.05$) difference between the eight day control group and the eight day treatment group. The only groups which had significantly ($p > 0.05$) different oxygen consumption rates were the 24h control and the eight day treatment groups.

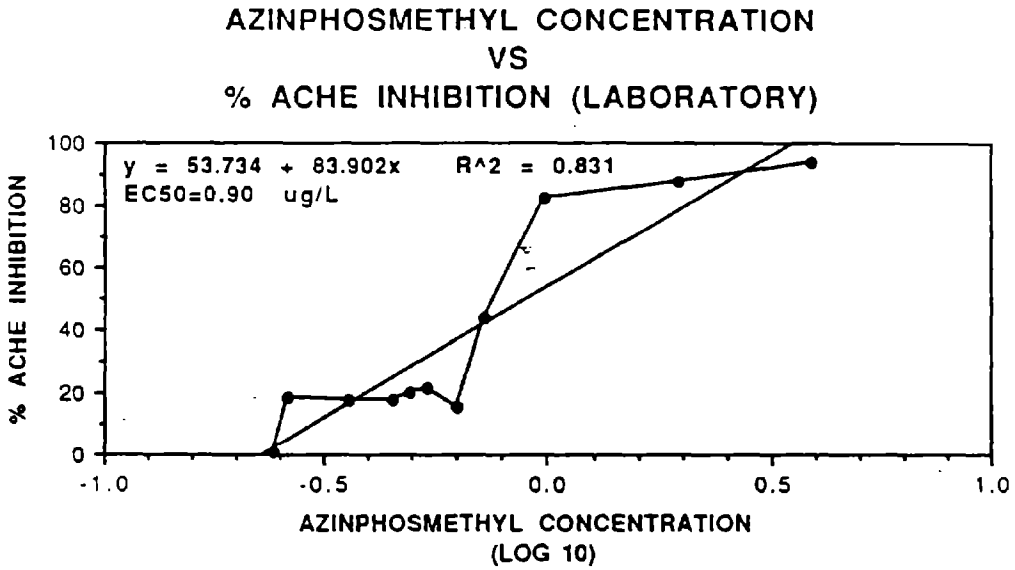


Figure 46. Laboratory predicted 24h EC 50 (ug/L) based on 50 % brain AChE inhibition in *F. heteroclitus* exposed to azinphosmethyl for 24h.

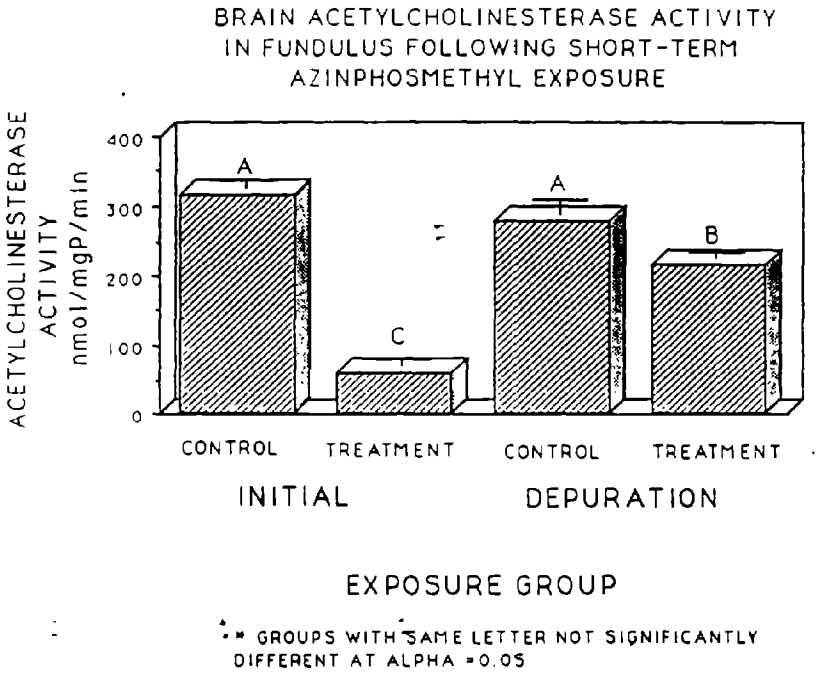


Figure 47. Brain AChE levels in *F. heteroclitus* exposed to a sublethal dose of azinphosmethyl in the laboratory for 24h. Note the significant AChE inhibition following initial exposure and the partial recovery some 7 days later.

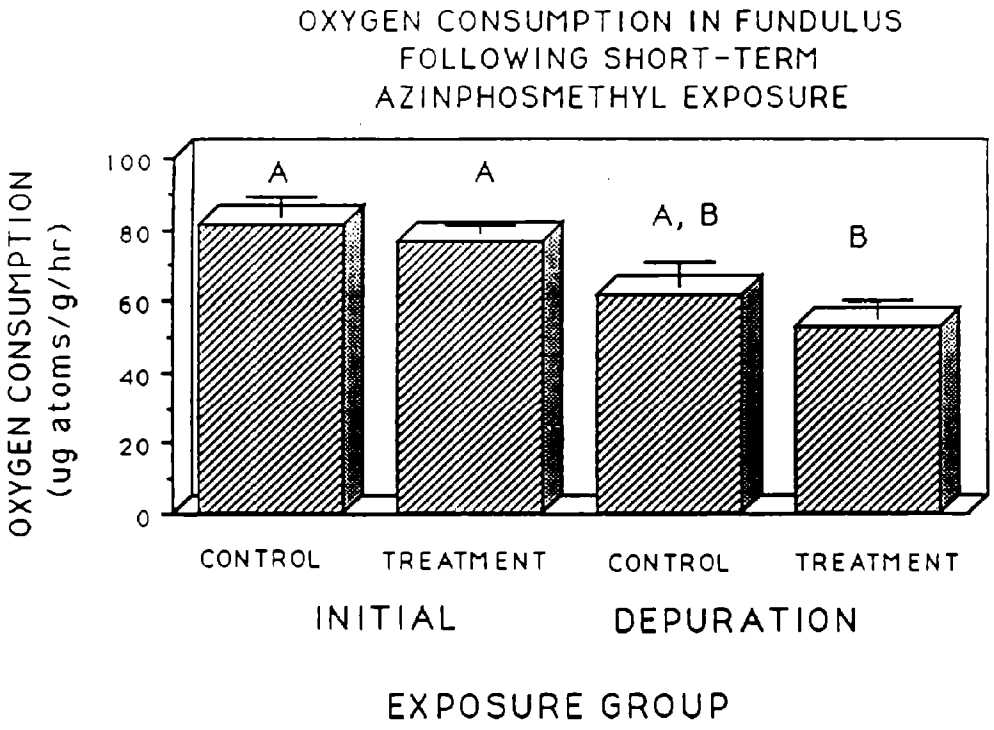


Figure 48. Respiration rates (ug atoms O2/g/h) in *F. heteroclitus* exposed to a sublethal dose of azinphosmethyl for 24h followed by a 168 hour depuration period. Exposure to azinphosmethyl did not affect respiration rates in mummichogs acutely exposed, despite the high levels of brain AChE.

This difference did not appear to be related to insecticide exposure, but may have been due to the effect of the experimental confinement on the eight day groups or the handling of stress experienced by the 24h groups. One of these possibilities seems most likely, since oxygen consumption rates tended to be lower in both the eight day treatment and eight day control groups than in the corresponding 24h groups. Oxygen consumption rates ranged from 81.01 μg atoms O_2/g dry weight/h in the 24h control group to 53.00 μg atoms O_2/g dry weight/h in the eight day treatment group.

Figure 49 shows the nitrogen excretion rates observed in control mummichogs and those exposed to azinphosmethyl for 24h at 2.4 μg/L. Nitrogen excretion rates were significantly ($p \leq 0.05$) lower in the 24h treatment group and both the treatment and control depuration groups than in the initial control group. Mean nitrogen excretion rates ranged from 11.38 μg atoms N/g dry weight/h in the 24h control group to 3.67 μg/g dry weight/h in the eight day control group.

The O/N ratios determined for the control mummichogs and those exposed to azinphosmethyl at 24h and eight days are shown in Figure 50. Mean O/N ratios ranged from 7.40 in the 24h control group to 25.65 in the 24h treatment group. There was no significant ($p > 0.05$) difference in the O/N ratio for any of the four groups.

C. Discussion and Conclusions:

Relationship Between Specific Levels of Azinphosmethyl - Induced Brain AChE Inhibition and Sublethal Effects on Respiration, Nitrogen Excretion and O/N Ratios

The results of these experiments indicated that short-term exposure (24h) of mummichogs to azinphosmethyl at 2.4 μg/L resulted in ~ 81% inhibition of AChE immediately following exposure. Following eight days of depuration this activity had recovered to ~ 70% of normal but was still significantly lower than that in control animals. No significant effect on oxygen consumption was observed in the fish exposed to azinphosmethyl at 2.4 μg/L for 24h, either immediately following exposure or after eight days of depuration.

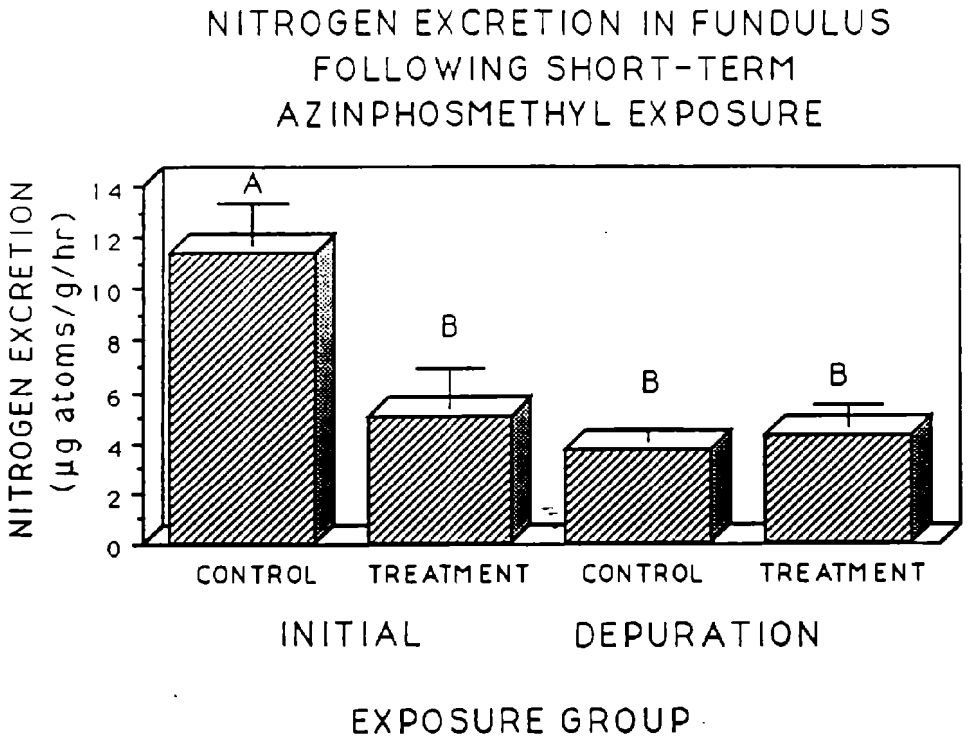


Figure 49. Nitrogen excretion rates (ug atoms N/g/h) in *F. heteroclitus* exposed to a sublethal dose of azinphosmethyl for 24h, followed by a 168 h depuration period. Exposure to azinphosmethyl resulted in significantly decreased nitrogen excretion rates. This effect was not evident in depuration phase organisms.

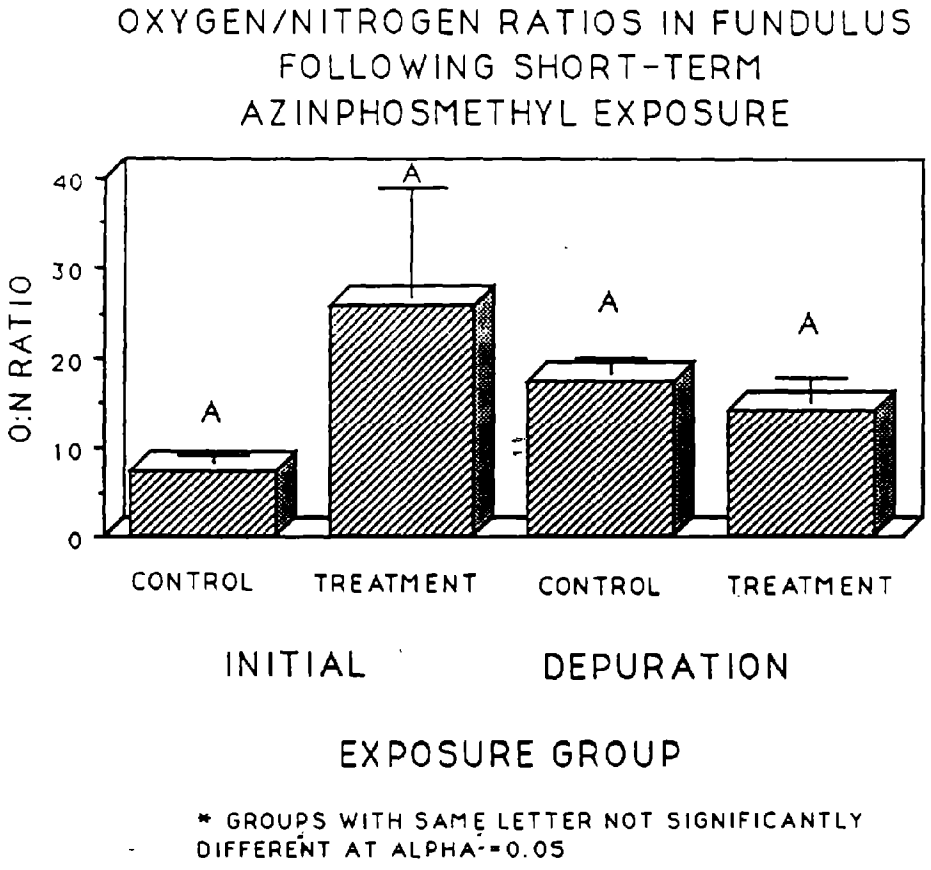


Figure 50. Mean O/N Ratios in *F. heteroclitus* exposed to a sublethal concentration of azinphosmethyl for 24h followed by a 168h depuration period. Azinphosmethyl exposure caused no significant effect on O/N ratios in mummichogs.

Nitrogen excretion was significantly lower in the 24h treatment group and in both the treatment and control depuration groups than in the initial control group. It is possible that the effect observed in the 24h treatment group may have been a result of the insecticide exposure while those seen in the control and treatment depuration groups may have resulted due to the stress of environmental confinement.

O/N ratios were not significantly different in any of the four groups although these ratios were generally lower in the 24h treatment groups and both the control and treatment depuration groups than in the 24h control group.

It is of interest to note that the azinphosmethyl concentration (2.4 μg/L) which produced ~ 81% inhibition following 24h of exposure in these experiments is about 0.065 times the 96h LC_{50} for this compound in mummichogs of 36.95 μg/L reported by Fulton and Scott (1991). This suggests, together with the fairly minor metabolic alterations observed in these experiments concurrent with high levels of AChE inhibition, that a fairly large reserve of brain AChE activity exists in this species at least as it relates to acute lethality and the sublethal metabolic parameters examined in these experiments.

IV. Biomarker Studies

A. Brain AChE in Mummichogs

1. Field Exposures

Ninety-six hour field exposure tests with mummichogs were conducted during June of 1989 and May-June of 1990. Four field exposure tests were conducted during each of these years at the CTL, TRT and KWA Sites.

Rainfall data for the field exposure tests conducted during 1989 and 1990 are shown in Tables 39 and 40. There were six periods of rainfall during the 1989 field exposure at each of the three field sites. Four of these events resulted in total rainfall amounts >1.27cm/24h at the CTL and TRT Sites while five of the rain events resulted in total amounts >1.27cm/24h at the KWA Site. During the 1990 field exposures,

Table 39. Dates of significant rainfall (> 1.27 cm/day) during the 1989 field study.

1989		Rainfall Amount (cm/day)		
Site	Date	Range	Average	(±SE)
CTL	6/5/89	4.70 - 4.83	4.75	(0.05)
	6/6/89	3.30 - 3.56	3.43	(0.08)
	6/9/89	1.27 - 1.52	1.35	(0.08)
	6/16/89	0.89 - 0.89[B]	0.89	(0.00)
	6/19/89	2.97 - 3.05	3.02	(0.03)
	6/24/89	0.25 - 0.25[B]	0.25	(0.00)
TRT	6/5/89	4.83 - 4.95	4.90	(0.05)
	6/6/89	3.30 - 3.53	3.43	(0.08)
	6/9/89	1.52 - 1.65	1.57	(0.05)
	6/16/89	2.03 - 2.10	2.30	(0.02)
	6/19/89	1.21 - 1.27[B]	1.22	(0.03)
	6/24/89	0.19 - 0.32[B]	0.25	(0.03)
KWA	6/5/89	7.37 - 7.62	7.54	(0.08)
	6/6/89	8.38 - 8.64	8.46	(0.08)
	6/9/89	2.03 - 2.29	2.11	(0.08)
	6/16/89	1.40 - 1.52	1.47	(0.03)
	6/19/89	0.00 - 0.00	0.00	(0.00)
	6/24/89	4.57 - 4.57	4.57	(0.00)

A = Range between three rain gauges
B = Rainfall < 1.27 cm/day but included for comparative purposes
X = Mean
SE = Standard error

Table 40. Dates of significant rainfall (> 1.27 cm/day) during 1990 field study.

1990		Rainfall Amount (cm/day)		
Site	Date	Range	Average	(±SE)
CTL	5/28/90	3.00 - 3.05	3.02	(0.03)
	6/15/90	1.40 - 1.52	1.45	(0.05)
TRT	5/28/90	2.67 - 3.05	2.90	(0.13)
	6/15/90	1.98 - 2.03	2.01	(0.03)
KWA	5/28/90	2.21 - 2.31	2.24	(0.03)
	6/15/90	1.78 - 1.78	1.78	(0.00)

A = Range between three rain gauges
B = Rainfall < 1.27 cm/day but included for comparative purposes
X = Mean
SE = Standard error

there were only two periods of rainfall at each of the sites. Each of these rain events resulted in total rainfall amounts > 1.27cm/24h at each of the three field sites. Results of insecticide analysis of water samples collected during the 1989 and 1990 field exposure tests are shown in Tables 7 - 13 and 15 - 17 and Figures 3 - 5 and 6 - 8. In general the highest measured insecticide concentrations were associated with periods of significant rainfall (> 1.27cm/24h) and depressed salinities in the tidal creeks.

The maximum insecticide concentrations, cumulative rainfall and minimum salinities measured at each of the field sites during the first field exposure test (June 3-7) of 1989 are shown in Table 41. The maximum insecticide concentration measured at the CTL Site during this field test was 0.014 μg/L of endosulfan while at the TRT Site measurable concentrations of three insecticides were detected. The highest insecticide concentration measured at the TRT Site was fenvalerate at 0.093 μg/L. Endosulfan and azinphosmethyl were measured at 0.020 μg/L and 0.016 μg/l, respectively. The highest insecticide concentration measured at the KWA Site was azinphosmethyl at 1.730 μg/l. Endosulfan and fenvalerate were detected at 0.163 μg/l and 0.054 μg/l, respectively.

The maximum insecticide concentrations measured at the CTL and TRT Sites during the second field exposure test (June 11 - 15) of 1989 were endosulfan at 0.012 μg/l and 0.010 μg/l, respectively. Measurable concentrations of three insecticides were again detected at the KWA Site. The highest insecticide concentration measured at this site was azinphosmethyl at 0.368 μg/l, respectively.

During the field test of June 15-19, 1989 endosulfan was detected at the CTL Site at 0.012 μg/l while endosulfan and fenvalerate were measured at the TRT Site at 0.010 μg/l and 0.015 μg/l, respectively. The highest insecticide concentration measured at the KWA Site was azinphosmethyl at 2.457 μg/L while endosulfan was detected at 0.038 μg/L.

During the final field exposure test of 1989, only trace amounts of endosulfan (≤0.010 μg/l) were detected at the CTL and TRT Sites. At the KWA Site, however, azinphosmethyl was measured at 7.002 μg/L. Additionally, endosulfan was detected at 0.065 μg/L.

Table 41. Summary of maximum measured insecticide concentrations, minimum salinity and cumulative rainfall measured during the 1989 field study.

A. Field Exposure (June 3-7, 1989)

Exposure Site	Total Rainfall (cm)	Minimum Salinity (0/00)	Maximum Insecticide Concentrations (μg/L)
CTL	8.18	5	Endosulfan (0.014)
TRT	8.33	5	Endosulfan (0.020) Azinphosmethyl (0.016) Fenvalerate (0.093)
KWA	16.00	0	Endosulfan (0.163) Azinphosmethyl (1.730) Fenvalerate (0.054)

B. Field Exposure (June 11-15, 1989)

Exposure Site	Total Rainfall	Minimum Salinity (ppt)	Maximum Insecticide Concentrations (μg/L)
CTL	0	26	Endosulfan (0.012)
TRT	0	18	Endosulfan (0.010)
KWA	0	4	Endosulfan (0.064) Azinphosmethyl (0.368) Fenvalerate (0.031)

C. Field Exposure (June 15-19, 1989)

Exposure Site	Total Rainfall (cm)	Minimum Salinity (0/00)	Maximum Insecticide Concentrations (μg/L)
CTL	5.00	23	Endosulfan (0.012)
TRT	3.91	7	Endosulfan (0.010) Fenvalerate (0.015)
KWA	2.21	3	Endosulfan (0.038) Azinphosmethyl (2.457)

D. Field Exposure (June 23-27, 1989)

Exposure Site	Total Rainfall (cm)	Minimum Salinity (0/00)	Maximum Insecticide Concentrations (μg/L)
CTL	5.00	23	Endosulfan (0.010)
TRT	3.91	7	Endosulfan (0.006)
KWA	2.21	3	Endosulfan (0.038) Azinphosmethyl (2.457)

No rainfall occurred at either of the field sites during the first field exposure test (May 24-28) of 1990. No water samples from either of the field sites were analyzed for insecticide residues for this time period. The maximum measured insecticide concentrations, cumulative rainfall and minimum salinities measured at each of the field sites during the second (May 28 and June 1, 1990) field test of 1990 are shown in Tables 15 -17 and 40. No insecticides were measured at concentrations above the detection limit at either the CTL or KWA Sites. At the TRT Site, endosulfan and fenvalerate were measured at 0.014 μg/L and 0.123 μg/L, respectively. The maximum measured insecticide concentrations, cumulative rainfall and minimum salinities measured at each of the field sites during the third (June 13-17) field exposure test of 1990 are shown in Tables 15 - 17 and 40. Endosulfan was detected at the CTL and TRT site at 0.009 μg/L and 0.005 μg/L, respectively. At the KWA Site, azinphosmethyl was measured at 0.062 μg/L. No rainfall occurred at either of the field sites during the final (June 21-23) field test of 1990. No water samples from either of the field sites were analyzed for insecticide residues for this period.

B. Field Effects on Brain AChE in Mummichogs

Brain AChE specific activity levels in mummichogs deployed at the field sites during the field studies conducted in 1989 and 1990 are shown in Figures 51 and 52, respectively.

Brain AChE specific activity levels in mummichogs deployed at the field sites during the first field deployment (June 3-7) of 1989, ranged from 321.70 nmol mgP^{-1} min^{-1} at the TRT Site to 123.02 nmol mgP^{-1} min^{-1} at the KWA Site. Brain AChE levels were significantly ($P \leq 0.05$) depressed in the animals deployed at the KWA Site when compared to those deployed at the other two field sites. There was no significant ($P > 0.05$) difference between activity levels in the animals deployed at the other two sites. During the second deployment (June 11-15) of 1989 there was no significant ($P > 0.05$) difference between brain AChE levels in animals deployed at the TRT Site (379.05 nmol mgP^{-1} min^{-1}) and those deployed at the KWA Site (352.59 nmol mgP^{-1} min^{-1}). Brain AChE levels were not determined for animals deployed at CTL Site during this test because of high mortality in this group that occurred as a result of depressed DO in the holding tank after the removal of these animals from

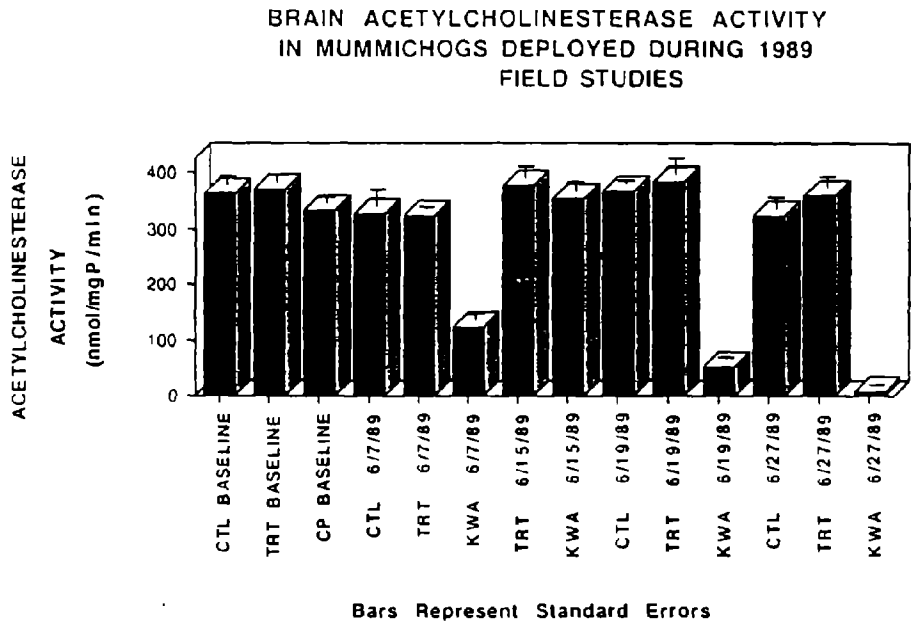


Figure 51. Brain AChE levels measured in *F. heteroclitus* exposed in the field during the 1989 field study. Note the significant reductions (*) in AChE levels in fish exposed to azinphosmethyl at the KWA Site during 1989.

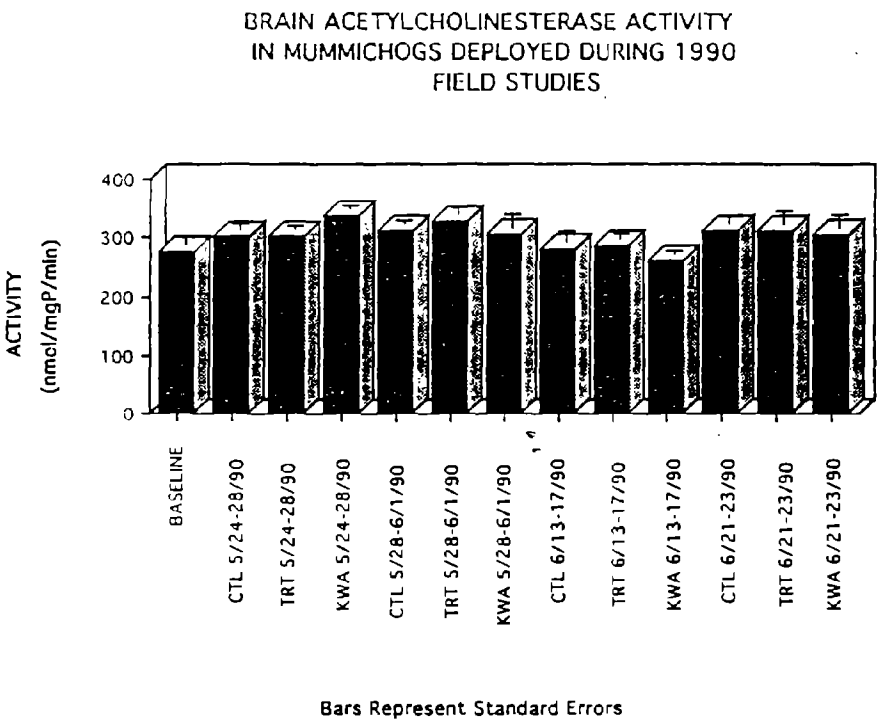


Figure 52. Brain AChE levels measured in *F. heteroclitus* exposed in the field during the 1990 field study. Note the similarities in fish brain AChE levels for all sites during 1990.

the field site. Brain AChE specific activity level in animals deployed at the field sites from June 15-19 ranged from 384.53 nmol mgP^{-1} min^{-1} in animals from the TRT Site to 51.44 nmol mgP^{-1} min^{-1} in animals from the KWA Site. Specific activity was significantly ($P \leq 0.05$) lower in the animals deployed at the KWA Site than in animals deployed at either the CTL or TRT Site. There was no significant ($P > 0.05$) difference between the levels at the TRT (384.53 nmol mgP^{-1} min^{-1}) and CTL (366.55 nmol mgP^{-1} min^{-1}) Sites. During the final (June 23-27) field deployment of 1989, AChE specific activity levels ranged from 361.87 nmol mgP^{-1} min^{-1} at the TRT Site to 5.90 nmol mgP^{-1} min^{-1} at the KWA Site. Brain AChE specific activity was significantly ($P \leq 0.05$) lower in animals deployed at the KWA Site than in animals deployed at either of the other two sites. Once again there was no significant difference between the AChE activity levels in animals deployed at the CTL and TRT Sites.

During the first deployment of 1990 (May 24-28) brain AChE levels in mummichogs deployed at the field sites ranged from 300.24 nmol mgP^{-1} min^{-1} at the TRT Site to 326.24 nmol mgP^{-1} min^{-1} at the CTL Site. There was no significant ($P > 0.05$) difference in the level of brain AChE activity in animals deployed at any of the three field sites. A similar pattern was observed during the second field deployment (May 28-June 1) of 1990. Brain AChE activity in animals deployed at the three sites ranged from 304.59 nmol mgP^{-1} min^{-1} in the KWA animals to 326.24 nmol mgP^{-1} min^{-1} in animals deployed to the TRT Site. Once again there was no significant ($P > 0.05$) difference in the level of brain AChE activity among the animals deployed at any of the three sites.

During the field exposure test of June 13-17 brain AChE levels ranged from 260.90 nmol mgP^{-1} min^{-1} in animals deployed at the KWA Site to 283.32 nmol mgP^{-1} min^{-1} in the animals from the TRT Site. There was no significant ($P > 0.05$) difference in the level of brain AChE activity among fish deployed at any of the three field sites. The results of the final field deployment (June 21-23) of 1990 were quite similar to those from the earlier deployments conducted during the year. Mummichog specific activity levels for brain AChE ranged from 305.41 nmol mgP^{-1} min^{-1} in animals deployed at the KWA Site to 311.44 nmol mgP^{-1} min^{-1} in the animals from the CTL Site. There were no significant ($P > 0.05$) differences in the levels of brain AChE activity among animals from any of the three field sites.

C. Discussion and Conclusions - Field Exposure Tests

Climatic conditions during the field exposure tests conducted during 1989 and 1990 provided an extremely interesting contrast, with the 1989 studies being carried out during an extremely wet period and the 1990 studies conducted during a relatively dry period. During the field tests of 1989 (June 3 - 27, 1989), total rainfall amounts at the three field sites ranged from 16.36 cm at the CTL Site to 25.40 cm at the KWA Site. During 1990, total rainfall amounts (May 24 - June 23) ranged from 4.32 cm at the KWA Site to 5.31 cm at the TRT Site. These different rainfall characteristics most probably contributed to the very different sublethal impacts observed on brain AChE observed in animals deployed at one of the field sites during field studies conducted during these two years.

Much higher insecticide concentrations were observed in water samples collected at the KWA Site during the field exposure tests conducted in 1989 than at either of the other two field sites. Azinphosmethyl was the insecticide typically measured, with highest concentrations at the KWA Site. This compound was measured at the KWA Site during each of the field exposure test in 1989, with maximum concentrations ranging from 0.37 μg/L to 7.00 μg/L. Endosulfan was also detected at relatively high concentrations at the KWA Site during 1989. The maximum measured endosulfan concentration at this site ranged from 0.04 μg/L to 0.16 μg/L for the four exposure tests. Lesser amounts of fenvalerate (0.03 - 0.05 μg/L) were detected at the KWA Site during 1989.

In contrast to the high levels of insecticides measured at the KWA Site, only very small amounts of insecticides were detected in water samples from the CTL and TRT Sites during 1989. Endosulfan was the only insecticide detected in water samples collected at the CTL Site during 1989 and the highest concentration measured was endosulfan at 0.01 μg/L. Relatively small amounts of endosulfan, azinphosmethyl and fenvalerate were detected at the TRT Site with maximum measured concentrations of 0.02 μg/L, 0.02 μg/L and 0.09 μg/L, respectively.

Very high levels of AChE inhibition were observed in mummichogs deployed at the KWA Site during three of the four field exposure tests conducted during 1989 and the level of this inhibition was closely related to azinphosmethyl concentration measured in water samples collected at this site (Table 41). The level of AChE inhibition for the four field

exposures ranged from 0 - 98% while the corresponding maximum measured azinphosmethyl concentrations ranged from 0.37 - 7.00 μg/L.

During 1990, only very minor insecticide concentrations were measured at any of the three field sites. The maximum measured insecticide concentration at the CTL Site during 1990 was endosulfan at 0.01 μg/L while at the TRT Site the maximum measured insecticide concentration was fenvalerate at 0.123 ug/L. The maximum insecticide concentration measured at the KWA Site was azinphosmethyl at 0.062 μg/L. No significant effects on brain AChE activity were observed in mummichogs deployed at either of the field sites during 1990.

A comparison of the sublethal effects on brain AChE observed in mummichogs deployed at the KWA Site during the 1989 field studies and the lack of a similar effect in 1990 appear to demonstrate the importance of nonpoint source agricultural runoff as a transport mechanism for the movement of insecticides from agricultural fields into the adjacent estuarine tidal creeks. Total rainfall amounts at the KWA Site during 1989 field studies were more than five fold higher than for a similar period in 1990.

D. Discussion and Conclusions

Sublethal Effects of Azinphosmethyl on Brain AChE-Comparison of Field and Laboratory Effects

The results of field studies conducted in 1989 indicated that significant concentrations of insecticides entered the tidal creek at the KWA Site on several occasions following significant (> 1.27 cm/24h) rainfall events. Brain AChE activity was depressed in caged mummichogs deployed at this site on three separate occasions. In each of these events, water samples collected at the site contained residues of the OP insecticide, azinphosmethyl. The maximum concentrations measured during each of these events ranged from 1.73 -7.00 μg/L. These results are quite similar to those previously reported by Scott et al, 1990 for field studies conducted at the same site during 1988. In those studies they found significant inhibition of AChE in mummichogs deployed at the KWA Site when azinphosmethyl concentrations were ≥ 0.57 μg/L.

There was also excellent agreement between the sublethal effects on brain AChE observed in the field studies and the results obtained form the laboratory experiments. As previously discussed, a 24h EC_{50} for brain AChE inhibition of 0.90 μg/L was determined for azinphosmethyl based on laboratory exposures (Figure 46). This value was subsequently compared to azinphosmethyl concentrations and effects of AChE measured in the field studies. Table 42 shows the azinphosmethyl concentrations and the effects on AChE activity measured during field deployments conducted in 1988 and 1989. The 1989 data were described earlier in this report and the 1988 data were reported by Scott et al, 1990. The data shown in this table were used to calculate field derived EC_{50}'s for AChE inhibition. Three different approaches were utilized in the treatment of these data. First, an EC_{50} was calculated based on the maximum azinphosmethyl concentration measured for a particular field deployment. This approach produced an EC_{50} of 1.53 μg/L (Figure 53). Next, an EC_{50} of 0.63 μg/L (Figure 54) was calculated based on the 24h azinphosmethyl concentration (the residual azinphosmethyl concentration present in water samples collected ~ 24h after the sample containing the highest azinphosmethyl concentration). Finally, an EC_{50} of 1.13 μg/L (Figure 55) was determined based on the 24h average concentration (the average of the maximum measured azinphosmethyl concentration and the azinphosmethyl concentration remaining 24h later). This value was quite similar to the laboratory derived EC_{50} of 0.90 μg/L. These results suggest that a simple 24h laboratory exposure is a good predictor of the effects on brain AChE produced following exposure to azinphosmethyl residues present in nonpoint source agricultural runoff.

Table 42. Summary of Insecticide Related Effects on Brain AChE Activity Observed in Field Studies Conducted in 1988 and 1989.

Field Test Date	Maximum Measured Azinphosmethyl concentrations (μg/L)	Azinphosmethyl Concentration at 24h (μg/L)	% AChE Inhibition
6/7 - 11/88	3.44	0.57	47
6/11 - 15/88	0.57	0.55	22
6/3 - 7/89	1.73	1.12	63
6/11 - 15/89	0.37	0.21	0
6/15 - 19/89	2.46	0.72	85
6/23 - 27/89	7.00	1.60	98

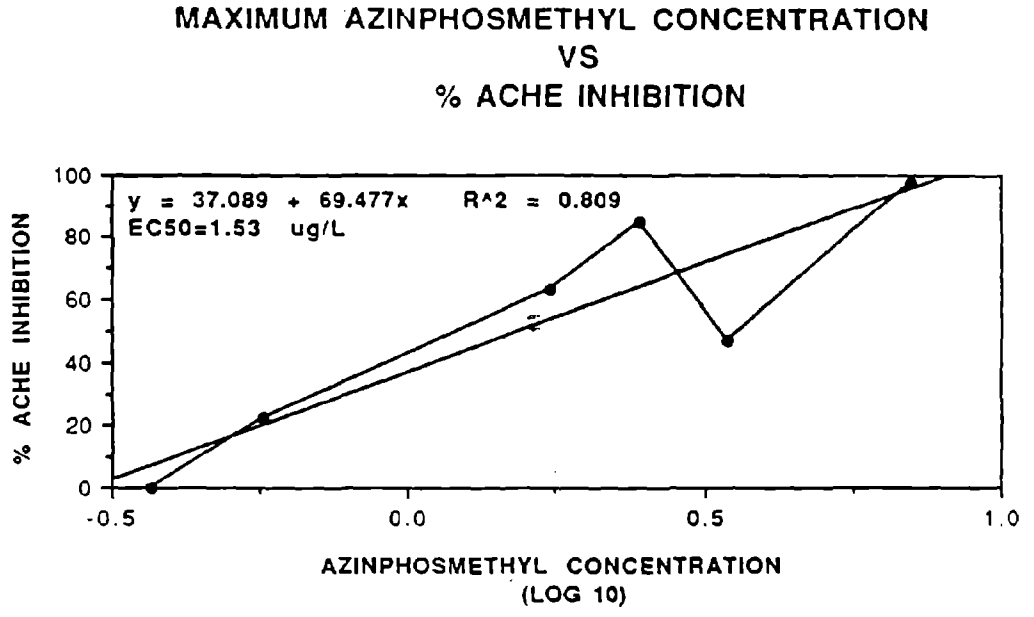


Figure 53. Predicted EC50 (ug/L) based upon field measured, brain AChE levels in *F. heteroclitus* exposed to azinphosmethyl. The maximum field exposure concentration was used to predict the EC50 value.

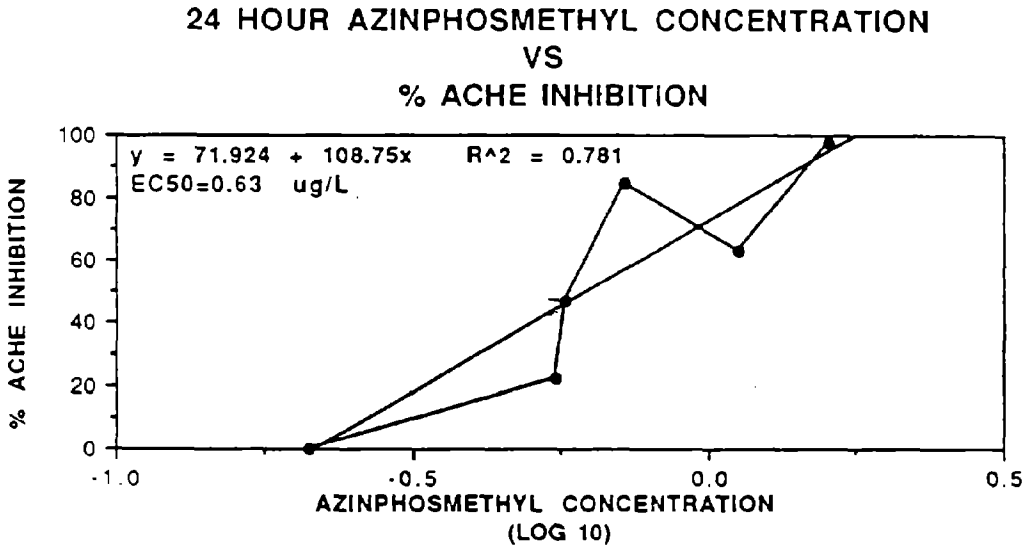


Figure 54. Predicted EC50 (ug/L) based upon field measured, brain AChE levels in *F. heteroclitus* exposed to azinphosmethyl. The azinphosmethyl concentrations measured 24h after the maximum concentration was observed, was used to predict the EC50 value.

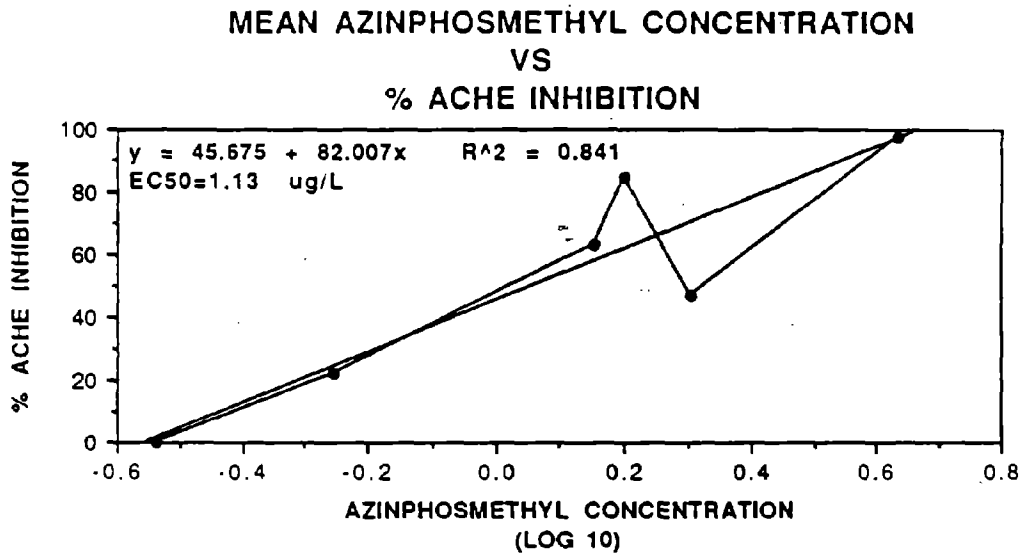


Figure 55. Predicted EC50 (ug/L) based upon field measured, brain AChE levels in *F. heteroclitus* exposed to azinphosmethyl. The mean azinphosmethyl concentration (maximum + 24h concentrations/2) was used to predict the EC50 value.

V. Ecotoxicological Studies

A. Block Seining 1989-90

1. Biomass

Results of total biomass (g/50m of stream) measurements for all macropelagic (> 15mm) fauna are given in Tables 43 - 44 and Figure 56. Table 43 lists the species observed in sample collected during this time period. Total biomass (Table 44) ranged from 524.3-7,066.7 g/50 m of stream at the CTL Site compared to a range of 1,510.0-10,666.7 g/50 m of stream at the TRT Site for the period of January, 1989 - September, 1990. Peak biomass was observed during September, 1989 and September, 1990 at the CTL Site and during August, 1989 and July, 1990 at the TRT Site.

Total mean biomass (January, 1989 - September, 1990) was 91,947.8 g/50 m of stream at the CTL Site versus 100,504.6 g/50 m of stream at the TRT Site. This indicated that total biomass for this period was 8.5% higher at the TRT Site than CTL Site.

Much of this between site difference in biomass may be attributed to Hurricane Hugo. Statistical analysis indicated that prior to Hurricane Hugo, biomass was higher at the TRT Site only in paired sample comparisons on two occasions, July and August, 1989. During these sampling periods biomass was increased primarily due to the greater abundance of *P. pugio* at the TRT Site (8,781-16,508.7/50 m of stream) compared to the CTL Site (2,031.7-7,266/50 m of stream). After Hugo, biomass was significantly different in both paired and unpaired sample comparisons during November, 1989 through January, 1990. Statistical analysis indicated that biomass measurements were 6,080.4 g higher during this time period at the TRT Site than at the CTL Site. This 6,080.4 g difference observed post Hugo would account for 71% of the 8.5% difference observed between sites for the entire study period (1/89 - 9/90). In fact, the biomass levels observed at the TRT Site during November, 1989 - January, 1990, were the highest ever observed at either site from 1985-1991, for winter

Table 43. List of pelagic species identified during ecotoxicological sampling 1989 - 1990.

MARINE SPECIES LIST	
Alectis crinitus	Pompano
*Anchoa mitchilli**	Bay Anchovy
*Bairdiella chrysoura**	Silver Perch
*Brevoortia tyrannus**	Atlantic Menhaden
*Callinectes sapidus**	Blue Crab
*Caranx hippo**	Jack Cravelle
*Centroprisis striata**	Black Sea Bass
*Cynoscion nebulosis**	Spotted Sea Trout
Cyprinodon varieagatus	Sheepshead Minnow
Dorsoma cepedianum	Gizzard Shad
Elops saurus	Lady fish
Eucinostomus gula	Silver jenny
Fundulus heteroclitus	Mummichog
Fundulus majalis	Striped Killifish
Gobiosoma bosci	Goby
*Lagodon rhomboides**	Pinfish
*Leiostomos xanthurus**	Spot
*Lolliguncula brevis**	Squid
Menidia menidia	Atlantic Silverside
*Micropogon undulatus**	Atlantic Croaker
Monocanthus hispidus	File Fish
*Mugil cephalus**	Mullet
Opsanus tau	Toad Fish
Palaemonetes pugio	Grass Shrimp
Palaemontes vulgaris	Grass Shrimp
Panopeus herbstii	Mud Crab
*Paralichthys dentatus**	Northern Flounder
*Paralichthys lethostigma**	Southern Flounder
*Penaeus aztecus**	Brown Shrimp
Penaeus duorarum	Pink Shrimp
Penaeus setiferus	White Shrimp
Poecilia latipinna	Salifin Molly
*Pomatomus saltatrix**	Blue Fish
Prionotus tribulus	Bighead Sea Robin
Selene vomer	Lookdown
Symphurus plagiusa	Tongue Fish
Sygnathus fuscus	Pipe Fish
Synodus foetens	Lizard Fish
Sphoeroides maculatus	Puffer Fish
*Sphyraena barracuda**	Barracuda
Uca pugilator	Fiddler Crab

* Denotes commercial and recreational species

Table 44. Summary of total biomass measurements (grams/50 m of stream) observed in block seining at the CTL and TRT Sites, 1989-90. Asterisks ("*";*) indicate dates when samples were significantly (0.05 - 0.10) different.

Parameter: Total Biomass (Grams/50 m of stream)				
	CTL Site		TRT Site	
Date	X	SE	X	SE
1/89	1,933.3	999.9	4,166.7	617.3
2/89	3,766.7	2,105.8	1,933.3	384.4
3/89	4,000.0	1,738.8	4,266.7	696.0
4/89	4,666.7	1,301.7	3,266.7	712.6
5/89	4,666.7	1,371.5	3,866.7	592.5
6/7/89	2,766.7	1,481.4	3,333.3	592.5
6/26/89	6,066.7	1,139.2	7,900.0	2,688.9
7/89	3,566.0	1,502.1	7,233.3*	896.9
8/89	5,200.0	1,951.9	10,666.7*	3,023.4
9/89	7,066.7	1,909.9	3,833.3	433.3
10/89	4,533.3	600.9	4,666.7	961.5
11/89	1,220.0	240.2	3,466.7**;*	39.0
12/89	524.3	77.4	3,486.7**;*	1,548.6
1/90	639.3	103.6	1,510.0**;*	97.1
2/90	4,504.3	2,246.8	4,833.3	902.5
3/90	6,066.7	3,658.0	2,500.0	500.0
4/90	2,055.7	633.3	4,593.7	2,461.9
5/11/90	5,333.3	1,965.0	6,000.0	763.8
5/30/90	2,621.3	321.5	2,774.7	868.2
6/90	4,616.7	696.4	3,516.7*^	1,140.3
7/90	4,166.7	578.3	6,058.0	1,810.7
8/90	5,600.0	529.2	3,000.0**;*	0.0
9/90	6,366.7	1,271.9	3,631.4	1,028.8
Total	91,947.8		100,504.6	

← Hugo

** = Significantly ($p \leq 0.05$) Different in Unpaired Test; N=6
* = Significantly ($p \leq 0.10$) Different in Paired Test; N=6
*^ = Significantly ($p \leq 0.075$) Different in Unpaired Test; N=5

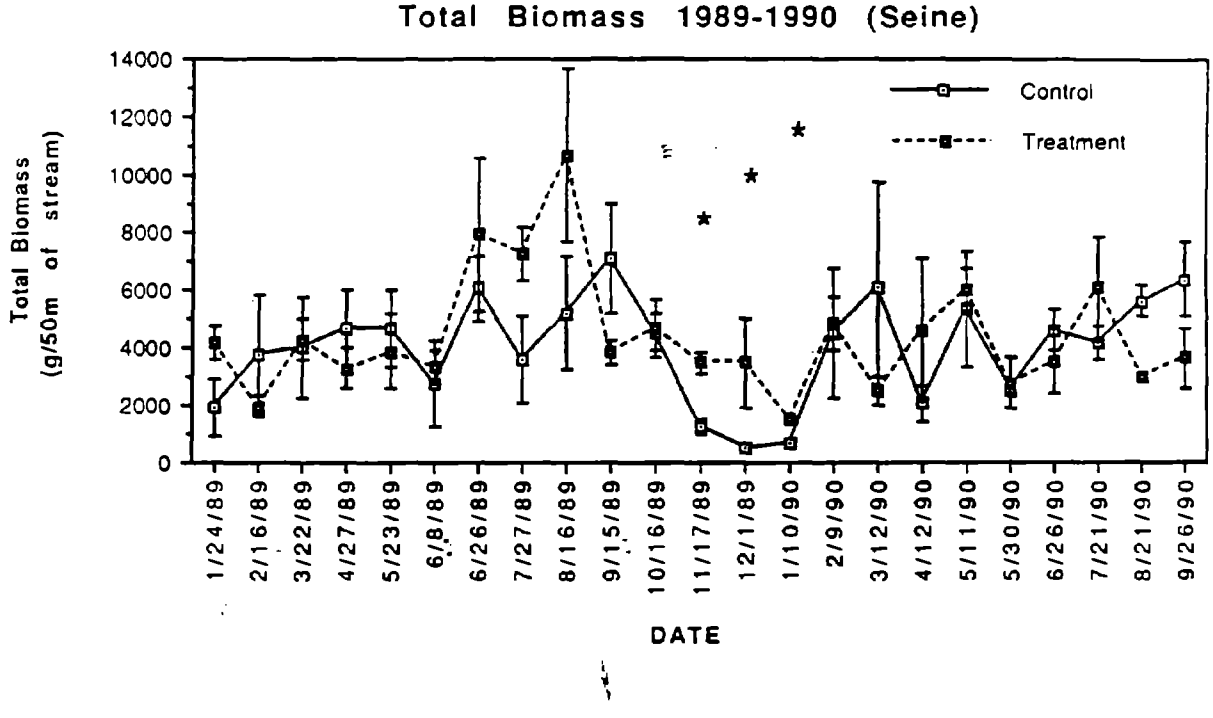


Figure 56. Total biomass (g/50m of stream) measured in block seining, 1989-90. Note the general similarities in biomass at the CTL and TRT Sites during this study. Asterisks (*) indicate samples which were significantly (0.05) different in statistical comparisons between the TRT and CTL Sites. Arrow (↑) denotes Hurricane Hugo.

(December-January) sampling periods. Generally during the winter sampling, biomass is < 1000 g/50 m of stream at both sites due to the reduced temperatures observed. December, 1989 was an extremely cold month with record snowfall (> 20 cm of snow) and a period of nearly one week when maximum daily air temperatures were below freezing. Despite this, record biomass levels were observed at the TRT Site. Dispersal of organisms by Hurricane Hugo may have accounted for this.

During Hurricane Hugo, winds on the back side of the hurricane eye caused extremely low tides at Leadenwah Creek (Jimmy Green, personal communication), as the storm surge occurred well to the north of this site. Winds would have blown from the northwest → southeast, pushing water and possibly small organisms from the CTL Site towards the TRT Site and other down wind portions of Leadenwah Creek. Sampling at the CTL and TRT Sites during September was conducted 2-3 days prior to Hugo and indicated relatively equivalent biomass at each site. One month after Hugo, again biomass was equivalent at both sites. Two to four months after Hugo, elevated biomass levels were observed at the TRT Site, mostly the result of significantly higher levels of *P. pugio* and *F. heteroclitus* and increased levels of total fish. Many of the grass shrimp measured in December, 1989 and January, 1990 would have been small post larvae (particularly poor swimmers) at the time of Hugo. In all likelihood, these grass shrimp were displaced from the CTL Site and other down wind habitats, as the wind direction would have prevented grass shrimp from being as readily displaced at the TRT Site.

Runoff of fenvalerate during June 1989, at the TRT Site appeared to have no effect as biomass was not statistically different in between site comparisons during May - June, 1989. Biomass was significantly ($p < 0.10$) higher at the TRT Site during July - August, 1989 primarily the result of increased levels of *P. pugio* and *F. heteroclitus*.

Similarly, runoff of fenvalerate during 28 May, 1990, appeared to have no immediate effect as biomass measurements, two days post rain (30 May, 1990) were not significantly different. Biomass in June was significantly ($p \leq 0.10$) lower at the TRT Site; however, reduced crustacean densities (species most sensitive to fenvalerate) did not account for these differences. Similarly in August, 1990, three months post fenvalerate runoff, biomass was significantly ($p \leq 0.05$) lower at the TRT Site, mostly the result of reduced mummichog densities (3651 vs 658/50 m of stream). Similarly

total fish densities were significantly ($p \leq 0.05$) reduced at the TRT Site in August, 1990 (4639 vs 1,109/50 m of stream). Many of these fish species would have been juvenile fish at the time of this rain event. Deployed juvenile *C. variegatus* had very poor survival at both the TRT (64% survival) and CTL (60.9%) Sites during this rain event. As a result ascribing effects due to fenvalerate to juvenile fish species and resulting post runoff effects on biomass, seem tenuous at best.

2. *P. pugio* Density

Results of *P. pugio* density (#/50 m of stream) measurements and statistical comparisons are given in Table 45 and Figure 57. Mean grass shrimp densities ranged from 23.7 (± 4.9) - 11,514.7 (± 7062.8)/50m of stream at the CTL Site compared to a range from 125.9 (± 46.5) - 19,763.4 (± 4,228.1)/50 m of stream at the TRT Sites.

During 1989, peak grass shrimp densities at the CTL Site were observed in February, March, June, August, September and October. At the TRT Site during 1989, peak grass shrimp densities were observed during February, March, April, May, June, July, August, September and December. These time periods corresponded with periods of recruitment (i.e., usually February, June, August, and November). During 1989, significant runoff of fenvalerate was observed during 6/5-6/89 runoff event at the TRT Site. A 30% reduction in *P. pugio* density was observed during this period at the TRT Site. Following this time period, no additional reductions in *P. pugio* density were observed during 1989.

For 1990, during the nine months reported, peak grass shrimp densities were observed at the CTL Site during February, March, July and September. At the TRT Site during 1990, peak grass shrimp densities were observed during January, February, April, and July. The absence of peak densities at the CTL Site during April, 1990 resulted from significant predation by mummichogs and other fish species. The reduced densities at the TRT Site during September, 1990 may have resulted from significant fenvalerate runoff observed on the 28 May 1990.

Table 45. Summary of *P. pugio* density measurements (number/50 m of stream) observed in block seining at the CTL and TRT Sites, 1989-90. Asterisks (**;*) indicate samples which were significantly ($p \leq 0.05 - 0.10$) different.

Parameter: *P. pugio Density* (#/50 m of stream)				
Date	CTL Site		TRT Site	
	$\bar{X}$	SE	X	SE
1/89	986.7	573.	1,672.7	1199.2
2/89	4,759.7	3,686.3	3.554.0	1,682.0
3/89	4,735.3	4,671.9	9,288.3	3,508.5
4/89	611.3	553.1	5,630.3**,*	1,515.6
5/89	169.0	63.7	5,045.3**,*	482.3
6/7/89	345.7	119.9	3,526.0**,*	1,473.7
6/26/89	4,304.7	1,285.6	9,970.7	5,085.8
7/89	2,031.7	890.0	8,781.3**,*	609.7
8/89	7,266.0	2,554.0	16,508.7	6,899.5
9/89	8,058.3	2,303.7	17,045.3	5,131.1
10/89	7,414.2	5,026.9	11,099,8*	5,244.5
11/89	1,920.4	755.6	1,857.9	272.5
12/89	986.7	199.6	15,469.5*	10,874.7
1/90	1,178.0	276.5	5,510.3**,*	876.8
2/90	11,514.7	7,062.8	19,763.4	4,228.1
3/90	9,135.8	9,073.5	3,309.1	1,554.6
4/90	23.7	4.9	7,426.2**,*	6,162.0
5/11/90	236.2	217.8	125.9	46.5
5/30/90	461.3	270.8	829.2	161.2
6/90	3,895.7	1,717.2	1,272.1	242.6
7/90	6,568.7	2,966.7	6,004.0	2,750.9
8/90	1,900.3	881.6	1,905.9	119.6
9/90	5,965.3	3,026.3	2,591.1	496.3
Total	84,469		158,187	

← Hugo

** = Significantly ($p \leq 0.05$) Different in Unpaired Test; N=6
* = Significantly ($p \leq 0.10$) Different in Paired Test; N=6

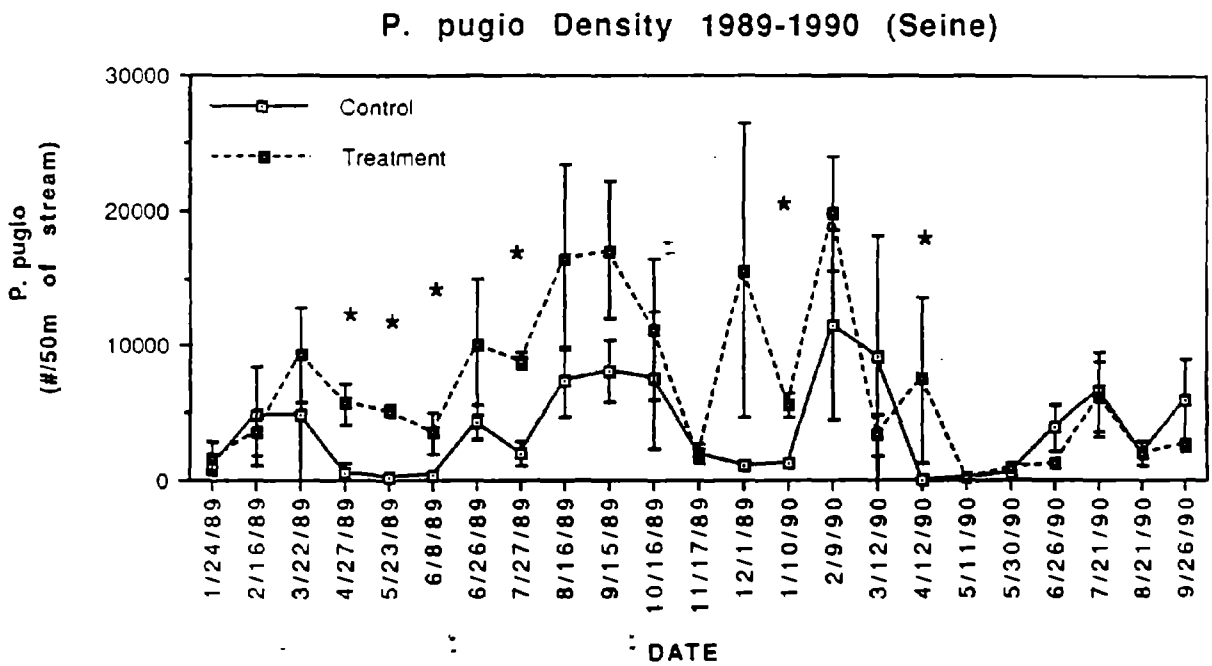


Figure 57. *P. pugio* densities (#/50m of stream) measured in block seining, 1989-90. Note the general similarities in grass shrimp densities during 1989-90 at both sites. Asterisks (*) indicate samples which were significantly ($p \leq 0.05$) different in statistical comparisons between the CTL and TRT Sites.

Although no toxicity was observed in caged adult *P. pugio*, larval *P. pugio* are four to five times more sensitive to low salinity, fenvalerate exposure than adults. Measured fenvalerate levels were more than 15 times greater than the 96h LC_{50} values for larval *P. pugio* exposed to fenvalerate at low salinities.

A total of 84,469 grass shrimp/50 m of stream were collected at the CTL Site versus 158,187 grass shrimp/50 m of stream at the TRT Site, during the 21 months of this study. Previous studies (Scott et al, 1990; Hampton, 1987) conducted during 1986-87, reported annual grass shrimp densities ranging from 55,293 - 114,000/50 m of stream at the CTL Site, compared to densities ranging only from 26,200-54,000/50 m of stream at the TRT Site. During 1986-87, there were significant impacts to *P. pugio* at the TRT Site observed in both caged toxicity tests and in biomonitoring studies (Scott *et al.*, 1990).

During 1989, annual *P. pugio* densities were 44,764 at the CTL Site compared to 109,448 at the TRT Site. In comparing these results, generally *P. pugio* densities at the CTL Site have remained fairly constant. (Mean densities varied by less than a factor 2.5 at the CTL Site versus 4.3 at the TRT Site). At the TRT Site, during episodes of significant agricultural pesticide runoff during 1986-87, *P. pugio* densities declined dramatically. During 1989, despite one period of significant fenvalerate runoff at the TRT Site, *P. pugio* densities dramatically increased, approaching peak annual densities observed at the CTL Site. These data may be suggestive that *P. pugio* populations are extremely resilient, being able to flourish despite some low concentrations of fenvalerate runoff occurring at the site. During 1986-87, fenvalerate concentrations were much higher (> 100 - 890 ng/L) than were measured during 1989 (< 100 ng/L). Another factor may be that fenvalerate concentrations measured during 1989 were residues of Asana rather than pydrin. Fenvalerate results from 1986-87, were measured as pydrin residues, rather than Asana.

An additional factor which may explain the higher *P. pugio* densities observed included the much lower predator pressures at the TRT Site, as evidenced by low populations of mummichogs and other fishes when compared to the CTL Site. This would allow *P. pugio* to flourish at the TRT Site due to the reduced predator pressures.

During the 9 months (January - September, 1990) sampled during 1990, total mean *P. pugio* densities were 39,705/50 m of stream at the CTL Site compared to a mean density of 48,739/50 m of stream at the TRT Site. During 1989, total grass shrimp densities at the CTL Site were only 40% of densities measured at the TRT Site. During 1990, grass shrimp densities at both sites were quite similar, with levels at the CTL Site approaching 81% of the TRT Site population densities. The much higher densities of mummichogs and other fish at the TRT Site during 1990, resulted in greater predatory pressures in *P. pugio* populations. As result *P. pugio* densities at the TRT Site were greatly reduced in 1990 as fish populations began to re-establish higher population abundance.

3. *F. heteroclitus* Density

Results of *F. heteroclitus* density measurements (#/50 m of stream) are listed in Table 46 and depicted in Figure 58. Mean mummichog densities ranged from 18-4391/50 m of stream at the CTL Site compared to levels ranging from 12 - 2,419/50 m of stream at the TRT Site. During 1989, peak mummichog densities at the CTL Site were observed during March, April, May, July, August, September and October with densities > 1000/50 m of stream. At the TRT Site during 1989, peak mummichog abundance was measured during July, August and September. These time periods generally correlated with entry of young of the year from late spawning during 1988 entering size classes measurable by our seining technique (March - May) and recruitment of first spawning (March) of 1989 young of the year entering measurable size cohorts. During 1990, peak abundance at the CTL Site was observed during June - September. At the TRT Site, peak densities were measured during June - July, 1990.

Total mean mummichog densities for the 21 months sampled during 1989-90, were 31,651.6/50 m of stream at the CTL Site versus 15,520.7/50 m of stream at the TRT Site. Previous studies during 1986-88 (Scott *et al.*, 1990; Hampton, 1987) reported annual mummichog densities ranging from 17,224 - 24,100/50 m of stream at the CTL Site, compared to levels ranging from 5,600 - 5,802.3/50 m of stream at the TRT Site. During 1986-87, significant runoff of azinphosmethyl, endosulfan and fenvalerate at the TRT Site resulted in depressed mummichog densities there, only 23-33% of CTL Site populations.

Table 46. Summary of *F. heteroclitus* density measurements (number/50 m of stream) observed in block seining at the CTL and TRT Sites, 1989-90. Asterisks (**;*) indicated samples which were significantly ($p \leq 0.05$ - 0.179) different.

Parameter: *F. heteroclitus* Density (#/50 m of stream)					
Date	CTL Site		TRT Site		
	X	SE	X	SE	
1/89	163.7	82.6	12.0	5.1	
2/89	861.7	311.7	77.3$^{**;*}$	30.8	
3/89	1,426.7	785.6	142.3$^{**;*}$	63.7	
4/89	1,601.3	1,352.0	364.3$^{**;*}$	102.2	
5/89	1,215.0	363.0	163.3$^{**;*}$	111.4	
6/7/89	1,490.3	1,437.0	282.3	74.4	
6/26/89	264.0	64.4	155.3	34.2	
7/89	1,012.0	501.5	2,328.0*	1,005.4	
8/89	2,111.0	1,378.8	2,419.0	1,420.7	
9/89	3,705.3	1,353.8	1030.0*	522.4	← Hugo
10/89	2,094.2	1,044.1	995.8*	533.8	
11/89	497.9	111.2	742.1	210.1	
12/89	130.7	70.9	319.7	114.7	
1/90	18.0	14.5	119.3*	49.8	
2/90	570.1	168.9	116.0$^{**;*}$	78.5	
3/90	733.5	309.1	202.4*	66.7	
4/90	611.1	216.8	475.6	88.3	
5/11/90	396.7	226.8	474.7	81.7	
5/30/90	923.6	453.0	352.5*	157.7	
6/90	1,336.5	832.7	1,947.1	700.1	
7/90	4,391.3	1,305.9	1,817.3*	799.7	
8/90	3,650.7	198.9	657.8$^{**A;*A}$	52.1	
9/90	2,446.3	840.2	326.6$^{**;*}$	213.2	
Total	31,651.6		15,520.7		

** = Significantly ($p \leq 0.05$) Different in Unpaired Test; N=6
* = Significantly ($p \leq 0.10$) Different in Paired Test; N=6
^{**A} = Significantly ($p \leq 0.08$) Different in Unpaired Test; N=5
^{*A} = Significantly ($p \leq 0.179$) Different in Paired Test; N=5

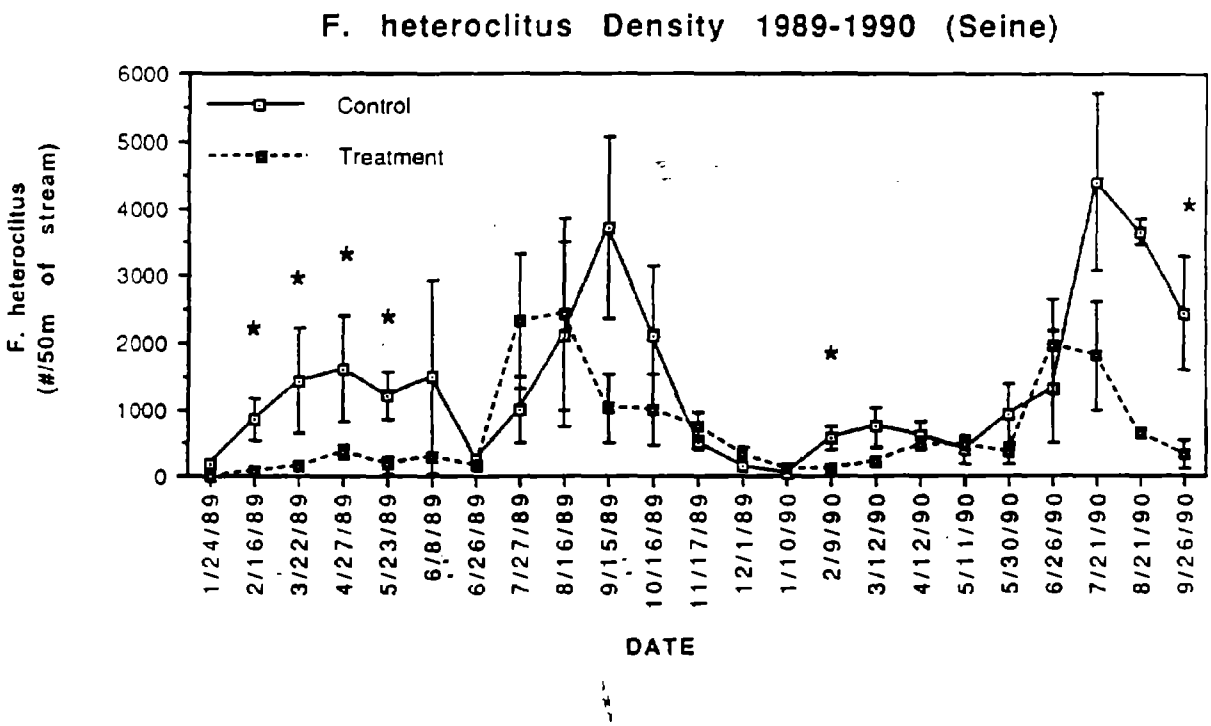


Figure 58. *F. heteroclitus* densities (#/50m of stream) measured in block seining, 1989-90. Note the higher densities at the CTL Site during most of 1989-90. Asterisks (*) indicated samples which were significantly ($p \leq 0.05$) different in statistical comparisons of the TRT and CTL Sites.

During 1989, total mean mummichog densities were 16,753.8 at the CTL Site compared to 9150.7 at the TRT Site. Mummichog densities at the TRT Site during 1989 were only 55% of CTL Site populations. Statistical analysis indicated that populations densities were significantly ($p \leq 0.05$-0.10) higher at the CTL Site during February - May, September and October, 1989. During July, 1989 mummichog densities at the TRT Site were significantly ($p \leq 0.10$) higher than CTL Site levels. The higher TRT Site populations observed during July were probably the result of recruitment of young-of-the-year fish whose densities flourished due to the higher grass shrimp densities measured at the TRT Site. Significant runoff of fenvalerate at the TRT Site during June, 1989 caused no toxicity in caged mummichogs deployed at that Site. Measured fenvalerate concentrations were below levels toxic to adult mummichogs. Similarly, biomonitoring results for 1989 appeared to support these results, as no toxicity directly attributable to runoff events was observed during June, 1989 sampling results. The increased population densities observed during 1989 at the CTL Site are likely the residual effects of previous fish kills at the TRT Site (June, 1985; May, 1986 and August, 1988).

During 1990 (January - September), total mean mummichog densities ranged from 15,077.8 at the CTL Site compared to 6,370 at the TRT Site. Mummichog densities at the TRT Site during 1990 were only 42% of CTL Site populations. Statistical analysis indicated that CTL Site densities were significantly ($p \leq 0.05$ - 0.10) higher than TRT Site densities during February, March, May, July, August and September, 1990. During January, 1990, mummichog densities at the TRT Site were significantly ($p \leq 0.10$) higher than CTL Site levels. Significant fenvalerate runoff during late May, 1990 at the TRT Site caused no toxicity in caged mummichogs as levels were below concentrations acutely toxic to adult mummichogs. Although mummichog populations were significantly lower at the TRT Site, two days post runoff, it is not likely that these differences were directly attributable to fenvalerate runoff. Rather these differences were probably related to earlier pesticide runoff effects at the TRT Site (1985-88).

Results from 1988-89, indicated that despite significant reductions in pesticide runoff at the TRT Site for 1989-90, mummichog densities remained significantly lower than at the CTL Site. These data are suggestive that mummichog populations at the TRT Site were slower to recover than other species (i.e. *P. pugio*).

4. Total Fish Density

Results of total fish density measurements (#/50 m of stream) are listed in Table 47 and depicted in Figure 59. Mean total fish densities ranged from 26.3 -4,723.9/50 mg of stream at the CTL Site compared to levels ranging from 108.3 - 3,787.0/50 m of stream at the TRT Site. During 1989, peak total fish densities (> 1500/50 m of stream) were observed at the CTL Site during March - early June, August - October, and in December, 1989. At the TRT Site, peak total fish densities were observed in early June, July and August, 1989. These peak periods of total fish density generally coincided with periods of time when juvenile mummichog young-of-the-year entered size cohorts measurable by our sampling methods. Mummichogs were the dominant fish species observed accounting for 72.7% of total fish density at CTL Site and 65.1% of total fish abundance at the TRT Site during 1989.

During 1990, peak abundances at the CTL Site were observed during March, June, July, August and September, 1990. At the TRT Site during 1990, peak densities were observed during June and July, 1990. These periods of peak densities observed during 1990, generally coincided with peaks of mummichogs density, especially intervals when juvenile mummichog young-of-the-year entered size cohorts measurable by our sampling methods. In 1990, mummichogs were the dominant fish species observed accounting for 70.2% of total fish density at the CTL Site and 57.1% of total fish abundance at the TRT Site.

Total mean total fish density for the 21 months sampled during 1989-90, was 44,274.5/50 m of stream at the CTL Site and 25,213.3/50 m of stream at the TRT Site. Previous studies during 1986-88 (Scott *et al.*, 1990; Hampton, 1987), reported annual total fish densities ranging from 24,314.3 - 39,060/50 m of stream at the CTL Site and from 12,255.7 - 17,505/50 m of stream at the TRT Site. During 1986-87, significant runoff of endosulfan, azinphosmethyl, and fenvalerate at the TRT Site resulted in several fish kills which reduced annual total fish populations to densities only 44.8 - 50.4% of CTL Site total fish densities.

Table 47. Summary of Total Fish Density measurements (number/50 m of stream) observed in block seining at the CTL and TRT Sites, 1989-90. Asterisks (**;*) indicate when samples were significantly ($p \leq 0.05$ - 0.179) different.

Parameter: Total Fish Density (#/50 m of stream)				
Date	CTL Site		TRT Site	
	$\bar{X}$	SE	X	SE
1/89	538.5	291.3	108.3	52.1
2/89	1,207.3	421.6	431.7*	170.7
3/89	2,107.7	775.7	726.3*	296.9
4/89	2,422.3	584.0	700.0**;*	206.5
5/89	1,989.3	384.9	449.7**;*	227.7
6/7/89	1,913.7	1,618.8	1,562.0	325.5
6/26/89	1,132.3	349.8	605.0	108.1
7/89	1,295.7	622.8	2,446.7*	1,067.3
8/89	2,716.0	1,618.3	3,787.0	1,199.0
9/89	4,284.0	1,668.5	1,109.0*	498.5
10/89	2,400.3	1,027.9	1,087.0*	535.6
11/89	611.3	105.5	682.1	138.7
12/89	165.7	67.2	360.2	118.5
1/90	26.3	13.2	191.7**;*	71.0
2/90	955.7	327.0	711.0	255.9
3/90	2,632.3	1,208.3	978.1	384.8
4/90	1,045.3	348.7	813.7	187.6
5/11/90	848.6	338.7	822.1	440.7
5/30/90	1,149.7	380.4	898.3	304.6
6/90	1,741.1	780.4	2,164.7	782.6
7/90	4,723.9	1,094.7	2,242.7*	974.9
8/90	4,639.2	381.4	1,109.4**A;*A	6.9
9/90	3,728.5	1,277.3	1,226.6	490.4
Total Density	44,274.5		25,213.3	

← Hugo

** = Significantly ($p \leq 0.05$) Different in Unpaired Test; N=6
* = Significantly ($p \leq 0.10$) Different in Paired Test; N=6
**A = Significantly ($p \leq 0.08$) Different in Unpaired Test; N=5
*A = Significantly ($p \leq 0.179$) Different in Paired Test; N=5

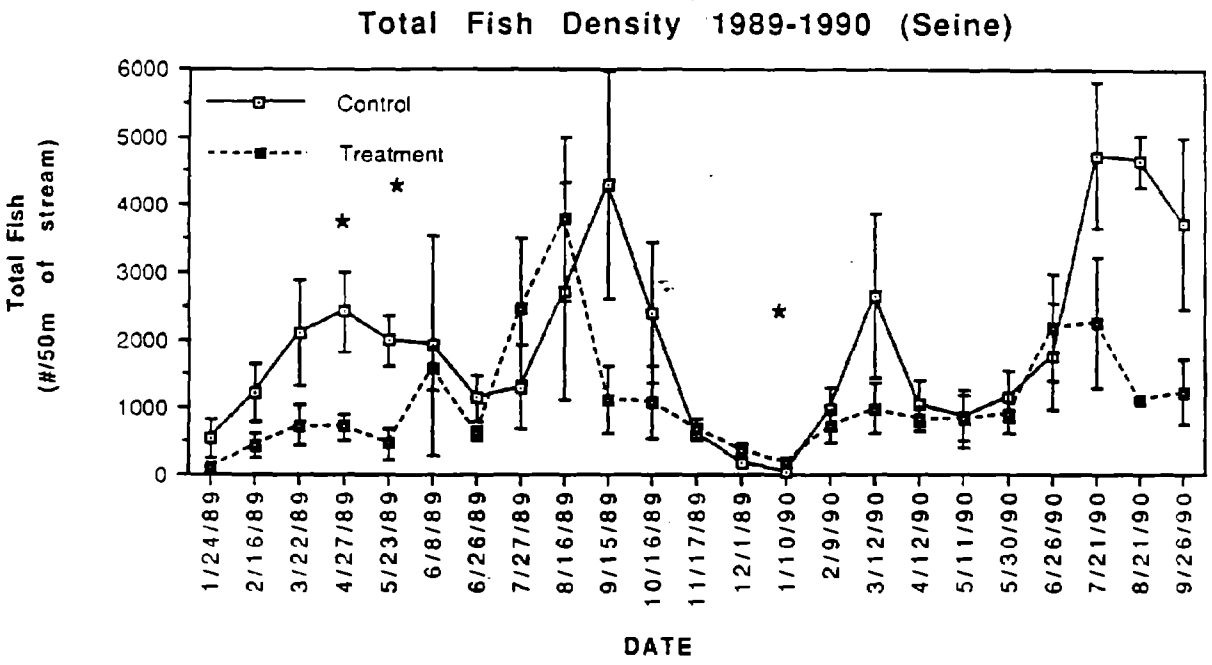


Figure 59. Total fish densities (#/50m of stream) measured in block seining during 1989-90. Note the higher densities at the CTL Site during much of the sampling period. Asterisks (*) indicated samples which were significantly ($p \leq 0.05$) different in between site comparisons of the CTL and TRT Sites.

During 1989, total fish densities at the CTL Site were 22,783/50 m of stream compared to 14,055/50 m of stream at the TRT Site. Total fish densities at the TRT Site during 1989 were only 57% of the CTL Site populations. Statistical analysis indicated that population densities were significantly ($p \leq 0.05 - 0.10$) higher at the CTL Site during February - May, September and October, 1989. During July, 1989 total fish densities at the TRT Site were significantly ($p \leq 0.10$) higher than CTL Site levels. This same pattern was observed in mummichogs during 1989.

During 1990 (January - September), total fish densities ranged from 21,490.6/50 m of stream at the CTL Site compared to 11,158.3/50 m of stream at the TRT Site. Total fish densities at the TRT Site were only 52% of CTL Site populations. Statistical analysis indicated that CTL Site densities were significantly ($p \leq 0.05 - 0.10$) higher than TRT Site total fish densities during January, July and August, 1990.

Significant fenvalerate runoff during June, 1989 and May, 1990 appeared to have little effect, as total fish density comparisons were not significantly different in both intra- and inter-site comparisons

5. Penaied Shrimp Densities

Results of penaied shrimp [*Penaeus aztecus* (brown), *P. duorurum* (pink), and *P. setiferus* (white)] density (#/50 m of stream) measurements and statistical comparisons at each site are listed in Table 48 and depicted in Figure 60. It was noted that juvenile brown shrimp first migrated into both branches of Leadenwah Creek during May, 1989 and generally remained at each site until November (TRT Site) or December (CTL Site). Juvenile white and pink shrimp first appeared in mid-June, 1989 and also remained until November, 1989. In other months of the year, penaied shrimp were not detected. A similar pattern of penaied shrimp migration was observed in 1990.

Mean penaied shrimp densities during 1989 ranged from 0.7 - 5,580.7/50 m of stream at the CTL Site and from 82.6 - 8,499.7/50 m of stream at the TRT Site. During 1990 (January - September), penaied shrimp densities ranged from

Table 48. Summary of *Penaeus species* density measurements (number/50 m of stream) observed in block seining at the CTL and TRT Sites, 1989-90. Asterisks (**) indicate when samples were significantly ($p \leq 0.05$ - 0.08) different.

Parameter: *Penaeus Species* Density (#/50 m of stream)					
Date	CTL Site		TRT Site		
	X	SE	X	SE	
1/89	0.0	0.0	0.0	0.0	
2/89	0.0	0.0	0.0	0.0	
3/89	0.0	0.0	0.0	0.0	
4/89	0.0	0.0	0.0	0.0	
5/89	330.0	169.9	217.3	110.8	
6/7/89	285.3	128.1	213.0	94.7	
6/26/89	5,580.7	426.6	8,499.7	3,388.2	
7/89	590.0	405.5	2,280.3	1,659.3	
8/89	800.3	264.7	2,357.7	518.9	
9/89	1,290.7	320.4	227.7**	137.2	← Hugo
10/89	61.7	11.3	138.7	65.7	
11/89	0.7	0.7	82.6**	56.6	
12/89	1.0	1.0	0.0	0.0	
1/90	0.0	0.0	0.0	0.0	
2/90	0.0	0.0	0.0	0.0	
3/90	0.0	0.0	0.0	0.0	
4/90	0.0	0.0	0.0	0.0	
5/11/90	50.5	18.1	96.9	50.7	
5/30/90	48.6	16.1	102.8	22.2	
6/90	117.1	16.4	361.7	232.7	
7/90	145.3	84.6	1,465.0	677.1	
8/90	21.0	5.3	148.7**A	18.4	
9/90	26.7	13.4	43.3	8.4	
Total	10,403.6		15,235.4		

** = Significantly ($p \leq 0.05$) different in Unpaired Test; N=6.

**A = Significantly ($p \leq 0.08$) different in Unpaired Test; N=5.

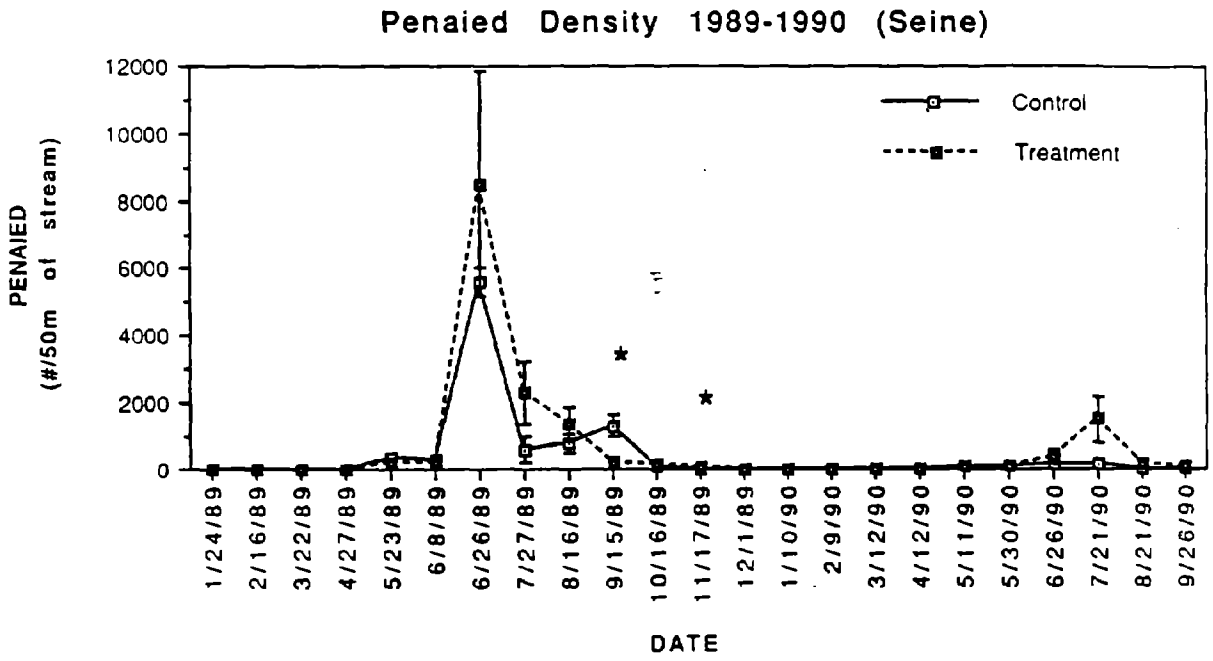


Figure 60. Penaied shrimp (*Penaeus aztecus*, *Penaeus duorarum*, and *Penaeus setiferus*) densities (#/50m of stream) measured in block seining during 1989-90. Asterisks (*) indicated samples which were significantly ($p \leq 0.05$) different in statistical comparisons between the CTL and TRT Sites.

21.0 - 117.1/50 m of stream at the CTL Site and from 43.3 - 1,465/50 m of stream at the TRT Site.

Peak shrimp densities during 1989 were observed during late June and September at the CTL Site and during late June, July and August at the TRT Site. Statistical analysis indicated penaied shrimp densities were significantly ($p \leq 0.05$) higher at the CTL Site compared to the TRT Site, during September, 1989. During November, 1989 penaied shrimp densities at the TRT Site were significantly ($p \leq 0.05$) higher than at the CTL Site. Between site density differences observed during 1989 were noted and related in part to significant fenvalerate runoff observed at the TRT Site during June, 1989. Differences in recreational fishing pressure between the CTL (higher) and TRT (lower) sites probably account in part for some of the observed between site differences.

During 1990, peak penaied shrimp densities were observed during June and July at both the CTL and TRT Sites. Statistical analysis indicated that penaied shrimp densities were significantly ($p \leq 0.08$) higher at the TRT Site during August, 1990, when compared to the CTL Site. Between site density differences observed during 1990 were not related to significant fenvalerate runoff observed at the TRT Site during May, 1990. Differences in recreational fishing pressures between the two sites may in part account for most observed between site differences.

A total of 10,403.6 penaied shrimp/50 m of stream at the CTL Site versus 15,235.4/50 m of stream at the TRT Site were observed during the 21 months of this study. Annual penaied shrimp densities ranged from 1463.2 (1990) to 8,940.4 (1989) at the CTL Site and from 2,218.4 (1990) to 13,017 (1989) at the TRT Site. Generally penaied shrimp densities at the CTL Site were reduced 31.3-33.4% compared to the TRT Site. These results compared favorably with earlier results (Scott *et al.*, 1990; Hampton, 1987) which reported penaied shrimp densities during 1986-88 ranging from 2,000 - 11,605/50 m of stream at the CTL and TRT Sites. During 1986-88, penaied shrimp densities at the CTL Site were reduced by 43-83% compared to the TRT Site, primarily due to higher recreational fishing pressure at the CTL Site.

6. Blue Crab Densities

Results of blue crab (*Callinectes sapidus*) density (#/50 m of stream) measurements and statistical comparisons at each site are listed in Table 49 and depicted in Figure 61. Generally highest blue crab densities were observed during the spring - early summer months and lowest densities were observed the late fall - early winter months. This pattern was observed at both sites during 1989 and 1990.

During the study period (1/89 - 9/90) mean blue crab densities ranged from 0.7 - 37.0/50 m of stream at the CTL Site and from 0.0 - 31.3/50 m of stream at the TRT Site. During 1989, peak blue crab densities at the CTL Site were observed during April, May, June and July, 1989. At the TRT Site, peak densities were observed during May, July and August, 1989. Statistical analysis indicated significantly ($p \leq 0.05 - 0.10$) higher densities at the CTL Site during March, April, June, September and December, 1989. Blue crab densities were significantly ($p \leq 0.10$) higher at the TRT Site during August and October, 1989.

During 1990, peak blue crab densities were observed during February, March, April, May and June at the CTL Site and during May, June and July at the TRT Site. Statistical analysis indicated significantly ($p \leq 0.05 - 0.10$) higher blue crab densities at the CTL Site during February, April and May, 1990. At the TRT Site, blue crab densities were significantly ($p \leq 0.05 - 0.10$) higher during July - August, 1990.

Total mean blue crab densities of 275.5/50 m of stream at the CTL Site and 188.5/50 m of stream at the TRT Site were measured during the 21 months of this study (1/89 - 9/90). Annual blue crab densities ranged from 118 (1990) - 157.5 (1989)/50 m of stream at the CTL Site and from 87.6 (1989) - 100.9 (1990)/ 50 m of stream at the TRT Site. Generally, annual blue crab densities were reduced by 14.5 - 44.4% at the TRT Site compared to the CTL Site during 1989-90. These results agree favorably with earlier results (Scott *et al.*, 1990; Hampton, 1987) which reported that from 1986-88 blue crab densities, ranged from 111 - 138.6/50 m of stream at the CTL Site and from 55 - 61.6/50 m of stream at the TRT Site. From 1986-88, blue crab densities were 50 - 55.4% lower at the TRT Site compared to

Table 49. Summary of *Callinectes sapidus* Density measurements (number/50 m of stream) observed in block seining at the CTL and TRT Sites, 1989-90. Asterisks (**, *, *A) indicated when samples were significantly ($p \leq 0.05$ - 0.10) different.

Parameter: *Callinectes sapidus* Density (#/50 m of stream)					
Date	CTL Site		TRT Site		
	$\bar{X}$	SE	X	SE	
1/89	3.3	1.5	1.3	1.3	
2/89	9.7	6.9	0.0	0.0	
3/89	8.3	3.8	1.7**	0.9	
4/89	25.0	6.1	5.7**	2.2	
5/89	37.0	22.5	12.7	8.6	
6/7/89	14.7	0.9	5.3**	2.3	
6/26/89	19.7	2.2	5.3**	3.2	
7/89	13.3	8.3	11.0	4.6	
8/89	5.7	3.3	31.3*	17.0	
9/89	7.7	0.7	4.0**	0.6	
10/89	2.7	2.2	5.3*	2.9	← Hugo
11/89	0.7	0.7	2.3	0.3	
12/89	9.7	3.3	1.7	1.2	
1/90	2.3	1.2	3.0	1.7	
2/90	22.0	9.9	0.3**	0.3	
3/90	11.0	5.1	4.7	3.3	
4/90	17.0	4.0	9.0*	2.1	
5/11/90	25.7	9.2	26.7	0.7	
5/30/90	12.7	3.3	8.7*	3.8	
6/90	9.3	1.9	13.0	5.1	
7/90	6.3	0.3	20.3**	2.3	
8/90	6.7	1.5	8.5*A	0.5	
9/90	5.0	0.6	6.7	3.3	
Total	275.5		188.5		

** = Significantly ($p \leq 0.05$) Different in Unpaired Test; N=6
* = Significantly ($p \leq 0.10$) Different in Paired Test; N=6
*A = Significantly ($p \leq 0.079$) Different in Unpaired Test; N=5

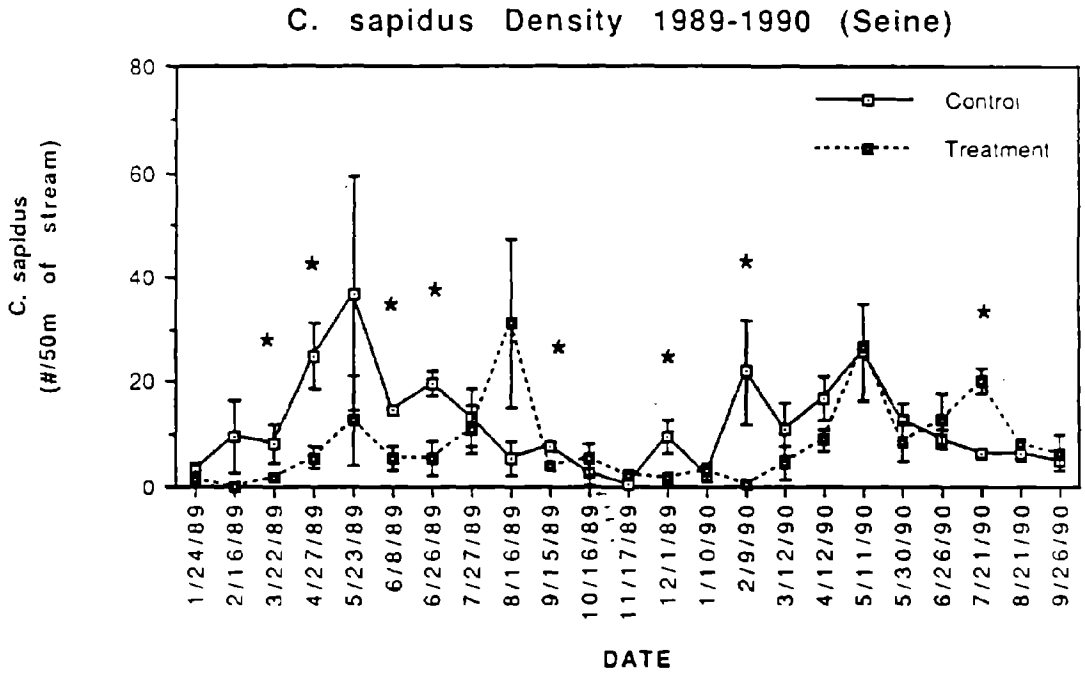


Figure 61. *Callinectes sapidus* densities (#/50m of stream) measured in block seining during 1989-90. Blue crab densities were quite similar at the CTL and TRT Sites during 1989-90. Asterisks (*) indicated samples which were significantly ($p \leq 0.05$) different in statistical between site comparison of the TRT and CTL Sites.

the CTL Site despite heavy recreational fishing pressures at the CTL Site. From 1986-90, blue crab densities at the TRT Site have steadily recovered from impacts resulting from significant runoff of azinphosmethyl, endosulfan, and fenvalerate. By 1990, annual blue crab densities only varied by 14.7% between the CTL and TRT Sites.

7. Discussion and Conclusions of Ecotoxicological Studies, 1989-90

During 1989-90, significant runoff of fenvalerate was observed during 5-7 June, 1989 (< 100 ng/L) and 28-29 May, 1990 (122 ng/L) at the TRT Site. Measured fenvalerate concentrations were below levels considered acutely toxic to adult fish and blue crabs but were considered potentially toxic to adult and juvenile grass shrimp, juvenile penaied shrimp and possibly juvenile fish.

In situ toxicity tests conducted at the TRT Site during 1989 indicated:

1) *P. pugio* survival was 28.5% (± 13.8%) at the TRT Site;

2) *Penaeus aztecus* survival was 51.9% (± 6.06%) at the TRT Site; and

3) No mortality (high survival) was observed in caged adult mummichogs and juvenile sheepshead minnow.

These results clearly indicated acute toxicity in caged grass shrimp and penaied shrimp following this rain event. Analysis of ecotoxicological data for *P. pugio* during May - June, 1989 indicated between site mortality of 34% compared to within site estimated mortality rates of 69%. These results agree favorably with the 72% mortality observed in field toxicity tests. Laboratory toxicity tests for fenvalerate predicted similar levels of toxicity in *P. pugio* (Scott et al, 1990). Additionally if one examines *P. pugio* recruitment 90 days post rainfall (September, 1989) and extrapolates the number of grass shrimp recruited into the population during the 90 day time period at both sites relative to the standing stock of adults at the time of the rain event, a mortality estimate of 75.2% is obtained. This estimate assumes equal growth and predation rates at both sites.

Analysis of 1989 penaied shrimp ecotoxicological data indicated that no significant toxicity in penaied shrimp was observed at the TRT Site, using both within and between site comparisons. These results may suggest in part, that juvenile penaied shrimp may

actively avoid pesticide runoff following periods of heavy rainfall unlike grass shrimp which appeared to be adversely affected.

During 1990, results of *in situ* toxicity tests indicated:

1) High survival in *P. pugio* at both sites;

2) High survival in *P. aztecus* at both sites: and

3) High survival in adult mummichogs and juvenile sheepshead minnows at both sites.

Analysis of 1990 ecotoxicological data indicated that grass shrimp and penaied shrimp populations at the TRT Site were unaffected by fenvalerate runoff. While grab samples collected at dead low tide indicated potentially toxic levels of fenvalerate (122 ng/L) composite samples collected during this same time period, indicated nondetectable fenvalerate levels (< 3 ng/L). These findings suggest that although significant fenvalerate runoff occurred, it may have only been a small volume of runoff which was diluted quickly with the incoming flood tide. As a result, no mortality was observed in field populations and caged bioassay organisms. The retention ponds at the TRT Site may have served to reduce overall runoff volume; hence preventing field mortality and large transboundary pesticide movement.

B. Push Netting, 1990

1. Total Biomass

Results of total biomass (g/50 m of stream) estimates from push netting are listed in Table 50 and depicted graphically in Figures 62 (CTL vs TRT Sites) - 63 (CTL vs KWA Sites). Mean monthly biomass ranged from 2.4 - 80.7 g/50 m of

Table 50. Summary of Total Biomass (grams/50 m of stream) measured in push net sampling during the 1990 field study. Asterisks (*) indicate when samples were significantly different from the CTL Site.

Parameter: Total Biomass (g/50 m of stream)						
Date	CTL		TRT		KWA	
	X	(SE)	X	(SE)	X	(SE)
3/26/90	11.9	9.2	13.2	3.5	12.0	7.8
4/20/90	27.1	18.0	90.1	39.7	41.2	21.0
6/4/90	70.6	57.4	72.3	41.1	27.7	3.4
6/20/90	80.7	47.0	170.2	96.6	51.6	20.5
7/20/90	63.9	30.3	56.5	23.7	50.8	15.2
8/22/90	42.7	17.4	43.8	10.1	27.3	11.2
10/13/90	31.7	2.9	10.2*	7.3	63.7*	8.5
11/20/90	11.2	5.5	3.2*	1.3	24.4	16.7
12/13/90	2.4	1.3	9.9	7.1	2.6	2.2
Total Biomass (3/26-12/13/90)	342.2		469.4		301.3	

* = Significantly ($p \leq 0.05$) different from ControlsTable 50

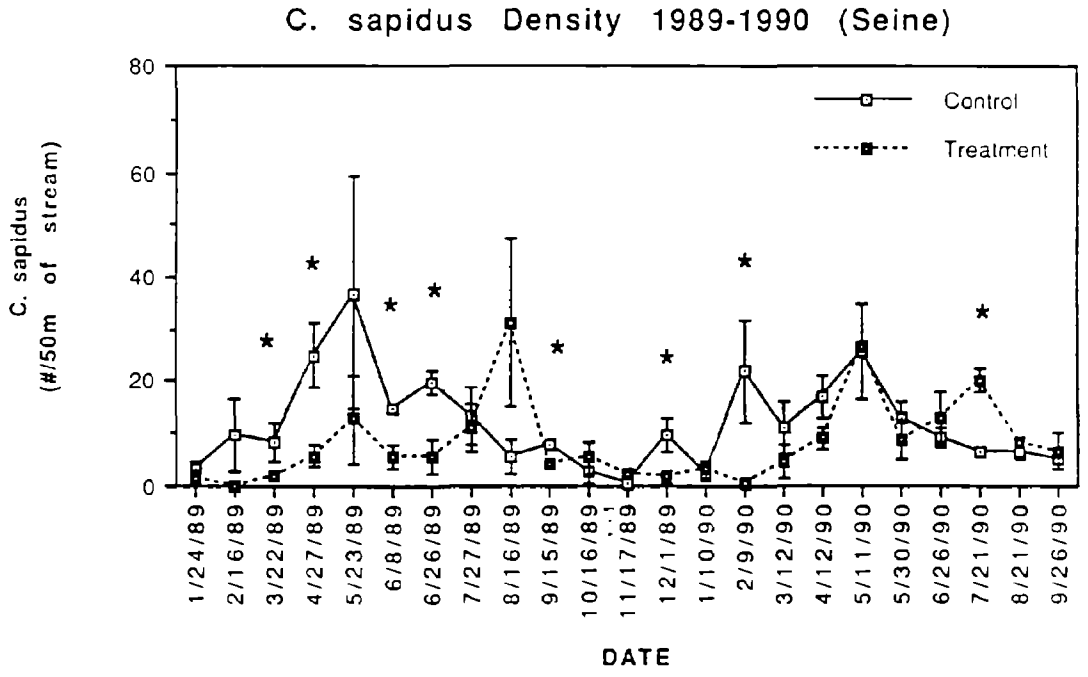


Figure 62. Total biomass (g/50m of stream) measured in push netting during 1990 at the CTL and TRT Sites. Generally total biomasses were quite similar at both sites during much of 1990. Asterisks (*) indicate samples which were significantly ($p \leq 0.05$) different in statistical between sites comparisons of the TRT and CTL Sites.

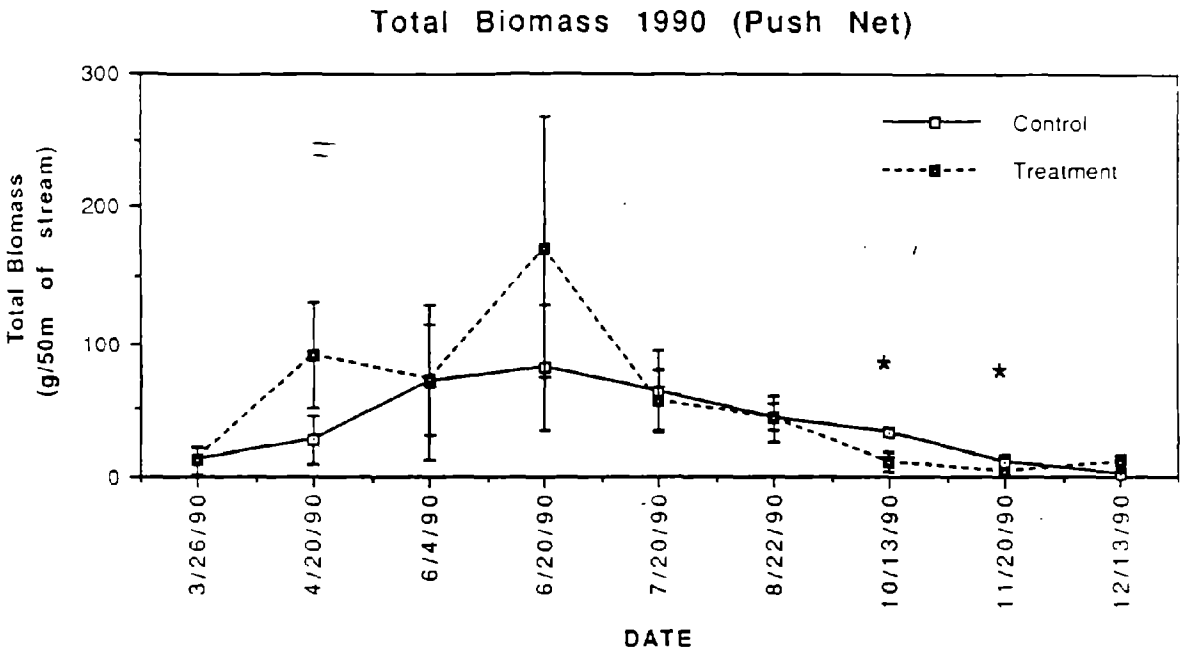


Figure 63. Total biomass (g/50m of stream) measured in push netting at the CTL and KWA Sites during 1990. Generally total biomasses were quite similar at both sites during much of 1990. Asterisk (*) indicated samples which were significantly ($p \leq 0.05$) different in statistical comparisons between the CTL and KWA Sites.

Total mean biomass for March - December, 1990 was 342.2 g/50 m of stream at the CTL Site, 464g/50 m of stream at the TRT Site, and 301.3 g/50 m of stream at the KWA Site. Statistical analysis indicated:

1) Significantly ($p \leq 0.05$) higher biomass at the CTL Site compared to the TRT Site during October and November, 1990; and

2) Significantly ($p \leq 0.05$) higher biomass at the KWA Site compared to both the CTL and TRT Sites during October, 1990.

Peak biomass was observed during June-July at the CTL Site, April - June at the TRT Site, and June, July and October at the KWA Site. These periods of peak biomass at each site generally coincided with peaks in *P. pugio* biomass which accounted for 78.4 - 83.2% of total biomass. Other species, including *Penaeus aztecus*, *Penaeus setiferus*, *Penaeus duorarum*, *F. heteroclitus*, *Mugil Cephalus*, *Poecilia latipinna*, *C. variegatus*, *M. menidia*, *A. mitchilli*, and *Callinectes sapidus*, accounted for the other 16.8 - 21.6% of the total biomass.

2. Total Density

Results of total density (#/50 m of stream) estimates from push netting are listed in Table 51 and depicted in Figures 64 (CTL vs. TRT Sites) and 65 (CTL vs KWA Sites). Mean monthly total density ranged from 22.7 - 531.7/50 m of stream at the CTL Site, 15 - 724.7/50 m of stream at the TRT Site, and 21.7 - 468.0/50 m of stream at the KWA Site. Total mean densities for March - December, 1990 were 1,947.7/50 m of stream, at the CTL Site, 2,003.1/50 m of stream at the TRT Site, and 1743/50 m of stream at the KWA Site. Statistical analysis indicated:

1) Significantly ($p \leq 0.05$) higher faunal densities at CTL Site compared to the TRT Site during October and November, 1990; and

2) Significantly ($p \leq 0.05$) higher faunal densities at the KWA Site compared to both the CTL and TRT Sites during October, 1990.

Table 51. Summary of Total Faunal Density (number/50 m of stream) measured in push net sampling during the 1990 field study. Asterisks (*) indicate when samples were significantly ($p \leq 0.05$) different from the CTL Site.

Parameter: Total Faunal Density (#/50 m of stream)						
Date	CTL		TRT		KWA	
	X	(SE)	X	(SE)	X	(SE)
3/26/90	57.3	47.4	44.0	18.2	30.3	15.7
4/20/90	22.7	16.9	194.7	94.5	123.7	83.6
6/4/90	470.7	390.8	355.7	165.6	154.7	18.2
6/20/90	531.7	289.7	724.7	412.9	253.3	82.2
7/20/90	371.3	167.6	316.0	139.2	152.0	76.5
8/22/90	215.7	113.2	239.0	48.0	199.7	94.9
10/13/90	192.0	25.3	31.3*	23.1	468.0*	50.4
11/20/90	63.0	31.0	15.0*	5.5	144.3	101.7
12/13/90	23.3	14.0	82.7	61.4	21.7	16.2
Total Density (3/26-12/13/90)	1,947.7		2,003.1		1,743.0	

* = Significantly ($p \leq 0.05$) different from Controls.

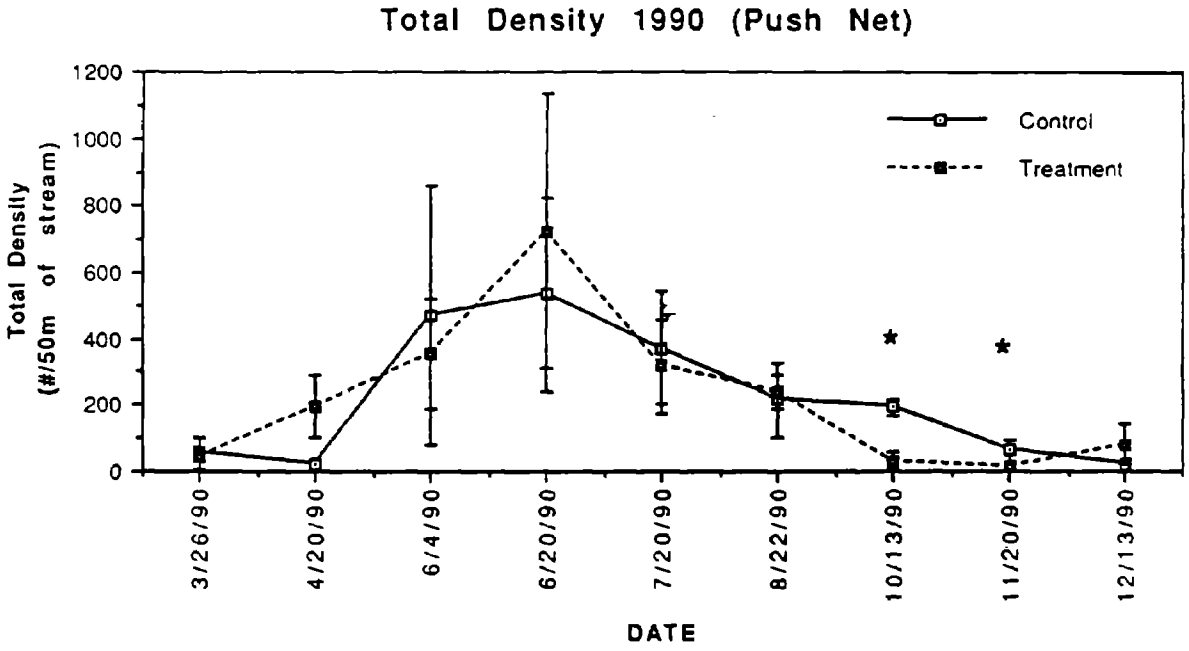


Figure 64. Total densities (#/50m of stream) measured in push netting at the CTL and TRT Sites during 1990. Generally, total densities were quite similar at both sites during much of 1990. Asterisks (*) indicated samples which were significantly ($p \leq 0.05$) different in between site statistical comparisons of the CTL and TRT Sites.

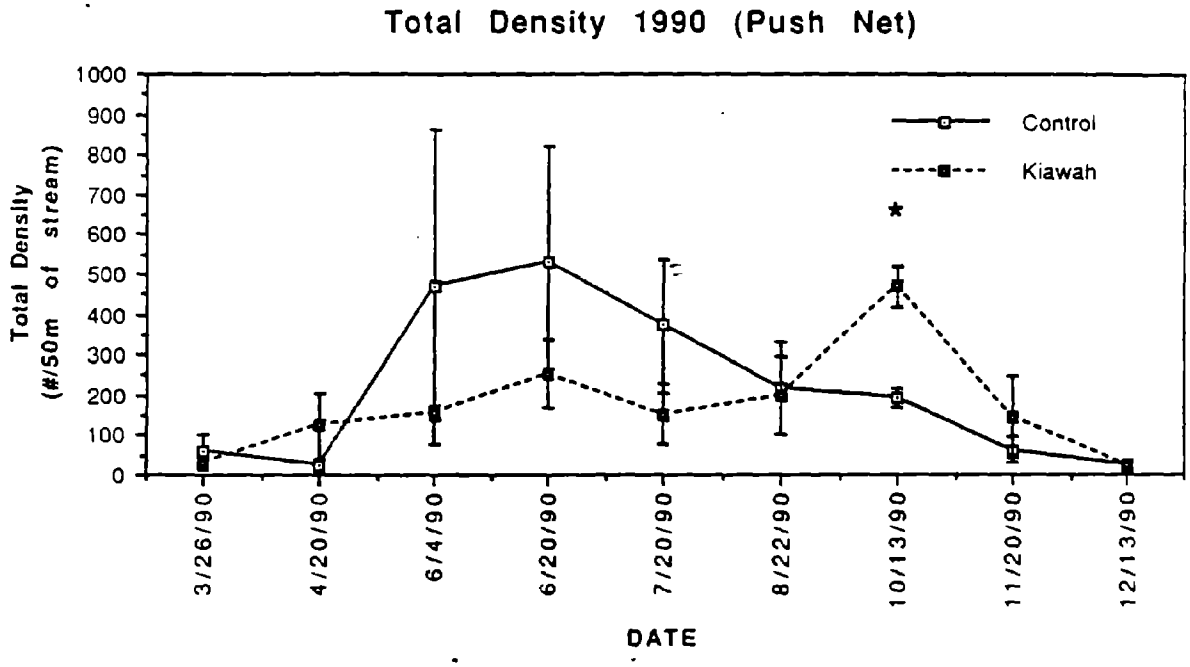


Figure 65. Total densities (#/50m of stream) measured in push netting at the CTL and KWA Sites during 1990. Total densities were generally similar at both sites during 1990. The asterisk (*) indicated the one sample which was significantly ($p \leq 0.05$) different in between site statistical comparison of the CTL and KWA Sites.

Peak total faunal densities were observed during June and July at the TRT and CTL Sites and during June and October at the KWA Site. These periods of peak faunal densities generally coincided with peaks in *P. pugio* density which accounted for 83.2 - 96.4% of all total faunal densities. At CTL and TRT Sites, *P. pugio* accounted for 95.2 and 96.4%, respectively of the total faunal density. At the KWA Site, *P. pugio* accounted for only 83.2% of total faunal densities. Species other than *P. pugio* accounted for 3.6, 4.8 and 16.8% of the total faunal densities, respectively at the TRT, CTL and KWA Sites.

3. *P. pugio* Density

Results of *P. pugio* abundance or density (#/50 m of stream) estimates from push netting are listed in Table 52 and depicted in Figures 66 (CTL vs TRT Sites) and 67 (CTL vs KWA Sites). Mean monthly *P. pugio* densities ranged from 15 - 520.7/50 m of stream at the CTL Site, 14 - 705.7/50 m of stream at the TRT Site, and 19 - 462.3/50 m of stream at the KWA Site. Total mean densities for March -December, 1990 were 1,854.4/50 m of stream at the CTL Site, 1931.1/50 m of stream at the TRT Site, and 1449.6/50 m of stream at the KWA Site. Statistical analysis indicated:

1) Significantly ($p \leq 0.05$) higher *P. pugio* densities at the CTL Site compared to the TRT Site during October and November, 1990; and

2) Significantly ($p \leq 0.05$) higher *P. pugio* densities at the KWA Site compared to the TRT Site during October, 1990.

Peak *P. pugio* densities were observed during June - July, 1990 at the CTL and TRT Sites while peak densities at the KWA Site were observed during June and October, 1990.

Earlier studies (Welch, 1975) reported *P. pugio* densities ranging from 20-300/m^2 using push netting in small tidal creeks in the Gulf Coast. Converting measured *P. pugio* densities/50 m of stream into densities/m^2 (using a conversion factor of 30 x measured density/50 m of stream) results in estimated *P. pugio* densities ranging from 0.5 - 15/m^2 at the CTL Site, 0.5 - 23.6/M^2 at the TRT Site,

Table 52. Summary of *P. pugio* Biomass (grams/50 m of stream) measured in push net sampling during the 1990 field study. Asterisks (*) indicate when samples were significantly ($p \leq 0.05$) different from the CTL Site.

Parameter: *P. pugio* Biomass (grams/50 m of stream)						
Date	CTL		TRT		KWA	
	X	(SE)	X	(SE)	X	(SE)
3/26/90	11.0	9.7	8.8	4.1	6.2	2.9
4/20/90	3.4	3.3	40.4	16.9	16.9	12.8
6/4/90	61.8	53.5	56.7	29.9	17.9	4.2
6/20/90	76.5	46.4	154.1	96.8	34.7	9.8
7/20/90	52.9	32.6	50.2	22.2	18.1	8.9
8/22/90	35.1	18.7	39.4	8.8	24.7	12.1
10/13/90	30.5	3.5	7.3*	4.4	59.1*	5.1
11/20/90	11.2	5.5	1.9*	0.8	24.2	16.8
12/13/90	2.2.	1.4	9.1	6.6	2.1	1.7
Total *P.pugio* Biomass (3/26-12/13/90)	284.6		367.9		238.6	

* = Significantly ($p \leq 0.05$) different from Controls.

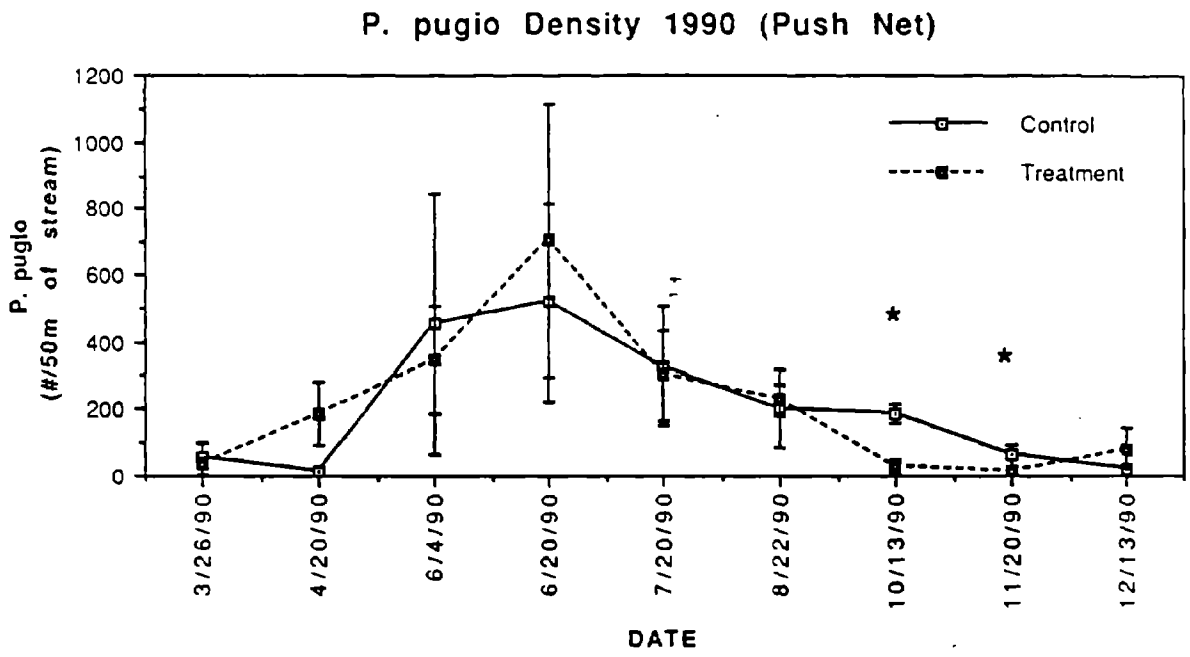


Figure 66. *P. pugio* densities (#/50m of stream) measured in push netting at the CTL and TRT Sites during 1990. Generally, grass shrimp densities were quite similar at both sites during much of 1990. Asterisks (*) indicated samples which were significantly ($p \leq 0.05$) different statistical comparisons.

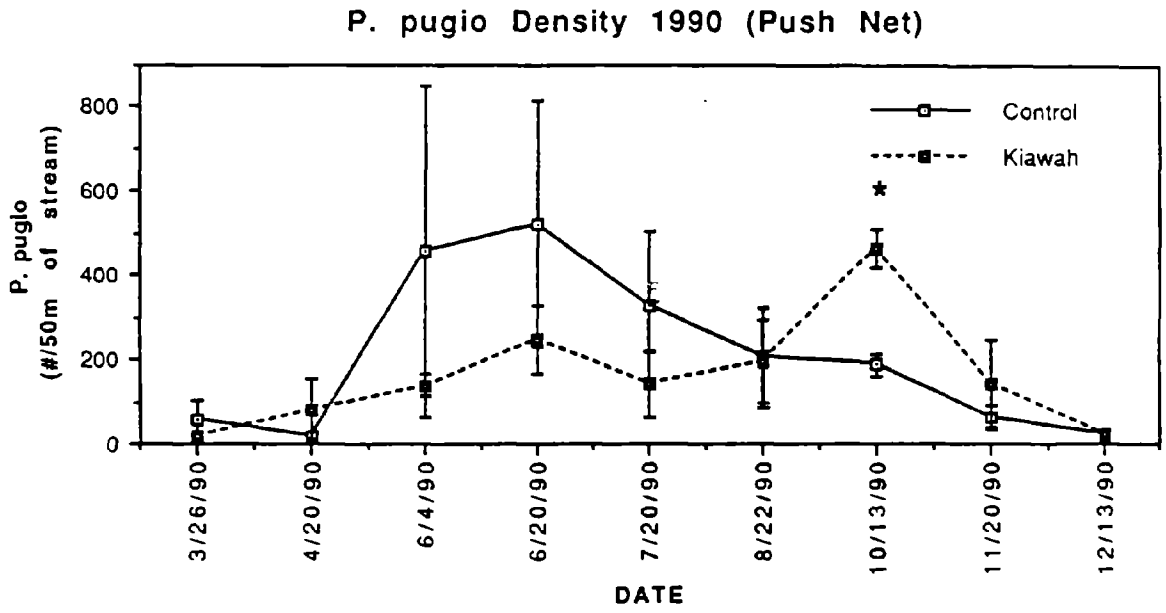


Figure 67. *P. pugio* density (#/50m of stream) measured in push netting at the CTL and KWA Sites during 1990. Generally, grass shrimp densities were quite similar at both sites during much of 1990. Asterisks (*) indicated samples which were significantly ($p \leq 0.05$) different in between site statistical comparisons.

and 0.7 - 15.4/m^2 at the KWA Site. On an annual basis, *P. pugio* densities would be 82.3/m^2 at the CTL Site, 85.1/m^2 at the TRT Site, and 64.2/m^2 at the KWA Site. Results from this study in mesotidal, (1 - < 3m tidal range) creeks are in agreement with those reported for microtidal (< 1m) creeks in terms of *P. pugio* densities.

4. *P. pugio* Biomass

Results of *P. pugio* biomass (g/50 m of stream) estimates from push netting are listed in Table 53 and depicted in Figures 68 (CTL vs TRT Sites) and 69 (CTL vs KWA Sites). Mean monthly *P. pugio* biomass ranged from 2.2 - 76.5g/50 m of stream at the CTL Site, 1.9 - 154.1 g/50 m of stream at the TRT Site, 2.1 - 59.1 g/50 m of stream at the KWA Site. Total mean *P. pugio* biomass for March - December, 1990 was 284.6 g/50 m of stream at the CTL Site, 367.9 g/50 m of stream at the TRT Site, and 238.6 g/50 m of stream at the KWA Site. Statistical analysis indicated:

1) Significantly ($p \leq 0.05$) higher *P. pugio* biomass at the CTL Site compared to the TRT Site during October and November, 1990; and

2) Significantly ($p \leq 0.05$) higher *P. pugio* biomass at the KWA Site compared to the CTL and TRT Sites during October, 1990.

Peak *P. pugio* biomass was observed during June - July, 1990 at the both the CTL and TRT Sites while peak *P. pugio* biomass at the KWA Site was observed during June and November, 1990.

5. Discussion: Comparisons of Estimated *P. pugio* Densities Using Block Seining and Push Netting Methodologies.

Tables 54 - 55 list results comparing estimated *P. pugio* densities using block seining and push netting methodologies at the CTL and TRT Sites from March - December, 1990. At the CTL Site, *P. pugio* densities ranged from 900 - 31,242/50 m of stream using push netting compared to densities ranging from 24 - 9,136/50 m of stream using block seining (Table 54). At the TRT Site, *P. pugio* densities ranged 1,842 - 42,342/50 m of stream using push netting compared to densities ranging from 829 - 7,426/50 m of stream using block seining (Table 54). These results suggest that

Table 53. Summary of *P. pugio* Density (number/50 m of stream) measured in push net sampling during the 1990 field study. Asterisks (*,*A) indicated when samples were significantly ($p \leq 0.05$) different.

Parameter: *P. pugio* Density (#/50 m of stream)						
Date	CTL		TRT		KWA	
	X	(SE)	X	(SE)	X	(SE)
3/26/90	55.0	48.5	39.0	16.9	19.0	6.1
4/20/90	15.0	14.0	187.3	93.5	83.0	70.6
6/4/90	456.7	392.7	344.7	159.4	140.3	24.3
6/20/90	520.7	293.8	705.7	409.2	245.7	80.5
7/20/90	328.7	178.6	300.0	133.3	141.0	76.3
8/22/90	204.3	116.6	229.0	45.7	194.0	95.8
10/13/90	188.0	26.3	30.7*	22.5	462.5*A	46.7
11/20/90	63.0	31.0	14.0*	5.5	144.0	102.0
12/13/90	23.0	14.2	80.7	60.4	20.3	14.9
Total Density (3/26-12/13/90)	1,854.4		1,931.1		1,449.6	

* = Significantly ($p \leq 0.05$) different from the CTL Site

*A = Significantly ($p \leq 0.05$) different from the TRT Site but not CTL Site.

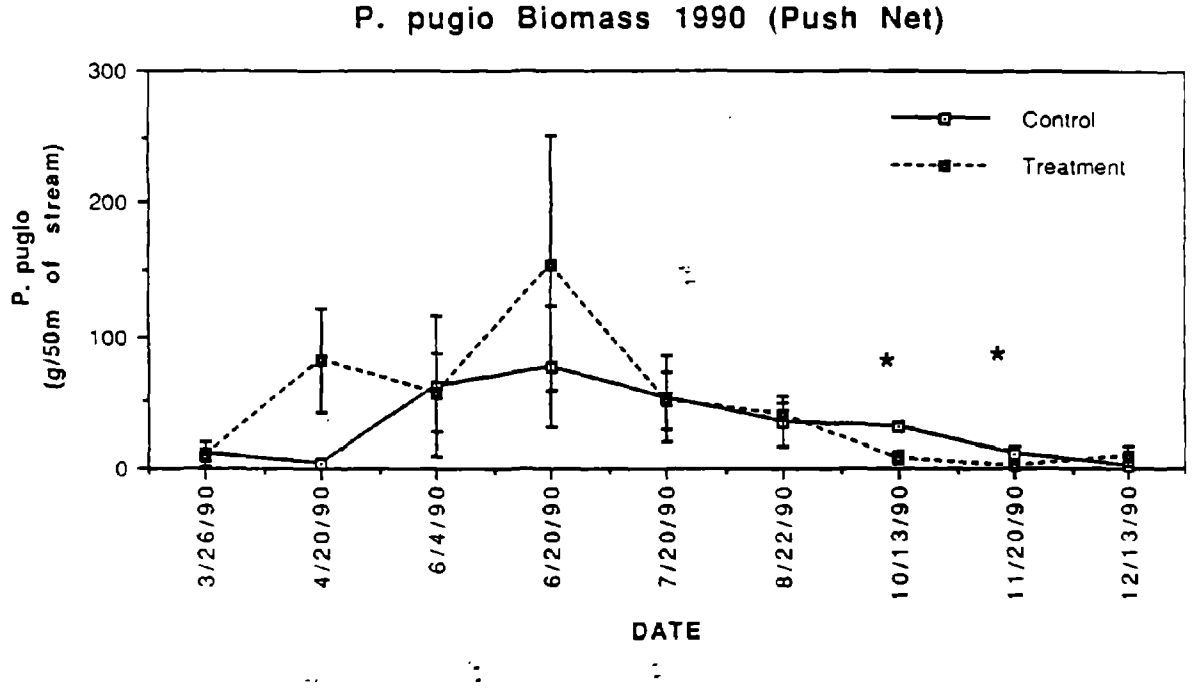


Figure 68. *P. pugio* biomass (g/50m of stream) measured in push netting at the CTL and TRT Sites during 1990. Generally, grass shrimp biomass was quite similar at both sites during much of 1990. Asterisks (*) indicated samples which were significantly ($p \leq 0.05$) different in statistical between site comparisons.

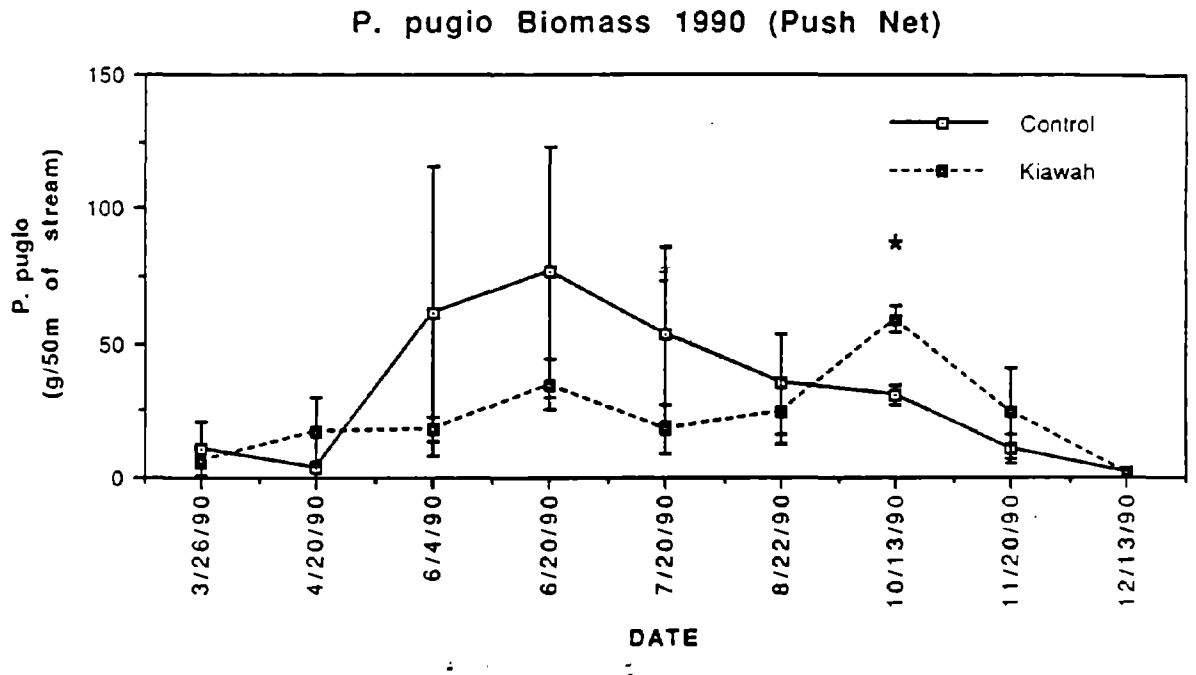


Figure 69. *P. pugio* biomass (g/50m of stream) measured in push netting at the CTL and KWA Sites during 1990. Generally, grass shrimp biomass was quite similar at both sites during much of 1990. Asterisks (*) indicated the one sample that was significantly ($p \leq 0.05$) different in between site comparisons.

Table 54. Summary of *P. pugio* Density (#/50 m of stream) measured in push net sampling during the 1990 field study.

Parameter: *P. pugio* Density (#/50 m of stream)				
Date	CTL		TRT	
	Push Net[1]	Seine	Push Net[1]	Seine
	X (SE)	X (SE)	X (SE)	X (SE)
3/90	3,300 (± 2,910)	9,136 (± 9,074)	2,340 (± 1,014)	3,309 (± 1,555)
4/90	900 (± 840)	24 (± 5)	11,238 (± 5,610)	7,426 (± 6,162)
6/4/90	27,402 (± 23,562)	461 (± 271)	20,682 (± 9,564)	829 (± 161)
6/30/90	31,242 (± 17,628)	3,896 (± 1717)	42,342 (± 24,552)	1,272 (± 243)
7/90	19,722 (± 10,716)	6,569 (± 2,967)	18,000 (± 7,998)	6,004 (± 2,751)
8/90	12,258 (± 6,996)	1,900 (± 882)	13,740 (± 2,742)	1,906 (± 120)
9-10/90	11,280 (± 1,578)	5,965 (± 3,026)	1,842 (± 1350)	2,591 (± 496)

1 = Conversion Factor of 60 used based on gear size differences between push net and seine net gear.

push netting *P. pugio* densities do not directly correspond numerically to absolute densities obtained using block seining; however, relative abundance comparisons are possible.

In statistical comparisons of *P. pugio* density differences between the CTL and TRT Sites using block seining and push netting, 86% of the conclusions reached in statistical tests were the same using both methods (Table 55). In 7% of the comparisons, block seining was more sensitive in detecting statistically significant differences than push netting ($p \leq 0.05$ seine vs. $p \leq 0.10$ push netting). In 14% of the comparisons, push netting detected significant differences when block seining did not. When this occurred mean *P. pugio* densities were numerically higher by 56.5% using block seining at the CTL Site, but were not statistically different due to skewness among replicates (i.e., most of the grass shrimp were congregated in one stream stretch rather than being randomly distributed). An error rate of 7-14% may be expected using the push net method. When one considers the added cost, time and effort required for block seining, push netting provides a reasonable sampling alternative, particularly in situations requiring rapid and large scale sampling such as oil/hazardous substance spills and for large regional-scale sampling efforts (i.e., E-MAP).

1989-90 Discussion and Conclusions

A. Correlating Laboratory and Field Toxicity Test Results with Field Ecotoxicological Biomonitoring

Earlier studies by Scott *et al.* (1990) have reported that the integration of field laboratory toxicity testing with ecotoxicological and ecophysiological biomonitoring provides a holistic method of environmental risk assessment for pesticides. Similarly, Swartz *et al.* (1986) and others (Chapman *et al.* 1983, 1984; Olla *et al.* 1984) have defined a Triad of toxicity tests, utilizing a combination of field assessments and laboratory toxicity testing to accurately define sediment toxicity and develop sediment quality criteria. The approach used in these methods involves: 1) Standard laboratory toxicity testing to define initial toxicity benchmarks and a battery of nonconventional toxicity tests to define an array of toxicant-ecological (abiotic-physicochemical and

Table 55. Summary of statistical results for *P. pugio* densities using seining and push netting during 1990. Note the excellent agreement between the two sampling methods.

Date	Seine	Push Net
3/90	C=T[1]	C=T
4/90	T>C ($p \leq 0.05$ unpaired) T>C ($p \leq 0.10$ paired)	T>C (unpaired $p \leq 0.12$) T>C (paired $p \leq 0.10$)
6/4/90	C=T	C=T
6/30/90	C=T	C=T
7/90	C=T	C=T
8/90	C=T	C=T
9-10/90	C=T (x = 5965 vs 2591)	C>T ($p \leq 0.05$ unpaired) C>T ($p \leq 0.10$ paired)

1 = All C = T are for paired and unpaired tests
C = Control Site; T = Treatment Site
I. Number of Sampling Date Comparisons = 14 (7 paired; 7 unpaired)
II. Number of Times of Agreement = 12 = 86%
III. Number of Time Seining was more sensitive than Push Net (False Negative) = 1 = 7%
IV. Number of Times Push Netting was more sensitive than seining (False Positive) = 2 = 14%

biotic-species, sex, lifestage differences) interactions; 2) A battery of field toxicity tests to define potential field effects; and 3) Biomonitoring to address field population effects and confirm field and laboratory toxicity test results. The approach used in this present study utilized a similar approach but added a series of ecophysiological studies to assess sublethal effects in field populations using both specific and nonspecific physiological parameters. The goals of these approaches are to develop protocols for establishing laboratory toxic tests which accurately predict field effects and to establish a paradigm for field validations in assessing both acute toxicity and acute/chronic sublethal effects.

Acute, laboratory toxicity tests provide the initial benchmark for the environment risk assessment process in determining pesticide safety. Most laboratory toxicity tests are designed to expose an organism to a number of sequential concentrations, over a defined (usually 96h) continuous exposure period. The results of such tests provide information on the no effect concentration, the lowest concentration causing 100% mortality, and the LC_{50} concentration. Additionally, these laboratory tests may provide identification of toxic threshold concentrations. Extrapolation of environmentally safe concentrations for a compound is possible if a number of different animal species (fish and invertebrates) are tested.

Acute toxicity testing in previous studies (Chandler and Scott, 1990; Scott *et al.*, 1990; Chandler, 1989; Fulton, 1989; Williams, 1989; Moore, 1988; Baughman, 1986; and Trim, 1986) have focused on the acute toxicity of azinphosmethyl, acephate, endosulfan and fenvalerate on the grass shrimp (*P. pugio*) and the mummichog (*F. heteroclitus*). Toxicity tests in these earlier studies were designed to:

1) Differentiate between the toxicity of EC and TG pesticides formulations (this is important since most conventional laboratory tests are conducted with TG material whereas field exposures are to various formulations).

2) Differentiate between different life history stage sensitivities. (This is important in both the selection of the most sensitive test species in field toxicity tests and in the prediction of impacts in field populations.)

3) Differentiate between acute toxicity in continuous and intermittent, pulsed exposures (field toxicity testing in semidiurnal mesotidal estuaries has indicated that a 6h pulsed dose exposure is representative of most field

exposures. Clark *et al.* (1987) have used 12h pulsed exposures in simulating fenthion toxicity in diurnal, microtidal environments).

4) Determine the interactive effects of low salinity conditions, which accompany pesticide exposure during runoff events (field toxicity testing has identified concomitant low salinity conditions generally accompany pesticide exposure during runoff events in small estuarine tidal creeks).

5) Determine the joint or additive toxicity potential of pesticide mixtures present in nonpoint source agricultural runoff. (Field toxicity testing has identified the presence of endosulfan/fenvalerate and azinphosmethyl/fenvalerate mixtures.)

6) Evaluate differences in toxicity between 6h pulsed dose and 96h continuous, dose exposures, by evaluating the entire dose-response curve. (Hazard Analysis)

7) Evaluate differences in pesticide toxicity at high and low salinities with continuous (96) and pulsed (6h) does exposures, by evaluating the entire dose-response curve. (Hazard Analysis)

8) Evaluate the effects of intrinsic factors (body length-size) on pesticide toxicity.

9) Determine the sublethal (respiration, nitrogen excretion, and O/N ratios) effects of fenvalerate exposure at high and low salinities.

10) Determine specific enzyme (AChE) responses to organophosphorus insecticide (azinphosmethyl) exposure.

11) Determine the lethal (acute toxicity) and sublethal (egg production and % hatch=fecundity) effects of sediment-bound fenvalerate and endosulfan in benthic invertebrates (copepods) and polychaete larvae.

Results from ecotoxicity tests in these earlier studies (Table 56) indicated:

1) Azinphosmethyl, endosulfan and fenvalerate were supertoxic (LC_{50} value < 10 μg/L) in tests with *P. pugio*.

2) Endosulfan and fenvalerate were supertoxic in tests with *F. heteroclitus* while azinphosmethyl was extremely toxic (LC_{50} value > 10 and < 100 μg/L). The SP formulation of acephate was rated relatively harmless (LC_{50} value > 1 x 10^6 μ/L) to *F. heteroclitus*.

3) There were no significant differences in LC_{50} values between TG and EC formulations of azinphosmethyl, endosulfan, and fenvalerate in tests with adult *P. pugio* and *F. heteroclitus*.

4) Adults and zoeal *P. pugio* were more sensitive to endosulfan exposure than post larval forms. Similarly juvenile *F. heteroclitus* were more sensitive to endosulfan exposure than adults.

5) Adults and zoeal *P. pugio* were more sensitive to fenvalerate exposure than post larvae. Adult and juvenile *F. heteroclitus* were equally sensitive to fenvalerate.

6) The 6h pulsed dose LC_{50} values for azinphosmethyl, endosulfan and fenvalerate in *P. pugio* ranged from 4.31-6.24 times the 96h LC_{50} value for these insecticides. The 6h pulsed dose LC_{50} values in *F. heteroclitus* exposed to endosulfan and fenvalerate ranged from 5.02-6.90 times the 96h LC_{50} value. The 6h Maximum Tolerated Pulse Does (MTPD) values for these three insecticides ranged from 0.48-1.12 times the 96h LC_{50} value in *P. pugio* and from 2.18-3.45 times the 96h LC_{50} value in *F. heteroclitus*. These findings indicate field toxicity would occur at concentrations ranging from 4-6 times the 96h LC_{50} values for these three insecticides.

Table 56. Summary of 96h static renewal and 6h pulsed dose acute toxicity tests exposing adult *P. pugio* to azinphosmethyl, endosulfan and fenvalerate; zoeal *P. pugio* to fenvalerate, and adult *F. heteroclitus* to azinphosmethyl, endosulfan, fenvalerate and acephate at high (20 ppt) and low (5-10 ppt) salinities. Note that the most any intrinsic or extrinsic factor affected acute toxcity (when compared to 96h LC50 at 20 ppt at 20°C) was ≤ a factor of 2.86. (From Scott et al., 1990).

Insecticide	Test Organisms[1]	Type of Toxicity Test	Salinity (ppt)	96h LC_{50} (95% CL) in µg/L	Toxicity Ratio Values[3]
EC Azinphosmethyl	*P. pugio* (A)	96h, SR	20	1.05 (0.91-1.21)	96h LC50 at 20 ppt / 96h LC50 at 5 ppt = 1.08
			5	0.97 (0.77-1.24)	
		6h, PD	20	6.68 (5.83-7.66)	6h PDLC50 at 20 ppt / 6h PDLC50 at 5 ppt = 0.82
			5	8.14 (7.25-9.13)	
	F. heteroclitus (A)	96h, SR	20	36.95 (28.30-48.24)	96h LC50 at 20 ppt / 96h LC50 at 5 ppt = 1.32
			5	28.00 (20.23-38.76)	
EC Endosulfan	*P. pugio* (A)	96h, SR	20	1.01 (0.72-1.43)	96h LC50 at 20 ppt / 96h LC50 at 5 ppt = 1.63
		6h, PD	20	4.35 (3.09-6.14)	6h PDLC50 at 20 ppt / 6h PDLC50 at 5 ppt = 1.14
			5	3.81 (3.01-4.83)	
	F. heteroclitus (A)	96h, SR	20	1.45 (1.32-1.59)	96h LC50 at 20 ppt / 96h LC50 at 5 ppt = 1.12
			5	1.29 (1.21-1.37)	
EC Fenvalerate	*P. pugio* (A)	96h, SR	20	0.052 (0.043-0.063)	96h LC50 at 20 ppt / 96h LC50 at 5 ppt = 0.87
			5	0.060 (0.037-0.097)	
		6h, PD	20	0.314 (0.260-0.380)	6h PDLC50 at 20 ppt / 6h PDLC50 at 5 ppt = 1.34
			5	0.235 (0.106-0.522)	
	P. pugio (Z)	96h, SR	20	0.020 (0.013-0.031)	96h LC50 at 20 ppt / 96h LC50 at 5 ppt = 2.86
			10	0.007 (0.005-0.009)	
	F. heteroclitus (A)	96h, SR	20	2.86 (2.02-4.06)	96h LC50 at 20 ppt / 96h LC50 at 5 ppt = 1.75
			5	1.63 (1.08-2.47)	
		6h, PD	20	14.35 (11.15-18.48)	6h PDLC50 at 20 ppt / 6h PDLC50 at 5 ppt = 1.68
			5	8.55 (5.88-12.45)	
SP Acephate	*F. heteroclitus* (A)	96h, SR	20	26.79×10^3 ($21.61-33.21 \times 10^3$)	96h LC50 at 20 ppt / 96h LC50 at 5 ppt = 0.75
			5	35.36×10^3 (29.35×10^3-42.58×10^3)	

EC = Emulsifiable Concentrate
SP = Soluble Powder
1 = Test Organism: *P. pugio* (A) = Adult (15-25 mm); *P. pugio* (Z) = Zoeal stage larvae 1-2 days old; and *F. heteroclitus* (A) = Adults (35-70 m)
2 = Type of Toxicity Tests: 96h. SR = 96 hour static renewal and 6h. PD = Six hour, pulsed dose.
3 = Toxicity Ratio Value: 1) 96h LC50 at 20 ppt / 96h LC50 at 5 ppt Ratio = the potency or enhancement of toxicity by low salinity (5 or 10 ppt), insecticide exposure during 96h toxicity tests.
2) 6h PDLC50 at 20 ppt / 6h PDLC50 at 5 ppt Ratio = The potency or enhancement of toxicity by low (5 ppt) salinity, insecticide exposure during 6h, pulsed does toxicity tests.

7) Low salinity did not significantly affect the toxicity of azinphosmethyl or endosulfan in continuous 96h exposures of adult *P. pugio* and *F. heteroclitus*. In continuous exposures to fenvalerate, low salinity slightly enhanced the toxicity of fenvalerate to adult *F. heteroclitus* and significantly enhanced the toxicity of fenvalerate to zoeal *P. pugio* but not adult *P. pugio*.

8) Low salinity did not significantly enhance the acute toxicity of azinphosmethyl, endosulfan and fenvalerate to adult *P. pugio* in 6h pulsed does exposures.

9) Endosulfan/fenvalerate insecticide mixtures were slightly less than additively toxic in exposures of adult and zoeal *P. pugio* and adult *F. heteroclitus*. In exposures of post larval *P. pugio*, this insecticide mixture was additively toxic. In toto, these findings indicate that endosulfan/fenvalerate mixtures were slightly less than additively toxic.

10) The fenvalerate/azinphosmethyl insecticide mixture was slightly less than additively toxic to adult *P. pugio*.

11) The azinphosmethyl/endosulfan mixture was slightly more than additively toxic to *F. heteroclitus* at 20 ppt salinity and approached simple additive toxicity at 5 ppt salinity.

12) The acephate/fenvalerate mixture was simply additively toxic to *F. heteroclitus* at 20 ppt salinity and less than additively toxic at 5 ppt salinity.

It is interesting to note that no intrinsic (life stage) or extrinsic (salinity, exposure duration) factors enhanced toxicity $\geq$ a factor of 2.86 of the 96h LC_{50} value at 20 ppt salinity and 20°C.

Results of earlier laboratory toxicity tests with benthic copepods exposed to sediment bound fenvalerate (22.5-90 µg/kg) indicated significant ($p \leq 0.05$-0.01) reductions in both the incidence of egg production and number of eggs produced/female for several species (*Microarthridion littorale* and *Paronychocamptus wilsoni*) at fenvalerate concentrations as low as 22.5 µg/kg (Chandler, 1990). Similarly, Chandler and Scott (1991) reported that exposure of benthic copepods and larval polychaetes to sediment-

bound endosulfan resulted in significant ($p \leq 0.05 - 0.01$) effects on survival (*Nannopus palustris*) and reductions in larval settlement, feeding and growth (*Streblospio benedicti*) at endosulfan concentrations ranging from 50-200 μg/kg. Results from these laboratory toxicity tests using field collected sediments clearly indicated that agricultural pesticide runoff may result in significant impacts to benthic copepods and polychaetes by adversely affecting survival, growth, settlement, feeding and reproduction.

The results of these earlier studies summarized by Scott *et al.*, (1990) clearly demonstrated the utility and practicality of using a battery of toxicity tests to assess acute and chronic, lethal and sublethal effects in aquatic and benthic marine species. Laboratory toxicity tests were designed to not only establish acute toxicity baselines but also to better define environmental risk assessment models by evaluating intrinsic and extrinsic factors affecting toxicity.

Laboratory toxicity experiments conducted in this present study were designed to:

1) Refine the EC_{50} for azinphosmethyl in mummichogs based upon brain AChE inhibition; and

2) Evaluate the effects of azinphosmethyl exposure and resulting brain AChE inhibition on general physiological performance in the mummichog.

Earlier studies (Scott *et al.*, 1990; Fulton, 1989) had reported a 24 EC_{50} based upon % AChE inhibition of 0.81 μg/L for mummichogs. Additional toxicity testing has further refined this 24h EC_{50} estimate to 0.90 μg/L. By conducting experiments using a larger number of exposure concentrations, a more precise 24h EC_{50} for azinphosmethyl AChE inhibition in mummichogs was achieved. The greater precision obtained with these laboratory experiments will enable more accurate field and laboratory comparisons to be made.

Results of laboratory toxicity tests exposing mummichogs to a subacute, 24h dose of azinphosmethyl and then assessing resulting general physiological responses indicated that:

1) 24h exposure of mummichogs to 2.4 μg/L azinphosmethyl resulted in 81% brain AChE inhibition;

2) After 8 days of depuration in clean seawater brain AChE had recovered to only 70% of controls ($p \leq 0.05$);

3) Whole animal oxygen consumption was unaffected by azinphosmethyl exposure;

4) Whole animal nitrogen excretion rates were significantly ($p \leq 0.05$) lower in azinphosmethyl exposed mummichogs after 24h exposure; however, after 8 days of depuration nitrogen excretion rates were not significantly different in comparisons between treatment and control groups;

5) O/N rates were not significantly different in comparisons between control and treatment group fish either immediately following 24h azinphosmethyl exposure or after 8 d of depuration.

Results of these experiments clearly indicated that exposure of mummichogs to 2.4 μg/L azinphosmethyl (6.5% of the 96 LC_{50}) resulted in significant ($p \leq 0.05$) inhibition of brain AChE (81%). While significant brain AChE inhibition was noted, no significant effect on respiration was observed, although nitrogen excretion was inhibited at 24h. After 8d of depuration, however, nitrogen excretion rates were comparable to those in control animals. Previous studies by Scott *et al.*, (1987) and Trim (1987) have reported a similar phenomena in mummichogs exposed to a subacute dose of endosulfan. Following 96h of exposure, nitrogen excretion was significantly reduced in endosulfan exposed fish. After 7 days of depuration, nitrogen excretion rates returned to levels comparable to control fish in a manner similar to what was observed in this study. O/N ratios were not significantly different in comparisons between treatment and control fish, suggesting that the rigors of azinphosmethyl exposure and resulting brain AChE inhibition did not cause major alterations and shifts in substrate utilization (i.e., from carbohydrate to protein or lipid to protein) and resulting whole animal physiology. The decreased nitrogen excretion rate observed in azinphosmethyl exposed fish may either reflect a shift in substrate utilization away from normal proportions of protein, or may signify inhibition of normal nitrogen excretion at the gill. Previous studies with endosulfan exposed mummichogs (Scott *et al.*, 1987; Trim, 1987) reported reduced

nitrogen excretion may have resulted in a build up of blood ammonia levels which may ultimately be a factor in the acute toxicity of endosulfan. Whether the decreased nitrogen levels observed in this study signify a similar inhibition of nitrogen excretion and resulting buildup of blood ammonia concentrations is unclear. Given the fact that the head-kidney of the mummichog is located in the gill, it is possible that azinphosmethyl may potentially affect the function of $Na^+K^+Mg^+$ pump which serves the dual function of ion and osmotic regulation in the mummichog. Additionally, given the high levels of brain AChE inhibition and only minor metabolic (i.e., nitrogen excretion) alterations observed at azinphosmethyl concentrations of only a fraction (6.5%) of the 96h LC_{50}, is suggestive that a fairly large reserve of brain AChE activity exists, as it relates to acute lethality.

Results from these laboratory toxicity tests and bioassays with azinphosmethyl are extremely important in better defining risk assessments for aquatic organisms exposed to this pesticide. When reduced brain AChE levels are found in azinphosmethyl exposed fish in the field, are they indicative of only azinphosmethyl exposure, or are they indicative of both azinphosmethyl exposures and significant physiological effects? In a more general sense, the real underlying hypothesis is: Does AChE inhibition measure organophosphorus pesticide exposure, effects, or both? Clearly results from this study indicate that for azinphosmethyl, AChE inhibition is an indicator of exposure. The relatively minor metabolic effects observed associated with AChE inhibition in this study, suggest additional work is needed to better explain the relationship between AChE inhibition and other metabolic bioenergetic perturbations. Additionally, given the short term persistence of many organophosphate insecticides in the environment and the apparent greater persistence of AChE inhibition following exposure, suggests that AChE inhibition may provide a reliable and moderately persistent biomarker of organophosphorus insecticide exposure.

Field toxicity tests provide the second tier in the environmental risk assessment process. Field toxicity tests are designed to expose an organism to the compound being studied at different geographical sites. Each site will typically have a wide range of physicochemical environmental exposure conditions. Pesticide exposure regimes at each site will be intermittent and discontinuous. Additionally, physicochemical water quality factors such as salinity, temperature, pH, and dissolved oxygen which are held constant in the lab, may vary significantly during field exposure and thus may potentially enhance

pesticide toxicity. Another factor to consider in assessing agricultural discharges is that runoff may contain pesticide mixtures rather than individual compounds per se. Results of field toxicity tests generally indicate: 1) field mortality rates at different toxicant concentrations; 2) provide evidence for the potential interaction of physicochemical water quality parameters; 3) evidence of the water solubility, bioavailability, persistence, and degradation potential for various toxicants; and 4) some prediction of relative toxicity in field populations.

Results of earlier field toxicity studies (Scott *et al.*, 1990) conducted during 1985-88 at the same field sites used in the present study indicated (See Table 57):

1) A total of 10 major dates of rainfall (> 1.25 cm/day) were observed which resulted in significant runoff of azinphosmethyl (0.0005-3.920 μg/L), endosulfan (0.003-0.998 μg/L), and fenvalerate (0.011-0.890 μg/L).

2) A total of three fish kills were observed at two field sites (TRT and KWA Sites).

3) During fish kills both dead *P. pugio* and *F. heteroclitus* were observed among endemic fauna.

4) In *P. pugio*, field mortality was observed in seven out of 10 rain events with mortality rates ranging form 0-100%.

5) In *F. heteroclitus*, field mortality was observed in four out of 10 rain events with mortality rates ranging from 0-100%.

During 1989-90, a total of eight days of significant (> 1.25 cm/day) rainfall was observed, which resulted in significant runoff of azinphosmethyl (< DL - 7.002 μg/L), endosulfan (< DL - 0.163 μg/L), and fenvalerate (< DL - 0.123 μg/L) (Table 58). A total of three fish kills were observed, two at the KWA Site and one at the adjacent Haulover Creek Site. During these fish kills both dead *P. pugio* and *F. heteroclitus* were observed as well as other fish species (*M. cephalus*, crustaceans (Uca and penaied Shrimp) and other invertebrates (polychaetes and other annelids).

Table 57. Summary of field toxicity tests results from 1985-88 for *P. pugio* and *F. heteroclitus* following dates of significant (> 1.27 cm/day) rainfall.

Rainfall Date	Insecticide	Insecticide Concentration[1]	% Mortality	
			P. pugio	*F. heteroclitus*
6/8/85	Endosulfan	0.003	52	NM
	Fenvalerate	0.107		
6/27/85	Endosulfan	0.249	100.0	NM
	Fenvalerate	0.079	(Fish Kill = 189 dead fish and crustaceans/50 m of stream)	
5/14-15/86	Azinphosmethyl	3.920	NM	NM
			(Fish Kill = Dead *F. heteroclitus* observed in endemic fauna)	
6/9/86	Azinphosmethyl	0.560	90	65-100
	Fenvalerate	0.032		
6/4/87	Endosulfan	0.012	0	0
	Fenvalerate	0.031		
6/19/87	Endosulfan	0.004	0	0
6/23/87	Azinphosmethyl	0.005[A]-0.024	53	0
	Endosulfan	0.005[A]-0.012		
	Fenvalerate	0.011[A]-0.013		
6/24/87	Endosulfan	0.024[A]-0.058	93-100	0
	Fenvalerate	0.110[A]-0.890		
6/25/87	Endosulfan	0.018	22-50	0
	Fenvalerate	0.070		
6/9-10/88	Azinphosmethyl	3.440	50	0
	Endosulfan	0.998	(Fish Kill = 150 dead fish and crustaceans/50m of stream)	

NM = Not Measured
1 = Peak concentrations measured in grab samples unless otherwise noted
A = Composite Sample

Table 58. Summary of field toxicity test results in *P. pugio* and *F. heteroclitus* measured during periods of significant (> 1.27 cm/day) rainfall for the 1989-90 field study.

Rainfall Date	Insecticide	Insecticide Concentration[1] (ug/L)	% Mortality	
			P. pugio	*F. heteroclitus*
6/5 - 6/89	Azinphosmethyl	1.730	80.0 (± 20.0)	0.0 (± 0.0)
	Endosulfan	0.163		
	Fenvalerate	0.064		
	Fenvalerate	0.065^A - 0.093	71.5 (± 13.8)	0.0 (± 0.0)
6/9/89	Azinphosmethyl	0.368	0.0 (± 0.0)	0.0 (± 0.0)
	Endosulfan	0.054		
	Fenvalerate	0.022^A - 0.021	36.7 (± 3.3)	0.0 (± 0.0)
6/16/89	Azinphosmethyl	2.457	100.0(±0.0)-62.6(±8.12)	0.0 (± 0.0)
	Endosulfan	0.038	(Fish Kill = Dead *F. heteroclitus*, *M. cephalus*, penaied shrimp, *P. pugio*	
	Fenvalerate	0.015	6.6 (± 6.6)	0.0 (± 0.0)
6/19/89	Azinphosmethyl	1.351	40.0 (± 15.28)	3.3 (± 3.3)
	Endosulfan	0.027		
	Fenvalerate	<0.003	10.7 (± 6.43)	0.0 (± 0.0)
6/24/89	Azinphosmethyl	7.002	100.0 (± 0.0)	16.6 (± 6.7)
	Endosulfan	0.065	Fish Kill = Dead *F. heteroclitus*, *P. pugio*, *Uca*, and *Polychaetes*)	
	Fenvalerate	<0.003	3.3 (± 3.3	0.0 (± 0.0)
5/28/90	Fenvalerate	<0.003^A - 0.123	3.3 (± 3.3)	3.3 (± 3.3)
6/15/90	Azinphosmethyl	0.024^A - 0.062	3.3 (± 3.3)	0.0 (± 0.0)
	Endosulfan	0.005^A - 0.004	0.0 (± 0.0)	0.0 (± 0.0)

[1] = Peak concentrations in grab samples unless otherwise denoted.
A = Composite Samples

In *F. heteroclitus*, significant (> 5%) field mortality was observed in one out of eight rain events with mortality ranging from 0 - 16.6% (± 6.7%). In *P. pugio*, significant (> 5%) mortality was observed in six out of eight rain events with mortality ranging from 0-100%. All observed mortality was attributed to insecticide exposure as all physicochemical parameters such as dissolved oxygen and salinity, were within known tolerance ranges for the fish and crustacean species studied.

During 1989, six periods of significant rainfall were observed. Significant mortality occurred at the KWA Site and TRT Site during six and two of the six rain events, respectively. During the first, two rain events (6/5-6/89 - combined due to two days of consecutive rain) deployed organisms were exposed to insecticide runoff. Significant concentrations of azinphosmethyl (1.73 μg/L) and endosulfan (0.163 μg/L) at the KWA Site and fenvalerate (0.093 μg/L) at the TRT Site caused 80 and 71.5% mortality, respectively, in *P. pugio* deployed during field toxicity tests. No mortality was observed in *F. heteroclitus* at all sites during these two initial rain events. All mortality in this rain event was attributed to insecticide exposure, as all physicochemical parameters, such as dissolved oxygen and salinity, were within known tolerance ranges for the fish and crustacean species studied.

During the third 1989 rain event (6/9/89), significant concentrations of azinphosmethyl (0.368 μg/L) and endosulfan (0.054 μg/L) were observed at the KWA Site and significant levels of fenvalerate (0.022 μg/L) at the TRT Site. In *P. pugio*, significant mortality (36.7%) was observed at the TRT Site but not at the KWA Site. No mortality was observed in *F. heteroclitus* at all sites during the third rain event. All *P. pugio* mortality was attributed to insecticide runoff, as all physicochemical parameters such as dissolved oxygen and salinity, were within known tolerance ranges for this crustacean.

During the fourth 1989 rain event (6/16/898), significant concentrations of azinphosmethyl (2.457 μg/L) and endosulfan (0.038 μg/L) were observed at the KWA Site, along with a fish kill involving dead *F. heteroclitus, M. cephalus,* penaied shrimp, and *P. pugio*. At the TRT Site, significant runoff of fenvalerate (0.015 μg/L) was observed. In *P. pugio*, significant mortality was observed at the KWA Site which ranged from 62.6 - 100%. No *P. pugio* mortality occurred at the TRT Site. In *F. heteroclitus*, no mortality was observed during the fourth rain event. All crustacean mortality was attributed to insecticide exposure as all physicochemical parameters such as dissolved oxygen and salinity, were within known tolerance ranges for these species.

During the fifth rain event of 1989 (6/19/89), significant runoff of azinphosmethyl (1.35 μg/L) and endosulfan (0.027 μg/L) was observed at the KWA Site. Significant mortality (40%) was observed in *P. pugio* deployed at the KWA Site. At the TRT Site slight *P. pugio* mortality (10.7%) was observed, despite the absence of detectable pesticide levels. No mortality attributable to pesticides was observed in field deployed *F. heteroclitus* (0 - 3.3%). All *P. pugio* mortality was attributed to insecticide exposure, as all physicochemical parameters, such as dissolved oxygen and salinity, were within known tolerance ranges for this organism.

During the sixth and final rain event of 1989 (6/24/89), significant runoff of azinphosmethyl (7.002 μg/L) and endosulfan (0.065 μg/L) was observed at the KWA Site, which resulted in significant mortality in field deployed *P. pugio* (100%) and *F. heteroclitus* (16.6%). Immediately following this rain event, a fish kill was observed at the KWA Site, and later at a tidal creek (Haulover) adjacent to the KWA Site. No mortality attributable to insecticide exposure was observed at the TRT Sites in either *P. pugio* or *F. heteroclitus*. All mortality was attributed to insecticide exposure, as all physicochemical parameters, such as dissolved oxygen and salinity, were within known tolerance ranges for these species.

During 1990, only two periods of significant ($>$ 1.25 cm/day) rainfall were observed (5/28 and 6/15/90). No significant mortality was observed among field deployed *P. pugio* and *F. heteroclitus*, despite the presence of a significant concentration of fenvalerate ($<$ DL - 0.123 μg/L) at the TRT Site (5/28/90) and azinphosmethyl (0.024 - 0.062 μg/L) at the KWA Site (6/15/90). The absence of toxicity in *P. pugio* exposed to potentially toxic levels of fenvalerate (0.123 μg/L) was puzzling. Analysis of composite water samples for the initial $\approx$ 12h post rain period indicated that fenvalerate levels were $<$ DL. The high fenvalerate levels (0.123 μg/L) were observed at dead low tide. These data suggest that only a small amount of low salinity runoff water containing high fenvalerate concentrations was discharged at the TRT Site during this runoff event. The retention pond at the TRT Site, by retaining a large portion of tomato field runoff, may have reduced the overall runoff volume sufficient that, the incoming flood tide was able to rapidly dilute fenvalerate concentrations to levels $<$ DL. The potential runoff loading capacity (volume of runoff/volume of stream) may decrease by 86% from ebb tide to flood tide, due to the simple increase in stream volume associated with normal mesotidal tidal exchange. These data are suggestive that the retention ponds at the TRT Site may in part help enhance the assimilative capacity of a watershed, by reducing runoff volumes and in turn resulting pesticide concentrations.

Biomonitoring or ecotoxicological studies of endemic field populations provide a third tier in the environmental risk assessment of pesticides. The approach used in most biomonitoring studies is quantitative, replicated ecological (usually pelagic or benthic) sampling. The results of such studies provide estimates of field population changes in response to toxicant exposure. Biomonitoring provides an independent mechanism to confirm the validity of toxicity test results. In addition, direct linkage between biomonitoring and laboratory/field toxicity tests is needed and should include the use of species in toxicity tests which are endemic to the habitat being studied. The two organisms used in laboratory toxicity tests in this present study, *P. pugio* and *F. heteroclitus*, are the most dominant crustacean and fish species, respectively in the Leadenwah tidal creek drainage basin, accounting for over 80% of the annual total abundance.

Results of earlier ecotoxicological biomonitoring studies (Scott *et al.*, 1990; Hampton, 1987; and Patterson, 1986) conducted from 1985-88, at the TRT and CTL Sites (See Table 59) indicated:

1) In the absence of significant pesticide runoff, traditional ecological comparisons such as species richness, evenness, diversity, index of similarity, total abundance, and total biomass were virtually identical, from January - May of each year, prior to peak periods of agricultural runoff (late May - June);

2) From 1985-88, ecotoxicological biomonitoring indicated significant mortality in eight out of the 10 rain events, with mortality rates ranging from 0 - 99% in *P. pugio* and from 0 - 95% in *F. heteroclitus*; and

3) Following periods of significant pesticide runoff during 1985-88, significant ($p \leq 0.05$) reductions in total biomass, total abundance, and densities of *P. pugio, F. heteroclitus,* Penaeus species, *Callinectes sapidus*, and total fish were observed at the TRT Site.

During 1989-90 (Table 6), a total of eight days of significant (> 1.25 cm/day) rainfall were observed, which resulted in significant runoff of azinphosmethyl (< DL - 7.002 μg/L), endosulfan (< DL - 0.163 μg/L), and fenvalerate (< DL - 0.123 μg/L). A total of three fish kills were observed, two at the KWA Site and one at the adjacent Haulover Creek Site. During these rain events, significant (> 5%) mortality was observed in three (including fish kills) out of the eight rain events in *F. heteroclitus*, with mortality ranging from 0 - 24.4% based upon block seining. *F. heteroclitus* results for 1990, indicated significant mortality in push netting during both rain events, with mortality estimates ranging from 21.2 - 45.4%. In *P. pugio*, block seining results for 1989-90, indicated significant (> 5%) mortality in five out of the eight rain events (including fish kills), with mortality estimates ranging from 0 - 43.1%. *P. pugio* push netting results for 1990, only indicated significant mortality in one out of the two rain events, with mortality estimates ranging from 0 - 24.6%.

Table 59. Summary of ecotoxicological biomonitoring estimates of field mortality in *P. pugio* and *F. heteroclitus* following dates of significant (> 1.27 cm/day) rainfall.

Rainfall Date	Insecticide	Insecticide Concentration[1] (μg/L)	% Predicted Mortality[2]	
			P. pugio	*F. heteroclitus*
6/8/85	Endosulfan	0.003	72	75
	Fenvalerate	0.107		
6/27/85	Endosulfan	0.249	98	80
	Fenvalerate	0.079	(Fish Kill)	
5/14-15/86	Azinphosmethyl	3.920	61	69
			(Fish Kill)	
6/9/86	Azinphosmethyl	0.560	99.9	83
	Fenvalerate	0.032		
6/4/87	Endosulfan	0.012	0	0
	Fenvalerate	0.031		
6/19/87	Endosulfan	0.004	0	0
6/23/87	Azinphosmethyl	0.005[A]-0.024	82[B]-94	69[B]-95
	Endosulfan	0.005[A]-0.012		
	Fenvalerate	0.011[A]-0.013		
6/24/87	Endosulfan	0.024[A]-0.058	82[B]-94	69[B]-95
	Fenvalerate	0.110[A]-0.890		
06/25/87	Endosulfan	0.018	82[B]-94	69[B]-95
	Fenvalerate	0.070		
6/9-10/88	Azinphosmethyl	3.440	NM	NM
	Endosulfan	0.998	(Fish Kill)	

1 = Peak Concentrations measured in grab samples unless otherwise noted.
2 = Between site mortality estimates unless otherwise noted.
A = Composite Sample
B = Within site mortality estimate.
NM = Not Measured

Table 60. Summary of ecotoxicological estimates of mortality in *P. pugio* and *F. heteroclitus* observed during significant (> 1.27 cm/day) rainfall events monitored during the 1989-90 field study.

Rainfall Date	Insecticide	Insecticide Concentration[1] (ug/L)	% Mortality*	
			P. pugio	*F. heteroclitus*
6/5 - 6/89	Azinphosmethyl	1.730	NM	NM
	Endosulfan	0.163		
	Fenvalerate	0.064		
	Fenvalerate	0.065[A] - 0.093	43.1	0
6/9/89	Azinphosmethyl	0.368	NM	NM
	Endosulfan	0.054		
	Fenvalerate	0.022[A] - 0.021	22.7	0
6/16/89	Azinphosmethyl	2.457	NM	NM
	Endosulfan	0.038	Fish Kill	
	Fenvalerate	0.015	0	0.0 (± 0.0)
6/19/89	Azinphosmethyl	1.351	NM	3.3 (± 3.3)
	Endosulfan	0.027		
	Fenvalerate	<0.003	0	0.0 (± 0.0)
6/24/89	Azinphosmethyl	7.002	NM	16.6 (± 6.7)
	Endosulfan	0.065	Fish Kill	
	Fenvalerate	<0.003	0	0
5/28/90	Fenvalerate	<0.003[A] - 0.123	0[2] - 24.6[3]	21.2[3] - 24.4[2]
6/15/90	Azinphosmethyl	0.024[A] - 0.062	0	0[2] - 45.4[3]
	Endosulfan	0.005[A] - 0.004	0.0 (± 0.0)	0.0 (± 0.0)

* = Between site mortality estimate unless otherwise noted.
A = Composite Sample
[1] = Peak concentrations in grab samples unless otherwise denotes
[2] = Block Seine Estimate
[3] = Push Net Estimate

During the six days of significant rainfall during 1989, the only significant mortality observed at the TRT site was in the crustacean, *P. pugio* exposed to fenvalerate at concentrations ranging from 0.065 (composite) - 0.093 (peak grab) μg/L during the rain events of 6/5-6/89 and at concentrations ranging from 0.021 (peak grab) - 0.022 (composite) μg/L during the rain event of 6/9/89. *P. pugio* predicted mortalities from block seining were 43.1% and 22.7% respectively, for these two rain events. Unfortunately, during 1989, biomonitoring was not conducted at the KWA Site.

During the two days of significant rainfall during 1990, significant runoff of fenvalerate at concentrations ranging from < DL (Composite) - 0.123 (peak grab) μg/L during the rain event of 5/28/90 and significant runoff of azinphosmethyl at concentrations ranging from 0.024 (Composite) - 0.062 (peak grab) μg/L on the rain event of 6/15/90, were observed. In *F. heteroclitus*, block seining predicted mortalities ranged from 0 - 24.4%, while push netting predicted mortalities ranged from 21.2 - 45.4%. During the first rain event, the two estimates of mortality in *F. heteroclitus* were quite similar (21.2 - 24.4%); however, during the second rain event, push netting predicted mortality while block seining did not. Push netting is generally intended to accurately estimate relative *P. pugio* densities and not *F. heteroclitus* densities. *F. heteroclitus* is not accurately censused by push netting and as a result, spurious conclusions may be reached.

In *P. pugio*, block seining predicted no mortality (0%) in either rain event, while push netting results predicted mortalities ranging from 0 - 24.6%. The 24.6% mortality predicted in the first rain event by push netting was higher than 0% mortality predicted by block seining; however, statistical analysis between *P. pugio* push net densities indicated that the 24.6% reduction in *P. pugio* densities at the TRT Site was actually not significantly different from CTL Site densities. These findings suggest that estimated *P. pugio* densities for push netting may only provide relative abundances, as opposed to the absolute abundance estimates block seining provides. Block seining samples a much larger proportion of the population (> 70%) of a defined area, than does push netting. As a result, when predicting *P.pugio* mortality from push netting, it is imperative to view any predicted mortality estimate within the context of statistical analysis for relative *P. pugio* abundance. Unless statistical analysis indicates that the relative *P. pugio* abundances are significantly different, then predicted mortality estimates are unreliable and may instead reflect simple population variability.

Comparisons between observed mortality in field toxicity tests and ecotoxicological biomonitoring studies with mortality estimates predicted for laboratory toxicity tests are difficult. Earlier studies by Scott et al, (1990) have indicated that each method of environmental risk assessment (acute laboratory toxicity test, *in situ* toxicity tests, and ecotoxicological biomonitoring) was useful in the assessment of the acute toxicity for three classes of pesticides (organochlorine, organophosphates, and pyrethoids) commonly used in agriculture. Generally most methods were similar in their prediction of the acute toxicity of azinphosmethyl, endosulfan, and fenvalerate on *P. pugio* and *F. heteroclitus*. Linear regression analysis for all pesticides (azinphosmethyl, endosulfan, and fenvalerate) and all species (*P. pugio* and *F. heteroclitus*) were significantly ($p \leq 0.01 - 0.05$) correlated (R - 0.47 - 0.64) in comparisons between field and laboratory toxicity tests ($R = 0.63$, $p \leq 0.03$), field toxicity tests and ecotoxicity estimates ($R = 0.64$, $p \leq 0.01$), and laboratory toxicity tests versus ecotoxicity estimates ($R = 0.47$, $p \leq 0.05$). Regression analysis for individual species, rather than combined species, gave much higher correlations in comparisons between the various toxicity assessment methods. For example, regression analysis for all pesticides and *P. pugio* were significantly ($P \leq 0.01 - 0.001$) correlated ($R = 0.78 - 0.95$). In further comparing these regression results, Scott *et al.* (1990) reported that:

1) The % mortality results comparisons for all pesticides and all species indicated that the mean differences in mortality estimates ranged from 19-50% for the various methods (lab, field, and ecotoxicity).

2) In *P. pugio*. the mean difference in % mortality estimates by the various methods (lab, field, and ecotoxicity) was much smaller, ranging from 7-18%.

3) The major source of error in making accurate predictions from laboratory toxicity tests is in the precision of field dose determination (peak versus composite insecticide concentrations).

4) The major sources of error in making accurate predictions from field toxicity tests include:

   a) Limited size class estimates of mortality (generally only adults are utilized);
   b) Confounding physicochemical factors such as low salinity, total filterable residue, dissolved organic carbon, and temperature; and

c) Presence and potential interaction of other insecticides.

5) The major sources of error in making accurate predictions from ecotoxicological biomonitoring include:

   a) Inter. vs Intrasite comparisons
   b) Cumulative (multiple rain events) in field populations versus single rain event/dose response effects in field and laboratory.

6) Comparisons between field derived and laboratory toxicity tests LC_{50} values indicated generally excellent agreement between field results and 96h, static renewal laboratory test results. Laboratory 6h pulsed dose tests results greatly underestimated field mortality effects.

Tables 61-62 (*F. heteroclitus* - 1989 and 1990, respectively) and 63-64 (*P. pugio* - 1989 and 1990, respectively) list the predicted mortality in mummichogs and grass shrimp from various laboratory toxicity tests at measured field concentration of azinphosmethyl, endosulfan, and fenvalerate detected during runoff events for 1989-90. Also listed are the mortality rates in caged *P. pugio* and *F. heteroclitus* observed in field toxicity tests and estimated mortality rates for *P. pugio* and *F. heteroclitus* from ecotoxicity sampling (block seining - 1989-90; push netting - 1990).

In *F. heteroclitus*, laboratory toxicity testing predicted no significant (< 5% = Control) mortality in all 1989-90, rain events but one. During the rain event of 24 June, 1989, (Table 61), significant runoff of azinphosmethyl (7.002 μg/L) was observed at the KWA Site. Laboratory toxicity tests with *F. heteroclitus* predicted 10% mortality which compares with observed field mortality of 16.6% ($\pm$ 6.7%). The laboratory-derived NOEC for azinphosmethyl of 4.95 μg/L, which was exceeded in the field with resulting mortality (16.6 $\pm$ 6.7%), again suggests significant agreement between the laboratory and field.

The only measured field effects in *F. heteroclitus* were observed during 1990 rain events (Table 62). During the first rain event significant fenvalerate concentrations (0.123 μg/L) were observed. Ecotoxicity measurements (block seining and push netting) predicted mortality ranging from 21.2 - 24.4% mortality. No significant mortality was observed in field toxicity testing nor was any significant mortality predicted in laboratory toxicity tests. Laboratory and

Table 61. Summary of field and laboratory toxicity tests results and ecotoxicological estimates of mortality in *F. heteroclitus* during the 1989 field study.

Rainfall Date	Insecticide	Insecticide Concentration[1] (ug/L)	% Mortality in *F. heteroclitus*		
			Lab	Field	Ecotox[2]
6/5 - 6/89	Azinphosmethyl	1.173	0	0.00 (± 0.0)	NM
	Endosulfan	0.163	0		
	Fenvalerate	0.064	0 - 3%		
	Fenvalerate	0.065[A]-0.093	0 - 3%	0.00 - (± 0.00)	0
6/9/89	Azinphosmethyl	0.368	0	0.00 (± 0.00)	NM
	Endosulfan	0.054	0		
	Fenvalerate	0.22[A]-0.021	0	0.00 (± 0.00)	0
6/16/89	Azinphosmethyl	2.457	0	0.00 (± 0.00)	NM
	Endosulfan	0.038	0		
	Fenvalerate	0.015	0	0.00 (± 0.00)	0
6/19/89	Azinphosmethyl	1.351	0	3.3 (± 3.3)	NM
	Endosulfan	0.027	0		
	Fenvalerate	<0.003	0	0.00 (± 0.00)	0
6/24/89	Azinphosmethyl	7.002	10	16.6 (± 6.7)	NM
	Endosulfan	0.065	0		
	Fenvalerate	<0.003	0	0.00 (± 0.00)	0

A = Composite Samples
[1] = Peak concentrations in grab samples unless otherwise denoted
[2] = Block Seine Estimate

Table 62. Summary of field and laboratory toxicity tests results and ecotoxicological estimates of mortality in *F. heteroclitus* during the 1990 field study.

Rainfall Date	Insecticide	Insecticide Concentration[1] (ug/L)	% Mortality in *F. heteroclitus*		
			Lab	Field	Ecotox[2]
5/28/90	Fenvalerate	<0.003[A]-0.123	0 - 3%	3.3 (± 3.3)	24.4[3] - 21.2[2]
6/15/90	Azinphosmethyl	0.024[A] - 0.062	0 - 0	0.00 (± 0.00)	0[2] - 45.4%[3]
	Endosulfan	0.005[A] - 0.004	0		

A = Composite Samples

[1] = Peak concentrations in grab samples unless otherwise denoted

[2] = Block Seine Estimate

[3] = Push Net Estimate for non *P. pugio* Total Faunal Density

field toxicity tests are based upon results for adult *F. heteroclitus* where as ecotoxicity estimates include adult and juvenile *F. heteroclitus*. Juvenile *F. heteroclitus* may be more sensitive to fenvalerate exposure, although earlier tests exposing larval mummichog (1-2 day old larvae) to fenvalerate did not indicate increased sensitivity (i.e., adult and larval LC_{50}s were comparable). These laboratory tests with larval *F. heteroclitus* were at 20 ppt salinities and not at the low salinities (5-10 ppt) observed in the field during this rain event. At lower salinities fenvalerate may be more toxic to larval mummichogs. Another factor to consider is that laboratory toxicity tests with fenvalerate were considered with pydrin where as all field exposures during 1989-90 for fenvalerate were Asana. Additional toxicity testing with larval *F. heteroclitus* is needed to better resolve these differences.

During the second 1990 rain event (15 June, 1990), only ecotoxicity estimates predicted from push netting were suggestive of potential toxic effects in *F. heteroclitus*. As earlier discussed, push netting does not provide accurate censusing of mummichogs and other fish species and as a result spurious conclusions may be reached. During this second rain event, this appeared to be the case as only push netting indicated potential *F. heteroclitus* mortality. Other assessment methods (lab and field toxicity testing and block seining) indicated no significant toxicity occurred.

In *P. pugio*, laboratory toxicity tests predicted significant (> 5%) mortality in seven out of the eight rain events observed during 1989-90. Field toxicity tests with *P. pugio* measured significant mortality in six out of the eight rain events. Ecotoxicity sampling (block seining - 1989-90; push netting - 1990) predicted significant mortality in seven out of the eight rain events observed during 1989-90.

During the first two rain events (5-6 June, 1989) significant runoff of azinphosmethyl (1.73 μg/L), endosulfan (0.163 μg/L) and fenvalerate (0.064 μg/L) was observed at the KWA Site (Table 63). Also, significant runoff of fenvalerate (0.093 μg/L) was observed at the TRT Site. Laboratory toxicity tests predicted mortalities of 51% (azinphosmethyl), 6-15% (endosulfan), 53-80% (fenvalerate), and combined pesticide mortality of 100% (assuming simple additive toxicity) in *P. pugio* at the KWA Site. At the TRT Site, *P. pugio* mortality from laboratory toxicity tests was predicted at 85-95% (fenvalerate). Observed mortalities in field toxicity tests were 80% (± 20%) at the KWA Site and 72% (± 13.8%) at the TRT Site. No ecotoxicity sampling was conducted at the KWA Site during 1989, but block seining at the TRT Site predicted 43.1% mortality in *P. pugio*. These results between field, lab and ecotoxicity

Table 63. Summary of field and laboratory toxicity tests results and ecotoxicological estimates of mortality in *P.pugio* during the 1989 field study.

Rainfall Date	Insecticide	Insecticide Concentration[1] (ug/L)	% Mortality in *P. pugio*		
			Lab	Field	Ecotox[3]
6/5 - 6/89	Azinphosmethyl	1.173	51	80 (± 20)	NM
	Endosulfan	0.163	06 - 15		
	Fenvalerate	0.064	53 - 80		
	Fenvalerate	0.065^-0.093	85-95	72 (± 13.8)	43.1
6/9/89	Azinphosmethyl	0.368	0	0.00 (± 0.00)	NM
	Endosulfan	0.054	0 - 6		
	Fenvalerate	0.022^-0.021	0 - 6	36.7 (± 3.3)	22.7[2]
6/16/89	Azinphosmethyl	2.457	96	100.00(±0.00)-62.6(±8.12)	NM
	Endosulfan	0.038	0 - 6		
	Fenvalerate	0.015	0 - 6	6.6 (± 6.6)	22.7[2]
6/19/89	Azinphosmethyl	1.351	51	40 (± 15.3)	NM
	Endosulfan	0.027	0 - 6		
	Fenvalerate	<0.003	0	10.7 (± 6.4)	22.7[2]
6/24/89	Azinphosmethyl	7.002	100	100.0 (± 0.0)	NM
	Endosulfan	0.065	0 - 6		
	Fenvalerate	<0.003	0	3.3 (± 0.00)	22.7

[1] = Peak concentrations in grab samples unless otherwise noted.
[2] = Mortality Estimate in *P. pugio* for period 6/9 - 27/89.
[3] = Ecotox Estimates Derived from Block Seining Data
[A] = Composite Samples

estimates of mortality closely agree. Additionally, these results were very similar to results obtained during the rain event of 9 June 1986 at the TRT Site. When similar concentrations of azinphosmethyl (0.58 μg/L) and fenvalerate (0.032 μg/L) caused significant mortality in field toxicity tests (90%) and field populations of *P. pugio* (90%).

During the third rain event (9 June, 1989), significant runoff of azinphosmethyl (0.368 μg/L) and endosulfan (0.054 μg/L) was observed at the KWA Site. Also, significant runoff of fenvalerate (0.022 μg/L) was observed at the TRT Site. Laboratory toxicity tests predicted *P. pugio* mortalities ranging from 0%(azinphosmethyl) to 0-6% (endosulfan) at the KWA Site which were very similar to 0% observed in field toxicity tests. Laboratory toxicity tests with *P. pugio* also predicted mortalities ranging from 0-6% (fenvalerate) compared to observed mortalities of 36.7% in field toxicity tests and 22.7% in ecotoxicity sampling. This lack of close agreement between field and laboratory toxicity tests may have resulted in part from poor characterization of peak fenvalerate concentrations during this rain event. The fenvalerate concentration measured in the composite sample exceeded the peak grab concentration, suggesting that the grab sampling schedule employed may have missed the actual peak fenvalerate concentration. As reported by Scott *et al.* (1990), the primary factor affecting correlation between the laboratory and the field is accurate characterization of field pesticide concentrations to use in laboratory toxicity dose response curves. Another factor may be that laboratory toxicity tests with fenvalerate utilized Pydrin where as field exposures were from Asana.

During the fourth rain event (16 June, 1989), significant runoff of azinphosmethyl (2.457 μg/L) and endosulfan (0.038 μg/L) was observed at the KWA Site and significant runoff of fenvalerate (0.015 μg/L) was observed at the TRT Site. Laboratory toxicity tests with *P. pugio* predicted mortalities ranging from 96% (azinphosmethyl), 0-6% (endosulfan) and combined mortality of 96-100% (azinphosmethyl and endosulfan) at the KWA Site. Field toxicity tests with *P. pugio* measured mortalities ranging from 62.6% (2 days post rain deployment) to 100% (initial post rain deployment) at the KWA Site, which were very similar to predictions from laboratory results. At the TRT Site, observed *P. pugio* mortalities ranged form 6.6% (field toxicity tests) to 22.7% (ecotoxicity sampling) compared to laboratory toxicity tests which predicted mortalities of 0-6%. Excluding the ecotoxicity predicted mortality of 22.7%, (which was an integrated mortality prediction for the time period of 6/7-6/27/89 which encompassed four rain events), there was close agreement between field and laboratory results at the TRT Site during the fourth rain event.

During the fifth rain event of 19 June, 1989, there was significant runoff of azinphosmethyl (1.351 μg/L) and endosulfan (0.027 μg/L) at the KWA Site. Laboratory toxicity tests with *P. pugio* predicted mortalities ranging from 51%(azinphosmethyl), 0-6% (endosulfan), and 51-57% (combined azinphosmethyl and endosulfan) compared to field toxicity tests with *P. pugio* which measured 40% ($\pm$ 1.53%) mortality. This suggests excellent agreement between field and laboratory toxicity testing for this rain event. At the TRT Site, no detectable levels of pesticides were observed. As a result, laboratory toxicity tests predicted *P. pugio* mortality was 0% compared to field toxicity test mortalities of 10.7% ($\pm$ 6.4%), which was just above maximum field control mortality (5%). This is in close agreement between field and laboratory results.

During the sixth rain event of 21 June, 1989, there was significant runoff of azinphosmethyl (7.002 μg/L) and endosulfan (0.065 μg/L) at the KWA Site. Laboratory toxicity tests with *P. pugio* predicted mortalities ranging from 100% (azinphosmethyl) to 0-6% (endosulfan), and combined mortalities of 100% (azinphosmethyl and endosulfan) compared to field toxicity tests results which reported 100% ($\pm$ 0.0%). This again suggests close agreement between field and laboratory toxicity tests results at the KWA Site. At the TRT Site, no detectable pesticide concentrations were observed. As a result, laboratory toxicity tests predicted *P. pugio* mortality was 0% compared to 3.3% ($\pm$ 0.0%) field toxicity tests.

During the seventh rain event (28 May, 1990-Table 64) significant runoff of fenvalerate ($<$ DL - 0.123 μg/L) was observed at the TRT Site. Laboratory toxicity tests predicted *P. pugio* mortalities ranging from 0% (composite sampling) to 100% (peak grab sample). Observed field mortality was 3.3% ($\pm$ 3.3%) in field toxicity tests and ranged from 0% (block seine) - 24.6% (push netting) in ecotoxicity sampling estimates. Results from this rain event graphically illustrate the problem in accurately characterizing the field exposure concentration to use in laboratory toxicity test dose response models. Using the composite concentration, significant correlation exists between field and laboratory results. Using peak grab concentration, field laboratory results do not agree. Which measured field concentration (peak or composite) is most

Table 64. Summary of field and laboratory toxicity tests results and ecotoxicological estimates of mortality in *P. pugio* during the 1990 field study.

Rainfall Date	Insecticide	Insecticide Concentration[1] (ug/L)	% Mortality in *P. pugio*		
			Lab	Field	Ecotox[2]
5/28/90	Fenvalerate	<0.003[A]-0.123	100	3.3 (± 3.3)	0[2] - 24.6[3]
6/15/90	Azinphosmethyl	0.024[A] - 0.062	0 - 0	3.3 (± 3.3)	0
	Endosulfan	0.005[A] - 0.004	0		

A = Composite Samples

[1] = Peak concentrations in grab samples unless otherwise denoted

[2] = Block Seine Estimate

[3] = Push Net Estimate

appropriate to use in laboratory toxicity tests predictions? Results from this study suggest that both composite and grab sampling are needed to accurately characterize field dose to obtain correlative results between the field and the laboratory. Geographical (acres of agricultural fields, etc.) and hydrological (stream volume, stream flow) factors and agricultural management practices (vegetative filter strips, retention ponds) may all ultimately affect and define the field pesticide exposure regime. During this rain event, perhaps only a small first flush "slug" of fenvalerate was discharged from retention ponds following the initial rain event. Very little additional pesticide discharge must have occurred after this initial runoff was observed, as evidenced by the nondetectable fenvalerate concentrations observed in composite samples. Results from this rain event are also suggestive that when "slugs" of pesticides are rapidly diluted with no resulting field toxicity , that the assimilative capacity of the stream has been maintained. The construction and operation of retention ponds along with IPM and BMP at the TRT Site may have contributed, in part to the return of the assimilative capacity in this water shed. The use of grab and composite sampling in conjunction with hydrolab measurements is an integrated procedure for not only accurately determining the pesticide field exposure regime, but also to evaluate the assimilative capacity of a watershed to predict its vulnerability to nonpoint source pesticide runoff. In the future the use of peak grab/composite sample ratios may provide some measure of a water shed's vulnerability to NPS agricultural runoff when evaluated with other hydrological and toxicological information.

During the eighth and final rain event of 15 June, 1990 (Table 64), significant concentrations of azinphosmethyl (0.062 μg/L) were observed the KWA Site. Laboratory toxicity tests with *P. pugio*, predicted 0% mortality which was highly correlated with field toxicity tests (0%) and ecotoxicity sampling results (0% in block seine and push netting).

Results from all field toxicity tests and ecotoxicity sampling for rain events studied form 1985-90 are listed in Table 65 as field derived LC_{50} values. These results are then compared with laboratory derived LC_{50} values for a variety of toxicity tests [96h static renewal (SR) and 6h pulsed dose (PD) at high (20 ppt) and low (5 ppt) salinities]. These results indicated generally excellent agreement between field and laboratory derived LC_{50} values for *P. pugio* and *F. heteroclitus* exposed to azinphosmethyl, endosulfan and fenvalerate.

Table 65. Comparison of field and laboratory derived LC_{50} values in *P. pugio* and *F. heteroclitus* exposed to azinphosmethyl, endosulfan and fenvalerate. Note the similarities between lab and field derived 96h LC_{50} values.

Insecticide	Test Organism	Field Derived[A] 96h LC_{50} (± 95% CL) in µg/L	Laboratory Derived LC_{50} Value in µg/L
Azinphosmethyl	*P. pugio*	0.95	0.97 - 1.05 SR
		(0.86 - 1.05)	
			6.68 - 8.14 PD
	F. heteroclitus	> 7.002	28.00 - 36.95 SR
		(NC) LOEC = 7.00	NOEC = 4.95
Endosulfan	*P. pugio*	0.28	0.25 - 1.01 SR
		(NC)	0.27 - 0.58 SR, Z
			3.81 - 4.35 PD
	F. heteroclitus	> 0.998	1.29 - 1.45 SR
		(NC)	0.14 - 0.40 SR, J
Fenvalerate	*P. pugio*	0.06	0.052 - 0.060 SR
		(0.05- 0.07)	0.013 - 0.031 SR, Z
			0.235 - 0.314 PD
	F. heteroclitus	0.23	1.63 - 2.86
		(0.19 - 0.28)	1.67 - 4.26 SR, J

A = Field Derived LC_{50} values were based upon a compilation of ecotoxicology and field toxicity test results.
NC = Confidence Limits Not Calculated.
SR = 96h Static Renewal Toxicity Tests at low (5 ppt) and high (20 ppt) salinities.
PD = 6h Pulsed Dose Toxicity Tests at low (5 ppt) and high (20 ppt) salinities.
Z = Zoeae, 1-2d; other values are for adults unless otherwise noted.
J = Juvenile; other values are for adults unless otherwise noted.

In *P. pugio* field derived LC_{50} values were:

1) 0.95 μg/L for azinphosmethyl (CL = 0.86 - 1.05 μg/L) versus 96h laboratory SR LC_{50} values ranging from 0.97 - 1.05 μg/L (CL = 0.77-1.24 μg/L):

2) 0.28 μg/L for endosulfan versus 96h laboratory SR LC_{50} values ranging from 0.25 - 1.01 μg/L (CL = 0.14 - 1.43 μg/L) in adults and 0.39 μg/L (CL = 0.27 - 0.58 μg/L) in zoeae; and

3) 0.06 μg/L (CL = 0.05 - 0.07 μg/L) for fenvalerate versus 96h laboratory SR LC_{50} values ranging from 0.052 - 0.060 μg/L (CL = 0.037 -0.097 μg/L) in adults and 0.007 - 0.020 μg/L (CL = 0.005 - 0.031 μg/L) in zoeae.

These results indicated significant agreement between field results and 96h SR laboratory toxicity tests. The 6h pulsed dose laboratory toxicity tests LC_{50} values for all three pesticides were not as highly correlated with field results, as they greatly underestimated field toxicity in *P. pugio*.

In *F. heteroclitus*, field derived LC_{50} values were:

1) > 7.002 μg/L for azinphosmethyl versus 96h SR LC_{50} values ranging from 28.00 - 36.95 μg/L (CL = 20.23 - 48.24 μg/L). Also the field derived LOEC was 7.00 μg/L versus a 96h SR NOEC of 4.95 μg/L;

2) 0.12 μg/L for endosulfan versus 96h SR LC_{50} values ranging from 1.29 - 1.45 μg/L (CL = 1.21 - 1.59 μg/L) for adults and 0.23 μg/L (CL = 0.14 - 0.40 μg/L) for juveniles; and

3) 0.10 μg/L (CL = 0.09 - 0.11 μg/L) for fenvalerate versus 96h SR LC_{50} values ranging from 1.63 - 2.86 μg/L (CL = 1.08 - 4.06 μg/L) for adults and 2.67 μg/L (CL = 1.67 - 4.26 μg/L) for juveniles.

These results generally indicated good agreement between field and laboratory results. Generally field derived LC_{50} values were lower than laboratory derived values. This was because field derived LC_{50} values used both field toxicity test results and ecotoxicity sampling

estimates, which included both adult and juvenile *F. heteroclitus*. Reported laboratory results, were for adults, except where indicated (i.e., endosulfan). For endosulfan, the juvenile *F. heteroclitus* LC_{50} value (0.23 μg/L) was almost 5 times lower than for adults (1.29 - 1.45 μg/L). As these results indicate, field derived LC_{50} values for *F. heteroclitus*, which include both juvenile and adults, may be lower than laboratory derived LC_{50} values for adults only. Additional toxicity testing with juvenile *F. heteroclitus* at high and low salinities and in pesticide mixture combination are needed to better define these field and laboratory comparisons.

Ecophysiological studies, using both specific (i.e., biomarkers - AChE inhibition) and nonspecific (i.e., general physiology - 0_2 respiration, nitrogen excretion, and O/N ratios) measures of sublethal effects, provides a fourth tier of ecological assessment which may be used to assess acute and/or chronic sublethal physiological stress responses in aquatic organisms exposed to pesticides and other toxic chemicals.

In this present study, during 1989-90, significant runoff of azinphosmethyl (1.73 - 7.00 μg/L) at the KWA Site resulted in inhibition of brain AChE in *F. heteroclitus*. Earlier studies by Fulton (1989) and also reported in Scott *et al.*, (1990) found significant brain AChE inhibition in mummichogs following exposure to azinphosmethyl at the KWA Site during 1988. Laboratory toxicity tests exposing *F. heteroclitus* to azinphosmethyl reported:

1) Reduced whole animal nitrogen excretion rates following 24h sublethal azinphosmethyl exposures; and

2) 24h EC_{50} (based upon % brain AChE inhibition) of 0.90 μg/L.

A comparison between field and laboratory derived EC_{50}s for *F. heteroclitus* exposed to azinphosmethyl is listed in Table 66. Note the similarities between the field derived EC_{50} (0.63 - 1.53 μg/L) and laboratory derived EC_{50} (0.90 μg/L). These findings clearly demonstrate field validation of laboratory-derived EC_{50} value in *F. heteroclitus* exposed to azinphosmethyl. As was previously mentioned in the acute toxicity field and laboratory comparisons section the greatest single factor affecting field and laboratory comparisons, is the accuracy of the field pesticide exposure concentration. This was reflected in the range of field derived EC_{50} estimates obtained (0.63 - 1.53, average = 1.13 μg/L). Never the less, field derived EC_{50} values using brain AChE were very close to the EC_{50} reported for laboratory toxicity tests with azinphosmethyl.

Table 66. Comparison of field and laboratory derived EC_{50} (% Brain AChE Inhibition in ug/L) in *F. heteroclitus*. Note the similarity between lab and field derived EC_{50} values.

Pesticide	Species	Laboratory Derived EC_{50}[1] in ug/L (95% CL in ug/L)	Field Derived EC_{50}[1] in ug/L (Range [2])
Azinphosmethyl	*F. heteroclitus*	0.90	1.13
		(NC)	(0.83 - 1.53)

[1] = Based upon Brain AChE Inhibition

[2] = Range based upon % inhibition using maximum insecticide concentation and 24h concentration.

NC = Not calculated.

Additionally AChE may be an excellent biomarker for AChE inhibiting pesticides such as organophosphate and carbamate insecticides. Many of these chemicals are difficult to monitor, due to their short half-lives in the environment.

Brain AChE may thus be useful as a surrogate biomarker of exposure for many AChE inhibiting pesticides with short half lives. This is particularly true given the persistence of AChE inhibition as observed during laboratory studies, which showed only partial AChE recovery following 7 days of depuration. Further studies with other AChE inhibiting pesticides and other fish species are needed to fully understand the usefulness of AChE as a field biomarker of exposure and sublethal physiological stress.

Nonspecific biomarkers such as bioenergetic metabolism were used to evaluate physiological alterations in basal metabolism in the lab (mummichogs - azinphosmethyl exposure) and field (oysters). In both field and laboratory studies, the nonspecific biomarkers (respiration rate, nitrogen excretion, and O/N ratio) assessed were useful in identifying effects in the species tested; however, interpretation of these results and identifying cause and effect relationships with pesticide exposure is an extremely difficult task using these tools. The concomittant exposure of pesticide and low salinity conditions particularly may confound interpretation of field results. Extensive laboratory studies to quantify and identify the importance of confounding factors, such as salinity, on bioenergetic metabolism are essential to better understanding and identifying pesticide and low salinity effects.

One limitation of this study, was that spatial, watershed-wide impacts (upstream and down stream) were not assessed. Of particular interest is the assessment of pesticide runoff impacts on the fauna of larger stream reaches, marine mammals, sea turtles, wading and shore birds, and benthic communties. Funding restraints obviously limited the scope of this project to address basin-wide impacts, although the limited studies conducted clearly indicated that pesticide transport up to two river miles away was observed along with significant toxicological impact at the KWA Site during 1989. Despite these limitations, results from these studies clearly indicate the need to protect estuarine ecosystems in the most vulnerable, upper reaches of small estuarine tidal creeks, which are the true nursery ground for many marine fish and invertebrate species. If water quality and environmental integrity is preserved in these nursery ground areas, water quality and environmental integrity will be maintained in larger tidal stream reaches, further downstream, assuring a safe and healthy ecosystem for most estuarine species.

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