United States
Environmental Protection
Agency

EPA

Research and Development

ANNUAL PROGRESS REPORT - YEAR 2

FATES AND EFFECTS OF HERBICIDES AND PESTICIDES ON ESTUARIES

COOPERATIVE RESEARCH PROGRAM

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MARCH 3, 1989

Progress Report
to

U.S. Environmental Protection Agency
Environmental Research Laboratory
Gulf Breeze, FL 32561

Fates and Effects of Herbicides and Pesticides on Estuaries

Year 2 - Contract #CR 813415-01

from

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February 24, 1989

Progress Summary

Introduction

The second year of a project titled "Studies of the Fates and Effects of Herbicides and Pesticides in Estuaries" has been completed in the South River estuary, Carteret County, North Carolina. The research is a cooperative effort among the following organizations: Duke University Marine Laboratory, the Department of Civil and Mineral Engineering of the University of Minnesota, Open Grounds Farm, Inc. and the Gulf Breeze Environmental Research Laboratory of the U.S. Environmental Protection Agency.

Field studies on the South River and adjacent Open Grounds Farm began in the fall of 1986. The first annual progress report to EPA covered the period August 1986 - July 1987. Research results presented here in this second annual report cover the period August 1987 - July 1988. Included below is a summary of the physical, chemical and biological studies which have been accomplished during this period. As a measure of project productivity we also include a list of papers which have been published, are "in press" or are "in preparation" that are, in part or in whole, a result of this project.

The South River estuary is a shallow non-tidal embayment of the Pamlico Sound, 11 km in length, typical of many southeastern coastal plains estuaries. Open Grounds Farm is a 44,186 acre agricultural development of the Ferruzzi Group located in Carteret County, NC on a peninsula adjacent to the Pamlico Sound and Neuse River estuarine system surrounding the South River. The farm was established in 1974 on undeveloped land covered by pine forest, swamp forest and pocosin. By 1980 development was completed with approximately one third of the area lying in the South River watershed. The farm grows grain crops (corn, soybeans, wheat) on 56% of the total area, has pasture for cattle on 26% and has forest on 12%. The remaining 6% is dedicated to roads, canals, field ditches, and buildings. An essential part of farm development was the construction of a one-mile grid of major drainage canals which serve to remove surface water and lower the seasonal water table below the land surface. Although a forested buffer was left around the South River the major drainage canals come together and empty directly into the uppermost headwaters of several tributaries of the estuary.

Based upon our two years of study we find that Open Grounds Farm and the adjacent South River Estuary provide an excellent field site for the study of the fates and effects of pesticides in estuaries. Farm management has cooperated fully and allows access to all planting and pesticide application records as well as providing physical access to the farm and the estuary at all times: day, night, and weekends. Data suggest that these studies will provide an excellent example of the influence on adjacent estuaries of row crop agriculture which uses best management practices (BMPs) in application of herbicides and pesticides.

Research Results

Physical studies during this year have been aimed at development and preliminary deployment of PSWIMS, a Profiling Shallow Water Instrument Mounting System, to be used to give detailed descriptions of the hydrography of the estuarine creeks receiving farm drainage. PSWIMS is a computer-driven, self-contained system for continuous profiling of water depth, salinity, and current velocity. Temperature and dissolved oxygen measurements are being added. At the sites (1-2 m deep) where the instrument is deployed it profiles the water quality parameter every 10 minutes using 7 depths scaled to give greatest resolution in surface layers. The instrument is designed to provide two to four weeks of continuous hydrographic data at upper estuarine sites where chemical and biological studies of the fates and effects of pesticides are in progress. The instrument is ideally suited for the temporal and spatial scales involved in rainfall-mediated runoff events. The instrument performed flawlessly for a one-month period in a research pond adjacent to the UNC IMS Laboratory. Field deployment has been less successful because of mechanical problems. However results to date from field studies have demonstrated the utility of the data collected by the instrument. As an example we observed velocity profiles on 4/7/88 which indicated surface (top 10 cm) low salinity (5 ppt) runoff water moving downstream in the estuary at a velocity of 2-10 cm/sec while there was an upstream movement of saline (15 ppt) estuarine water at velocities of approximately 2.5 cm/sec in the remaining 1 m of the water column. Such information is critical to our understanding of exposure of estuarine organisms to herbicides and pesticides and to interpreting chemical samples taken in surface and near bottom water samples. Development of PSWIM will continue in the fall and winter 1988-89 to ensure its successful operation in the spring 1989.

In chemical research two major field experiments examining the fates of herbicides and pesticides were done during the last year. Runoff of the pesticide permethrin was studied in August - September 1987 and runoff of the herbicide alachlor was examined in April - May 1988. Analysis of the alachlor experiments has not been completed and results of these studies will be detailed in the next (third) annual report. The results of the permethrin experiment are described below.

The pyrethroid insecticide permethrin was measured in the South River estuary, North Carolina, during and following summer application on adjacent farmland that drains into the estuary. Particulate material was separated by filtration, and the dissolved compounds were isolated by solid phase or liquid-liquid extraction and analyzed by GC-MS. The predominant form of permethrin in all samples was in the particulate phase, generally representing about 66% of the total. Maximum concentrations of 0.69 µg/L particulate permethrin and 0.36 µg/L dissolved permethrin were measured in farm drainage ditches immediately after application. Dilution of runoff with estuarine water and other farm runoff resulted in maximum concentrations of particulate permethrin of 0.08 µg/L in the estuary when rainfall two days after application resulted in increased farm runoff. Dissolved permethrin concentrations remained below detection limits at both estuarine sites during the sampling period. The concentration of the cis-permethrin isomer was greater than the trans-permethrin in all samples, representing 62-78% of the total. The measured distribution coefficients

of permethrin in water from farm drainage ditches ($1.2 - 7.0 \times 10^5$ L/kg) were similar to that predicted (1.3×10^5 L/kg) from a simple equilibrium adsorption model.

Biological studies in 1987-88 involved field and laboratory investigation of the effects of alachlor, permethrin, and farm runoff in general. Laboratory studies included (1) rearing of larval mud crabs exposed to runoff, (2) effects of alachlor on O_2 consumption in mud crabs, and (3) effects of alachlor on growth of phytoplankton (a dinoflagellate and a diatom). Field studies included: (1) exposure of grass shrimp to runoff, (2) nekton sampling in estuarine creeks, and (3) benthic community analysis of estuarine creeks.

In laboratory studies the larvae of the mud crab Rhithropanopeus harrisii were reared in two separate sets of experiments. In the first experiment gravid females were exposed for a period of 4 days in estuarine creeks, one receiving runoff from forest and the other receiving runoff from permethrin-sprayed soybean fields. Larval survival from animals held at the two sites did not differ from unexposed control animals. In a second set of experiments gravid crabs were held in the laboratory for four days in water from a ditch draining a permethrin-sprayed field and water collected from a forest stream. In both cases the salinity of the water was increased to 10 ppt by adding instant ocean. In neither case did larval survival differ significantly from survival of controls.

Although laboratory projects were not considered a focus for research in this current project we report here the results of three independent study projects which developed because of our ongoing research on effects of agricultural chemicals. Laboratory experiments with alachlor concentrations of 10 and 25 ppm were used to investigate herbicide effects on the respiration of adult mud crabs (Rhithropanopeus harrisii). While decreased salinities caused O_2 consumption to rise 30%, alachlor at concentrations of 10 and 25 ppm had no effect on respiration. In preliminary experiments the effects of alachlor on the growth of phytoplankton (the diatom Skeletonema costatum and dinoflagellates Prorocentrum micans) was examined. In Skeletonema studies concentrations of .001, .01, .1 and 1 ppm were used. After 96 hours only 1 ppm had significantly lowered growth rates compared to the control. In studies using Prorocentrum micans alachlor concentrations of 0.1, 1, 10, and 50 ppm were used in 96-h experiments. Significant inhibition of growth occurred at alchlor concentrations of 1 ppm or greater.

Grass shrimp (Palaemonetes pugio) were held in 3 experiments in estuarine creek runoff from fields sprayed with permethrin and a control creek receiving only forest drainage. Mortality ranged from minimum 40% in one control experiment to a high of 100% in two field runoff experiments. Effects could not be attributed to runoff because of extremes in salinity (runoff creek, 0 ppt) and dissolved oxygen (both creeks) experienced by the animals. These preliminary results were used in planning late summer 1988 bioassay which will include holding animals in cages with an air/water interface further downstream. In addition field bioassays are planned in which larval shrimp will be raised in runoff from fields receiving pesticide applications.

Nekton communities were sampled by trawl in six tributary creeks of the South River in the fall of 1987. Four creeks receive forest drainage while 2 creeks receive farm drainage. Communities were dominated by bay anchovy, spot and mullet. A comparison of farm runoff with forest runoff creeks indicated no significant differences in total catch by weight or numbers and that communities were basically identical. Preliminary analysis of trawls taken weekly in the summer of 1988 in six creeks (three with forest and three with farm runoff) suggest that nekton communities in all creeks (with one exception) were similar in number, biomass and community structure. The exception was one farm runoff creek which differed from the other in having dramatically fewer bay anchovies throughout the summer sampling period. Absence of anchovies remains unexplained at this time.

Benthic community studies were done during the summer of 1988 in the same six creeks as the nekton studies. Preliminary analysis of data indicates that there is no difference among creeks in fauna (species presence, abundance, or community structure) related to whether or not the creeks receive farm runoff. All communities were similar at the 85% level. At 94-97% similarity communities were related to each other in pairs by their geographic location within the South River Estuary.

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In preparation

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Sandstrom, M.S., S.J. Eisenreich and W. Kirby-Smith. Distribution of permethrin from agricultural runoff in South River estuary, North Carolina.

Sommer, C.H., W. Kirby-Smith and R.B. Forward. Toxicity of the herbicide alachlor to Atlantic silversides (Menidia menidia) and sublethal effects on schooling behavior.

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Fates and Effects of Herbicides and Pesticides on Estuaries: Chemical Analysis

Year 2-Contract # CR 813415-01

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Distribution of Permethrin from Agricultural Runoff in South River Estuary, North Carolina

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Abstract- The pyrethroid insecticide permethrin, 3-(phenoxybenzyl (1RS)-*cis*,*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate, was measured in the South River estuary, North Carolina, during and following summer application on adjacent farmland that drains into the estuary. Particulate material was separated by filtration, and the dissolved compounds were isolated by solid phase or liquid-liquid extraction and analyzed by GC-MS. The predominant form of permethrin in all samples was in the particulate phase, generally representing about 66% of the total. Maximum concentrations of 0.69 µg/L particulate permethrin and 0.36 µg/L dissolved permethrin were measured in farm drainage ditches immediately after application. Dilution of runoff with estuarine water and other farm runoff resulted in maximum concentrations of particulate permethrin of 0.08 µg/L in the estuary even after rainfall 2 d post-application resulted in increased farm runoff. The concentration of the *cis*-permethrin isomer was greater than the *trans*-permethrin in all samples, representing 62-78% of the total. The measured distribution coefficients of permethrin in water from farm drainage ditches (1.2-7.0 x 10^5 L/kg) were similar to that predicted (1.3 x 10^5 L/kg) from a simple equilibrium adsorption model.

Keywords- Pesticides Nonpoint source pollution Estuaries Permethrin GC-MS Distribution coefficient

INTRODUCTION

Nonpoint source agricultural pollution - the runoff losses of pesticides from agricultural fields - depends on a number of critical factors, including rainfall timing, soil type, and the chemistry, persistence and formulation of the pesticide (see Wauchope and Leonard 1980; and Wauchope,1978 for reviews). While reasonable estimates of the inputs of pesticides into adjacent rivers, lakes, or estuaries can be made, information about the environmental fate and effects of the pesticide is lacking. Assessments of the biological effects usually depend on extrapolation of laboratory toxicity studies. Similarly, predictions of the chemical behavior and fate are made from laboratory studies or estimates based on chemical structure and physical properties. These extrapolations from laboratory studies generally do not account for the dynamics of dilution, changes in ionic strength, or exchange with suspended particles and organic colloids that occur when agricultural runoff mixes with estuarine water.

The objective of this research was to characterize the concentration and predominant forms of selected agricultural chemicals in estuarine waters receiving agricultural runoff from farms using best management practices. The South River estuary in North Carolina was selected as the field study site. Most of the watershed of the South River lies within Open Grounds Farm (OGF), a 45,000 acre agricultural enterprise developed into farmland by construction of untiled drainage canals and field ditches. Information about chemicals used, application rates, timing, and access to the study site was providing by management of OGF. Initial fieldwork for the project was conducted in spring 1987 during the planting of corn and the application of alachlor and terbufos on fields adjacent to the estuary (Eisenreich and Sandstrom, 1987). The work described in this report was conducted during the summer of 1987 when permethrin was applied on soybean fields in the Southwest Creek study area for the control of insects.

Permethrin, 3-(phenoxybenzyl (1RS)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate, is a widely used synthetic pyrethroid insecticide sold under the trade names "Pounce" (FMC Agricultural Chemical Group) or "Ambush" (ICI Americas Inc.). Commercial formulations are mainly emulsifiable concentrates and contain variable mixtures of the isomers, generally 60% *trans*- and 40% *cis*-permethrin (Sine et al.1987). It is generally applied by ground or aerial spraying at a rate of about 0.2 kg/ha. Permethrin has a low water solubility, ca. 0.2 mg/L, and a log K_{ow} of 5.70 (Muir et al. 1985a). Permethrin is of low mammalian toxicity but is highly toxic to fish: the LC_{50} (96 h) for rainbow trout is 0.1-0.5 µg/L (Swain and Tandy, 1984).

MATERIALS AND METHODS

Study Area

Southwest Creek is a small tributary of the South River, which in turn is a shallow embayment of the lower Neuse River Estuary (Figure 1). Mean tidal range in the South River is about 1 m, although these are irregular tides caused by wind-induced set-up of Pamlico Sound and the Neuse River Estuary; lunar tidal oscillations are only 1-2 cm. The main study sites focused on two sites within Southwest Creek and one of the major drainage canals of a section of Open Grounds Farm. The section of the estuary at Site SW4 was about 20 m wide and 2 m deep, while Site SW4 was located further upstream where the creek was about 3 m wide and 1 m deep. Site DD12 was located in one of the large farm drainage ditches at the headwaters of the creek. While this drainage ditch drained most of the fields on which permethrin was applied in August 1987, drainage ditches from other fields (which were not treated with permethrin) also emptied into the headwaters of Southwest Creek. Also, this drainage ditch was connected to the South River through another outlet, so that the runoff from the fields in which permethrin was applied was both diluted and diverted from our sampling sites.

Chemicals. All solvents were distilled in glass grade (Burdick and Jackson Laboratories). An analytical reference standard of

trans-permethrin was obtained from the U.S. E.P.A. Pesticides and Industrial Chemicals Repository (MD-8), Research Triangle Park, NC. d-(cis,trans)-Phenothrin [3-phenoxybenzyl 2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate] was obtained from Chem Service, West Chester,PA. Stock solutions (1 mg/ml) were prepared in hexane-dichloromethane (9:1) and stored at -10°C in a freezer. Chrysene-d_{12} was obtained from Supelco Inc. Working calibration standards were prepared by dilution of the stock solutions with hexane-dichloromethane (9:1) at five concentrations, 0.5, 2.5, 5.0, 10.0, and 25.0 ng/µl, and 5.0 ng/µl for Chrysene-d_{12}.

Apparatus. A Hewlett-Packard 5985 mass spectrometer coupled to a 5840A gas chromatograph and 21 MX E-Series data system was used for all measurements. Calibration standards and samples were injected manually. Compound separations were performed on a fused silica capillary column 25 m x 0.31 mm i.d. (5% phenylmethyl silicone, 0.52 µm film thickness, Hewlett-Packard) at the following conditions: splitless injection at 80°C, isothermal for 2 min. followed by temperature programming at 20°C/min to 120°C and then 10 °C/min. to 280°C; injector temperature and transfer line temperatures of 250°C. The mass spectrometer operating conditions were ion source temperature at 200°C, electron energy of 70 eV, and selected ion monitoring mode (SIM) for characteristic ions at *m/z* 183, 123, and 240. Each ion was sampled for 75 ms for a total scan time of 600 ms.

Field Sampling Procedures Samples were collected in Southwest Creek from locations shown in Figure 1. Daily samples were collected from Sites SW4, SW3 and DD12 from 12-16 August. Application of permethrin on fields north of Southwest Creek by aerial spraying occurred on the morning of 13 August 1987.

Samples were collected for suspended particulate material (SPM), dissolved organic carbon (DOC) and chloride by pumping estuarine water directly into a 2 L bottle. An aliquot of this sample was filtered through pre-weighed 0.4 µm Nuclepore filters, and the weight of suspended solids determined after drying.

Six to eight liter water samples for XAD-2 resin adsorption were collected at Sites SW3 and SW4 by pumping estuarine water through 90 mm dia. GF/F glass fiber filters at a flow rate of 200 ml/min. using a submersible pump (March Manufacturing, Inc.; Model 893-02 seal-less magnetic pump). The pump inlet was located 2-4 cm below the water surface in order to sample the runoff water above the halocline. Dissolved pesticides that passed through the filters were stored in 20 L glass bottles until isolation in the field laboratory, generally within 2-3 hrs after collection. The filters, which retained particulate pesticides, were spiked with approximately 500 ng of the field surrogate phenothrin, wrapped in aluminium foil and stored in a freezer until subsequent extraction and analysis.

Samples for liquid-liquid extraction (LLE) of pesticides were collected at Site DD12 by submerging 2 L glass bottles below the water surface and removing the caps. These samples were returned to the field laboratory and stored in a refrigerator until filtration through 47 mm dia GF/F glass fiber filters. Most samples were processed within 2 hrs of collection.

Laboratory Procedures In the field laboratory, the 6-8 L samples were spiked with the field surrogate phenothrin (about 300 ng/L) and the dissolved pesticides were isolated by pumping through glass columns (2.5 x 30 cm) containing 150 ml of XAD-2 resin at a flow rate of 100-120 ml/min . The columns were capped, returned to the laboratory, and Soxhlet extracted with methanol for 24 hrs followed by dichloromethane for 24 hrs. The 1-2 liter samples from Site DD12 were spiked with the field surrogate permethrin (400-600 ng/L), extracted with 20 ml dichloromethane-methanol (2:1) followed by 2 X 20 ml dichloromethane. The dichloromethane layers were combined, stored in a refrigerator, and returned to the laboratory.

The dichloromethane layers and Soxhlet extracts were partitioned against water, reduced in volume on a rotary evaporator, and dried by passage through a column of Na_2SO_4. The total extracts

were concentrated to about 100 µl with a gentle stream of nitrogen at 25 °C, and then the external GC-MS quantitation standard (chrysene-d_{12}) was added prior to GC-MS analysis.

The wet filters containing particulate pesticides were placed in in 50 ml glass centrifuge tubes and extracted by vigorous agitation (1-2 min) with hexane:isopropanol (HIP) 3:2 (Hara and Radin,1978). The tubes were centrifuged and the solvent extract poured off, and the extraction was repeated 2-3 times. The extracts were combined, dried by passage through short columns of Na_2SO_4, and then reduced to approximately1 ml using a rotary evaporator and transferred to a glass vial. The total extract was taken to a few µl under a stream of nitrogen, and immediately dissolved in a known volume of n-hexane.

The total particulate extracts were then fractionated on columns (10 mm i.d. x 300 mm) of neutral aluminum oxide (3.5 g; Fisher 70-200 mesh; deactivated with 1% H_2O) over silica (7.0 g; Fisher 100-200 mesh, deactivated with 5% H_2O) prepared by slurry packing in n-hexane. The particulate extract was transferred to the column and washed with 25 ml n-hexane to remove aliphatic hydrocarbons (alkanes and alkenes). The pesticides were eluted with diethyl ether: hexane 1:1 (25 ml). Each fraction was transferred to clean glass vial, concentrated to about 100 µl with a gentle stream of nitrogen at 25 °C, and then the external GC-MS quantitation standard (chrysene-d_{12}) was added prior to GC-MS analysis.

DOC was determined on a separate aliquot filtered through GF/F glass fiber filters. DOC was measured using a Dohrmann Model DC-80 total organic carbon analyzer and a Horiba Model PIR-2000 infrared analyzer. Inorganic carbon was removed from acidified water samples by purging with CO_2-free nitrogen. Organic carbon was then oxidized to CO_2 with potassium persulfate in an ultraviolet irradiation chamber and the resultant CO_2 was analyzed by non-dispersive infrared spectroscopy. Chloride was measured on diluted samples by an automated spectrophotometric technique (US EPA, 1976) and then converted to salinity using the relationship between

salinity and chlorinity for standard seawater (Grasshoff et al., 1983). Estimates of operational accuracy and precision for all of these techniques are less than ±10 %.

Quantitation. The most intense ions in the mass spectra of permethrin (m/z 183) and phenothrin (m/z 123) were chosen for quantitation using the selected ion-monitoring (SIM) technique. Each of the 5 calibration mixtures was analyzed each day during a sample run and the mean relative response of each compound was determined from quantitation of ion peak areas and injected quantities of calibration mixtures and the external standard, chrysene-d_{12}.

Method Characteristics Recoveries of the field surrogate phenothrin from filtered material averaged about 44% (range 28-105%). Low recoveries can most likely be attributed to losses from adsorption to silica-alumina columns in the sample clean-up step. Phenothrin recoveries from 1-2 L liquid-liquid extracts were from 44-55%. However, phenothrin recoveries were less than 1% from the 6-8 L samples passed through XAD-2 columns. Previous work has demonstrated good recoveries from reagent water and spiked samples using this technique (Eisenreich and Sandstrom,1987). GC-MS analysis of the XAD extracts indicated the samples contained fatty acids and alcohols derived from estuarine organisms, suggesting that there were no problems with the efficiency of the XAD-2 columns in isolation of hydrophobic organic compounds. One possibility for low recoveries may be that the 20 L glass sample bottles were not rinsed with solvent after the samples were pumped through the resin columns. Phenothrin (as well as permethrin and other synthetic pyrethroids) is hydrophobic and will adsorb onto glass surfaces from aqueous solution (Swain and Tandy 1984, Sharom and Solomon, 1981b).

RESULTS

Hydrology

Previous fieldwork (Kirby-Smith and Barber, 1979, Eisenreich and Sandstrom, 1987) has shown that runoff from rainfall results in

increases in salinity stratification and an overall decrease in bottom salinity in the estuary. The thickness and salinity of the surface layer varies in response to freshwater runoff, tides (water level), and wind speed and direction.

A 12 mm rainfall event on 10 August resulted in a significant amount of freshwater in the estuary at the start of the fieldwork (Figure 2). During this period the water column at Site SW3 was characterized by a salinity gradient consisting of a 10-20 cm thick layer of low salinity water (salinity = 2-4 ppt) above more saline bottom water (salinity = 10-13 ppt). At Site SW4 the entire 1 m water column was fresh throughout the sampling period. The surface salinity at Site SW3 gradually increased as freshwater runoff subsided and in response to northerly winds until another rainfall event of 8-10 cm occurred on 14 August after application of permethrin (Figure 2).

Concentrations of suspended particulate matter (SPM) at Sites SW3 and SW4 during the August sampling period ranged from 11-24 mg/L, with a mean value of 16 mg/L (Table 1). Suspended solids increased slightly at SW3 and decreased at SW4 after the rainfall event on 15 August. Dissolved and colloidal organic carbon (DOC) was extremely high and variable in the farm drainage waters at Site DD12 with daily values varying between 33 and 196 mg/L. DOC concentrations were lower in the estuarine waters, with mean concentrations of 50 ± 14 mg/L at Site SW4 and 33 ± 8 mg/L at Site SW3. Since the salinity values at SW4 indicated very little dilution with estuarine water, the lower values of DOC at SW4 compared to DD12 may be the result of dilution by mixing with drainage from other fields with farm waters having low DOC. For example, during April 1987 DOC levels in farm drainage water from an adjacent area of OGF were only about 10-12 mg/L (Eisenreich and Sandstrom, 1987).

Permethrin concentrations

Permethrin concentrations at all sites were below detection limits (2-20 ng/L) prior to application. Daily concentrations of

dissolved and particulate permethrin at Site DD12 are shown in Figure 3. About 5 h after application permethrin concentrations in farm drainage water at Site DD12 were 692 ng/L particulate permethrin and 356 ng/L dissolved permethrin, consisting of approximately 66% and 61%, respectively, of the *cis* -permethrin isomer. The following day particulate permethrin concentrations had decreased to 93 ng/L, and dissolved permethrin to 44 ng/L, each consisting of about 77 % of the *cis* -permethrin isomer. Drift from the aerial application may have resulted in the initial transfer of the permethrin to the drainage water, in addition to shallow seepage flow from the fields.

Shallow groundwater flow was apparently sufficient to transport particulate permethrin from the drainage ditch to the estuarine sites also. Particulate permethrin concentrations at Site SW4 increased from 6 ng/L about 4 h after aerial application on 13 Aug to 53 ng/L on 14 Aug (Figure 4). The percentage of the cis-permethrin isomer was similar to that at Site DD12, about 65 % on 14 August and 88 % on 15 August. At Site SW3 further downstream, particulate permethrin concentrations were below or just above detection limits on 13 and 14 August. Dissolved permethrin concentrations remained below detection limits at both estuarine sites during the sampling period.

A 10 mm rainfall on the evening of 14 August resulted in increased runoff of farm drainage water into the estuary on 15 August, as shown by the dramatic decrease in surface salinity at Site SW3 (Figure 2). Runoff resulted in increased concentrations of particulate permethrin at both estuarine Sites SW4 and SW3 (Figure 4), consisting of about 67 % of the *cis* -permethrin isomer. However, dissolved permethrin concentrations remained below detection limits at both estuarine sites. At the headwaters of the estuary at Site DD12 the pesticide concentrations decreased to 52 ng/L particulate permethrin (consisting of about 65 % of the *cis* -permethrin isomer) and 6 ng/L dissolved permethrin (consisting of about 56 % of the *cis* -permethrin isomer) after the rainfall event.

Discussion

These results have demonstrated that the concentration of total (dissolved plus particulate) permethrin in agricultural runoff was as high as 1 µg/L immediately after application. Such high concentrations in farm drainage water were most likely due to drift from the aerial application, since there was no intervening rainfall to wash the pesticide from the soybean plants. However, there was sufficient flow of shallow groundwater from the fields to transport the particulate permethrin to the estuary, where concentrations were much lower because of dilution. This low flow of water from the drainage ditch into the estuary probably also caused a gradual flushing of the permethrin from the drainage ditch as indicated by the decrease in concentration of particulate permethrin at Site DD12 and increase at Site SW4 on 14 August after application.

Dissolved permethrin concentrations in the drainage ditch immediately following application were similar to levels known to be toxic to aquatic organisms. Permethrin is acutely toxic to macrozooplankton at levels of 0.5 µg/L (Kaushik et al. 1985). A concentration of 0.39 µg/L permethrin killed 50% of newly hatched crawfish in 96 h in sediment-free water.

This work also demonstrated that the permethrin occurred predominantly in the particulate phase (66%) less than 5 h after application on the adjacent fields. Previous field studies have demonstrated that deltamethrin, another synthetic pyrethroid, rapidly partitioned into suspended sediments when applied directly to water (Muir et al. 1985b). The percentage of particulate permethrin in the drainage ditch increased to 90% after the rainfall event, suggesting that pessticide in runoff after the rainfall event consisted mainly particulate permethrin. This is consistent with the fact that only particulate permethrin was observed in the estuarine samples. However, low recoveries of the field surrogate prevents any definitive interpretation of the particulate-water partitioning of permethrin in these samples.

For samples from the drainage ditch at Site DD12 where results of dissolved and particulate determinations are more reliable we can compare the observed partitioning into the particulate phase with that predicted from a simple equilibrium sorption model. The partition coefficient K_p can be expressed as

$$K_p = \frac{(C_p/SS)}{C_d}$$

where C_p is the concentration of pesticide on particles (µg/kg), C_d is the dissolved concentration (µg/L), and SS is the dissolved solids concentration (kg/L). Suspended solids in Southwest Creek ranged from 9 to 25 mg/L during the sampling period (Table 1), and an average value of 15 mg/L was used to calculate the particulate permethrin concentration in µg/kg, and the K_p values for permethrin at Site DD12 during August. The calculated K_p values for *cis*- and *trans*-permethrin, which are shown in Figure 5, ranged from 1.2 to 7.0×10^5 L/kg (log K_p = 5.1-5.8).

These values can be compared to partition coefficients predicted from simple reversible adsorption. The sorption of hydrophobic organic compounds from water by sediment consists primarily of partitioning into the sediment organic phase. Karickhoff et al. (1979) expressed the partition coefficient of aromatic and chlorinated hydrocarbons in terms of the organic carbon content of the solids:

$$K_p = K_{oc} * f_{oc}$$

where K_{oc} is the partition coefficient expressed on an organic carbon basis, and f_{oc} is the fractional organic carbon concentration. The value of K_{oc} was further related to the octanol-water partition coefficient according to the following relationship:

$$K_{oc} = 0.63\ K_{ow}$$

Using a value of 5.70 for log K_{ow} (Muir et al. 1985a), the K_p can be estimated for a range of organic carbon concentrations (f_{oc}). The predicted log K_p values for f_{oc} values from 0.1 to 0.6 are 4.5 to 5.3, and are shown in Figure 5. These predicted values are similar to the log K_p values measured at Site DD12.

The results of this fieldwork also demonstrated that *cis*-permethrin was the predominant isomer found in all samples, consisting of 61-88% of the total, even though technical mixtures generally contain *trans*-permethrin as the predominant isomer (Sine et al.,1987). This suggests very rapid degradation of the trans isomer soon after application. Other field studies have demonstrated that the *cis* isomer is more stable than the *trans* isomer toward chemical and biological degradation in soils (Sharom and Solomon, 1981a; Jordan and Kaufman, 1986). The percentage of the *cis* isomer increased on the second day after application, supporting the hypothesis of rapid degradation of the *trans* isomer in the dissolved and suspended particle phases. Samples collected after the rainfall event had higher percentages of the *cis* isomer, similar to that the day of application. This may have been the result of slower degradation of the permethrin in the soils compared to that in the water or suspended solids, assuming that particulate permethrin in runoff after the rainfall event was derived mainly from the soils.

Previous studies have shown that timing of rainfall after application is one of the most important factors in determining runoff losses of pesticides from agricultural fields. In this study, rainfall occurred 2 d after application, increasing the potential for high concentrations of pesticide in the runoff (Wauchope, 1978). Although we have no information on the runoff volume and percentage loss of the applied pesticide, we can compare the maximum concentrations observed in runoff with that predicted from a simple empirical model. Wauchope and Leonard (1980) developed a semiempirical prediction formula from literature data on pesticides in runoff. The formula is based on the pesticide

formulation, application rate and time elapsed between application and runoff:

$$C_t = AR(1+ 0.44\ t)^{-1.6}$$

where C_t is the predicted concentration, A is the availability index A for different classes of pesticides, R is the application rate, and t is time (d) post-application.

Permethrin, as an emulsifiable concentrate applied to foliage, would be assigned a value of A of 300-1000 ppb ha/kg. Using the application rate of 0.21 kg/ha for permethrin during this field study, the predicted concentration of permethrin in runoff 2 d post-application would be 20-70 µg/L. This is about 2 orders of magnitude greater than that observed at Site DD12. While Wauchope and Leonard (1980) caution that this formula will generally overestimate concentrations, we know that the runoff at Site DD12 was diluted with water from other fields not treated with permethrin. The differences between that predicted and observed may give some indication of the magnitude of this dilution, and in turn our observations of maximum concentrations of permethrin in the estuarine sites.

ACKNOWLEDGEMENTS

We thank Bill Kirby-Smith for provision of facilities and generous support during all aspects of the fieldwork; Oregon, Suzanne, and Judy for field assistance; and Jay Powers for invaluable assistance in the laboratory.

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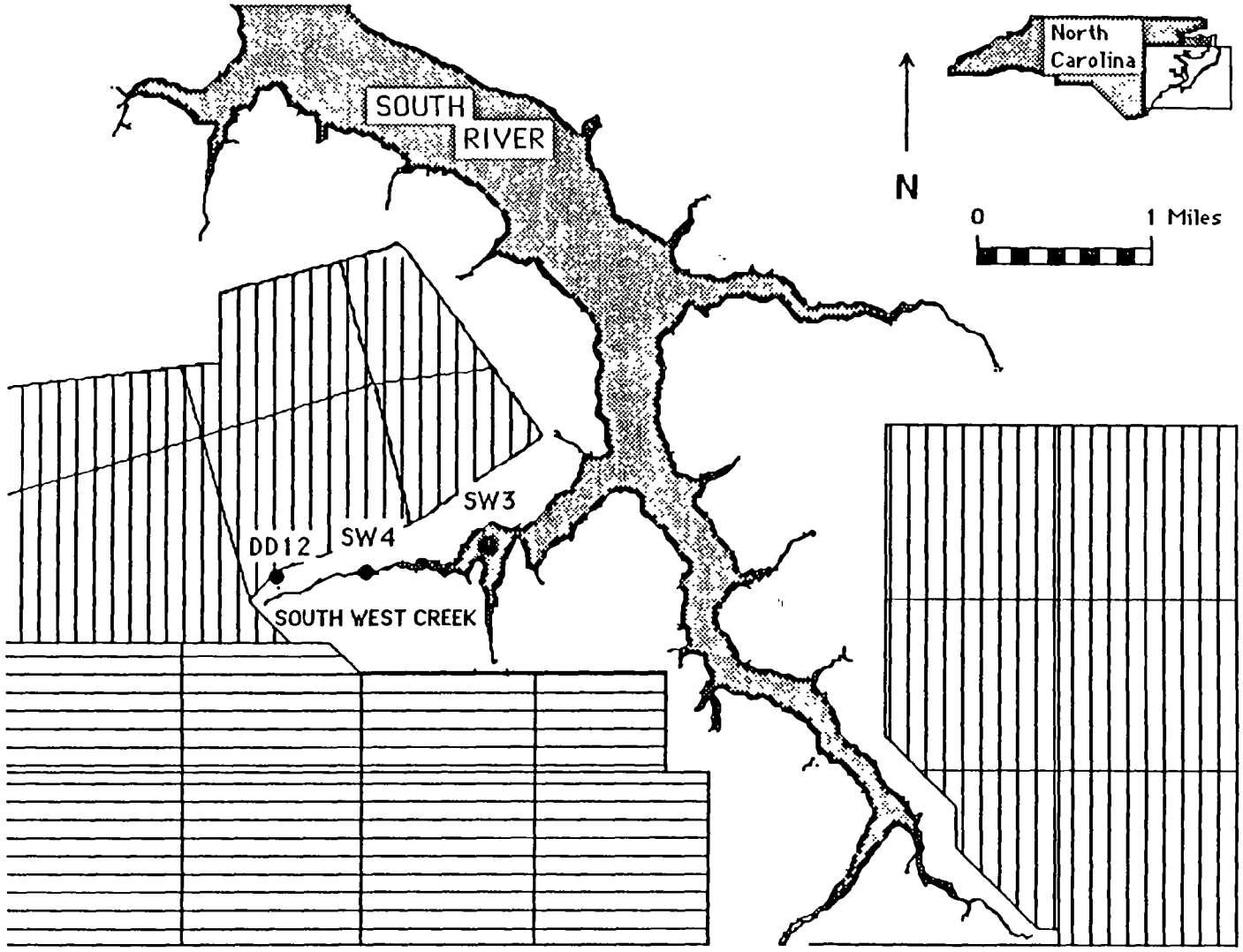
Table 1- Salinity, suspended solids (SS), dissolved organic carbon (DOC), and permethrin concentrations in Southwest Creek during August 1987.

Date	Salinity	SS (mg/L)	DOC (mg/L)	Particulate Permethrin *cis-* (ng/L)	Particulate Permethrin *trans-* (ng/L)	Dissolved Permethrin *cis-* (ng/L)	Dissolved Permethrin *trans-* (ng/L)
Site DD12							
12-Aug	0.1	6.9	40.3	0†	0	0	0
13-Aug	0.3	-	195.9	423.2	269.2	204.0	152.0
14-Aug	0.0	-	-	71.8	22.0	34.0	10.0
15-Aug	0.1	-	33.2	34.7	18.8	3.3	2.6
16-Aug	0.1	-	140.2	5.6	4.6	0	0
Site SW4							
12-Aug	0.0	24.0	33.9	0	0	0	0
13-Aug	0.1	20.9	72.2	4.1	2.2	0	0
14-Aug	0.1	12.8	53.4	41.7	11.7	0	0
15-Aug	0.0	13.1	52.2	52.1	26.8	0	0
16-Aug	0.1	11.3	42.5	5.8	2.4	0	0
Site SW3							
12-Aug	1.4	-	42.0	0	0	0	0
13-Aug	2.9	15.7	24.8	0	0	0	0
14-Aug	4.5	13.3	23.8	0.3	0.2	0	0
15-Aug	0.4	18.2	39.3	26.5	12.5	0	0
16-Aug	0.5	20.0	34.5	5.4	2.1	0	0

†0 concentration indicates below limit of detection.

Figure 1


SOUTH
RIVER
North
Carolina
N
0
1 Miles
SW3
DD12
SW4
SOUTH WEST CREEK

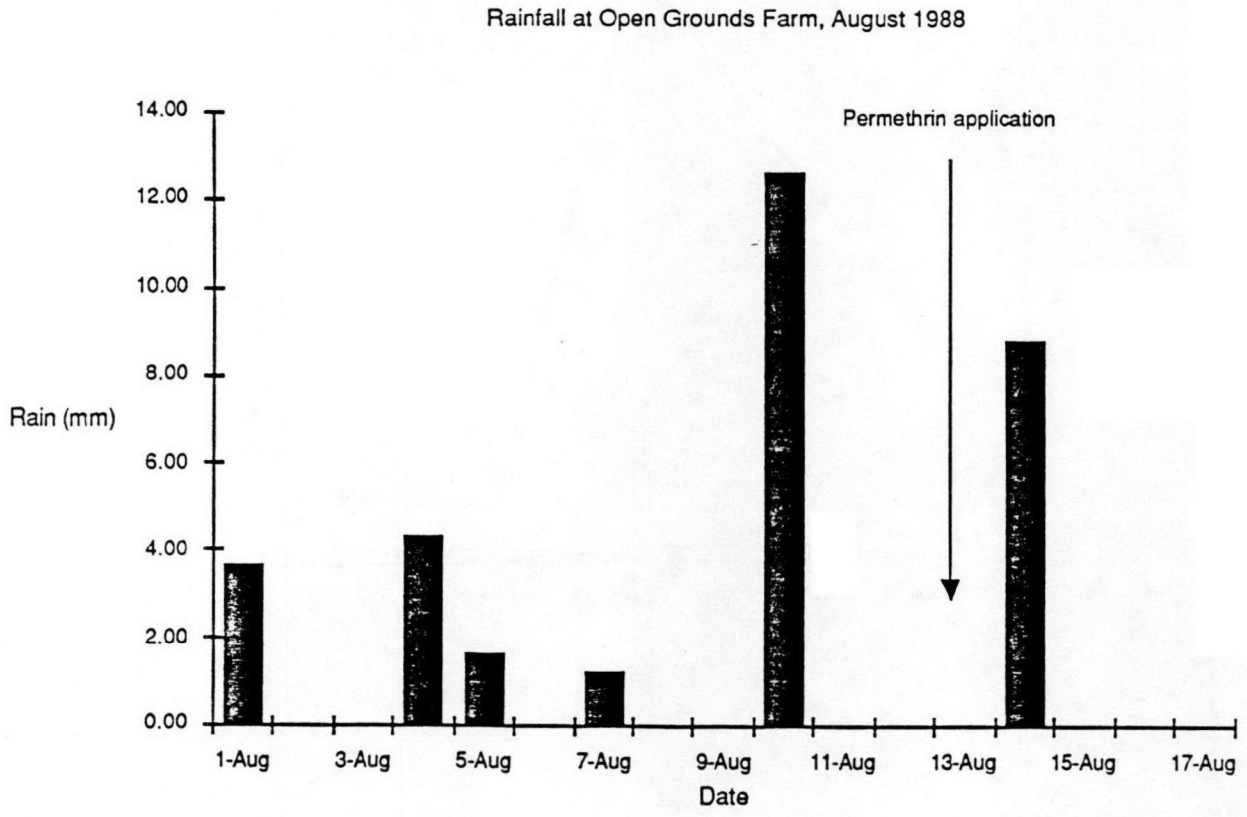

Rainfall at Open Grounds Farm, August 1988
Permethrin application
Rain (mm)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
1-Aug
3-Aug
5-Aug
7-Aug
9-Aug
11-Aug
13-Aug
15-Aug
17-Aug
Date

Figure 2a

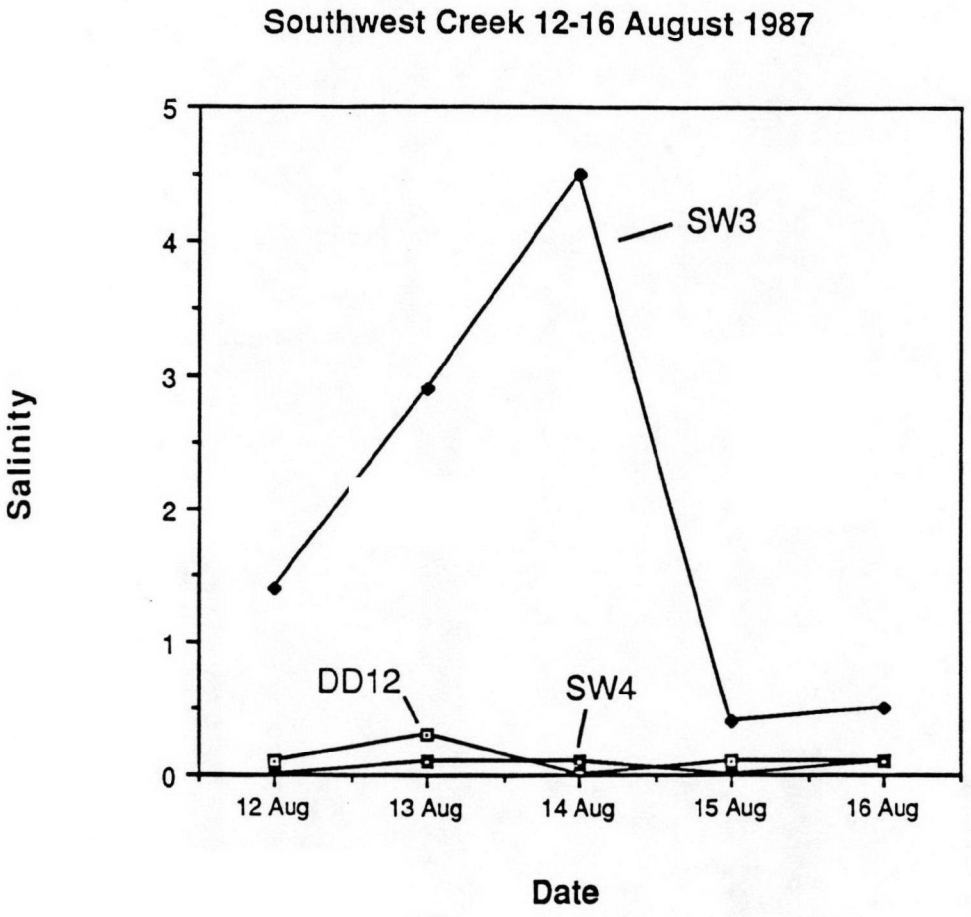

Southwest Creek 12-16 August 1987
Salinity
5
4
3
2
1
0
SW3
DD12
SW4
12 Aug
13 Aug
14 Aug
15 Aug
16 Aug
Date

Figure 26

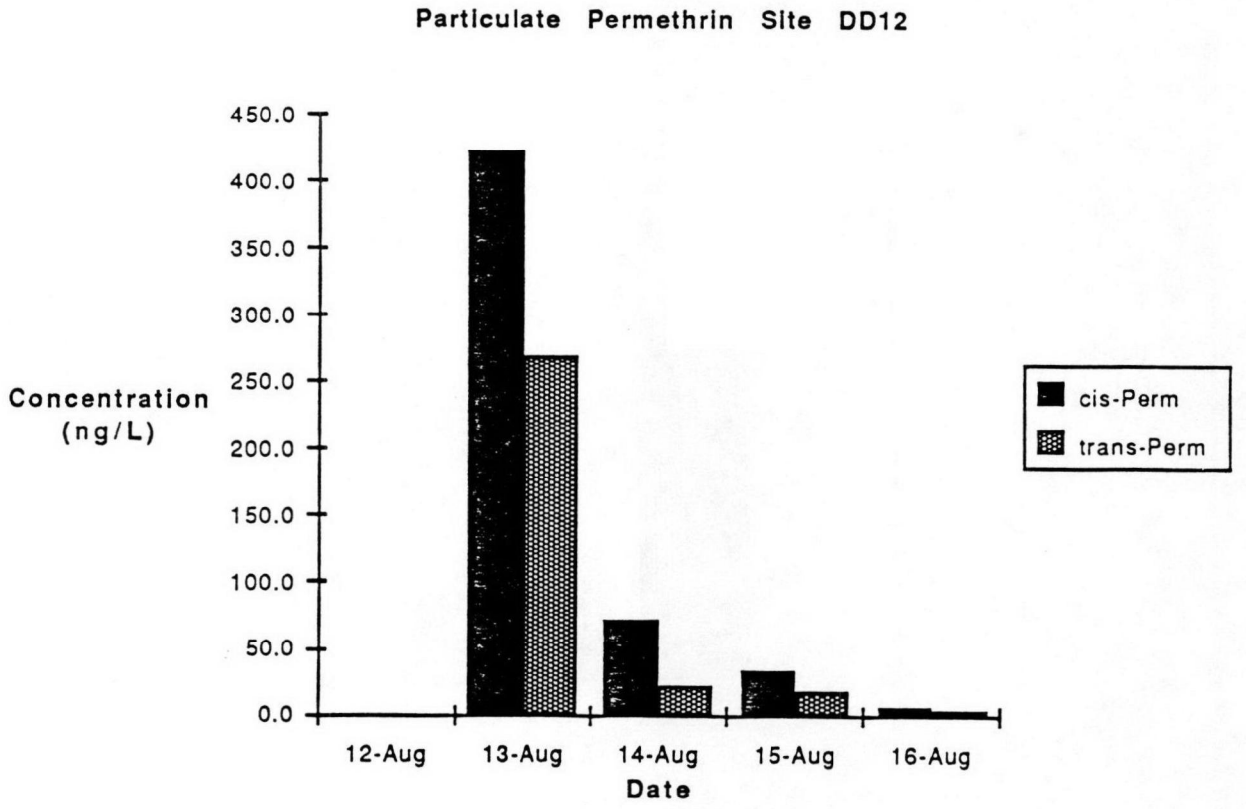

Particulate Permethrin Site DD12
Concentration (ng/L)
450.0
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
12-Aug
13-Aug
14-Aug
15-Aug
16-Aug
Date
cis-Perm
trans-Perm

Figure 3a

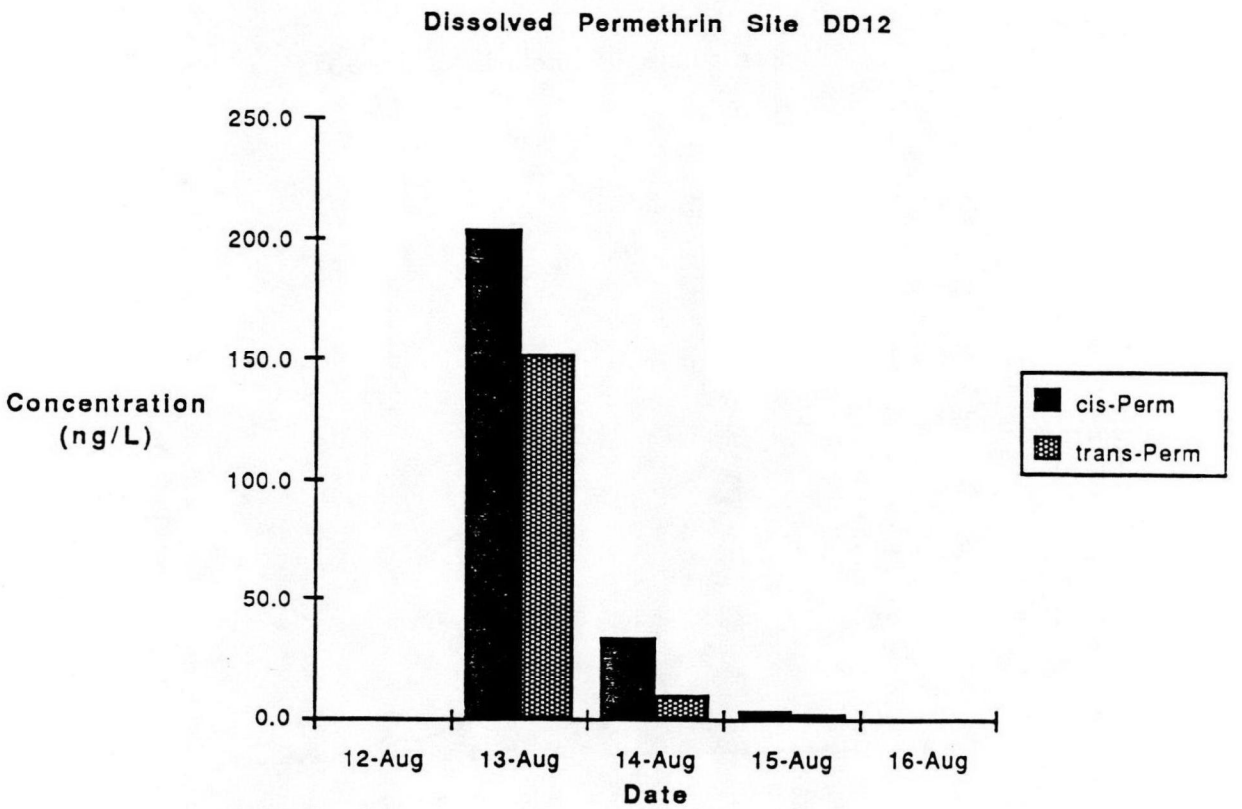

Dissolved Permethrin Site DD12
250.0
200.0
150.0
100.0
50.0
0.0
Concentration (ng/L)
12-Aug
13-Aug
14-Aug
15-Aug
16-Aug
Date
cis-Perm
trans-Perm

Figure 3b

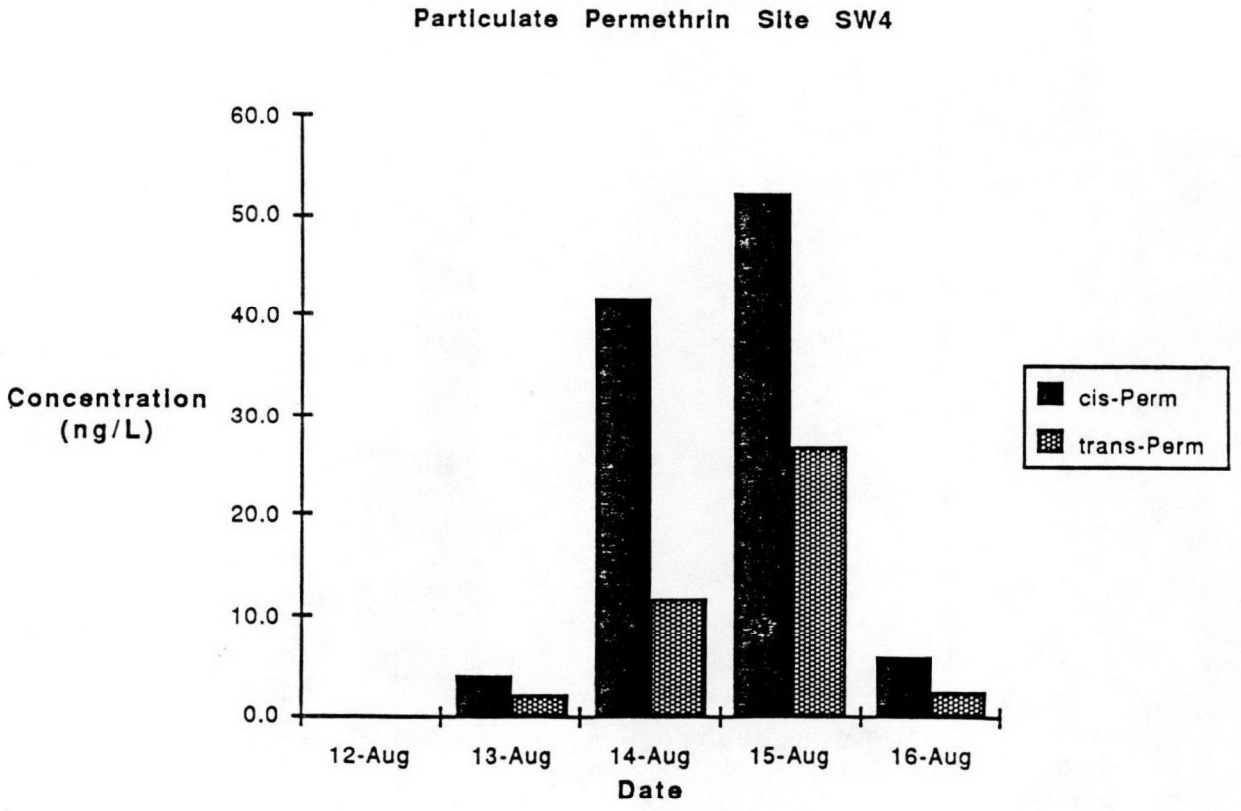

Particulate Permethrin Site SW4
Concentration (ng/L)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
12-Aug
13-Aug
14-Aug
15-Aug
16-Aug
Date
cis-Perm
trans-Perm

Figure 4 a

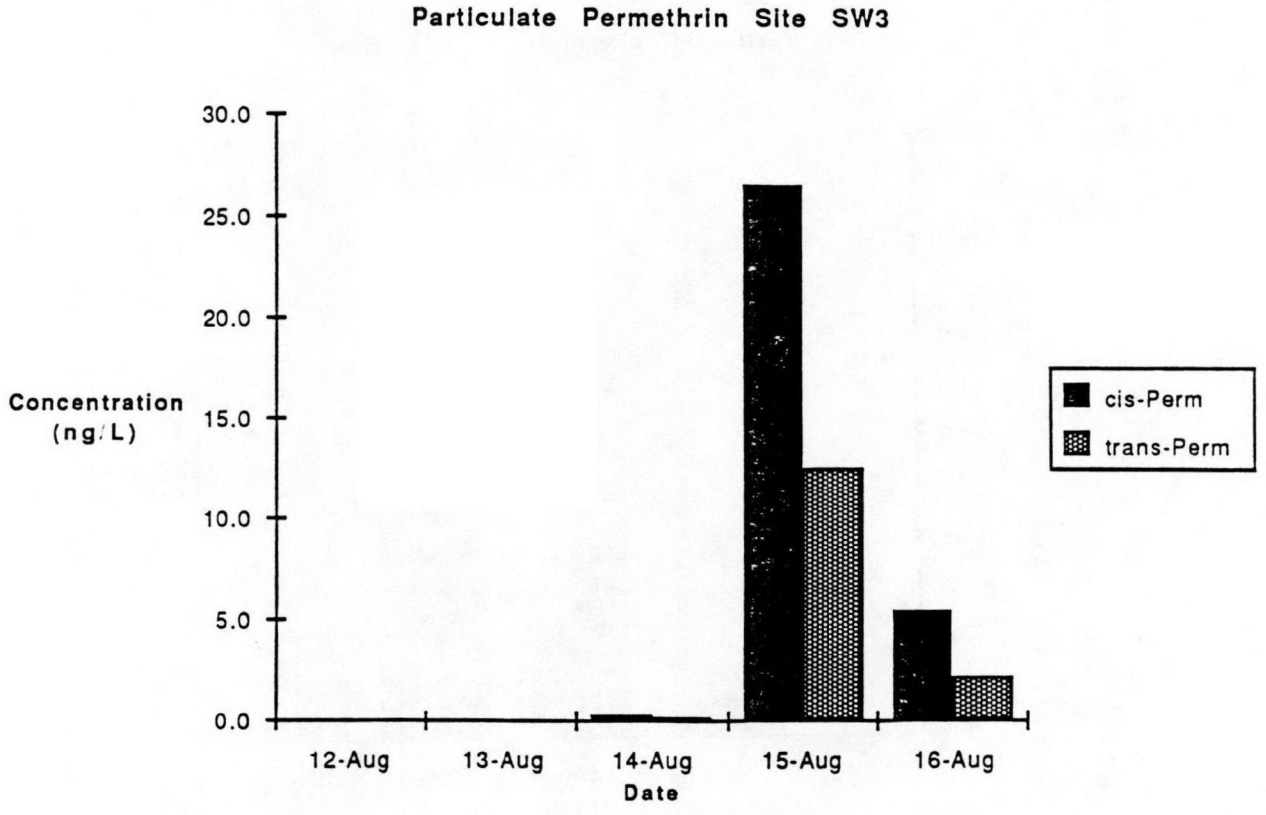

Particulate Permethrin Site SW3
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Concentration (ng/L)
12-Aug
13-Aug
14-Aug
15-Aug
16-Aug
Date
cis-Perm
trans-Perm

Figure 45

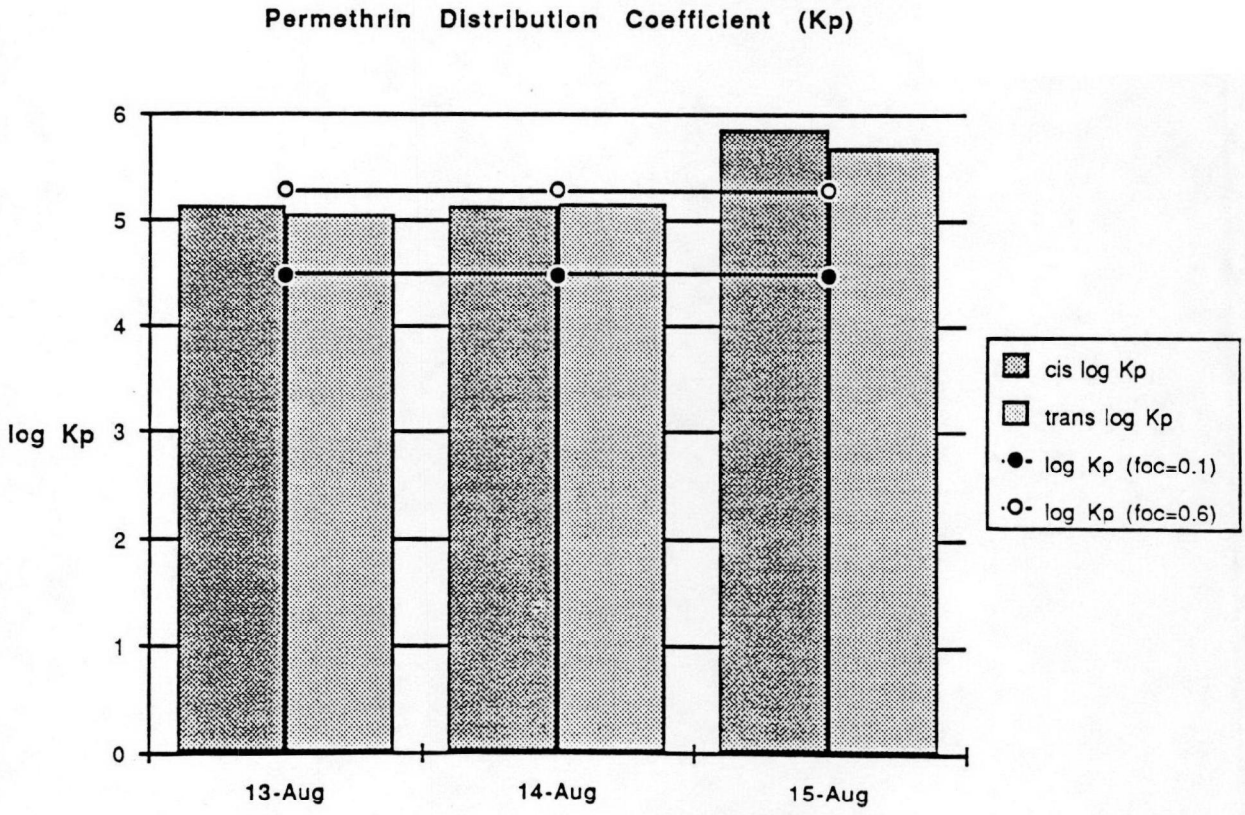

Permethrin Distribution Coefficient (Kp)
log Kp
6
5
4
3
2
1
0
13-Aug
14-Aug
15-Aug
cis log Kp
trans log Kp
log Kp (foc=0.1)
log Kp (foc=0.6)

Figure 5

DRAFT COPY

PROJECT ARIES PHYSICAL OCEANOGRAPHY COMPONENT - YEAR 2

Submitted by Rick Luettich, UNC Institute of Marine Sciences

This report is broken up into three sections:

(I) The development of PSWIMS, a Profiling Shallow Water Instrument Mounting System.

(II) Summary and analysis of physical data collected during April/May 1988.

(III) Summary and analysis of physical data collected during August 1988. (not completed)

(I) The development of PSWIMS, a Profiling Shallow Water Instrument Mounting System

It is a commonly observed and predictable phenomenon that the water column in a shallow waterbody does not behave as a layer which is uniform over its depth. On the contrary, friction forces horizontal velocities to zero at the bottom while they can be driven by the wind at the free surface. In closed basins, density driven flows, and highly transient flows, reversals in horizontal velocity may occur over the depth. Vertical transport is predominantly due to molecular or turbulent diffusion, both of which imply the existence of vertical gradients of the quantity being diffused. Stratification tends to inhibit vertical transport and further segregate parts of the water column. For example strong temperature or salinity stratification can

effectively isolate bottom water from contact with the atmosphere and allow it to become anoxic at the same time water above the gradient zone is oxygen enriched.

Observational programs which attempt to quantify the behavior of such waterbodies require instrumentation systems which can resolve these vertical structures. In the past this has been dealt with in one of two ways, either by manually raising and lowering a single set of instruments over the depth of the water column or by mooring a vertical array of instruments in the water column. Both methods have serious drawbacks. The use of manual labor becomes impractical in rough weather conditions and for long term studies which require frequent samplings. It is also difficult to accurately suspend sensors over the side of a boat in moderate currents or waves. Vertical arrays require the purchase of multiple instrument sets and quickly become very expensive. In these systems vertical resolution is often coarse and due to the available budget, although even with unlimited funds the physical dimensions of the sensor units ultimately limit their spacing. Also moored instruments are by design fixed at a constant depth. Thus they are inefficient in situations where temporal variations in the waterlevel are significant in comparison to the total depth or in which dense sampling is desired over a fraction of the water column (such as a gradient zone) whose position varies in time.

The first year of field measurements for the ARIES project illustrated these problems dramatically. Measurements were necessary in water having mean depths ranging from 1 - 2 meters which were routinely subject to 0.5 - 1.0 meter changes in water level over periods as short as one day. Fresh water runoff typically occurred in a surface layer of

variable depth and salinity and at velocities which were difficult to resolve from a boat using a hand held electromagnetic current meter. Also runoff from a particular rainfall event was variable in time and could extend for several days.

To overcome these problems PSWIMS, which stands for Profiling Shallow Water Instrument Mounting System, has been developed. The principal design considerations were the following:

(i) to be able to measure vertical profiles of physical and chemical parameters (e.g., velocity, salinity, dissolved oxygen, etc.) over intervals which are small in comparison to their timescale of variation,

(ii) to be able to make real time adjustments in measurement position in response to changes in water level or to changes in elevation of specific regions of interest (e.g., gradient zones),

(iii) to be light and portable enough to be deployed and recovered from a small boat (i.e., one which could easily be maneuvered in the narrow and shallow creeks under study) without hoists or scuba divers,

(iv) to be capable of deployments for periods of weeks or longer,

(v) to be simple to build and as inexpensive as possible.

The heart of PSWIMS consists of a computer controlled, motor driven sleeve which travels up and down a vertical shaft, Figure PO.I.1. The motor is a reversible, 12 volt DC motor geared to run at 9 rpm and is located in a water tight housing at the bottom of the vertical shaft. The motor drive turns a set of two pulley wheels which simultaneously take up and let out stainless steel wire. One end of the wire is attached to the bottom of the sleeve while the other end is attached to the top of the sleeve after passing over a single pulley wheel at the top of the vertical shaft. In this configuration the sleeve can be

raised by running the motor in one direction and lowered by reversing the motor.

The vertical shaft consists of 2 in. schedule 80 PVC pipe. The 1/4 in thick walls provide substantial rigidity while the PVC is light weight, easily available and inexpensive. The sleeve consists of a 3 in. PVC "T" which fits over the 2 in. pipe with about 1 in. of total clearance. Sixteen delrin ball bearings (eight at the top and eight at the bottom) are located around the inner circumference of the sleeve between it and the vertical shaft. The bearings are held in place by two collars which are glued inside the PVC "T" at the top and the bottom. The bearings serve as spacers to keep the sleeve parallel to the shaft and to minimize frictional resistance by minimizing the contact area between the sleeve and the shaft. A single thin strip of plexiglass is glued along the length of the vertical shaft and serves as a track to keep the sleeve from turning.

The water tight housing for the motor is constructed from a piece of 6 in. I.D. schedule 40 PVC pipe. The back end is sealed by gluing two circular pieces of 1/4 in. thick PVC over it. This provides a convenient surface for mounting standard electrical bulkhead penetrators to provide power and motor control. The front end consists of a 1/4 in. thick piece of plexiglass which is sealed against the end of the PVC pipe using an o-ring seal. It is held in place by four wing nuts which are threaded onto studs attached to the outside of the PVC pipe. A water pump from a 1974 Honda Civic automobile (after removing the impeller blades and the drive wheel) is used as the part of the drive shaft which penetrates the housing. This unit is mounted on the inside of the front end of the housing and provides a fixed alignment as well

as a double seal for the drive shaft. The water pump shaft is coupled on one end to the motor shaft and on the other end to the shaft through the drive wheels using collars machined from delrin.

The assembly is controlled by a Tattle Tale model II data logger (manufactured by Onset Computer Corp.) which also samples and stores data from the various instruments mounted on PSWIMS. This little marvel runs off a 6 - 10 volt DC supply, comes with an 8-channel, 8-bit A to D converter which reaches full scale at 5 volts, 14 digital I/O lines, 16K of program storage area, 224K of RAM for data storage, an RS-232C interface and a built in basic interpreter. Programs can be uploaded and data downloaded by connecting a PC directly to the RS-232C cable which is provided. In the field this is easily accomplished using a portable laptop computer.

The Tattle Tale digital lines are used to control relays which switch power on and off to the motor as well as control its direction. The sleeve position is determined using a 10K, 10 turn potentiometer which is geared to the motor. A 5 volt reference is provided to one side of the potentiometer and one of the Tattle Tale A/D channels is used to measure the voltage on the wiper. This voltage is linearly related to the sleeve position which is accurate to within the 8-bit resolution of the A/D. In the present application a 236 cm long vertical travel path is used allowing better than 1 cm accuracy.

As a safety feature, end of travel switches are mounted near the top and bottom of the vertical shaft. These consist of magnetic switches which are closed by magnets mounted on the top and the bottom of the sleeve, should it reach these points. Closing either of these switches shuts off the power to the motor.

The profiling system is mounted on an 8 ft. high by 6 ft. wide frame made from 2 in. PVC pipe. (Figure PO.I.2). The vertical shaft is located in one corner so that an arm attached to the sleeve projects the measurement sensors into the middle of the frame to minimize the effects of flow disturbance induced by the frame. The actual design of the frame is a compromise between stability and portability. PVC pipe is used because it is inexpensive, easy to work with and is relatively light weight. Each leg of the frame has a screw on cap at the top and when all are in place the frame is water tight. When PSWIMS is deployed the leg caps are removed and a valve at the base of the frame is opened allowing water to fill the structure. Iron bars are then placed inside the legs for weight. For recovery the iron bars are removed and the caps are placed on each leg. The frame is then pumped full of air (the water exits through the open valve at the bottom) using a battery operated air pump. This greatly facilitates lifting PSWIMS onto the recovery boat.

At present PSWIMS is instrumented with sensors to measure water level, salinity and horizontal water velocity. Water level is measured using a surface piercing sensor manufactured by the Metritape company. This measurement is made and recorded just prior to beginning a vertical profile and therefore is used to adjust the extent over which the sampling is to be conducted. Salinity is measured using an Aandera model 2975 salinity sensor and horizontal velocity is measured along two axes using a Marsh McBirney model 512 electromagnetic current meter. The electronics for the salinity meter come packaged in a small housing attached to the sensor itself while those for the current meter have been built into the arm on which the salinity and velocity sensors are

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL RESEARCH LABORATORY
SABINE ISLAND
GULF BREEZE, FLORIDA 32561-3999

February 28, 1989

SUBJECT: Pesticide Runoff Study at Beaufort, NC

FROM: James Clark
Acting Branch Chief-Ecotoxicology

TO: Bruce Barrett
Director, Water Management Division

THRU: Raymond Wilhour
Acting Laboratory Director

Enclosed are two copies of the second annual progress report by the staff at Duke University on the cooperative research project we have funded to investigate effects of pesticide runoff on estuarine biota. The report covers hydrographic, chemical, and biological factors covered in the fate and effect study of the South River Estuary near Beaufort, NC. As we have discussed previously, results of this study are directly applicable to Region IV interests in coastal, non-point source pollutant problems and the comprehensive investigation underway in the Albemarle-Pamlico Sound. We hope you find these data useful. We will continue to keep you appraised of our progress in this endeavor.

This research program is being operated on a shoe-string budget and could easily be expanded with additional resources. If you have interest in this project and see a spin-off benefit and have resources to cover changes to the project, please contact me. Or, if you wish to discuss the prospects of expansion, I have some ideas on how to approach non-point source nutrient inputs, siltation effects, and other non-pesticide related research questions.

Enclosure

mounted. The Tattle Tale, relays to turn the motor, the electromagnetic current meter and the water level gauge on and off, and several signal amplifying circuits are located in a small plexiglass and PVC housing which is strapped to one corner of the frame.

Power to PSWIMS is provided by two 12 volt 40 Amp. Hr. rechargeable batteries which are configured to provide + and - 12 volts to run the motor down and up respectively and power the electromagnetic current meter. The batteries are located in a watertight enclosure which can be mounted on the upper rung of the PVC frame or placed on shore, depending on the distance. A 9 volt radio battery in the data logger housing functions as a back up for the Tattle Tale to prevent the loss of any data stored in memory while the primary batteries are being changed or if they should expire prematurely. The principal battery drain occurs while the motor is raising the sleeve up the shaft. This can be as much as 1.2 amps while the sleeve and attached sensor arm are out of the water. However, because the sensor arm is nearly neutrally buoyant under water, this is reduced to about 0.6 amps when the arm is submerged. The Tattle Tale, water level sensor and electromagnetic current meter each typically use less than 20 mA of current and the power to the sensors is turned off when they are not in use. The Tattle Tale is capable of monitoring the battery supply voltage and shutting PSWIMS down if it drops below a preselected level.

The development of PSWIMS was begun in the latter part of year one of the ARIES project with the intention of having it ready for use during the year two April/May study. Early deployments of the system were plagued by randomly occurring "glitches" which either destroyed some of the amplifying circuitry associated with the vertical

positioning or even worse froze the Tattle Tale entirely. In the latter case the operation of PSWIMS was completely disrupted and unfreezing required that all power to the Tattle Tale be disconnected. This caused any data which was in the data logger memory to be lost. Unfortunately this problem was not fully solved until after the April/May study was completed and therefore very little useful PSWIMS data was obtained. However, the data which was obtained clearly showcases the capabilities of PSWIMS.

Figure PO.I.3 shows data collected from 16:00 on 4/6 to 12:00 on 4/7 at site HR1 in Home Road Creek (see section II for a further description). The variation in water level is shown along with plots of salinity and horizontal velocity measured 0.76m above the bottom. Unfortunately, a "glitch" had caused the loss of ability to position the arm vertically and therefore it was left at a constant elevation. The data in Figure PO.I.3 show why physical measurements at one elevation are of minimal use in estuarine studies. Initially the salinity and velocity data suggest that the sensors were measuring in a nearly fresh water runoff layer. However at the beginning of 4/7 a rapid increase occurred in water level. Correspondingly the salinity jumped to values characteristic of near bottom water and the water velocity became strongly upstream. Therefore it seems that the increase in water level was sufficient to lift the fresh water runoff layer above the sensor elevation and bring salty near bottom water which was flowing upstream into contact with the sensors. Thereafter each drop in water level brought the sensors into contact with the bottom of the downstream flowing surface layer and each rise imbedded them in the upstream flowing bottom layer.

The problem associated with the vertical positioning was corrected on 4/7 and for the following 18 hours PSWIMS functioned normally. Figure PO.I.4 presents the variation in water level from 16:00 on 4/7 to 10:00 on 4/8. For comparison water level measurements made simultaneously by a Stevens tide gauge located a short distance away at the mouth of South West Creek are also shown. The agreement is outstanding particularly considering the tide gauge data was recorded on a strip chart and later digitized by hand.

During this period PSWIMS was programmed to take a vertical profile every 10 minutes. Each profile consisted of 7 points over the vertical where the instruments were positioned and sampled for 30 seconds. Each 30 seconds of data was averaged and stored before proceeding to the next elevation. Sampling was somewhat concentrated near the surface to resolve the thin freshwater runoff layer which was expected. Figure PO.I.4 shows a plot of the points where measurements were made during this period. It illustrates clearly how PSWIMS is able to adapt its sampling points to account for the varying water depth.

Figure PO.I.5 shows three salinity and velocity profiles which were measured at the beginning of this period. They indicate downstream flow in a freshwater surface layer and a weaker upstream flow in a salty bottom layer.

Figure PO.I.6 is a composite vector plot of the velocity data from each profile. The data shows a clear picture of the horizontal velocity structure throughout the water column during this period. For the first 12 hours there is a mostly downstream surface flow and weak upstream bottom flow. The downstream layer deepens at about 20:00 on 4/7 which corresponds to a drop in water level (Figure PO.I.4) that

effectively pulls water downstream. Shortly after 4:00 on 4/8 the surface velocity reverses and the entire water column moves strongly upstream. This corresponds to a period of increasing water level which, much like a tidal surge, pushes water ahead of it over the entire depth.

Figure PO.I.7 is a contour plot of the salinity data from each profile. It shows the existence of a fresh water surface layer for the first 12 - 14 hours which corresponds to the period of downstream surface flow. It also shows that the rise in water level and the accompanying upstream flow occurring after 4:00 on 4/8 is strong enough to push vertically well mixed water from South West Creek up into Home Road Creek, at least past the measurement site. This process is discussed further in section II.

Further testing with PSWIMS during May uncovered the cause of the troublesome "glitches" and these have now been eliminated. Since that time PSWIMS was operated continuously for over 4 weeks in water 1 m deep in a cement pond behind the UNC Institute of Marine Sciences. At this point I am confident of its mechanical and electronic integrity and anticpate using it in August in conjunction with additional runoff studies.

(II) Summary and analysis of physical data collected during April/May 1988

Summary of Observations:

Data was collected in two separate tributaries feeding into South West Creek during the course of this study. From March 30 - April 21

sampling was conducted in Home Road Creek while from April 22 - May 9 it was concentrated in the upstream sections of South West Creek, (Figure PO.II.1).

(i) Home Road Creek Study, March 30 - April 21

Figure PO.II.2 indicates that one major rainfall occurred during this interval, leaving about 60 mm of rain over the two day period of 4/12 and 4/13. A moderate rainfall of between 15 and 20 mm fell on 4/6 while smaller events occurred on 4/4, 4/16 and 4/19.

Water level fluctuations measured at the mouth of the South West Creek are shown in Figure PO.II.3. The most striking feature is the high water which occurred on 4/13 and 4/14. At its peak it was more than 1.3 m above the average elevation for the period and more than 1.5 m above the lowest recorded elevation. Unfortunately, this extreme event, which overtopped the channel banks by more than 1.2 m, drowned the automatic samplers thereby terminating the collection of samples for salinity as well as other chemical analyses.

Six hour averages of the wind velocity measured about 30 km to the southwest at the UNC Institute of Marine Sciences are presented in Figure PO.II.4. While the distance between the wind measurement and the field sites precludes conclusions about the effects of high frequency wind variations at the field site, it is reasonable to expect that lower frequency wind components are coherent over this distance. The six hour averaging period used in Figure PO.II.4 was selected for clarity in presentation of the data as well as to filter out the high frequency part of the wind spectrum. Figure PO.II.4 shows that typically the strongest winds are aligned within about 30° of north and south and have

magnitudes which range up to 15 m/s (34 mph).

Horizontal water velocity was measured at sampling point HR0 (see Fig. PO.II.1) from 4/4 - 4/21 using an Inter Oceans S4 current meter. Measurements were made ~ 0.5 m above the bottom in a channel which was ~ 3 m wide and ~ 1 m deep below the top of the bank. Figure PO.II.5 shows vector plots of this data. Typical water movement in the upstream or downstream direction occurred at speeds of 0 - 10 cm/s. Maximum velocities reached 40 cm/s on 4/14 in the downstream rush of water following the high water peak.

Salinity was measured in water samples taken hourly by three automatic samplers, one of which collected surface water at point HR0 while the other two collected surface and bottom waters at point HR1. The results for the period up until the automatic samplers were drowned are presented in Figure PO.II.6. During this period the bottom water at point HR1 maintained a near constant salinity of about 13 ppt while the surface salinities at both points HR1 and HR0 ranged from 1 - 13 ppt, indicating that Home Road Creek varies from a stratified system to a vertically well mixed system. The cause and importance of this are discussed below.

Additional salinity measurements were made at daily intervals and are presented in Table PO.II.1. It is interesting to note that the water column at point HR2 at the mouth of Home Road Creek was seldom more than weakly stratified even when strong stratification was present at point HR1.

(ii) South West Creek Study, April 22 - May 9

Figure PO.II.2 indicates that one major rainfall occurred during

this interval, leaving about 40 mm of rain on 5/5. A moderate rainfall of between 15 and 20 mm fell on 5/3 while smaller events occurred on 4/24 and 5/9.

Water level fluctuations measured at the mouth of the South West Creek are shown in Figure PO.II.3. Variations during this period were limited to a range of about 0.65 m and are more typical of the area than those measured during the Home Road Creek study.

Wind velocity measurements in Figure PO.II.4 are similar in character to those during the Home Road Creek study with strongest winds typically aligned within about 30° of north and south and having magnitudes which range up to 15 m/s (34 mph).

Horizontal water velocity was measured at sampling point SW1-A (see Fig. PO.II.1) from 4/22 - 5/9 using an Inter Oceans S4 current meter. The instrument was anchored ~ 1.5 m above the bottom in a cross section approximately 6 m wide and 2.1 m deep below the top of the bank. Figure PO.II.7 shows vector plots of this data. Typical water movement in the upstream or downstream direction occurred at speeds of 0 - 10 cm/s. Maximum downstream velocities of ~ 20 cm/s were measured on 5/5 and were probably associated with runoff from rainfall falling earlier that day.

Salinity was measured in surface water samples taken every two hours by automatic samplers located at SW1-B and SW0, Figure PO.II.8. Surface salinities at point SW1-B typically ranged from 8 - 11 ppt while those upstream at point SW0 typically ranged from 2 - 7 ppt.

Additional salinity measurements were made at selected intervals and are presented in Table PO.II.1.

Discussion

It is well established that changes in waterlevel in Pamlico Sound are predominantly wind forced. Due to the low relief of the surrounding lands, these water level changes are easily propagated upstream and thus can be expected to dominate the levels in rivers and creeks. A comparison of Figures PO.II.3 and PO.II.4 illustrates this clearly. Almost all decreases in water level are accompanied by southerly winds (winds blowing from the south) which push water out of the southern end of Pamlico Sound and the associated rivers, while almost all increases in water level are associated with northerly winds. The extreme water level recorded on 4/13 and 4/14 was the result of a prolonged period of moderate northerly winds starting about 4/8 combined with 24-36 hours of strong northerly winds, in excess of 10 m/s, on 4/13 and 4/14.

It is noted that since the water level in South West Creek is dominated by the behavior of Pamlico Sound, it would be most appropriate to have wind measurements over Pamlico Sound to describe the dominant water level forcing. The close correspondence between Figures PO.II.3 and PO.II.4 provides some justification for the use of a 6-hour averaging interval to filter out the spatially incoherent part of the wind spectrum.

Estuaries are commonly separated into three categories based on their vertical density structure: strongly stratified estuaries (often called "salt wedge" estuaries), partially mixed estuaries and well mixed estuaries. These are shown schematically in Figure PO.II.9. A strongly stratified estuary is formed when the energy available for mixing is insufficient to overcome the buoyancy of the fresh water discharge. A well mixed estuary occurs when the mixing energy is much higher than

the potential energy associated with the buoyancy of the fresh water flow. Thus stratification is destroyed by strong mixing and enhanced by a large fresh water discharge. Sources of mixing energy are bottom friction, which is a function of the strength of the near bottom flow, and wind stress, which depends on the wind speed and the surface area of the water body.

Salinity measurements in Home Road Creek at points HR0 and HR1 indicate that at least part of the time this water body is strongly stratified. This is despite the relatively low fresh water discharges and is primarily due to the very small amount of energy available for mixing. As the fresh water leaves Home Road Creek and enters South West Creek, it spreads out laterally and, to conserve mass, must shrink in depth. The decreased layer thickness along with the increased surface area of South West Creek facilitate wind induced vertical mixing. Therefore, it can be expected that the strength of the stratification will decrease in South West Creek. This is consistent with the measurements at HR2, most of which show little or no stratification.

Salinity measurements at HR1 indicate that this site was also unstratified a significant portion of the time, having surface salinities of 13 - 14 ppt. A comparison of the salinity data with the water level data (Figure PO.II.10) shows that significant increases in surface salinity at HR1 are always associated with increases in water level. This behavior occurs because the pressure gradient associated with the rise in the water level of southern Pamlico Sound is stronger than that associated with the fresh water outflow, causing unstratified water from South West Creek to be forced back up into Home Road Creek. (The PSWIMS data in Figures PO.I.6 and PO.I.7 illustrate this quite

clearly. After about 4:00 on 4/8 a rise in water level drives a vertically uniform flow which pushes unstratified water past point HR1.)

This process is quite significant in effecting the impact of agricultural runoff in Home Road Creek. During water level rises, runoff is effectively trapped in the field ditches while during periods of falling water level, its entry into the estuary is enhanced. Chemicals entering Home Road Creek are confined to a thin fresh water layer at the surface. If the water column biota penetrate this surface layer they are exposed to high chemical concentrations. On the other hand if they remain in the lower regions, most of the runoff passes them by. Mixing in South West Creek provides a relatively large water mass for the dilution of these chemicals (compared to the water column in Home Road Creek). The biota in the lower part of the water column do not come in contact with the chemicals until a subsequent rising water level pushes South West Creek water back up into Home Road Creek or the density dirven circulation carries near bottom South West Creek water into Home Road Creek.

Surface salinity measurements made at point SW1-B in the headwaters of South West Creek are generally more constant that those measured in Home Road Creek. While the salinity measurements at SW0 reflect the rainfalls of 4/24, 5/3 and 5/5, only the latter one significantly affected the surface layer at SW1-B. Thus, with the exception of 5/6, site SW1-B appears to be only weakly stratified. The salinity drop on 5/6 seems to be a combination of a large amount of fresh water runoff from the previous day's rain and a rapid drop in water level at the end of 5/5 (Figure PO.II.11). During the following 48 hours the water level gradually increases and eventually forces unstratified water back up

past point SW1-B on 5/7. The water level peaks near the end of 5/7 and the following drop appears to allow another pulse of relatively fresh (and perhaps high in chemical concentration) water to reach site SW1-B on 5/8.

A major goal of the physical oceanographic component of project ARIES is to quantify the flux of fresh water runoff into the estuary, particularly following rainfalls which are strong enough to wash agricultural chemicals from the fields. For typical density driven estuarine circulation (Figure PO.II.9) the measurement of horizontal velocity at one location in the vertical will provide little help in accomplishing this since the velocity structure is not uniform over the depth. During these times, velocities measured with the S4 current meter at point HRO and SW1-A will be of little quantitative value. During times in which the circulation is dominated by the pressure gradient set up by water level changes in Pamlico Sound, the velocity should be unidirectional over the depth. An example of this is the period discussed above when unstratified South West Creek water was forced upstream in Home Road Creek on 4/8 (see Figure PO.I.6). During much of the Home Road Creek Study and some of the South West Creek study, a visual comparison of the horizontal velocities measured with the S4 and the water level measurements does suggest that rises in water level are usually accompanied by upstream velocities while drops in water level are usually accompanied by downstream flow. Unfortunately, we are still left without salinity profiles and therefore information about the thickness of the fresh water runoff layer. Order of magnitude calculations might be made using a typical fresh water layer thickness shown in Table PO.II.1.

The instruments deployed with PSWIMS are ideally suited for the purpose of determining fresh water flux in the estuary. Figure PO.II.12 shows the calculated fresh water and salt water fluxes/unit width for part of 4/7 and 4/8. It is the objective of future PSWIMS deployments to be able to make similar flux calculations during significant runoff events.

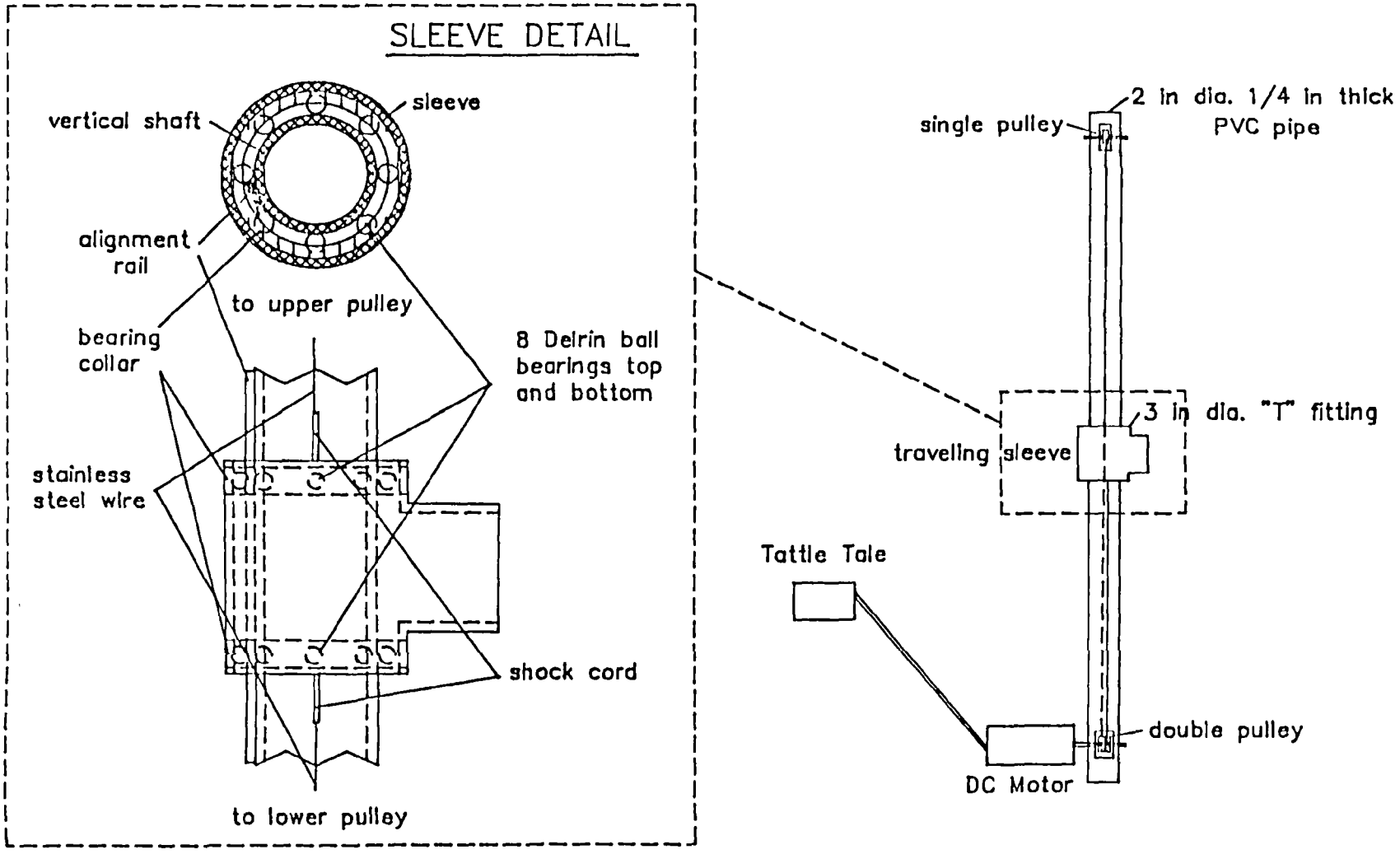


Figure PO.I.1. Principal components of PSWIMS

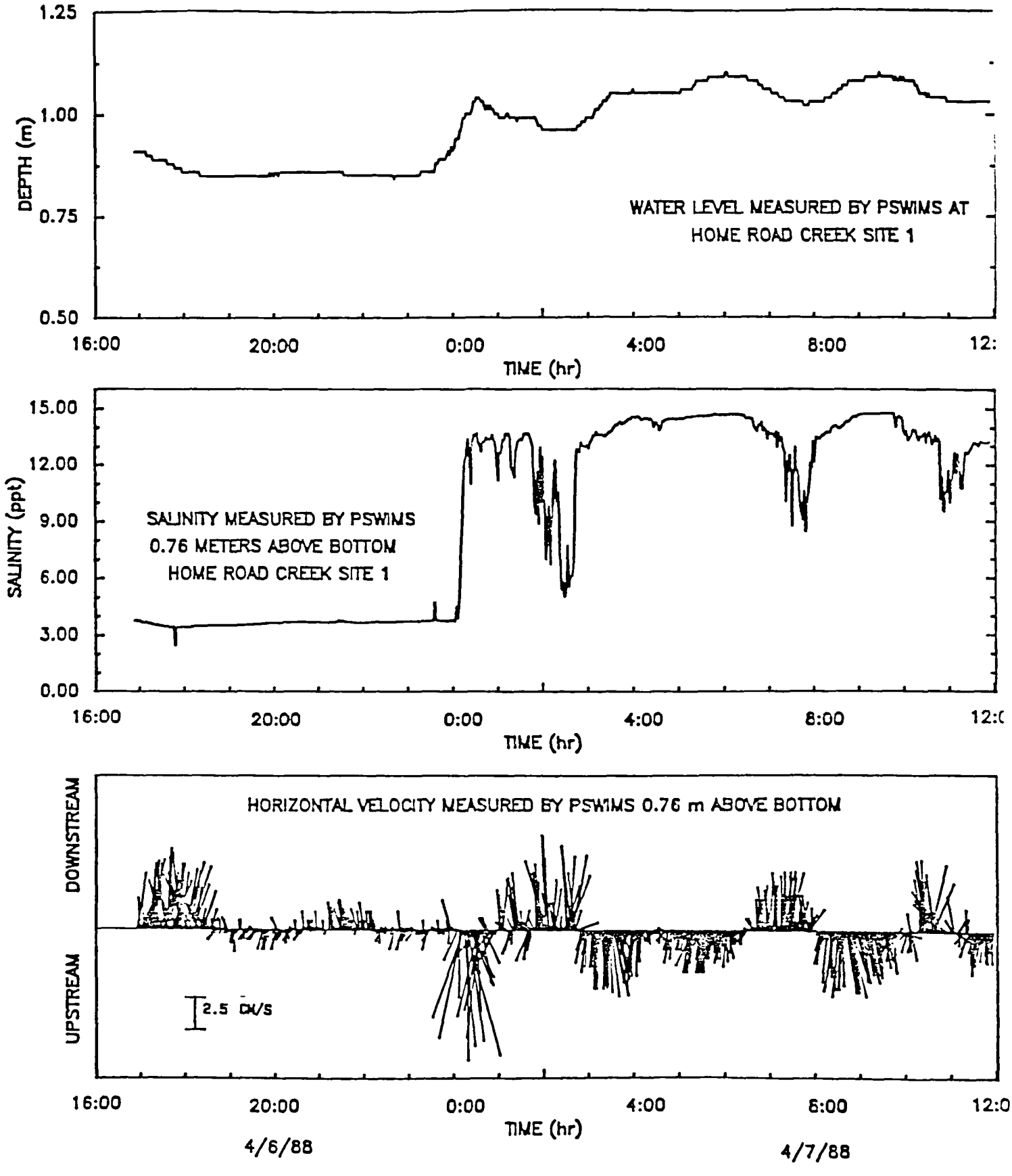
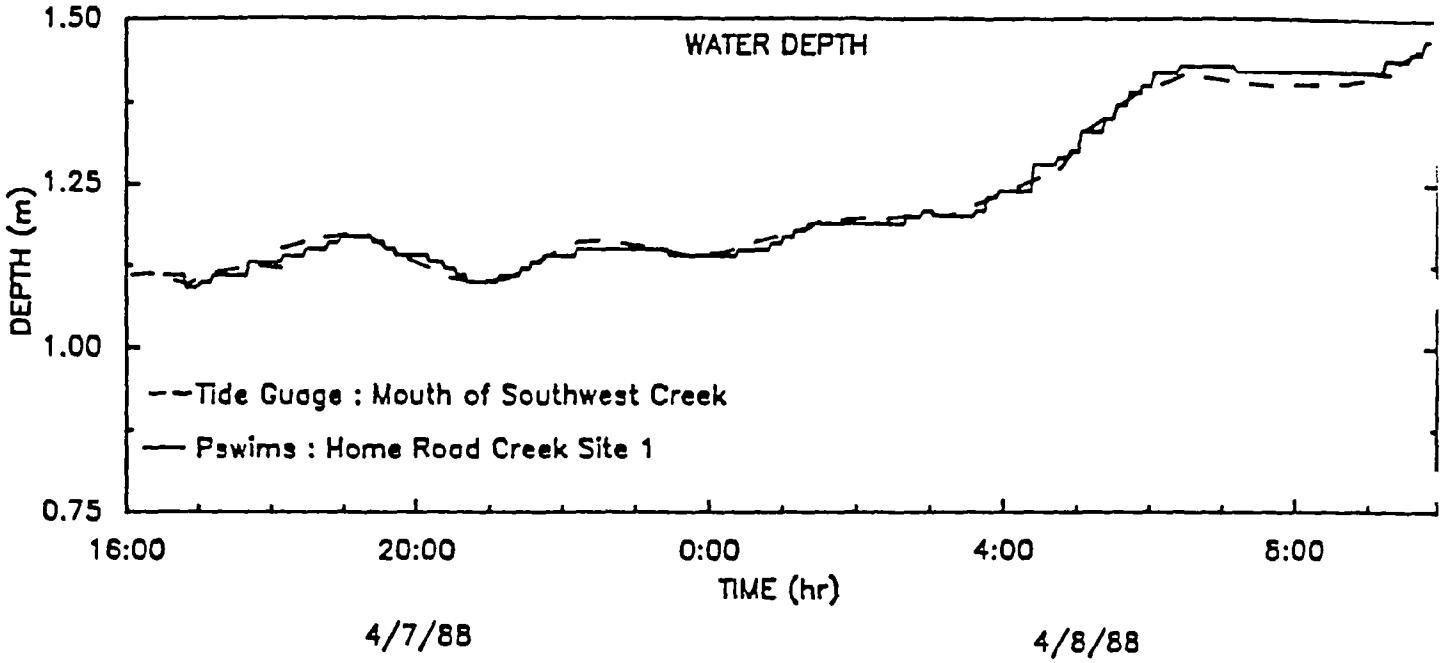


Figure PO.I.3. PSWIMS data collected at Home Road Creek site 1 at a fixed elevation 0.76 m above the bottom.



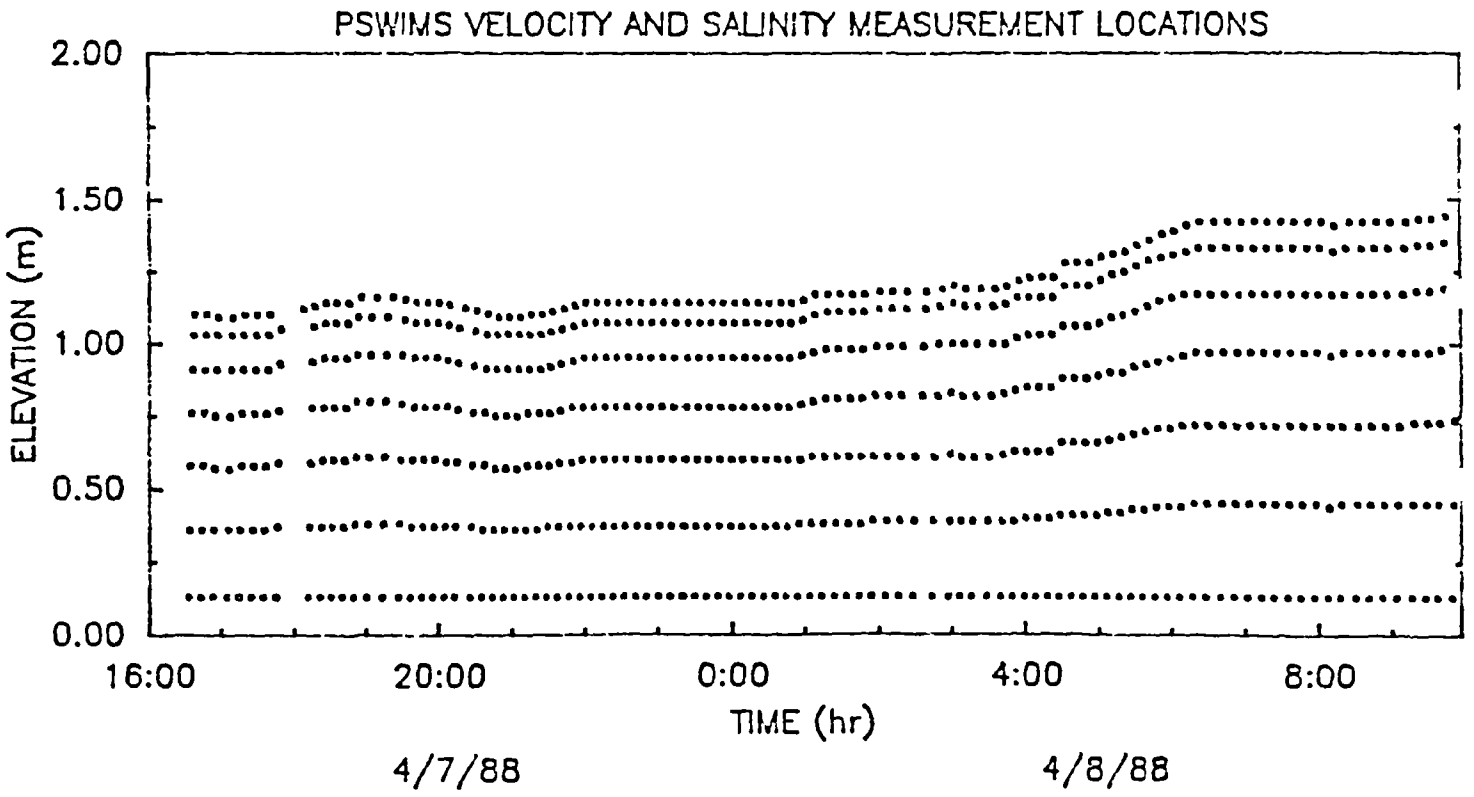


Figure PO.I.4. PSWIMS elevation measurements and sampling points

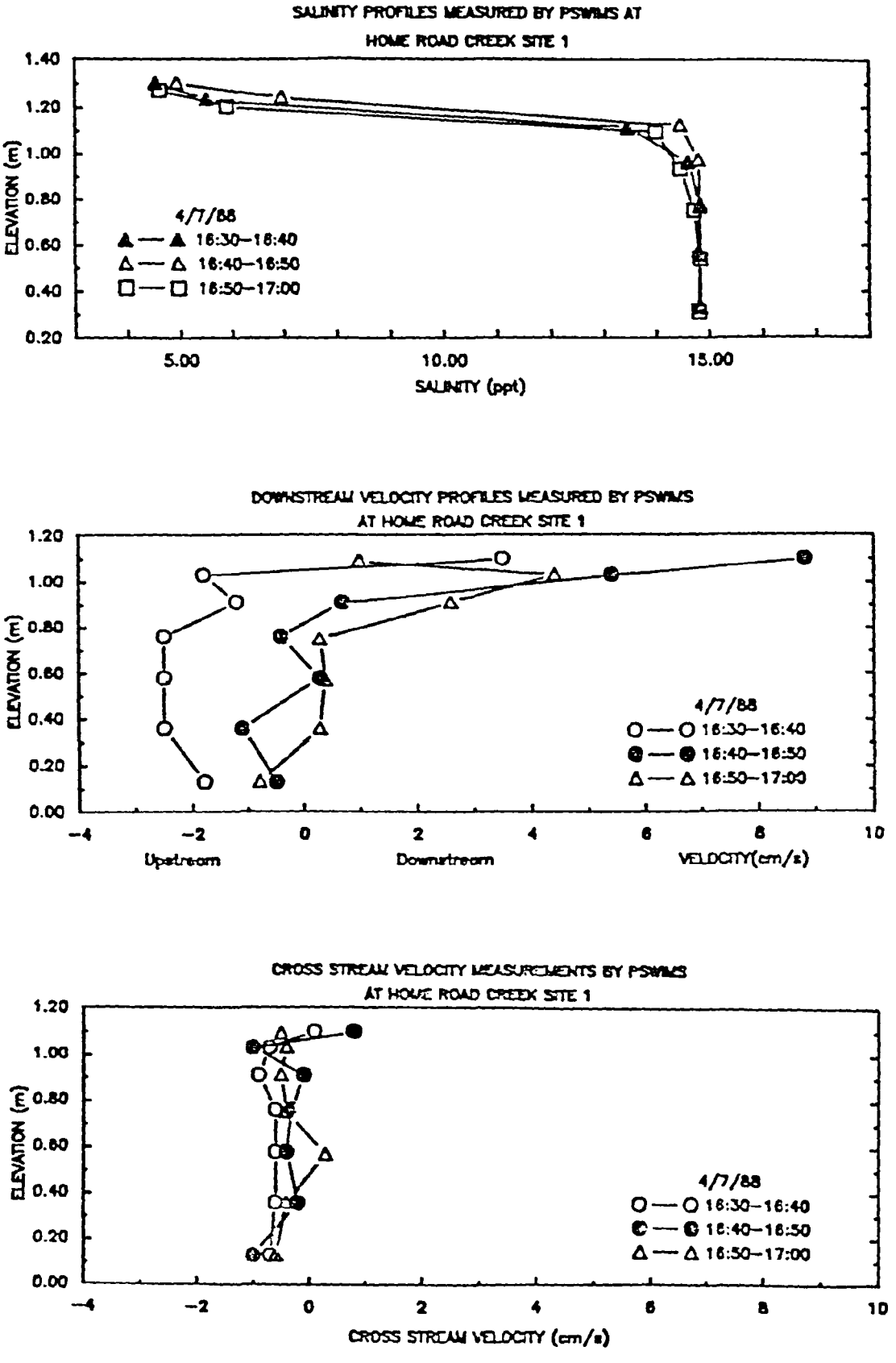


Figure PO.I.5. Sample salinity and velocity profiles measured by PSWIMS

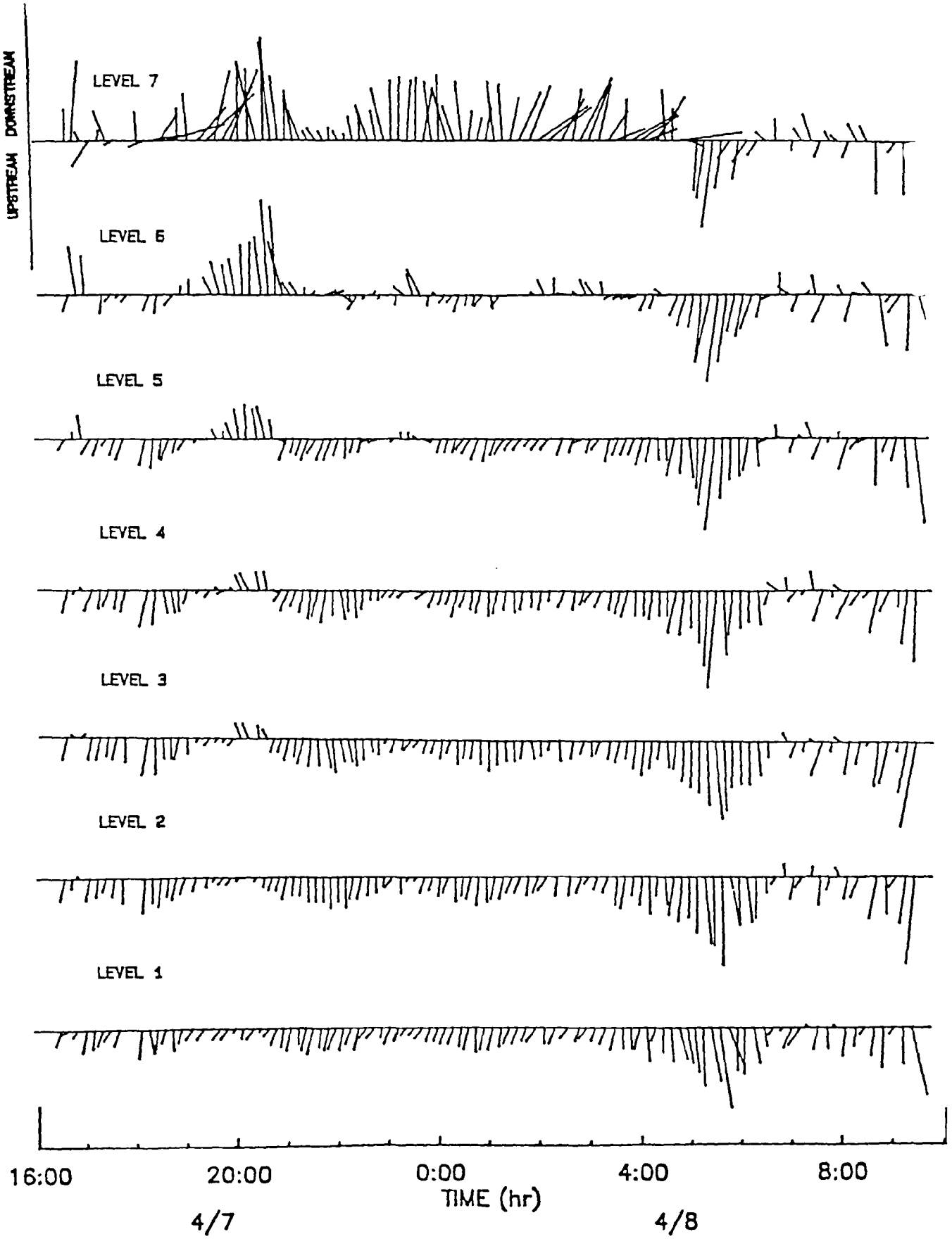


Figure PO.I.6. Vector plot of velocity data recorded by PSWIMS at Home Road Creek site 1. The scale is 1 inch = 15 cm/s.

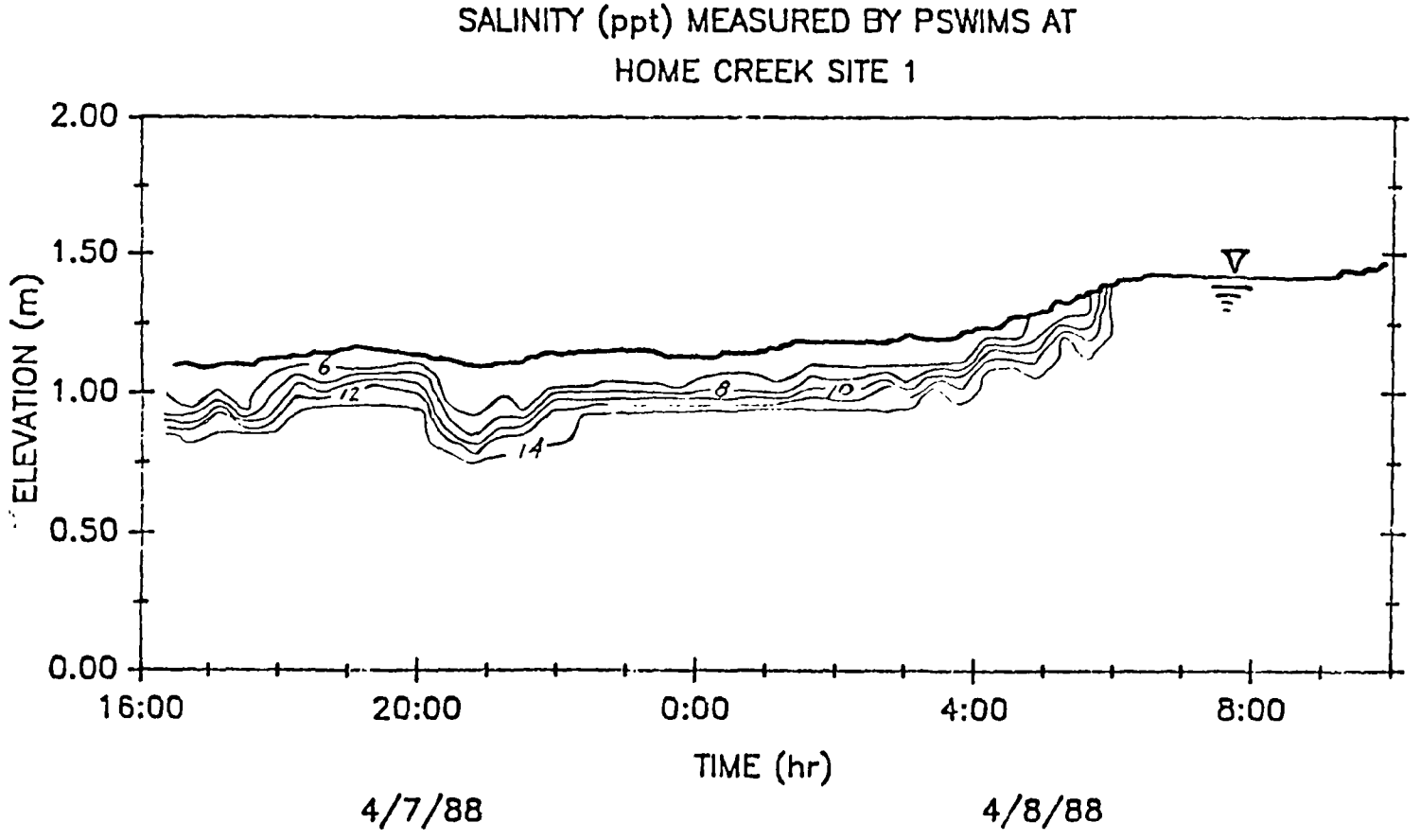


Figure PO.I.7. Contour plot of salinity measured by PSWIMS. The contour interval is 2 ppt.

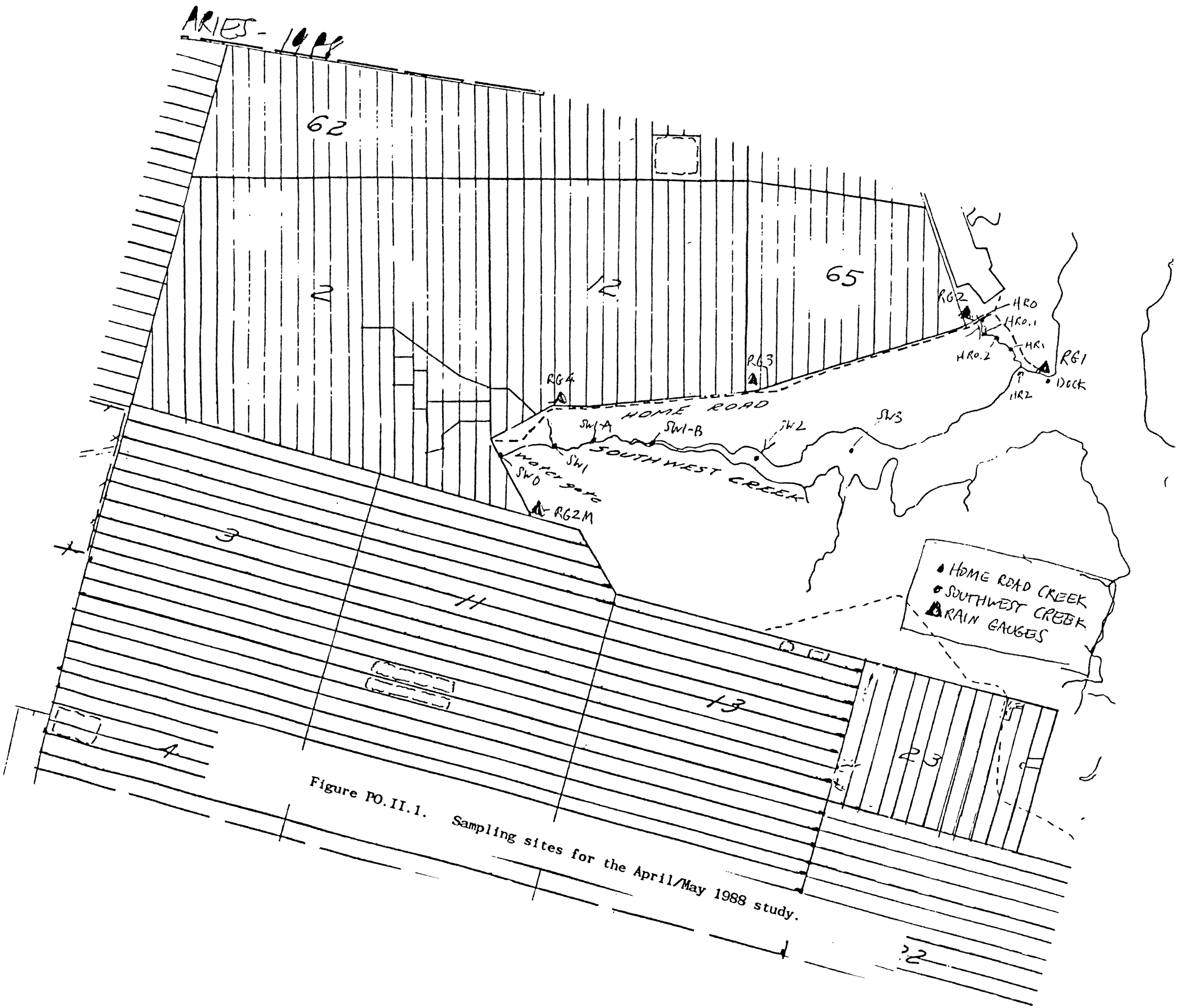


Figure PO.II.1. Sampling sites for the April/May 1988 study.

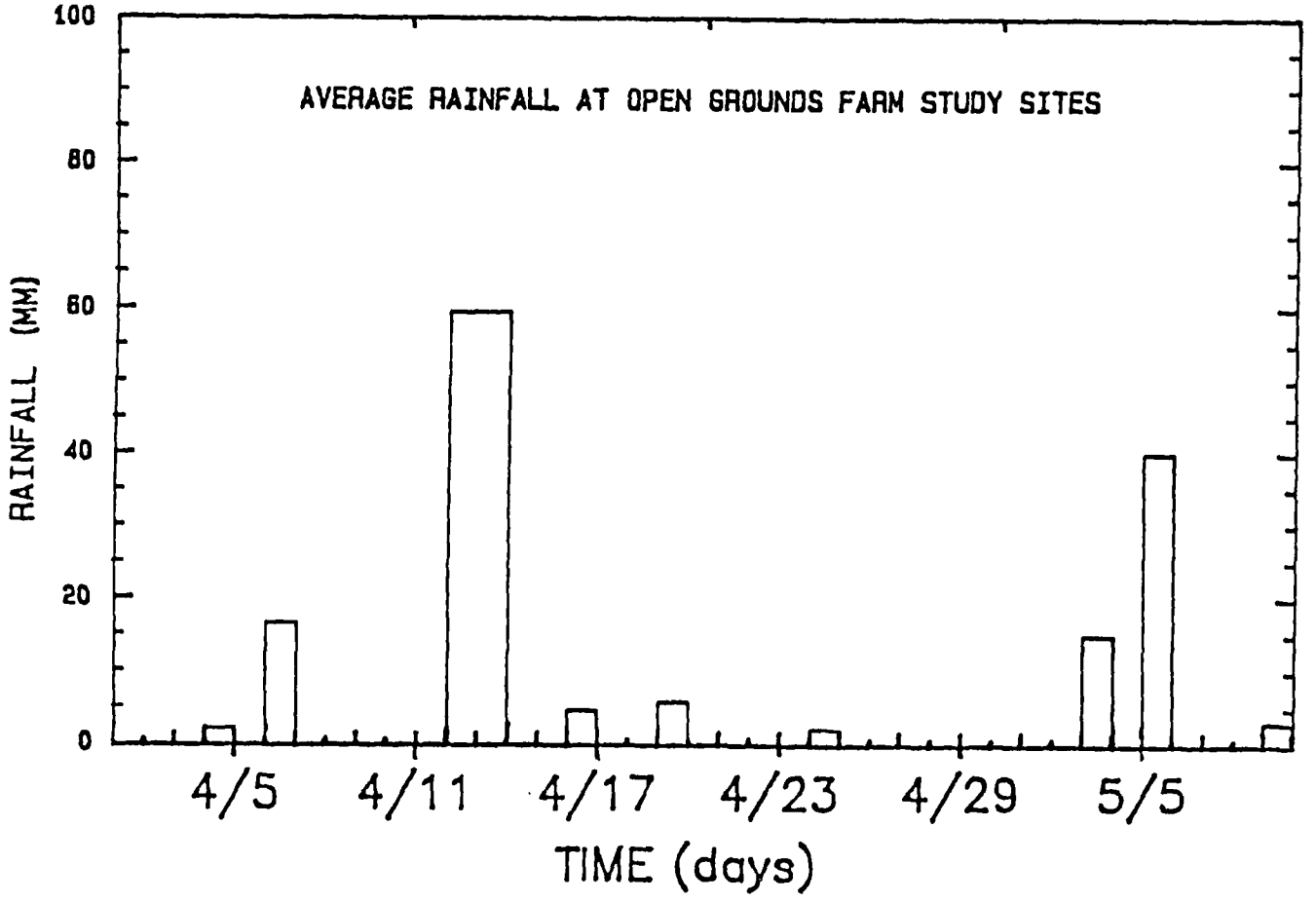


Figure PO.II.2. Rainfall recorded during the April/May study. Rainfall from 4/1 - 4/21 is an average from gauges at RG1, RG2, RG3, RG4. Rainfall from 4/22 - 5/9 is an average from gauges at sites RG1, RG2m, RG3, RG4.

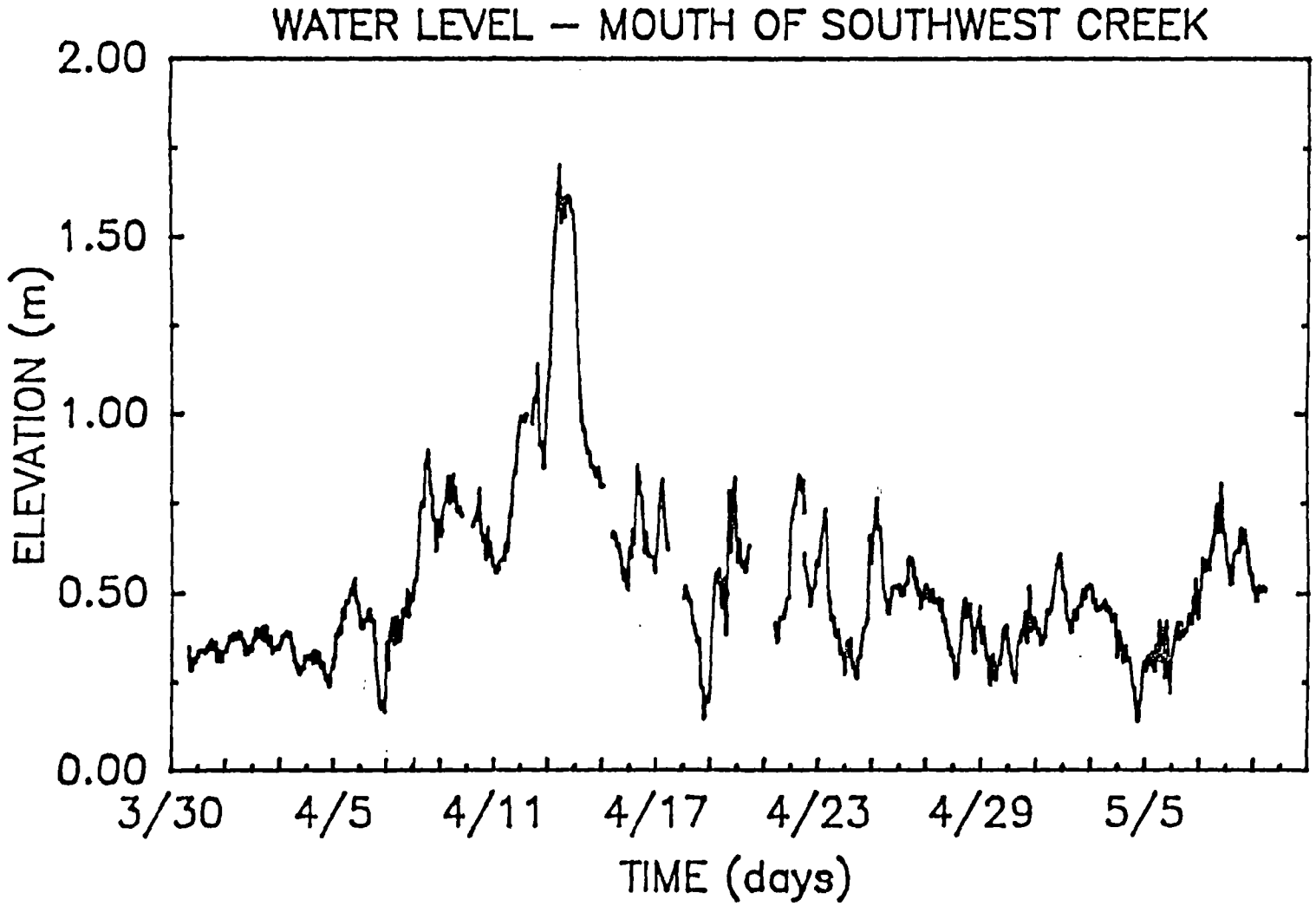


Figure PO.II.3. Water level measurements during the April/May 1988 study.

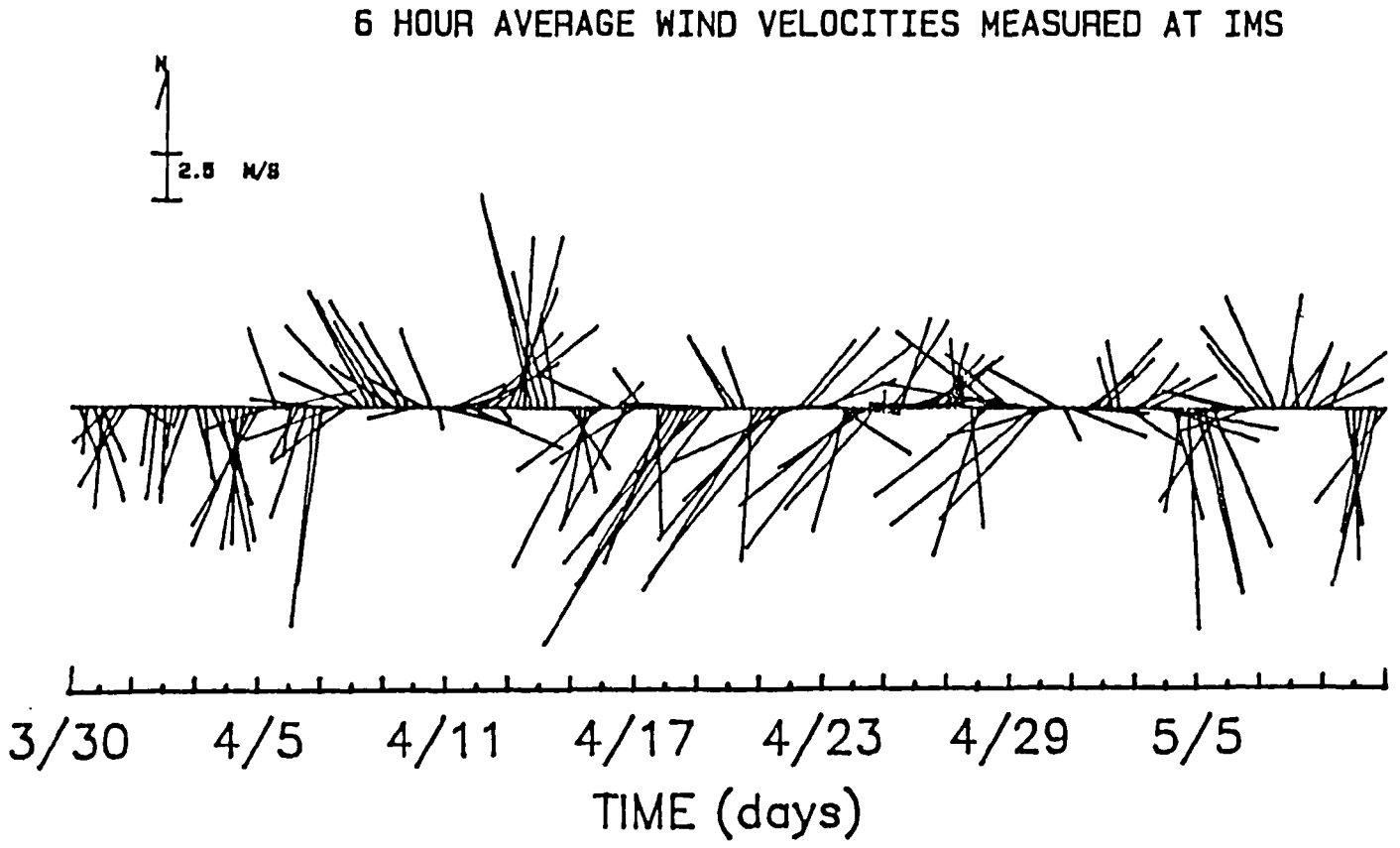


Figure PO.II.4. Six hour averaged wind velocities recorded at the UNC Institute of Marine Sciences during the April/May study.

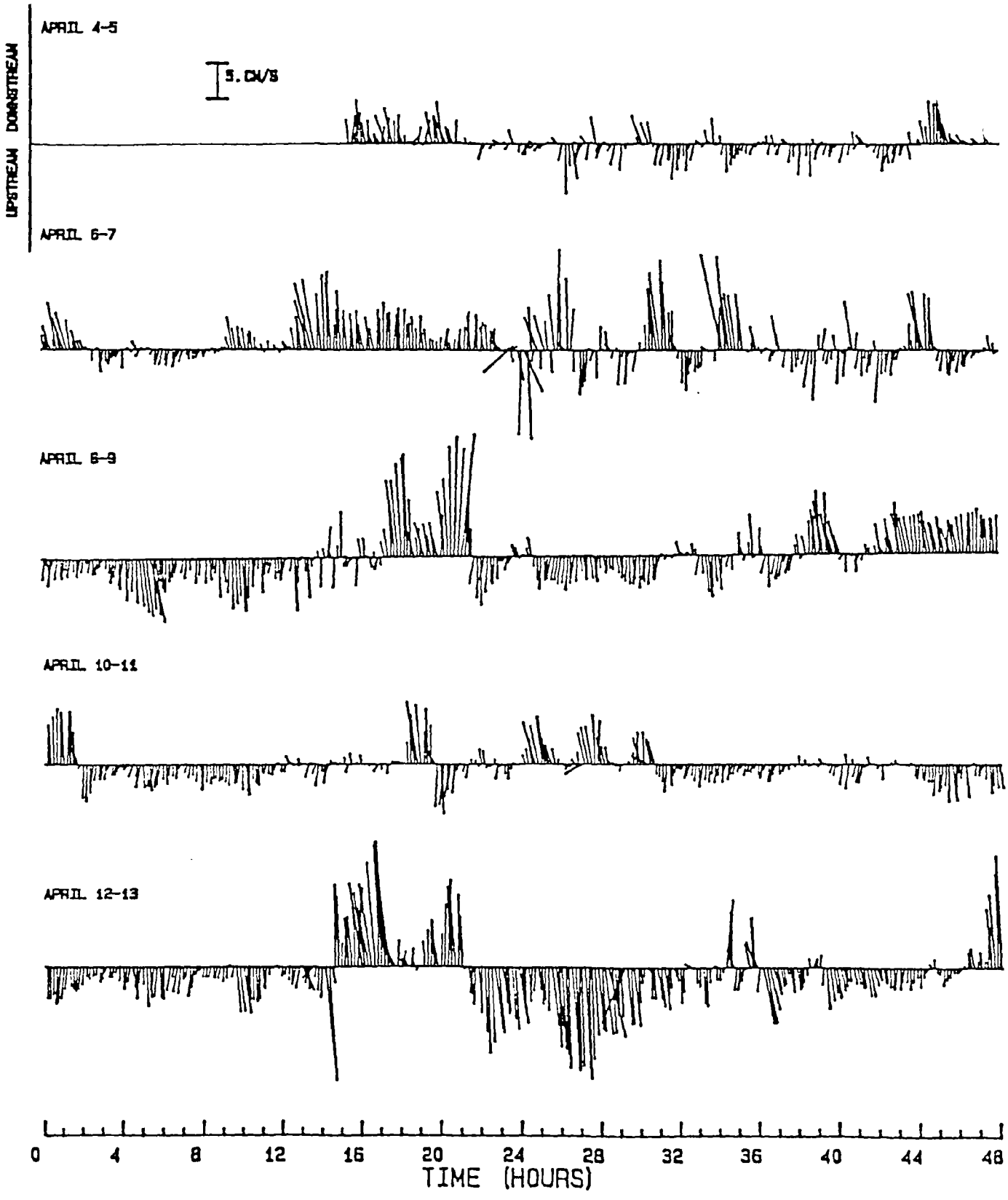


Figure PO.II.5. Vector plots of velocity data ~ 0.5 m above the bottom at site HRO during the Home Road Creek study. The scale is 1 in. = 20 cm/s.

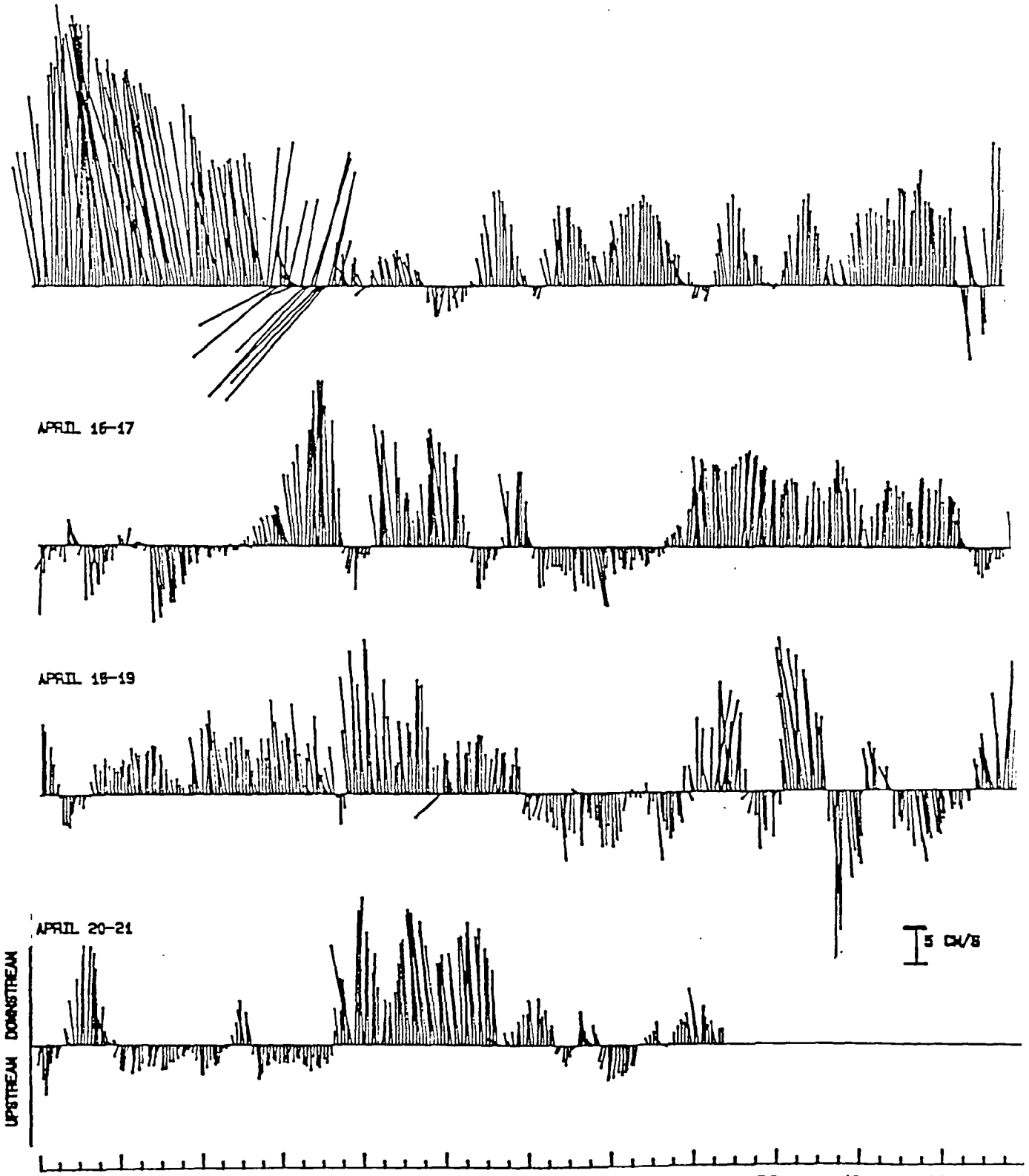


Figure PO.II.5. continued.

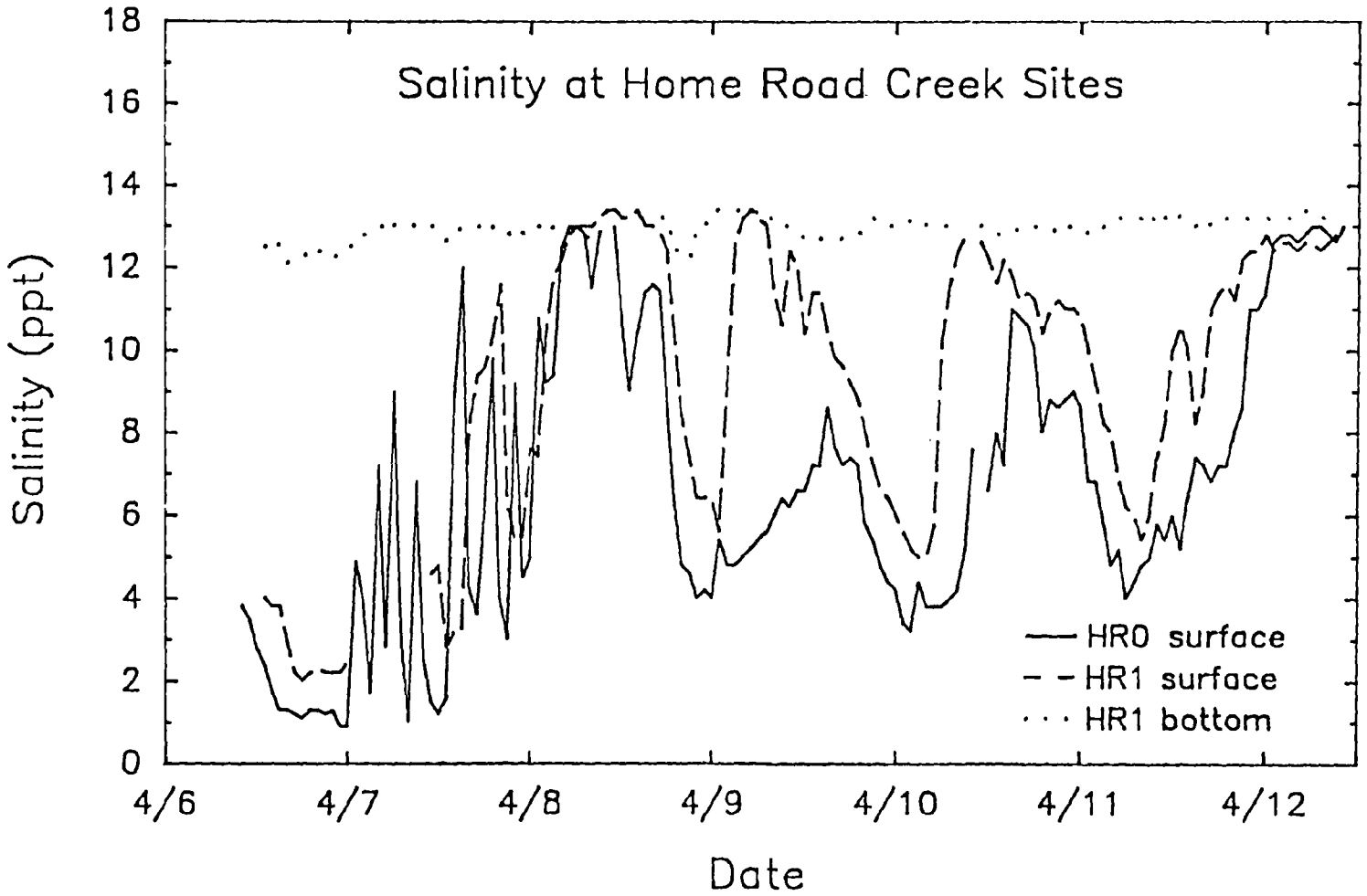


Figure PO.II.6. Salinity measurements taken from hourly water samples during the Home Road Creek study.

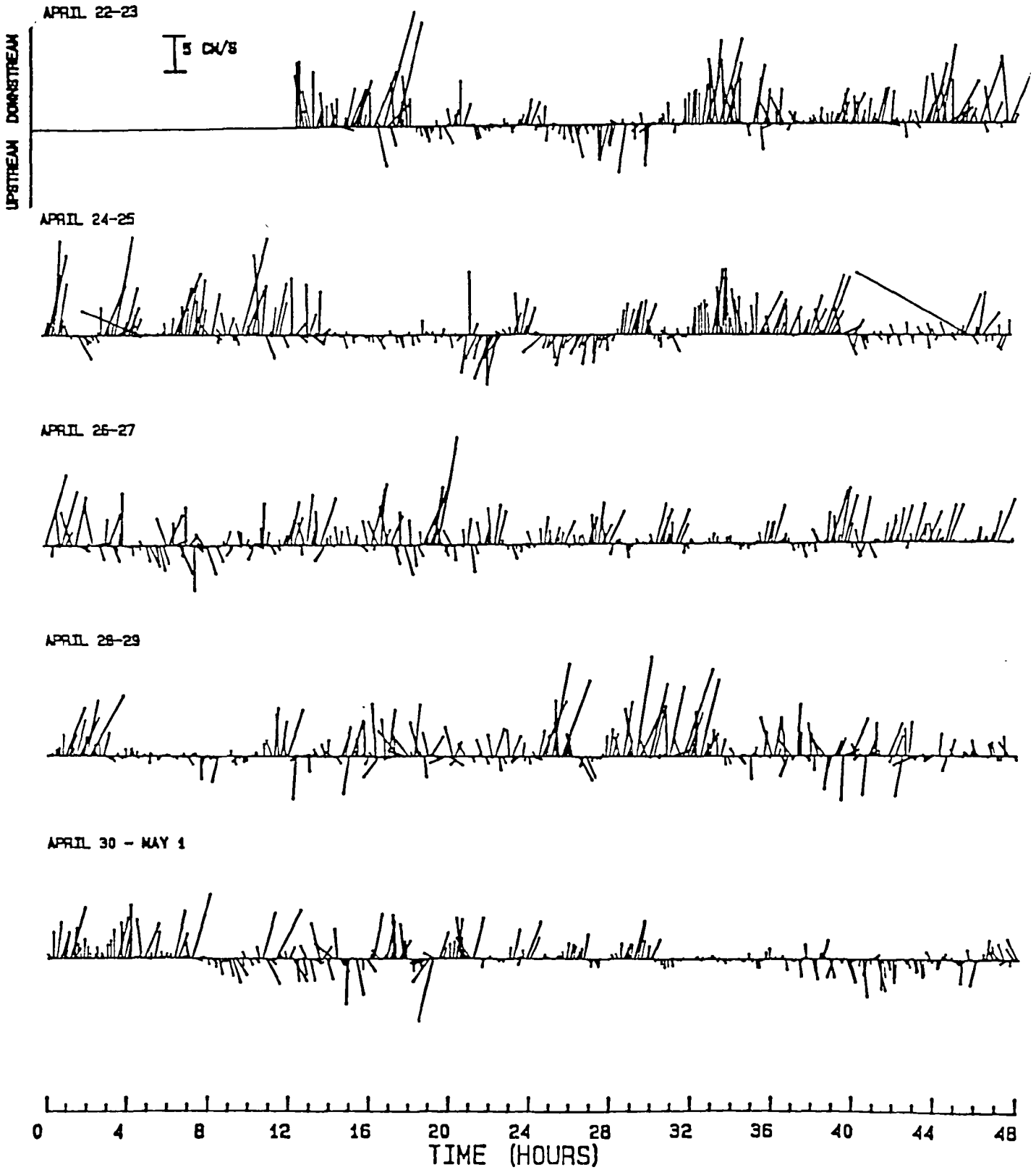


Figure PO.II.7. Vector plots of velocity data ~ 1.5 m above the bottom at site SW1-A during the South West Creek study. The scale is 1 in. = 20 cm/s.

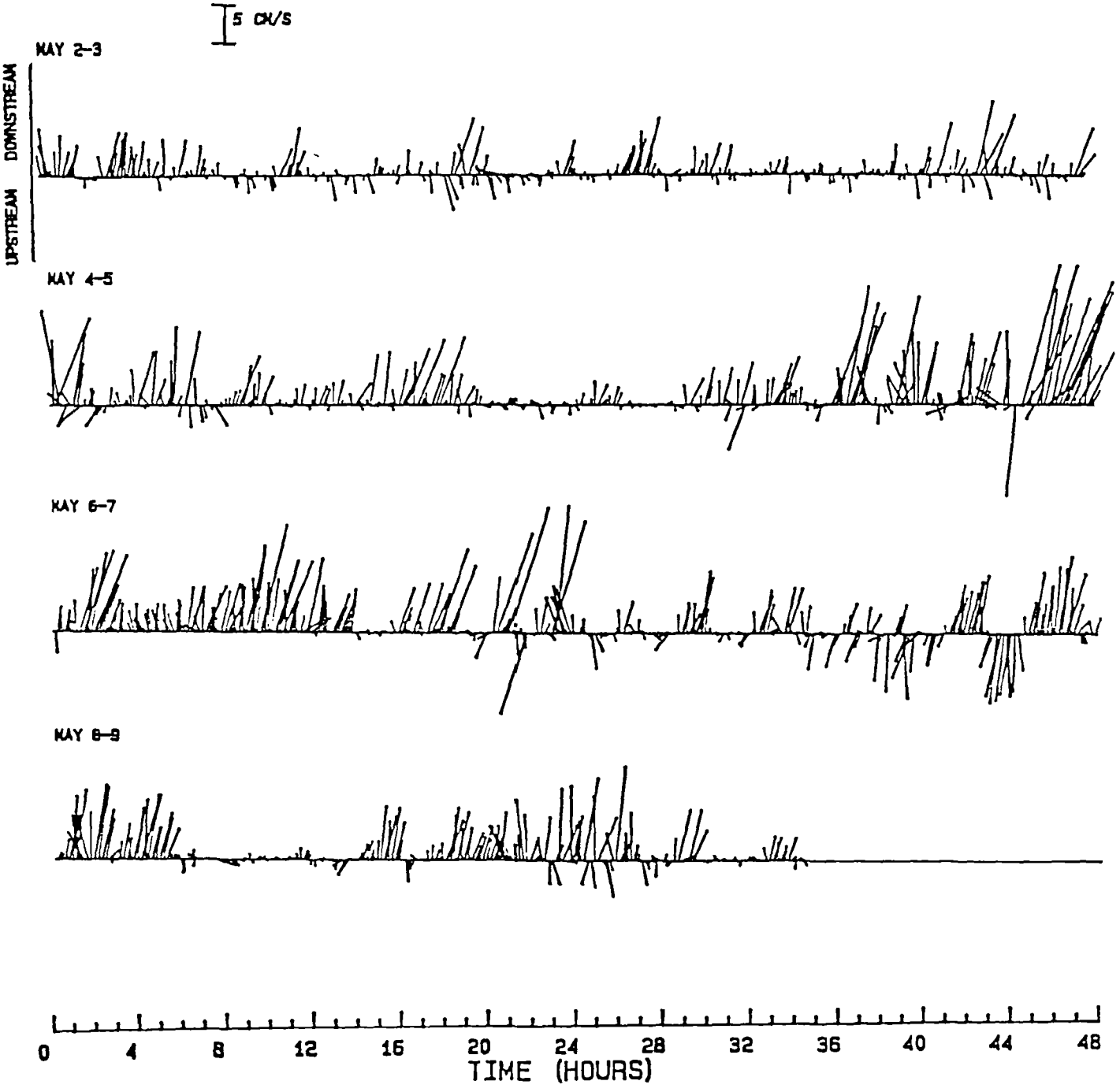


Figure PO.II.7. continued.

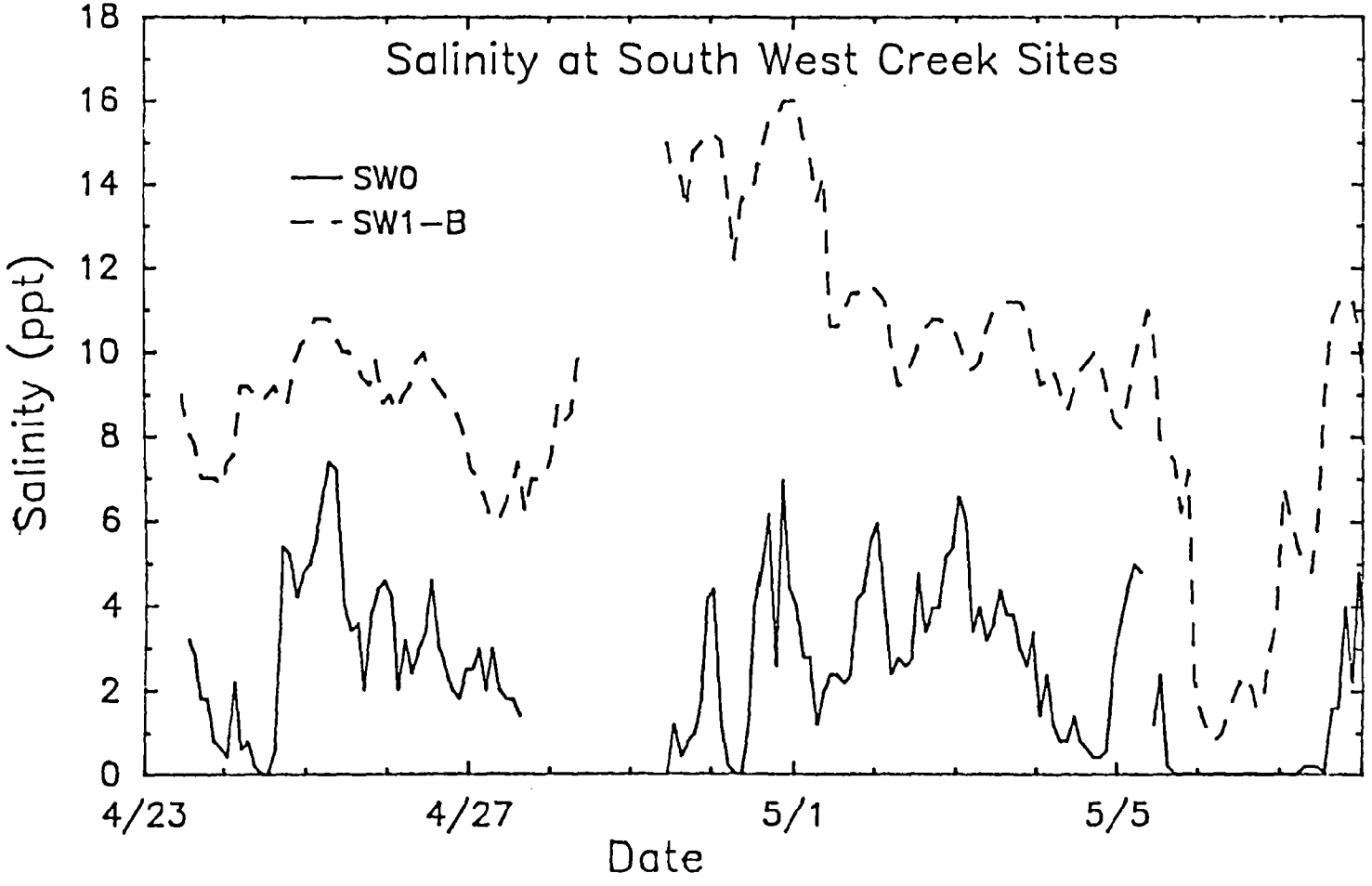
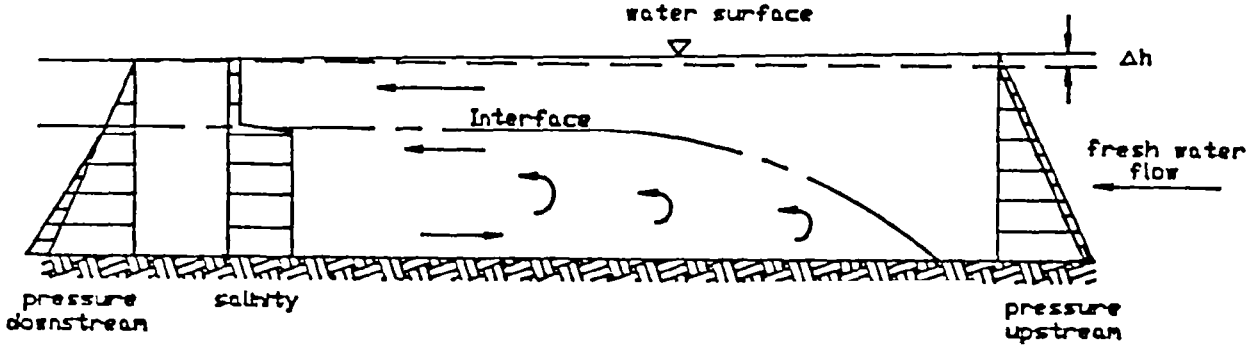
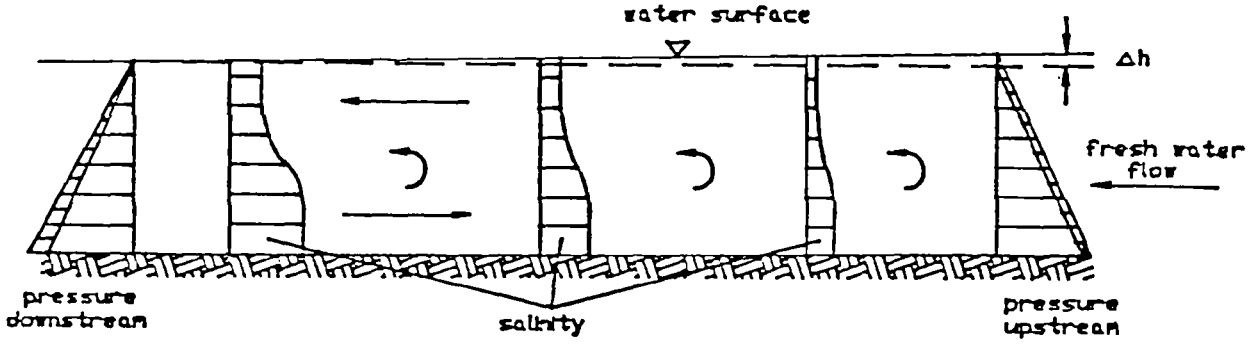


Figure PO.II.8. Salinity measurements taken from bi-hourly water samples during the South West Creek study.

STRONGLY STRATIFIED ESTUARY



PARTIALLY MIXED ESTUARY



WELL MIXED ESTUARY

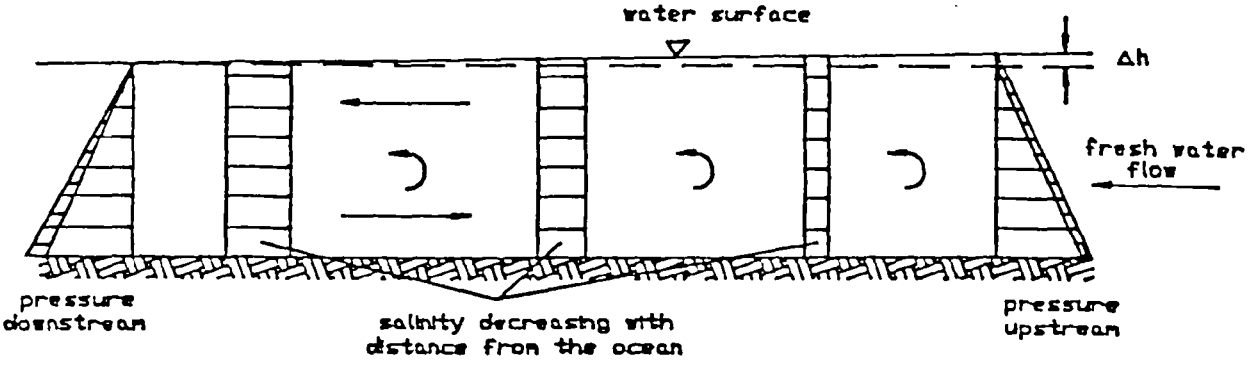


Figure PO.II.9. Estuarine classification scheme.

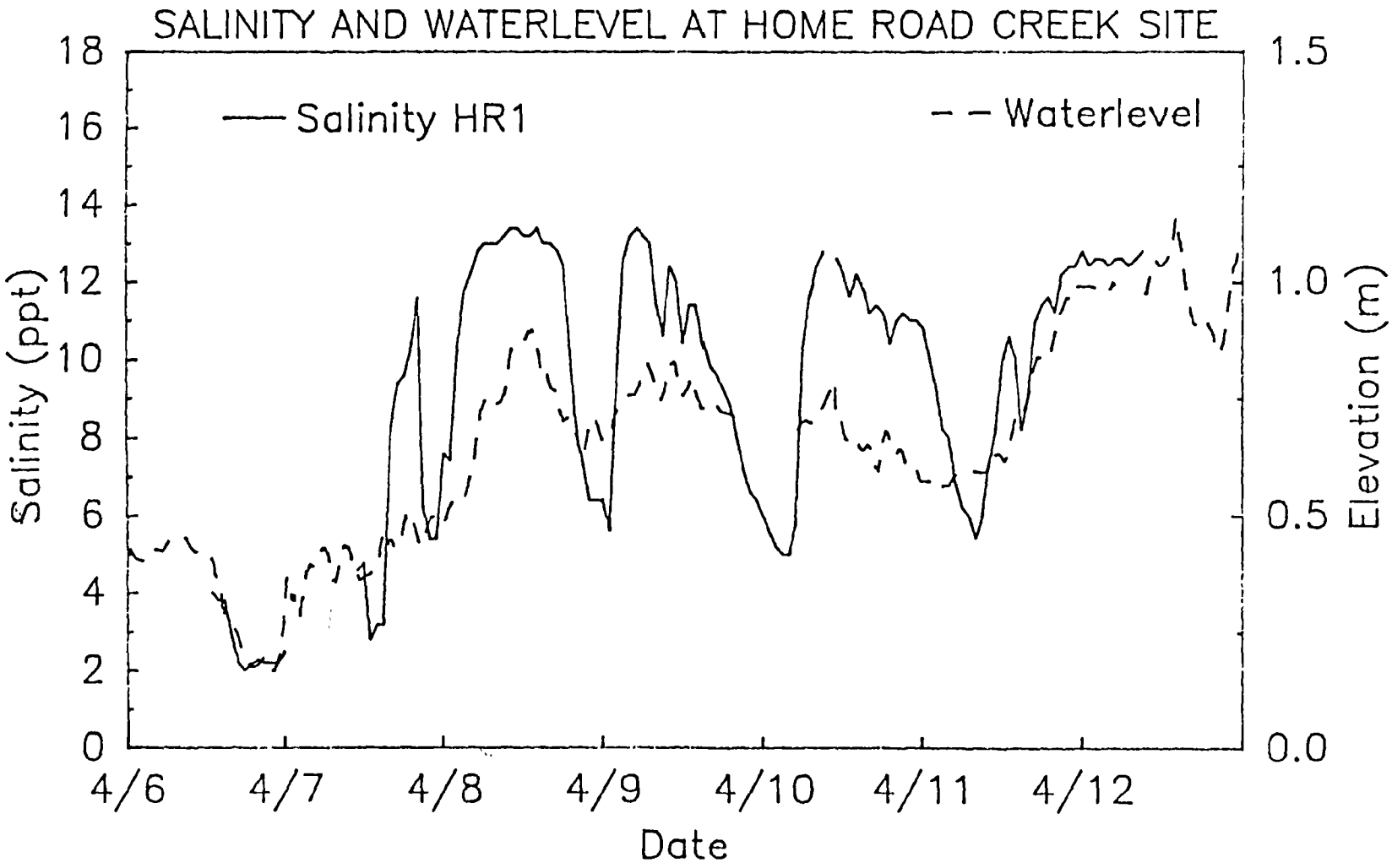


Figure PO.II.10. Comparison between surface salinity and water level during the Home Road Creek study.

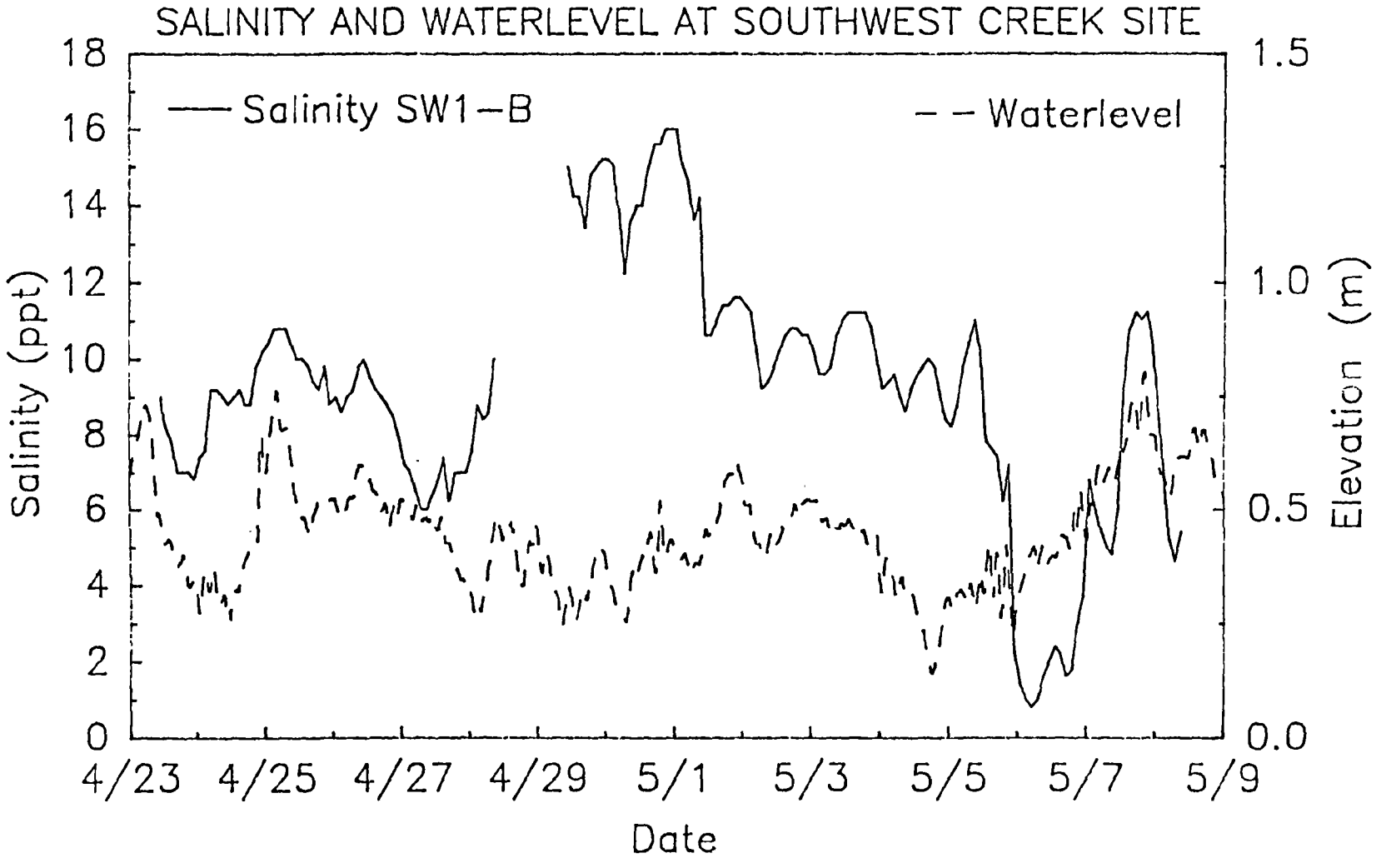


Figure PO.II.11. Comparison between surface salinity and water level during the South West Creek study.

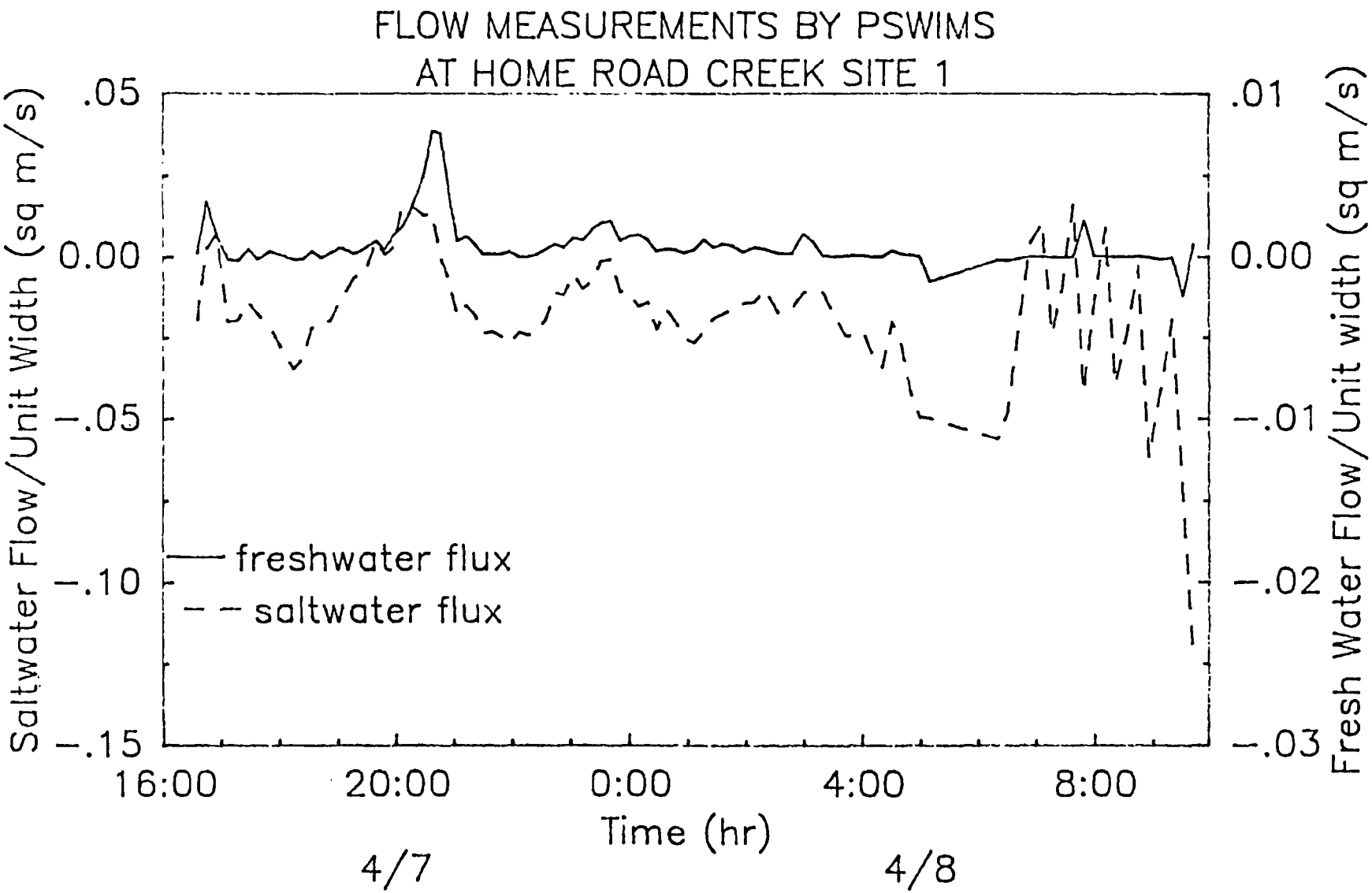


Figure PO.II.12. Computed flux/unit width of fresh water (0 ppt) and salt water (~ 14.8 ppt) using velocity and salinity data measured by PSWIMS. Note the difference in scale on the two vertical axes.

Table PO.II.1 Additional Salinity data measured during April/May 1988 study.

Depth	3-29-88			3-30-88			3-31-88			4-1-88		
(cm)	HRO	HR1	HR2	HRO	HR1	HR2	HRO	HR1	HR2	HRO	HR1	HR2
surface	0.0	2.6	9.8	0.5	2.0		0.0	0.0	19.0	1.0	1.0	9.0
10							0.2	2.0	19.0	1.0	3.0	12.0
20							2.0	12.0	19.0	6.0	5.0	12.0
30							4.5		19.0	9.4	11.0	12.2
40							7.1	13.0	19.0	10.5	12.0	12.8
50				6.0	13.0		9.0		19.0	11.1	12.8	12.8
60							9.8		18.8	12.2	12.8	12.8
70							9.8	13.0	19.0	12.5	12.6	12.8
80									19.0	12.5	12.6	12.8
90											12.6	12.8
100											12.8	12.6
110	2.2	13.0	12.2	9.8	13.0			13.0			12.5	

Depth	4-2-88			4-3-88			4-4-88			4-5-88		
(cm)	HRO	HR1	HR2	HRO	HR1	HR2	HRO	HR1	HR2	HRO	HR1	HR2
surface	1.2	1.5	12.0	0.5	1.0		0.0	0.8		1.5	5.0	7.8
10	1.5	3.0		1.5	2.0		0.2	2.0		3.6	13.0	12.3
20	7.0	7.5	12.5	4.8	4.0		1.2	6.0		6.0	13.5	13.2
30	12.4	12.4		11.3	10.0		4.2	9.0		9.5	13.5	13.2
40	12.6	12.8	12.7	11.9	11.0		6.5	10.1		10.8		
50	12.6			12.1	11.0		7.5	10.4		11.5	13.5	13.2
60	12.6			12.1	11.0		10.2	10.6		12.4		
70	12.6	12.8	12.9	12.2	11.0		10.5	10.9		12.5	13.5	13.2
80	12.6			12.2				10.9		12.0		
90		12.9	12.0		11.0			10.9			13.5	12.0
100			12.5		10.8			10.9				11.8
110		12.7	12.2								13.5	

Depth	4-6-88			4-7-88			4-8-88			4-9-88		
(cm)	HRO	HR1	HR2	HRO	HR1	HR2	HRO	HR1	HR2	~~HR-1~~	~~HRO~~	~~HR1~~
surface	1.5		11.0	0.4	1.5		6.8	6.4		2.0	6.2	13.4
10	3.0		12.2	1.2	3.5		13.2	13.4		2.0	6.4	13.4
20	3.5		12.6	1.3	11.2		13.4	13.4		4.6	8.4	13.4
30	4.3		12.8	5.5	12.2			13.4		8.0	13.2	
40	6.8			12.0	12.7		13.4			9.8	13.2	13.4
50	8.2		13.0	12.0	13.0			13.4				
60	9.5			12.2	13.4		13.4			10.4		13.4
70	10.5		13.0	12.3	13.4			13.8				
80	12.3			12.3	13.5		13.4			10.6	13.4	13.6
90			12.2		13.6							
100					13.5							
110					13.2		13.4					

Depth (cm)	4-10-88 HR-1	HRO	HR1	4-11-88 (No Data)	4-12-88 HR-1	HRO	HR1	4-13-88 'RUN-ON'!!!
surface	1.4	6.8	12.8		10.0	13.2	12.8	
10	1.8	7.4	12.8		12.2	15.0	13.2	
20	6.0	11.2	12.8		13.6	15.0	13.2	
30	10.0	13.2	12.8		13.8	15.0	13.2	
40	11.0	13.4	13.4		14.2		13.2	
50						15.0		
60	11.4	13.6	13.2		14.2		13.2	
70						15.0		
80	11.4	13.4	13.2		14.2		13.2	
90						14.8		
100					14.0			
110						14.8	13.2	
120							13.2	
130						14.6		
140							13.2	
150						14.4		

SALINITY PROFILES - Home Road Creek Sites
(post 'Run-On' event)

Depth (cm)	4-14-88						
	HR-1	HRO	HRO1	HRO2	HR1	HR2	D
surface	6.8	7.2	7.2	8.6	8.8	9.8	12.2
10	6.8	7.4	8.4	8.6	8.8	10.2	11.2
20	6.8	7.4	8.4	8.6	8.8	11.0	11.2
30	7.0	7.6	8.4	8.6	9.0	11.8	11.2
40							
50	7.0	7.6	8.6	8.8	9.0	12.0	11.2
60							
70	7.0	7.8	8.8	8.8	9.8	12.0	
80				9.4			
90	7.2	8.0	9.4		10.4	12.0	
100				9.8			11.2
110	7.4	8.6	9.8		12.2	12.0	
120							
130				9.8	12.8	12.0	
140		8.6					
150			10.2	10.0			
160					12.8	11.8	11.2
170			10.2				
180							
190							
200							

Depth (cm)	4-15-88						
	HR-1	HRO	HRO1	HRO2	HR1	HR2	D
surface	2.4	3.8	4.0	4.6	4.6	7.0	9.6
10	2.6	4.2	4.0	4.8	4.6	7.0	9.8
20	2.6	4.4	4.2	4.8	4.8	7.6	9.8
30	2.6	4.6	4.0	5.0	5.0	11.4	9.8
40			4.4	5.2	6.0	11.6	
50	2.8	4.8	4.4	5.4	6.4	11.6	11.6
60			6.0	5.8	7.0	11.6	
70	2.8	5.2	6.4	6.8	8.6	11.6	11.6
80			6.6	7.6	10.2	11.6	
90		5.8	8.0	9.4	11.2	11.6	11.6
100	2.8	5.8	10.4	11.6	11.4	11.6	
110		10.6	11.2			11.6	11.6
120	3.0	10.0	11.2	11.6	11.8	10.6	
130		10.8					
140							
150			11.6	11.6			11.6
160							
170							
180							
190							
200			11.6				

SALINITY PROFILES - Home Road Creek
Post 'Run-On' Sampling

Depth (cm)	4-16-88							4-19-88	
	HR-1	HRO	HRO1	HRO2	HR1	HR2	D	HRO	HR1
surface	1.0	5.6	8.4	9.6	10.4	11.0	11.6	3.0	7.0
10	1.0	5.8	10.6	10.2	11.4	11.8	12.0	4.0	7.4
20	1.4	7.8	11.0	11.0	11.6	11.8	12.0	4.0	7.6
30	3.0	10.6	11.4	11.0	11.6	12.0	12.0	4.0	7.6
40	6.0							4.6	7.8
50	7.8	11.6	11.4	11.4	11.8	11.8	12.0	5.0	11.0
60								6.6	12.2
70	7.2	11.6	11.6	11.6	11.8	11.6	12.0	10.0	12.6
80								10.0	12.8
90		11.6						10.0	12.8
100	7.8	11.6	11.6	11.6	11.8	11.4	12.0	10.0	12.8
110		11.6							
120									
130		11.6							
140			11.6	11.6		11.4	12.0		
150									
160									
170									
180				11.8					
190									
200									
210			11.8						

SALINITY PROFILES - Southwest Creek
(post 'Run-On' event)

Depth	4-14-88			4-15-88				4-16-88			
(cm)	SW1	SW2	SW3	SW1	SW1A	SW2	SW3	SW1	SW1A	SW2	SW3
surface	1.0	3.8	8.0	0.8	3.8	7.4	9.0	7.8	10.6	11.2	10.8
10	1.0	4.0	8.2	1.0	3.2	7.4	9.0	8.6	10.6	11.4	11.0
20	1.0	4.0	8.2	1.2	3.4	7.0	9.0	9.4	10.8	11.4	11.0
30	1.4	4.6	8.8	1.2	3.4		9.0	10.4	10.8	11.4	11.2
40		5.0		1.4	3.6	7.4	9.0				
50	1.8	5.8	9.4	1.8	5.8	9.8	9.2	10.4	10.8	11.4	11.2
60		6.8	9.4	2.0	6.6	9.8	9.4				
70	3.8	10.2	11.2	2.2	6.8	10.4	9.4	10.6	11.0	11.4	11.4
80		10.2		2.6	9.8	10.4	9.4				
90	3.8	10.2		3.4	11.0	10.4	9.6		11.0		
100		10.6	11.2	5.6	11.2	10.4	9.8	10.8		11.4	11.6
110				7.6	11.0	10.4	10.4		10.4		
120	3.8			8.8						11.4	
130		10.8		9.4		10.4					
140					10.4					11.2	
150		10.8	11.8	10.0			10.4	11.0			11.6
160											
170											
180											
190											
200				10.2			10.4	10.8			11.4
200+		10.6	11.0	10.2			10.6				11.2

SALINITY PROFILES - Southwest Creek

Depth	4-22-88			4-23-88			4-25-88		4-27-88	
(cm)	SWO	SW1	SW1B	SWO	SW1	SW1B	SWO	SW1B	SWO	SW1B
surface	2.5	10.2	8.5	3.0	6.0	9.2	2.2	9.8	2.0	6.0
10	3.0	10.2	8.5	3.0	6.0	9.2	3.2	10.2	2.2	8.0
20	3.0	10.2	9.5	3.0	6.0	9.2	4.0	10.2	2.2	9.5
30	3.0	10.2	10.0	3.0	6.0	9.4	4.4	10.2	2.5	10.0
40	3.5	10.2	8.0		6.0	9.4		10.2	2.5	11.0
50	4.0	10.2	8.0	3.0	6.0	9.4	4.0	10.2	3.0	11.0
60	5.0	10.2	8.0		6.0	9.4			3.2	11.0
70	5.5		8.5	3.2			4.6	10.2	3.6	11.0
80	5.5	10.2	9.5		6.0	9.6			3.8	11.0
90									4.0	11.0
100	6.0	10.2	9.5	3.2	6.0	9.6	4.8		4.0	11.0
110									4.2	11.0
120			9.5			9.8			4.2	11.0
130	7.0				6.0		5.4		4.2	11.0
140										11.0
150		10.4	9.0	3.2	6.0					11.0
160										
170										
180										
190										
200		10.4								
200+										

Depth	4-29-88		5-1-88		5-3-88		5-5-88	
(cm)	SWO	SW1B	SWO	SW1B	SWO	SW1B	SWO	SW1B
surface	0.4	No	1.8	No	3.4	No	1.4	10.4
10		Data		Data	3.8	Data	1.6	
20					4.8		1.6	
30					5.4		1.6	
40								
50					5.6		1.8	
60								
70					6.2		2.0	
80								
90					6.6			
100								
110					6.6		2.4	
120								
130								
140								
150								
160								
170								
180								
190								
200								
200+								
(Bottom)	0.4		9.2					11.0

SALINITY PROFILES - Southwest Creek

Depth	5-6-88			5-9-88	
(cm)	SW0	SW1	SW1B	SW0	SW1B
surface	0.0	0.0	2.0	0.2	5.4
10	0.0	0.0	2.4	0.2	9.4
20	0.0	0.2	2.4	0.2	11.2
30	0.0	0.4	10.2	0.2	11.4
40		0.6			
50	0.0	0.6	11.8	0.2	11.8
60		1.0			
70	0.0	7.4	12.0		11.8
80		9.8			
90	0.0				
100				0.2	12.0
110		9.8			
120	0.0			0.4	12.2
130					
140					
150			12.0		
160					
170					
180					
190					
200			12.0		11.8
200+			12.0		
(Bottom)					

BIOLOGICAL INVESTIGATIONS

Effects of Pesticides on the Mud Crab, Rhithropanopeus harrisii

In 1987 and 1988 three experiments were conducted using the common estuarine mud crab, Rhithropanopeus harrisii, as the experimental animal. Two of these were laboratory studies which, while not a direct part of this project, were a result of our focus upon alachlor. The first experiment was a laboratory study of the effects of the herbicide alachlor on larval development (see attached reprint), the second was a study of effects of alachlor on respiration of adult crabs (see attached preprint) and the third involved rearing larvae from ovigerous females which had been held in runoff from fields sprayed with the insecticide permethrin. The results of the first laboratory study has been published, the second has been accepted for publication and the third will be published in the Proceedings of the National Pesticide Research Conference.

Exposure of Gravid Mud Crabs to Farm Water (2 phases)

In phase one, ovigerous crabs (Rhithropanopeus harrisii) with immature eggs were held in farm runoff for 4 days at two sites. Site 3 (Home Road Creek; Figure 1) received runoff for soybean fields while site 6 (Grassy Knoll Creek; Figure 1) received runoff from mature corn fields. Crabs were held in porous polyethylene containers filled with oyster shell which were suspended in surface waters of the estuarine creeks by floats. Fields draining into site 3 were sprayed with permethrin on August 22, 1987 at 89 ml/acre. On August 26 and again on August 31 approximately 2.5 cm of rain fell but did not cause a runoff "event." On September 4 the ovigerous crabs were placed at sites 3 and 6. On September 5-7 7.4 cm of rain fell resulting in significant runoff from fields into the main canals.

Salinity at site 3 fell from 10 ppt to 0.5 ppt on September 5 and remained below 3 ppt until crabs were brought into the laboratory on September 8. The crabs returned to the laboratory were placed in clean 10 ppt sea water for 4 days, at which time the eggs hatched (9/12). Ovigerous crabs and larvae were maintained at 25°C under a 12:12 LD cycle. Fifty larvae from each of 3 crabs from each site were then reared. Larvae were separated into groups of 10 for rearing, thus there were 15 groups. Larvae were reared for 96 h in 10 ppt to determine larval survival. Rearing procedures consisted of placing larvae in clean water (10 ppt) each day, identifying and discarding dead individuals, and then feeding live larvae newly hatched Artemia sp. nauplii. After 96 h, larval survival in each group of 10 was determined and a percent survival calculated. The results for all 15 groups in each condition were combined after arcsine transforming the data and the mean standard deviation and standard error calculated. These data were statistically compared to a control using a Dunnett test for multiple comparisons with a mean. Control larvae came from crabs that were not exposed to farm water but were reared using the same procedure. The results were as follows:

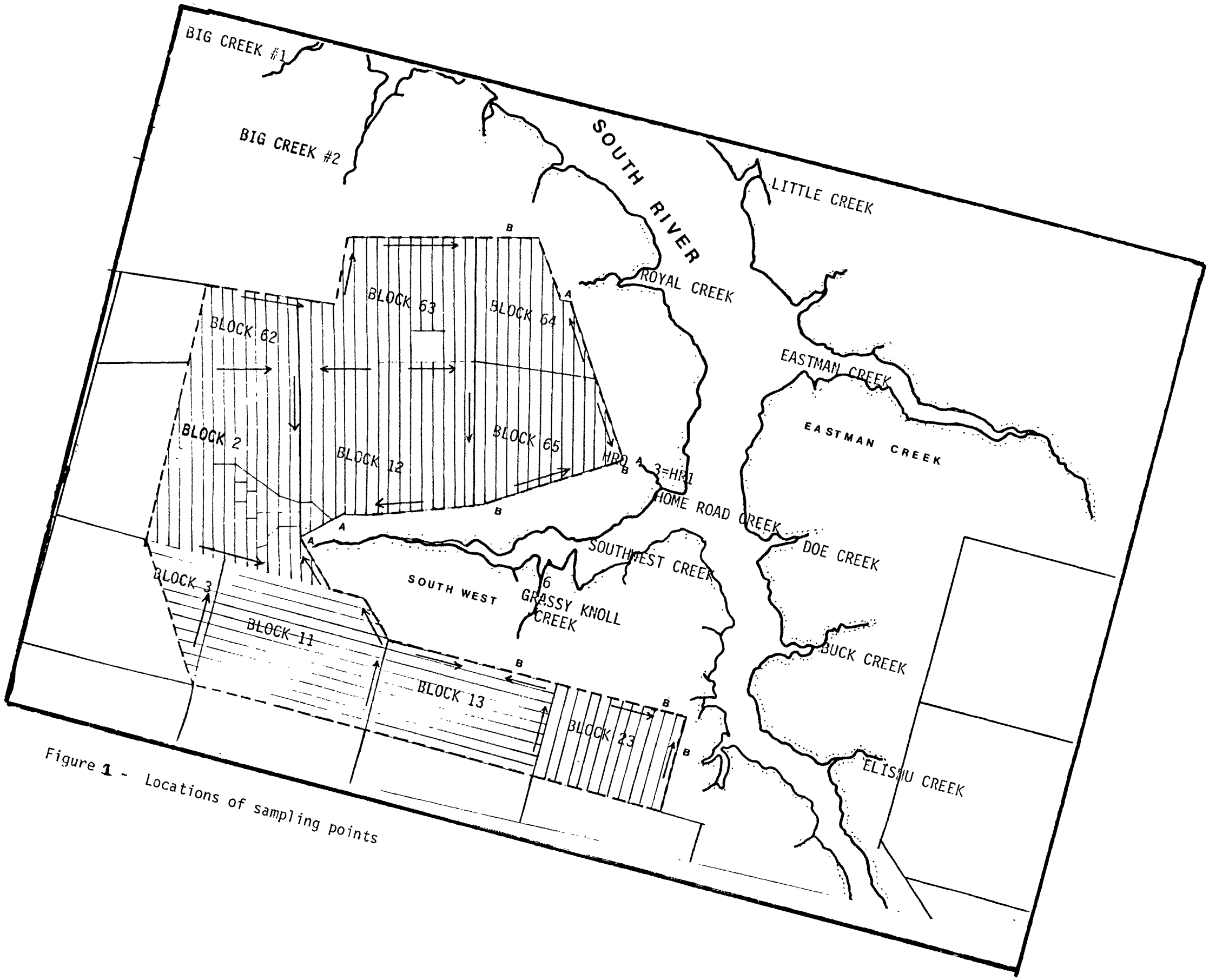


Figure 1 - Locations of sampling points

Condition	n	Transformed Data m	SD	Back Transformed Data m	M+SE	M-SE
Control	15	70.2%	15.7	88.6%	92.7	83.7
Site 3	15	72.7%	15.6	91.2%	94.7	86.8
Site 6	15	86.2%	10.9	99.5%	100	98.7

Analysis of results showed that larval survival after exposure at sites 3 and 6 was not significantly lower than that in the control conditions.

The phase two experiment consisted of exposing ovigerous crabs collected at an uncontaminated site (Pine Cliff on the Neuse River) for 4 days to water from fields sprayed with permethrin and to uncontaminated creek water and then rearing the larvae in these waters for 96 h. On 9/7/87 water (0 ppt) was collected from a ditch adjacent to the fields (Block 64; Figure 1) which had been sprayed with permethrin (8/22/87) and which had received 5.1-7.6 cm of rain on 9/6/87. Water (0 ppt) was also collected from a forest stream which drains an undeveloped watershed (Bridge Water). In both cases the salinity of the water was increased to 10 ppt by adding artificial sea salts. The procedures were as discussed above and the control consisted of maintaining ovigerous crabs and rearing larvae in clean 10 ppt. The results were as follows:

Condition	n	Transformed Data m	SD	Back Transformed Data m	+SE	-SE
Control	15	67.6%	14.1	85.5%	89.7	80.8
Bridge water	20	78.1%	12.9	95.7%	97.5	93.5
Block 64	20	74.0%	13.9	92.4%	95.0	89.3

Analysis of results showed that in no case did exposure to Bridge or Block 64 water significantly reduce survival compared with controls.

The results of the two phases of this experiment indicate that under the conditions of the experiment (14 days between spraying and runoff) there was no effect of runoff from permethrin-sprayed fields on mud crab development.

The Grass Shrimp, Palaeomonetes pugio (Field Bioassays)

1987 Experiments

In August 1987 individual grass shrimp (Palaeomonetes pugio) were held in cages in estuarine headwaters in a series of field bioassay experiments. Three experiments were done in creeks (sites 3 and 4; Figure 1) receiving

farm drainage from permethrin-sprayed soybean fields (Block 12; Figure 1) and three experiments were done in a creek receiving only forest drainage (Buck Creek; Figure 1). Thirty shrimp were held in experiments lasting two to three days. Permethrin (approximately 30 z/acre) was sprayed by air on August 13 (Block 12). Based upon dye studies, runoff from these fields could move to site 3 or site 4. Rainfall was 12 cm on August 10 and 8-10 cm on August 14. Experimental sites were visited daily between 10 a.m. and 1 p.m. and mortalities recorded. In addition temperature, salinity and oxygen measurements were made. The results were as follows.

	# Live	# Dead	% Mortality	T(°C)	Sal. (ppt)	D.O. (ppm)
Control						
Exp. 1 8/6-8/9	18	12	40	30.0	13.0 (12.1-13.7)	6.2 (2.9-11.8)
Exp. 2 8/10-8/13	11	16	59	28.0	8.3 (2.1-14.5)	4.8 (3.9-5.7)
Exp. 3 8/13-8/15	5	25	83	27.0	7.5 (2.1-15.2)	3.7 (3.2-3.9)
Runoff						
Exp. 1 (Site 3) 8/6-8/9	29	1	3	29.4	7.1 (3.2-13.7)	3.8 (3.4-4.7)
Exp. 2 (Site 3) 8/10-8/12	0	30	100	27.0	3.3 (1.0-5.6)	7.4 (3.2-11.5)
Exp. 3 (Site 4) 8/12-8/14	0	30	100	24.1	1.6 (0.2-4.2)	4.0 (3.1-5.1)

Mortality in pre-runoff experiments was moderate (40%) in the control site and low (3%) at the runoff site. Following a 13 cm rainfall on August 10 but before permethrin spraying, mortality was 59% at the control site and 100% at the experimental site receiving runoff. Following permethrin application (August 13) and another rain (9 cm on August 14) mortality rose to 83% in the control receiving forest runoff and was again 100% at the experimental site. These results suggested that significant mortality was due to natural causes (low salinity and, perhaps, early morning low oxygen) which would have masked any effects due to pesticide inputs even if they had been present. Based upon these preliminary bioassays, plans for 1988 included holding shrimp further downstream in the estuary to keep salinities higher and placing cages so that there was always an air/water interface to allow access to oxygen by caged animals.

1988 Experiments

In August and September 1988 gravid Palaeomonetes pugio were collected and placed in plastic boxes with 1 mm nylon mesh. Boxes were placed in wire cages with flotation attached to the sides to keep the boxes at the surface of the water to provide an air/water interface for each animal. Twenty-five animals were held separately at each site for each experimental period. Buck Creek and Doe Creek sites (Figure 1) received forest drainage while Grassy Knoll Creek (Figure 1) received drainage from soybean fields in farm Blocks 13 and 23. The animals were checked every two days and each animal was recorded as being either alive or dead. Oxygen and salinity measurements were taken each time the animals were checked. Environmental measurements and pesticide applications records were as follows:

Environmental conditions and pesticide applications during field experiments during the summer 1988. Sal=salinity (ppt); Oxy=oxygen (ppm) for spraying T=thiocarb, P=permethrin, B=farm block # draining into Grassy Knoll Creek and F=field ditch #.

Date	Rain (cm)	Grassy Knoll Cr.		Doe Creek		Buck Creek		Pesticides
		Sal	Oxy	Sal	Oxy	Sal	Oxy	
8/23		16.0	7.1	16.5	3.6			T(B23; F1-4)
8/24	3.6	8.8	6.3	15.4	2.6			
8/25		8.2	3.5					
8/26		6.0	4.7	12.4	2.5			T(B13,F3-5; B23, F-7) P(B13,F6-8)
8/27		13.5						
8/28	0.5	12.0	5.6	18.0	3.5			
8/30		12.0	5.2	17.5	4.1			
8/31	3.0	10.0						
9/1		13.8	7.9			18.0	7.2	
9/2	0.8	9.0						
9/3		2.0	5.4			9.0	7.2	T(B13,F1-2; B23, F9-12)
9/4	2.8	15.5						
9/5		12.2	7.2			6.0	6.4	
9/7	0.5	13.4	7.9			18.4	5.2	
9/8								P(B13,F9)
9/9	0.3							
9/10	1.3							
9/12		10.0	10.8					

During the first experiment (8/23-8/28) both thiocarb and permethrin were applied to fields in the drainage area. Rainfall of 3.6 cm on 8/24, which occurred prior to permethrin spraying, caused salinity to decrease from 16 ppt to 6 ppt at the treated site (Grassy Knoll Creek) but only caused a slight reduction at the untreated site (Doe Creek). Permethrin was applied on 8/26 but since salinities increased through 8/28 it appears that little or no runoff reached the caged animals. There were no deaths at either the treated or untreated sites.

The location of the untreated site was changed to Buck Creek in the second experiment because oxygen values were consistently low at Doe Creek during experiment 1. During the second experiment (9/2-9/7) additional thiocarb was applied to fields located in the treated drainage system on 9/3. Changes in water level in the South River estuary on 9/3 resulted in salinity decreasing from 13.8 on 9/1 to 2 ppt on 9/3 probably subjecting the animals to runoff water from the 8/24 and 8/31 events which would have transported permethrin into the estuary if it was present in the runoff (analysis of water samples for permethrin is underway). Salinity at the treated site increased again to 15.5 on 9/4 and then decreased again slightly following a 2.8 cm rain. Salinity at the untreated site (Buck Creek) decreased from 18 ppt to 6 ppt and then returned to 18 ppt during the experiment. There was no mortality grass shrimp noted at either site. Two of the twenty-five shrimp were missing at each site, presumably having escaped from the cages.

Effects of Alachlor on Marine Phytoplankton

Two species of marine algae (Prorocentrum micans and Skeletonema costatum) were the subject of preliminary experiments to examine the effects of alachlor on growth rates of populations of these phytoplankters. These projects were done by students for Independent Study course credit at the Duke University Marine Laboratory under the direction of W. Kirby-Smith and R. Forward. Abstracts of the results are presented below.

K. Condict. Alachlor acute toxicity tests using Prorocentrum micans

A dinoflagellate representative of phytoplankton of the South River estuary, Prorocentrum micans, was grown in cultures exposed to the following concentrations of alachlor: control, 0.1 ppm, 1.0 ppm, 10 ppm and 50 ppm. The mean growth rate in controls was 0.36 divisions/day. Significant (P=0.05) inhibition of growth occurred at alachlor concentrations of ≥ 1.0 ppm. Probit analysis yielded a 120-h EC_{50} value of 1.9 ppm. Chlorophyll a per cell was greater than controls at 0.1 ppm alachlor, not significantly different at 1 ppm and 10 ppm and significantly decreased at 50 ppm.

A.S. Horne. The effects of the herbicide alachlor on growth of the diatom Skeletonema costatum.

Skeletonema costatum was grown in cultures exposed to the following concentrations of alachlor: control, 0.001 ppm, 0.01 ppm, 0.1 ppm and 1.0 ppm. Concentration of 1.0 ppm alachlor reduced growth rates by 73% compared to controls and 65% to acetone controls. Chlorophyll a per cell and chain length of populations exposed to alachlor were no different from controls.

Nektonic Communities

Fall 1987

Fish communities were sampled by standard N.C. Division of Marine Fisheries trawl (two minute/137 m) weekly from mid-September through October, 1987. Six small creeks or embayments (Figure 1) were sampled on each of 7 sampling dates. Four creeks received forest drainage (Big Creek #1, Eastman, Doe and Buck) while two received farm drainage (Big Creek # and Southwest). Fish collected were identified, counted, weighed and measured.

During the fall eleven fish species were collected (Table 1). Three species were dominant (99.6% by number): bay anchovy (Anchoa mitchilli), spot (Leiostomus xanthurus) and mullet (Mugil cephalus) while seven were dominant in biomass (96.4%): bay anchovy (A. mitchilli), spot (L. xanthurus), striped mullet (M. cephalus), pinfish (Lagodon rhomboides), croaker (Micropogonias undulatus), southern flounder (Paralichthys lethostigma), and Atlantic menhaden (Brevoortia tyrannus). Numbers and biomass (Table 2) of fish remained relatively high until late October when the predictable fall decrease took place.

Table 1. Total number and total biomass (in grams) of each fish species encountered. For each species, all sites at all sample dates were combined (6 sites with 7 trawls lasting 2 min).

Species	Total Number	Total Biomass (g)
Bay Anchovy	13,787	2645
Spot	100	2254
Striped Mullet	65	1103
Pinfish	15	663
Croaker	14	716
Southern Flounder	10	317
Yellowfin Menhaden	8	373
Hogchoker	7	100
Silver Perch	4	115
Naked Goby	4	0.8
Ladyfish	1	85

Table 2. Total number and total biomass (in grams) for each sample date. All species for all sites are combined (6 sites; 2-min trawl at each site).

Date	Total Number	Total Biomass (g)
9/17	3692	1292
9/24	2528	1278
10/2	2117	1929
10/8	1557	1349
10/15	2697	1105
10/22	614	750
10/29	816	658

A comparison of forest with farm creeks indicated: (1) No significant differences in total catch, either number or biomass (Table 3); (2) No significant differences in number on any one date (Table 4); and (3) No

Table 3. Total number, total biomass means and standard deviations of fish caught in the two creek types. t-test results for number and biomass between creek types. Neither t-test is significant.

FOREST RUNOFF n=4	FARM RUNOFF n=2	t-TEST
	TOTAL NUMBER	
X=2616 s=996.96	X=1775 s=1352.70	t=1.102 df=4, n.s.
	TOTAL BIOMASS (g)	
X=1227.7 s=401.03	X=1740.8 s=43.44	t=1.719 df=4, n.s.

Table 4. Total number (of all species) means and standard deviations between creek types for each sample date. T-tests show no significance for any sample date.

DATE	FOREST RUNOFF	FARM RUNOFF	t-TEST
9/17	X=776 s=996.96	X=295 s=249.61	t=0.637 df=4, n.s.
9/24	X=513 s=251.02	X=239 s=76.37	t=1.241 df=4, n.s.
10/2	X=201 s=159.88	X=658 s=696.50	t=0.916 df=2, n.s.
10/8	X=243 s=220.21	X=292 s=158.39	t=0.274 df=4, n.s.
10/15	X=460 s=424.28	X=428 s=438.41	t=0.086 df=4, n.s.
10/22	X=118 s=210.12	X=71 s=95.46	t=0.288 df=4, n.s.
10/29	X=45 s=40.63	X=318 s=424.97	t=0.906 df=2, n.s.

significant difference in biomass on any one date except October 15 when significantly greater biomass was caught in the farm runoff creeks (Table 5). Cluster analysis to assess community similarities indicated no difference in fish communities among the creeks. Hydrographic data collected on each sampling date indicated no predictable differences in water temperature, salinity, dissolved oxygen or secchi depth. Water temperature decreased from approximately 28° in early September to 16° in late October. Salinity varied from 12 ppt to 17 ppt and dissolved O_2 values ranged from 3 to 9 ppm. Secchi depths increased as fall progressed with a range of 15 cm to 40 cm on 9/17 to 110 cm to 160 cm on 10/29. Rainfall was relatively light during the sample period probably resulted in only slight runoff during the period.

Table 5. Total biomass (of all fish species) means and standard deviations between creek types for each sample date. * significant $p > 0.05$.

DATE	FOREST RUNOFF	FARM RUNOFF	t-TEST
9/17	X=232.1 s=283.08	X=182.0 s=176.42	t=0.222 df=4, n.s.
9/24	X=235.0 s=125.49	X=169.1 s=142.41	t=0.586 df=4, n.s.
10/2	X=244.2 s=97.44	X=476.1 s=196.44	t=2.068 df=4, n.s.
10/8	X=208.6 s=19.29	X=263.1 s=6.86	*t=3.689 df=4, s
10/15	X=134.1 s=47.83	X=284.4 s=177.61	t=1.771 df=4, n.s.
10/22	X=82.0 s=75.54	X=210.9 s=247.91	t=1.062 df=4, n.s.
10/29	X=86.7 s=101.97	X=155.3 s=53.15	t=0.859 df=4, n.s.

Summer 1988

Fish communities in the upper South River were sampled by trawl on 9 occasions July through August 1988. Three replicate, one-minute trawls were taken in six creeks; three which received farm runoff and three which received forest runoff. Fish and shrimp were identified, counted, and weighed (wet). Trawling was done using a standard N.C. Division of Marine Fisheries nursery stock assessment trawl. The two-seam otter trawl had a 3.2 m headrope with 6.4 mm bar mesh wings and body and 3.2 mm bar mesh tailbag. Each trawl was 1 minute in duration and covered a distance of

67.5±8.5 m distance. The trawls swept an average area of 216 m^2. Farm and forest drainage creeks (Figure 1) were paired by proximity to each other and physical attributes. Elishu (farm) paired with Buck (forest), Southwest (farm) with Eastman (forest), and Royal (farm) with Little (forest).

The total catch data for the summer of 1988 (Table 6) show a community typical for upper estuarine creeks of the Pamlico Sound region. Most abundant by number were Bay Anchovy (Menidia menidia) which are a primary forage species food for larger predatory fish. The nursery function of these systems is demonstrated by the catch of juvenile spot, croaker, flounder, and shrimp. Spot were second in numbers to Bay Anchovy but were dominated by weight. Pinfish and Silverperch, also forage species, made up the remainder of the more abundant fish species. Penaeid shrimp (Penaeus spp. - mostly P. aztecus) were also present in moderate abundance, ranking 4th in number and 5th in weight.

Total catch by date (Table 7) showed a general increase in both number and biomass as the summer progressed. Although rainfall was moderate throughout the period, salinities remained high (15-18 pp) in surface waters measured at the time each collection was made. The 7/25 trawls were taken to see if a 6.4 cm rainfall (7/22-7/23) influenced catches. There was a drop in number and biomass however salinities did not decrease significantly. Rapid decreases in salinity or oxygen are known to cause temporary emigration of nekton from small estuarine creeks.

The total catches from each of the creeks is shown in Table 8. There were no patterns in the data based upon land use. One forest creek (Eastman) had lowest total number and biomass while another forest creek (Little) had highest numbers and biomass. In comparing paired creeks there were two cases (Elishu/Buck and Royal/ Little) in which farm creeks had somewhat lower numbers and biomass than forest creeks but the third pair (Southwest/Eastman) had the opposite pattern. These data illustrate one of the possible pitfalls in field research in which only one replicate "experimental" and "control" system is compared (pseudoreplication) for any specific attribute.

There were no predictable patterns in number and biomass when the data are summarized by creek on each date (Table 9). The catch from all creeks except Eastman did decrease following rain (compare 7/21 to 7/25) but there were other similar variations in comparing other dates.

The data summarizing the distribution of total catch by species relative to location are presented in Table 10. One farm drainage creek (Elishu) stood out as having an extremely low abundance of Bay Anchovy compared to other creeks. However abundance of other species (Spot, Croaker, Pinfish, shrimp) in Elishu was no different from other creeks. Another farm creek (Royal) had the greatest number of Bay Anchovy, more than twice the number of all creeks except Elishu. In addition Royal had the greatest number of species of any of the other creeks.

Table 6. Total catch from three replicate trawls at six locations on nine dates from upper South River tributaries during the summer 1988.

Species	Number	Biomass (g)
1. Anchovy	23,288	5029
2. Spot	3,038	21829
3. Croaker	327	3158
4. Pinfish	177	2635
5. Flounder	32	357
6. Hogchoker	18	100
7. Silver Perch	33	80
8. Menhaden	14	98
9. American Eel	9	346
10. Naked Goby	6	0.5
11. Pipefish	1	1.0
12. Bluefish	3	19
13. Lizardfish	2	128
14. Silverside	33	56
15. Brown Shrimp	200	2054

Table 7. Total fish catch by date. Data are the sum of three replicate trawls at six locations on each date.

Date	Number	Biomass (g)
7/1/88	969	5189
7/7/88	725	43459
7/14/88	1808	3906
7/21/88	3003	4574
7/25/88	2291	2429
7/28/88	4150	3123
8/4/88	4557	2926
8/12/88	2996	3425
8/25/88	6682	5973

Table 8. Total fish catch by location. Data are sum of three replicate trawls at each site on nine dates.

Station	Number	Biomass (g)
Elishu	1251	6021
Southwest	1791	9948
Royal	1305	6046
Buck	1575	9289
Eastman	630	2511
Little	2169	12888

Table 9. Total catch from 3 replicates at each location on each sample date.

Date	Station	Number	Biomass (g)	Date	Station	Number	Biomass (g)
7/1/88	Elishu	139	669	7/28/88	Elishu	82	715
	Southwest	199	1105		Southwest	51	174
	Royal	145	672		Royal	1857	718
	Buck	175	1032		Buck	632	499
	Eastman	70	279		Eastman	1268	327
	Little	241	1432		Little	260	690
7/7/88	Elishu	66	403	8/4/88	Elishu	86	571
	Southwest	91	294		Southwest	1316	421
	Royal	166	1159		Royal	1121	492
	Buck	118	722		Buck	623	415
	Eastman	57	354		Eastman	912	404
	Little	227	1412		Little	499	623
7/14/88	Elishu	58	439	8/12/88	Elishu	305	995
	Southwest	353	330		Southwest	24	158
	Royal	1013	1200		Royal	1220	1091
	Buck	80	540		Buck	47	144
	Eastman	88	362		Eastman	33	287
	Little	216	1035		Little	1367	750
7/21/88	Elishu	123	675	8/25/88	Elishu	172	1343
	Southwest	435	489		Southwest	1860	611
	Royal	1389	993		Royal	3248	1897
	Buck	286	1012		Buck	100	780
	Eastman	457	426		Eastman	119	224
	Little	313	979		Little	1183	1117
7/25/88	Elishu	41	287				
	Southwest	239	314				
	Royal	478	296				
	Buck	138	493				
	Eastman	1020	396				
	Little	375	642				

Table 10. Total number of individuals of each species (Table 1) caught in three replicate trawls from nine dates from each creek. H'=diversity

Creek	H'	Species														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Elishu	3.71	375	573	65	15	6	5	0	0	6	0	0	0	0	3	24
Southwest	1.20	4133	384	32	6	0	0	0	2	0	0	0	1	0	0	10
Royal	1.20	9844	493	114	46	11	11	24	8	1	1	0	0	1	7	76
Buck	2.73	1560	522	44	31	6	1	6	1	2	0	0	0	0	0	26
Eastman	1.07	3719	250	32	1	1	1	0	2	0	1	1	2	1	0	13
Little	2.34	3657	816	40	78	8	0	3	1	0	4	0	0	0	23	51

Diversity (H') was greatest (3.71) in a farm drainage creek (Elishu) because of the greater evenness of the number of individuals due to the low numbers of anchovies. Buck and Little, forest drainage creeks, had the second and third greatest diversity (2.73 and 2.34 respectively). Although Royal, a farm creek, had the greatest number of species (13) its diversity was relatively low due to the high dominance by Bay Anchovy, lowest diversity (1.07) was seen in Eastman, a forest drainage creek.

Throughout the estuarine systems catches of Bay Anchovy increased through time as did the number of species (Table 11). Spot remained relatively constant in abundance while croaker, shrimp and flounder decreased in abundance over the summer. Diversity decreased dramatically from a high of 3.20 on 7/7 to a low of 0.77 on 8/4 as Bay Anchovy abundance increased together with a decline in abundance of some of the other species.

Nektonic community similarities were compared using 3 sets of data based on species abundance (numbers) (Figure 2). In the first set the three replicate trawls at any one time and place were added together and treated as one sample, giving a total of 54 communities (six locations and nine dates). There were only two major groups which were distinct in comparisons among 54 communities. Group 1 contained those communities which had relatively low numbers of Bay Anchovy while Group 2 had high numbers. In the second analysis, data from the 9 dates were added together for each station to see if there were patterns of community structure related to location. Adding these data together in this fashion reduced the variability in catches such that all locations except Elishu Creek cluster tightly together as a single community type. Elishu differed in having very low abundance of Bay Anchovy. Finally, in the third analysis, when collections were lumped together by date there appeared to be a temporal pattern in community structure with 7/1 and 7/7 forming one group (Group 2), and all others forming a second group (Group 1). The 7/14 community appeared to serve as a link between the two groups. Again the difference was primarily related to the number of Bay Anchovies, with the increase in abundance as time progressed causing the two distinct community types.

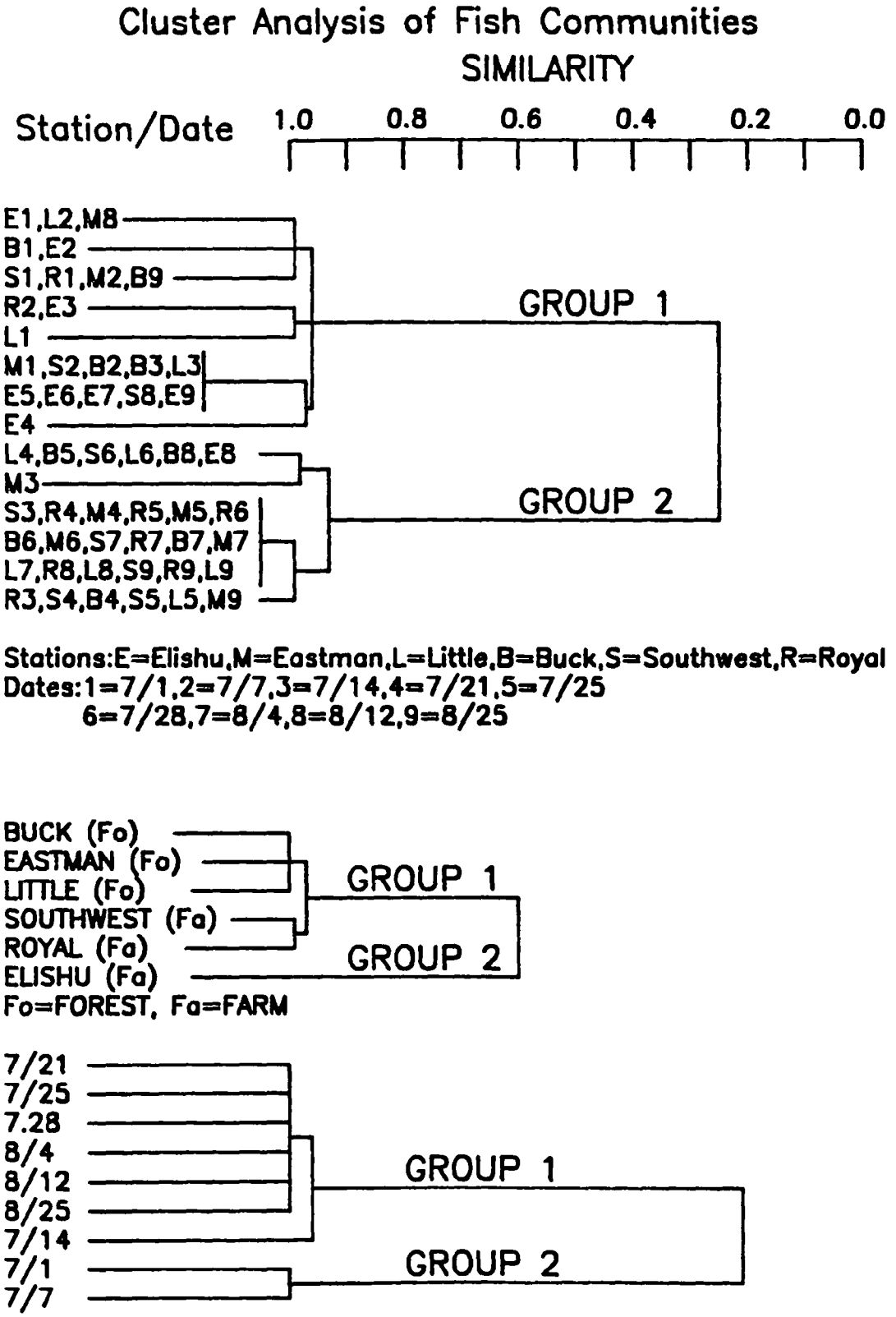


Figure 2. Community analysis of nekton collected in the summer 1988.

Table 11. Total number of individuals of each species (Table 1) caught in three replicate trawls from six locations on the date given. H'=diversity

Date	H'	Species														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
7/1	3.19	92	711	62	60	7	0	0	0	0	0	0	0	0	0	37
7/7	3.20	76	532	43	31	4	1	0	0	0	0	0	1	0	0	37
7/14	2.76	1310	385	43	16	4	4	1	2	0	3	0	0	1	0	39
7/21	1.97	2571	273	60	20	7	5	25	2	2	0	0	0	0	0	38
7/25	1.50	2041	182	36	14	3	1	1	2	1	1	1	0	0	0	8
7/28	1.00	3891	179	33	14	3	1	4	4	0	1	0	0	0	0	20
8/4	0.77	4328	191	13	6	1	1	2	0	0	0	0	0	0	0	15
8/12	1.18	2731	225	23	3	2	3	0	0	3	0	0	0	0	0	6
8/25	0.96	6248	360	14	13	1	2	0	4	3	1	0	2	1	33	0

In summary there were no patterns in fish communities which could be attributed to location with reference to farm versus forest drainage. Communities were more or less identical to those found at similar locations throughout the Pamlico Sound System. No large fluctuations in salinity were observed during the period in spite of the normal to moderate rainfall. It is well known that if there is displacement of the salt water/freshwater interface in shallow estuaries marine fish migrate to higher salinities but then return with the return of saltier water. Based on these data it is apparent that the farm activities have not caused a permanent change in the nekton community in creeks receiving runoff as compared to creeks in forested watershed. During the summer of 1989 nekton sampling will shift from bottom to surface trawls in the same set of creeks to assess surface nekton community which may be more quickly displaced as salinity of surface water decreases during rainfall events.

Benthic Communities

Benthic communities were sampled 5 times during the summer 1988 (July-September) at approximately 2-week intervals. Six replicate core samples were taken at each of six tributary creeks of the South River (3 farm runoff, 3 forest runoff) on each sampling date. These creeks were the same as sampled for nekton. The top 10 cm on each of each core was preserved in the field with 10% formalin and stained with Rose Bengal. In the laboratory samples were sieved (0.5 mm) and all organisms sorted from debris. Bivalves were not sorted from the first two sets of samples but were sorted thereafter. Species were identified and counted.

In analysis, the six replicates were summed for each creek and each date. Cluster analysis to determine community similarity was done using pooled data (all dates) for each station. Analysis has been completed for the first three of the five sampling dates.

Samples (Table 12) were dominated by the polychaetes Mediomastus californiensis, Streblospio benedicti, the oligochaete Peloscolex sp., the bivalve Macoma tenta and the mysid shrimp Mysidopsis bigelowi. Forest and

Table 12. Total species abundance of benthic animals collected from six replicate cores taken on three separate dates (18 samples) for each forest and farm creek (BU=Buck, EM=Eastman, LT=Little, EL=Elishu, SW=Southwest, RY=Royal).

	Forest			Farm		
	1 BU	2 EM	3 LT	4 EL	5 SW	6 RY
Mediomaster	59	47	200	81	26	250
Peloscolox	36	26	49	60	12	123
Stroblospio	38	50	62	22	29	41
Macoma tenta	11	10	5	12	12	19
Mysidopsis	17	6	13	16	18	10
Heteormastus	4	5	12	10	-	25
Nereis	16	9	8	16	1	5
Insect L	2	14	9	1	3	21
Melinna	4	1	7	1	-	10
Eulalia	2	-	4	3	5	5
Gyathura	1	2	6	5	-	1
Scyphiatoma	-	-	2	4	6	-
Novertina	-	-	4	2	-	3
Macomba B	1	3	1	1	1	1
Gammarus	-	-	6	1	-	-
Lethoscolopis	-	-	1	-	-	5
Edotea	1	1	1	-	1	-
Rangia	-	3	-	-	-	-
Syllidae	-	-	-	-	-	2
Spiosetosa	-	-	1	-	-	-
Corophium	-	-	1	-	-	-
# Species	13	13	19	15	11	15
# Individuals	192	177	392	235	115	521
H'	2.77	2.84	2.50	2.78	2.83	2.38

farm creeks differed little in the communities of benthic invertebrates. The greatest number of species (19) were found at Little Creek (forest runoff) with two farm runoff creeks (Royal and Elishu) second having 15 species each. Fewest species (11) were found in Southwest Creek which received farm runoff. The greatest density of animals (521) was found in Royal and least (115) at Southwest, both farm runoff creeks). Diversities were moderate and similar in all creeks ranging from a low of 2.38 at Royal Creek to a high of 2.84 in Eastman Creek. In cluster analysis all the creeks came together at a 0.91 similarity indicating no significant differences among them in community structure.

In summary there were no differences in benthic communities which could be correlated with differences in type of drainage, forest or farm. Furthermore the species present and the moderate diversity of these creeks make them very similar to other small creeks found in areas of low to moderate salinities throughout the Pamlico Sound system.

Experiment 1

Estuaries Vol. 11, No. 2, p. 79–82 June 1988

Effects of the Herbicide Alachlor on Larval Development of the Mud Crab, *Rhithropanopeus harrisii* (Gould)

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ABSTRACT: The effects of the herbicide alachlor, in both technical grade and commercial product form (Lasso), were tested for acute toxicity on larvae of the estuarine crab *Rhithropanopeus harrisii*. The generalized effect is a reduction in survival and a lengthening of developmental time with an increase in concentration. The LC_{50} values were inversely proportional to exposure time and ranged from 10 to 27 mg l^{-1}. Lasso was slightly more toxic than technical grade alachlor.

Introduction

Alachlor [2-chloro-2′-6′-diethyl-N-(methoxymethyl)acetanilide] is the active ingredient (45.1%) in Lasso, a widely used herbicide produced by the Monsanto Agricultural Products Company. The herbicide is used primarily with corn and soybean crops as a preemergent inhibitor of annual grasses, broadleaf weeds, and yellow nutsedge (Monsanto 1984).

Due to its high rate of application (1.68–4.48 kg ha^{-1}), high solubility in water (242 mg l^{-1}) and high stability, alachlor is persistent in soil and aquatic environments (Weed Science Society of America 1979). Detectable levels of alachlor can persist in soils for up to one year and in farm drainage water for up to four weeks (Skaggs et al. 1980). Because of these characteristics, alachlor can readily leach through soils during heavy rainfall. Although most concentrations of alachlor range between 0.078 and 0.184 mg l^{-1} in drainage streams (Wauchope 1978), Skaggs et al. (1980) found levels in farm ditch water as high as 2.7 mg l^{-1} immediately following a runoff event.

Much of the coastal plain wetlands that have been converted to agriculture border directly on ecologically sensitive and economically important estuarine water systems. These systems are susceptible to direct runoff from wetlands agriculture. In recent years, the fates and effects of herbicides and pesticides once they enter the aquatic ecosystem has been of great concern, but little lethal or sublethal toxicity data exist with direct estuarine applicability.

Larvae from the crab *Rhithropanopeus harrisii* were chosen for study because (1) this species is an abundant animal in low salinity headwaters of estuaries, (2) the technique for larval rearing is well known (Costlow et al. 1966), (3) the larval stages are typically the most sensitive to environmental variables (Thorson 1964), and (4) *R. harrisii* larvae have been shown to be sensitive to various pollutants (e.g., Christiansen and Costlow 1975; Forward and Costlow 1978; Bookhout et al. 1980).

In this paper toxicity tests and analysis for posthatch exposure are described for both Lasso alachlor and technical grade alachlor.

Methods and Materials

Preparation of Toxicant and Dilutions

Lasso, an emulsifiable concentrate (EC) of alachlor, was obtained commercially, while the technical grade alachlor was supplied in crystalline form by the Monsanto Agricultural Products Co., St. Louis, Missouri. For experiments on the effects of alachlor, a stock solution was made from each form of alachlor. Ten ml of Lasso EC alachlor (480 g l^{-1}) was pipetted into a glass 1 l bottle and allowed to evaporate. Certified A.C.S. acetone was then added to give a final volume of 480 ml stock solution, which produced a nominal concentration of 10 mg ml^{-1} (10,000 mg l^{-1}). Stock solution of the technical grade alachlor crystal was created by dissolving 3 g of the crystal in 300 ml of acetone, for a stock solution of 10 mg ml^{-1}. Stock solutions were stored in the dark at 5 °C and were replaced after each 30-day period.

Standard dilution techniques were used for both solutions. Acetone serial dilutions of 2,500, 1,250, 500, 50, and 5 mg l^{-1} were made daily. To each 7-cm diameter glass finger bowl, 1 ml of dilution

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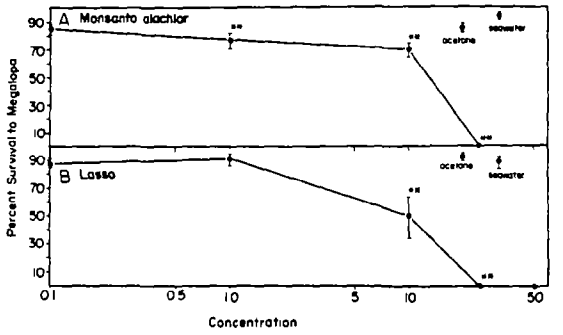

Fig. 1. The percent of larvae developing to the megalopa stage plotted against concentration (mg l^{-1}) of Monsanto alachlor (A) and Lasso (B). Means and standard errors are shown. The n for each determination was 15. Acetone and seawater indicate the percent survival in the acetone and seawater control solutions. The asterisk indicates mean percent development is significantly ($p < 0.05$) different from the seawater control.

was pipetted and allowed to evaporate in a hood. The remaining alachlor film was then resuspended in 50 ml of filtered (5 μm) seawater (salinity 20‰) to give final alachlor concentrations of 50, 25, 10, 1, and 0.1 mg l^{-1}. Acetone controls and seawater controls were also tested. Acetone controls were set up by pipetting 1 ml of acetone into each test bowl and allowing it to evaporate. This was designed to reveal any possible effects of an acetone residue following evaporation on the test bowls. The additional control was to expose larvae to filtered (5 μm) seawater (20‰).

All glassware to be used in herbicide toxicity tests was first washed in a 10% HCl bath, and then washed in a concentrated (37 N) H_2SO_4 bath, followed by a deionized water rinse and a final acetone rinse. Subsequent daily washes consisted of scrubbing bowls with a clean brush in deionized water, followed by an acetone rinse. Separate bowls were used at each concentration throughout the experiment to reduce the risk of cross contamination among concentrations.

Rearing of Larvae

Ovigerous females of *Rhithropanopeus harrisii* (Gould) were collected from the Neuse River in North Carolina. Females were held individually in an environmental chamber in large (19 cm diameter) finger bowls containing 20‰ filtered (5 μm) seawater. Chamber temperature remained at 25 °C (±1 °C), and a 12:12 LD cycle was maintained. Hatching normally occurred 2–3 h after the dark phase began (Forward et al. 1982).

Upon hatching, the adult female was removed from the bowl, and larvae were fed newly hatched *Artemia* sp. *nauplii*. Larvae of *R. harrisii* were also reared under the same environmental conditions as above (20‰; 25 °C; and a 12:12 LD cycle). These parameters have been shown to be optimum conditions for laboratory rearing and development in *R. harrisii* larvae (Costlow et al. 1966).

Approximately 12 h after hatching, larvae from each of three hatches (minimum hatch size: 400 larvae) were placed in the test solution. Five replicates of 10 larvae from each hatch were tested in each solution. Thus the total number of replicates for each condition was 15. Dilution and control solutions were renewed daily, and larvae were fed newly hatched *Artemia* sp. *nauplii*. Dead larvae and molts were counted and removed at this time.

Toxicity Experiments

Three experimental series were conducted. First, the chronic tests consisted of continuously exposing larvae to the test solution throughout larval development. Effects were evaluated as percent survival at the megalopa stage and time duration to reach this stage. Both technical alachlor and Lasso were tested.

In the second experiment larvae were exposed to test solutions continuously for 96 h beginning just after hatching. Survival after this time was recorded. This experiment used both technical alachlor and Lasso and was designed to determine the 96-h mean lethal concentration (LC_{50}).

Finally, the third experiment involved short-term exposure and was designed to test the effect of a simulated short duration runoff event. Larvae were exposed to test solutions of Lasso for 12 h just after hatching during the light phase and then transferred to clean seawater. Mortality was measured after 96 h.

For data analysis, mean percent survival and the standard errors were calculated after arcsine transformation of the data. Since all experiments consisted of a control and many test concentrations, differences were tested using a Dunnett *t* test for multiple comparisons with a control (Dunnett 1964). The LC_{50} values were determined by probit analysis.

Results

Chronic Exposure to Monsanto Alachlor and Lasso

When exposed to 25 and 50 mg l^{-1} of Monsanto alachlor (Fig. 1A), no larvae completed development to the megalopa stage. At concentrations of 1 and 10 mg l^{-1}, survival was significantly lower than in seawater alone; whereas, at 0.1 mg l^{-1} and in the acetone control, survival was similar to that in seawater (Fig. 1A). The LC_{50} level was 14 mg l^{-1}. For larvae that survived, the duration of larval

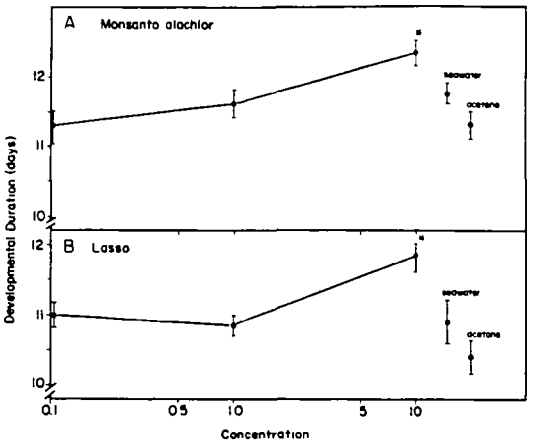

Fig. 2. The number of days for development from stage I zoea to megalopa plotted against concentration (mg l^{-1}) of Monsanto alachlor (A) and Lasso (B). Mean and standard error times are shown and the average n was 14 in A and 11 in B. Seawater and acetone indicate developmental times in these control solutions. Double asterisks indicate development time is significantly ($p < 0.01$) longer than in seawater.

development to the megalopa stage increased with Monsanto alachlor concentration and was significantly longer than that in seawater at 10 mg l^{-1} (Fig. 2A).

Similar results were obtained with Lasso. No larvae survived at 25 and 50 mg l^{-1}, and the lowest test concentration to significantly reduce survival was 10 mg l^{-1} (Fig. 1B). Survival in all other test conditions was not significantly different from levels in seawater. The LC_{50} was 10 mg l^{-1}. The length of larval development increased as survival decreased and was significantly longer at 10 mg l^{-1} (Fig. 2B).

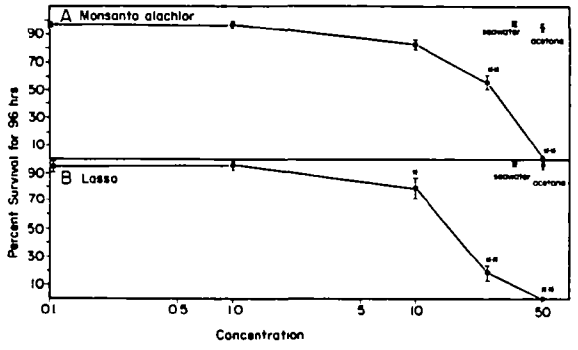

Fig. 3. The percent of larvae surviving for 96 h after exposure to various concentrations (mg l^{-1}) of Monsanto alachlor (A) and Lasso (B). Means and standard errors are plotted. The n for each determination was 15. Seawater and acetone indicate the percent survival in these control solutions. The single and double asterisks indicate survival was significantly lower than levels in the seawater control at the $p < 0.05$ and 0.01 levels, respectively.

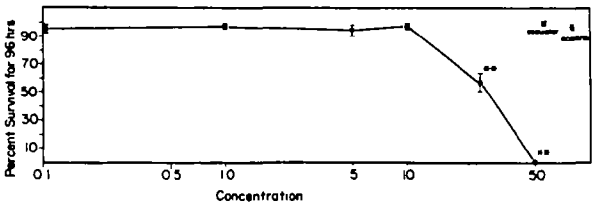

Fig. 4 The percentage of larvae surviving after 12-h exposure to various concentrations (mg l^{-1}) of Lasso. Means and standard errors are plotted and the n for each concentration was 15. Seawater and acetone indicate percent survival in these control solutions. Double asterisks show those concentrations at which the percent survival is significantly lower than that in the seawater control at the $p < 0.01$ level.

Acute Exposure for 96 h to Monsanto Alachlor and Lasso

Exposing larvae to the herbicide for the first 96 h of development also caused a significant reduction in survival. In Monsanto alachlor significant effects were seen at 25 and 50 mg l^{-1} (Fig. 3A). The LC_{50} was 26 mg l^{-1}. Larvae were more sensitive to Lasso, the lowest concentration to significantly lower survivorship was 10 mg l^{-1} (Fig. 3B). The Lasso LC_{50} occurred at 16 mg l^{-1}, a lower concentration than alachlor.

Acute Exposure for 12 h to Lasso

As larvae were more sensitive to short-term exposure to Lasso (Fig. 3B), larvae were exposed to various concentrations for 12 h and then placed in clean seawater. Mortality was determined after a total time of 96 h. Larval survival was significantly reduced at concentrations of 25 mg l^{-1} and greater (Fig. 4). The LC_{50} was 27 mg l^{-1}.

Discussion

The generalized effect of alachlor on *Rhithropanopeus harrisii* larvae is a reduction in survival and a lengthening of developmental time with an increase in concentration. Sensitivity was inversely proportional to exposure time; the estimated LC_{50} concentrations decreased as exposure time increased (Table 1).

The usefulness of larval crustaceans as test organisms for pollutants entering estuarine systems

TABLE 1. The estimated LC_{50} values upon exposure to Monsanto alachlor and Lasso for various time periods.

Exposure Time	LC_{50} Monsanto alachlor mg l^{-1}	LC_{50} Lasso mg l^{-1}
Continuous (Fig. 1)	14	10
96 h (Fig. 3)	26	16
12 h (Fig. 4)	—	27

has been discussed in detail by Epifanio (1979). The planktonic larval stages of crustaceans are considered the most sensitive to environmental perturbations in the life cycle (Thorson 1964). That larval crustaceans usually are much more sensitive to pesticides than adults has been dramatically illustrated by Wilson (1985), who found that grass shrimp (*Palaemonetes pugio*) had a 96-h LC_{50} for diflubenzuron (an insect growth regulator) of 1.4 $\mu g\ l^{-1}$ as larvae, 1.6 $\mu g\ l^{-1}$ as postlarvae, 202 $\mu g\ l^{-1}$ as males and nonovigerous females, and 6,985 as ovigerous females.

The LC_{50} concentrations for *R. harrisii* larvae range from 10 to 27 mg l^{-1} alachlor (Table 1). These values are similar to those for an adult nonmarine crustacean (crayfish), where the LC_{50} for alachlor was 19.5 mg l^{-1} (Weed Science Society of America 1979). The LC_{50} concentrations, however, vary with the type of alachlor tested. Lasso EC alachlor, with its inert detergent carriers, is slightly more toxic (lower LC_{50} values) to *R. harrisii* larvae than technical grade Monsanto alachlor (Table 1). This result is in partial agreement with past studies. Acute dermal LD_{50} studies on rabbits show Lasso to be more toxic than technical grade alachlor (Weed Science Society of America 1979). However, freshwater fish (bluegill, sunfish, trout) were more sensitive to technical grade alachlor than Lasso.

In field studies the alachlor concentration in water of ditches draining a coastal plains farm in North Carolina peaked at times of peak flow and usually ranged up to approximately 0.07 mg l^{-1} (Skaggs et al. 1980). These concentrations are well below those causing mortality in crab larvae. However, on several occasions Skaggs et al. (1980) reported concentrations up to 2.7 mg l^{-1}, values much closer to LC_{50} levels. Although there were no data available, they suggested that these concentrations may have resulted from wind drift spray falling directly into the ditches. Thus there is the possibility of rare-to-occasional short-term exposure to significant concentrations of alachlor by crab larvae in upper estuarine creeks which receive direct drainage from farms. The 12-h pulse experiments simulated a heavy runoff event where concentrations of the herbicide would be at high levels for a short period of time. *R. harrisii* adults inhabit low salinity areas in creeks, thus they are in a position to be exposed to undiluted runoff water. Since early stage larvae are sensitive to short-term exposure (12 h), reproductive success could be affected by alachlor.

Even though the present study indicates *R. harrisii* larvae are sensitive to alachlor, it is possible that even greater sensitivity exists during embryo development. Thus future experiments will consider what effect exposing the eggs to alachlor has on future larval survival. In addition, sublethal effects on larval behavior should be investigated as these might indicate ecological effects which could influence the crabs at concentrations well below that necessary to produce direct mortality.

Acknowledgments

This study was supported by the National Science Foundation's Research Experience for Undergraduates (to RLT) and funded in part by Environmental Protection Agency Contract #CR 813415-01. We thank Dr. D. Rittschof and A. Schmidt for designing the dilution techniques and laboratory assistance of C. Sommers. We also thank Open Grounds Farm, Inc. for supplying the Lasso and Monsanto Agricultural Products, Inc. for providing the technical grade alachlor.

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Received for consideration, December 7, 1987
Accepted for publication, March 15, 1988

Respiration and osmoregulation of the estuarine crab *Rhithropanopeus harrisii* (Gould): Effects of the herbicide alachlor

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Running title: Herbicide effects on crab respiration and osmoregulation

Abstract

1. The effects of a sudden decrease in salinity and exposure to sublethal concentrations of the herbicide Alachlor on osmoregulation and respiration of the crab Rhithropanopeus harrisii were studied.

2. Crabs were hyperosmotic regulators at salinities below 24 ppt and became hypoosmotic at higher salinities. Upon a salinity decrease from 20 to 1 ppt, crabs adjusted their haemolymph osmolality to a stable hyperosmotic level in 8 h. Alachlor concentrations to 50 ppm did not affect this adjustment.

3. A salinity decrease from 10 to 0 ppt elevated $\dot{V}O_2$ and the critical oxygen tension. This response was unaffected by Alachlor concentrations as high as 25 ppm.

Introduction

The crab Rhithropanopeus harrisii is perhaps the most abundant crab in the upper reaches of estuaries along the southeastern United States (Williams, 1984). Runoff from agricultural lands following rainfall can lead to a rapid decrease in salinity in estuarine areas and exposure to products used in agriculture. The present study was initiated because large numbers (unquantified) of dead R. harrisii were found following runoff from an agricultural area that was recently treated with the herbicide alachlor. Although many environmental factors could contribute to crab death, exposure to alachlor and a salinity decrease were chosen for study.

The herbicide alachlor (2-chloro-2'-6'diethyl-N-(methoxymethyl) acetanilide) is the active ingredient (45.1%) in Lasso^{R} produced by the Monsanto Agricultural Products Company (Monsanto, 1984). It is used as a preemergent inhibitor of annual grasses, broadleaf weeds and yellow nutsedge with corn and soybean crops.

Alachlor is very soluble in water (242 ppm) and is widely used in amounts of 1.68 - 4.48 kg/ha by the farming industry (Weed Sci. Soc. Amer., 1979). Previous research has found that alachlor can remain in the soils for up to 1 year (Skaggs et al., 1980). After heavy rainfall alachlor can easily be leached from soils into drainage systems, which transport it to estuaries. Concentrations of alachlor in drainage waters have been measured at 0.078 to 2.7 ppm (Wauchope, 1978; Skaggs et al., 1980).

A recent study of alachlor effects on larval development of an estuarine crab, Rhithropanopeus harrisii, found that there was a reduction in survival and a lengthening of developmental time with an increase in concentration (Takacs et al., 1988). Lasso^{R} was slightly more toxic than

technical grade alachlor, and the LC_{50} values ranged from 10 to 27 ppm depending upon the exposure time. Effects were evident after as little as 12 h exposure. Since these LC_{50} values exceed the upper levels measured in nature and adults usually have higher LC_{50} levels than larvae, adult R. harrisii will probably be exposed to sublethal levels of alachlor in nature. Osmoregulation and respiration represent two physiological processes that could be affected by sublethal stress, and if altered, could contribute to an animal's death.

Osmoregulation is well studied among crustaceans (recently reviewed by Gilles and Pequeux, 1983). Using inorganic ion concentration as a measure of the relationship between hemolymph and seawater osmolality, Smith (1967) suggested that R. harrisii was a hyperosmotic osmoregulator at salinities between 1 and 23 ppt but became hypoosmotic at higher salinities. The present study determined this relationship by directly measuring seawater and haemolymph osmolality and considered the effects of alachlor upon acclimation to a sudden reduction in salinity.

Respiration is also well studied in crustaceans (recently reviewed by Cameron and Mangum, 1983; Vernberg, 1983). In estuaries, the oxygen concentration fluctuates widely throughout the 24 h day, being highest during mid to late afternoon as a result of photosynthesis and being least at late night and early morning due to the combined respiration of aquatic plants and animals (e.g. Warner, 1977; Kenney et al., 1988). When the external oxygen tension is high many crustaceans exhibit independent weight specific oxygen consumption rates ($\dot{V}_{O2}$) and when external oxygen tension is low, dependent $\dot{V}_{O2}$ occurs. The inflection oxygen tension for the switch from independent to dependent respiration is the critical oxygen tension (P_c; Hill, 1976). Both $\dot{V}_{O2}$ and the P_c were used as an assay for the

effects of alachlor and salinity decrease upon respiration of R. harrisii. Results indicate that neither osmoregulation nor respiration were affected by sublethal concentrations of alachlor.

Materials and Methods

Rhithropanopeus harrisii (Gould) were collected without regard for sex or molt stage from the Neuse River, North Carolina from September to November. They were kept unfed in 10 ppt seawater in glass finger bowls (19 cm diameter) at a temperature of about 23°C. The water in the bowls was changed every two days, and all experiments were completed within 3 weeks of collection.

Herbicide preparation

Lasso^{R}, an emulsifiable concentrate (EC) of alachlor, was obtained commercially and used in the experiments because this form is slightly more toxic than pure alachlor to R. harrisii larvae (Takacs et al., 1988) and it is the form to which crabs are exposed in nature. A stock solution was made by pipetting 10 ml of Lasso^{R} EC alachlor (480 g/L) into a 1 L glass bottle and allowed to evaporate. Certified A.C.S. acetone was then added to give a final volume of 480 ml stock solution, which produced a nominal concentration of 10 mg/ml (10,000 ppm). The stock solution was stored in dark at 5°C. Unless otherwise specified in the remaining paper, the designation alachlor is derived from Lasso^{R}.

Standard dilution techniques were used to produce acetone dilutions of 2500 mg/L (ppm), 1250 ppm and 500 ppm. To each 7 cm diameter glass finger bowl, 1 ml of dilution was pipetted and allowed to evaporate to dryness in a hood. The remaining alachlor film was then resuspended in 50 ml of

filtered (5 µm) seawater at the test salinity to give final alachlor nominal concentrations of 10, 25 and 50 ppm. These concentrations were used because the LC_{50} values for R. harrisii larvae with identical techniques ranged from 10 to 27 ppm (Takacs et al., 1988), these values are sublethal for the adults and these values are the same order of magnitude as the highest levels reported in nature (Skaggs et al., 1980). Test crabs remained alive upon exposure to test concentrations of alachlor. An acetone control was not run because larval development was not affected by acetone (Takacs et al., 1988).

Osmoregulation experiments

An initial experiment determined the relationship between haemolymph osmolality and external salinity. Groups of five crabs were acclimated for one week to separate salinities ranging from 1 to 30 ppt at 4-5 ppt intervals. The different salinities were made up by mixing dechlorinated tap water with seawater and were measured with a refractometer (A.O.). Approximately 10 µl of haemolymph was withdrawn from the base of the crab's walking legs using a 1 cc syringe with a No. 27 needle. Haemolymph osmolality was measured immediately with a vapor pressure osmometer (Wescor, Inc., Model 5100B). The osmolalities of the acclimation waters were measured with the same osmometer.

To determine the rate of acclimation of haemolymph osmolality upon a sudden salinity change, crabs were acclimated for 3 days to 20 ppt and then placed in 1 ppt. These salinities were used because the crabs are approximately isosmotic at 20 ppt (Fig. 1) and a decrease to 0-1 ppt is a realistic salinity level after a heavy rain in estuarine areas inhabited by the crabs. The exact procedure was to acclimate 21 crabs to 20 ppt. Blood

osmolality was initially determined for 3 crabs. The remaining crabs were then transferred into 1 ppt seawater and 3 crabs were sampled thereafter at 1, 4, 8, 12, 24 and 48 h.

The tests for the effects of alachlor upon osmoregulation consisted of acclimating 18 crabs to 20 ppt for 3 days. Two separate groups of 3 crabs were initially sampled to indicate haemolymph osmolality upon acclimation to 20 ppt. Two groups of 6 crabs were then placed individually in separate finger bowls containing 50 ml of 25 ppm or 50 ppm alachlor made up in 1 ppt seawater. Haemolymph of three crabs was sampled at 12 and 24 h. These times were selected because acclimation occurs within 8 h and alachlor effects on larvae are evident after 12 h exposure time (Takacs et al., 1988).

Respiration experiments

Since R. harrisii is a small crab (average adult weight about 0.4 g) and the water volume of the respirometer flasks was relatively large, it was necessary to measure oxygen uptake for groups of crabs. For each trial, seven crabs of approximately equal size were placed in a clean bottle filled with air saturated seawater (approximate volume = 350 ml). A fiberglass screen was inserted into the bottle for the animals to hold onto during the trial. The bottle was then sealed with a self-stirring oxygen probe. This apparatus was placed in a water bath maintaining a temperature of 24.5°C. Crabs were allowed to settle for 15 min, after which measurements of the oxygen concentration in the water were made every 15 min using an oxygen meter (Model 54, Yellow Springs Instruments Co., Inc.) attached to the bottle probe. The trial was terminated when the meter reading remained unchanged for 1 h. The oxygen concentrations at

each measurement interval throughout the test period were then converted to weight specific oxygen consumption rate ($\dot{V}_{O2}$) and the average oxygen tension for each 15 min interval.

To study the effects of salinity on respiration, 4 trials were initially run for separate groups of crabs acclimated for 1 week to 10 ppt. The study of the effect of a salinity decrease upon respiration used the same procedure, except that the oxygen consumption of the crabs was determined 24 h after placement in 0 ppt water. This time was selected because crabs completed osmotic acclimation to a salinity decrease within 24 h (Fig. 2).

Two experiments examined the effects of prior exposure to alachlor and a sudden decrease in salinity upon respiration. First animals were acclimated to 10 ppt seawater for at least one week and then exposed to 10 or 25 ppm alachlor for 24 h. Crabs were then placed in herbicide-free 10 ppt seawater for 0.5 h, and respiration subsequently tested in 10 ppt seawater. Testing in herbicide-free water reduced contamination of the respiratory equipment. Second, crabs were acclimated to 10 ppt for at least one week and then placed in 0 ppt water containing 25 ppm alachlor for 24 h. Crabs were then placed in herbicide-free 0 ppt water for 0.5 h after which respiration was measured in another volume of herbicide-free 0 ppt water.

Each experiment was replicated two or four times and all data were used to plot $\dot{V}_{O2}$ at the various oxygen tensions because the crabs were all of approximately equal weight. A computer-derived 5th order polynomial best fit curve was drawn to approximate the respiratory rate as the oxygen tension decreased. Trials with different equations indicated this polynomial provided the best fit to the data. For each set of trials, the

average $\dot{V}_{O2}$ for the independent respiration was calculated between oxygen tensions of 50 and 100 mm Hg. This interval eliminated the initial elevated $\dot{V}_{O2}$ values caused by handling and disturbance of the animals when first placed in the bottle and also eliminated the possibility of including low $\dot{V}_{O2}$ values during dependent respiration, because the critical oxygen tension (P_c) was below 50 mm Hg for these crabs. This average $\dot{V}_{O2}$ line was also plotted on the graph. A vertical line was drawn from the point at which the best fit curve fell below the average $\dot{V}_{O2}$ line to determine the P_c value.

Results

Osmoregulation experiments

R. harrisii were acclimated to salinities ranging from 1 to 30 ppt. Haemolymph osmolality remained hyperosmotic until about 24 ppt (Fig. 1) and became hypoosmotic at higher salinities.

Crabs acclimated relatively rapidly upon a salinity decrease from 20 to 1 ppt (Fig. 2). Haemolymph osmolality decreased gradually for the first 8 h and then stabilized around 550 mOsm. Mean haemolymph osmolalities at 8 h and longer were not significantly different (Student *t* test) from the mean value upon acclimation for 1 week at 1 ppm (Fig. 1).

The test for the effect of alachlor on osmoregulation indicated that neither 25 nor 50 ppm affected this process (Fig. 3). When the salinity decreased from 20 to 1 ppt in the presence of alachlor, the haemolymph osmolality decreased and stabilized at about the same level as it did when alachlor was not present (Fig. 2). Since *R. harrisii* is a strong hyperosmotic regulator (Fig. 2), a decline in the level of hyperosmotic regulation was predicted if osmoregulation was affected.

Respiration experiments

The respiratory pattern was the same under all test conditions (Figs. 4-8). At high oxygen tensions there was a very high $\dot{V}_{O2}$, which probably resulted from an initial increase in activity upon being placed in the respirometer bottle. At progressively lower oxygen tension levels, the crabs displayed independent respiration followed by dependent respiration. At control conditions of 10 ppt and no alachlor, the average $\dot{V}_{O2}$ was 102.8 µl O_2/s/h, and the crabs switched to dependent respiration at 15.9 mm Hg (Fig. 4; Table 1). Prior exposure of alachlor in 10 ppt seawater caused an increase in the $\dot{V}_{O2}$ and P_c (Figs. 5,6; Table 1).

Upon a salinity decrease from 10 to 0 ppt both the $\dot{V}_{O2}$ and P_c were elevated (Fig. 7) as compared to respiration in 10 ppt. However, when subjected to this salinity decrease in the presence of 25 ppm alachlor, similar $\dot{V}_{O2}$ and P_c values were observed (Fig. 8; Table 1). Thus alachlor did not have any additional effect on the respiratory change upon a decrease in salinity.

Discussion

A pattern of hyperosmotic regulation at low salinities and hypoosmotic regulation at high salinities is typical of estuarine grapsoid crabs (Barnes, 1967) and species living in high intertidal areas (Jones, 1941). However, most subtidal estuarine crustaceans are hyperosmotic at low salinity and become isosmotic at high salinities (e.g. Callinectes sapidus, Ballard and Abbott, 1969; Panopeus herbstii, Blasco and Forward, 1988).

In salinities ranging from 1 to about 24 ppt, R. harrisii is an osmoregulator, remaining hyperosmotic to the external environment. At salinities greater than about 24 ppt, the crabs displayed hypoosmotic

regulation. These results agree with the previous study by Smith (1967). His measurements of haemolymph Cl^- concentration suggested that R. harrisii was a hyperosmotic regulator at salinities from 1 to 23 ppt and perhaps a hypoosmotic regulator at higher salinities.

R. harrisii acclimated its haemolymph osmolality to a decrease in salinity within 8 h. This relatively rapid rate of adjustment is similar to that observed for a related species Panopeus herbstii (Blasco and Forward, 1988), which stabilized its haemolymph osmolality within 4 h upon an 8 ppt decrease in salinity. However most crustaceans require 12 to 24 h to stabilize their osmolality (C. sapidus, Tagatz, 1971; Engel and Nichols, 1977; Callianassa jamaicense, Felder, 1978). Neither 25 ppm nor 50 ppm alachlor affected the rate or level of adjustment by R. harrisii upon a dramatic salinity change from 20 ppt to 1 ppt.

Upon exposure to declining oxygen tensions, R. harrisii shows the pattern of independent respiration at high tensions which changes to dependent respiration at low tensions. This pattern is typical of many crustaceans (Mangum and Van Winkle, 1973), especially those living in an environment having variable oxygen tensions.

Under possible normal conditions in an estuary (10 ppt, 0 ppm alachlor), Rhithropanopeus harrisii has a P_c of 15.9 mm Hg (Table 1) and is in no danger of tissue damage caused by insufficient oxygen because normally the minimum measured oxygen tensions that occur in estuarine creeks in the summer at night are approximately 25 mm Hg (unpublished data, W. Kirby-Smith). The animals probably never switch to dependent respiration under normal salinities and thus their blood is always saturated with oxygen and no stress is caused to their tissues (Redmond, 1955). Under conditions of heavy rainfall in herbicide-free runoff creeks,

however, the salinity may decrease to about 0 ppt, which causes the P_c to increase to approximately 24 mm Hg and the average $\dot{V}_{O2}$ to increase by about 30%. This increase in respiration may represent the energy needed for increased hyperosmotic regulation. In this situation the respiratory volume and cardiac output may not saturate the blood to keep up with the elevated oxygen demands of the body, and the organism may experience anaerobic damage resulting in death (Arudpragasam and Naylor, 1964; Taylor and Butler, 1973; Taylor, 1976; Warner, 1977).

When exposed to both 10 ppm and 25 ppm alachlor in 10 ppt seawater, both the $\dot{V}_{O2}$ and P_c are elevated, which indicates the animals are experiencing sublethal stress. However, this situation is unrealistic because exposure to alachlor will be accompanied by a salinity decrease due to rain water runoff. Under conditions of 25 ppm alachlor and a salinity decrease, the average $\dot{V}_{O2}$ and P_c increase by about 30% and 50%, respectively. This increase in P_c and $\dot{V}_{O2}$ must be caused by the hypoosmotic stress because the $\dot{V}_{O2}$ and P_c values are very similar to those upon a salinity decrease alone (Table 1). If the reduction in salinity occurs at night when there is no photosynthetic activity, the dissolved oxygen concentration may decline below the new critical oxygen tension and dependent respiration may result. If animals are prevented from reaching the air/water interface, or if they are unable to switch to anaerobic respiration for a long enough duration, then the animals may be stressed past their limits. This situation may explain the observed deaths of R. harrisii, which prompted this study.

Runoff events expose estuarine animals to low salinity water, which can potentially contain high concentrations of herbicides. Respiration and osmoregulation of adult R. harrisii are affected by rapid decreases in

salinity but alachlor did not affect hyperosmotic regulation or elevate respiration beyond the level due to the salinity decrease alone. Test concentrations of alachlor were at or above the LC_{50} levels for larvae (Takacs et al., 1988) and the same order of magnitude of the highest levels found in drainage areas (Skaggs et al., 1980). Thus it is unlikely that alachlor caused the observed death of R. harrisii in the field after a runoff event which prompted this study. A more likely explanation is that death resulted from elevated respiration in response to the salinity reduction and reduced oxygen levels at night (Kenney et al., 1988).

Acknowledgements

This study was funded in part by Environmental Protection Agency Contract #CR 813415-01. We thank Monsanto Agricultural Products, Inc. for providing the technical grade alachlor.

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Table 1. The $\dot{V}_{O2}$ and P_c for respiration under different salinities and alachlor concentrations

Conditions		Number	$\dot{V}_{O2}$	P_c	Figure
Salinity	Alachlor	of trials	(μl O_2/s/h)	(mm Hg)	
10 ppt	0 ppm	4	102.8	15.9	4
10 ppt	10 ppm	4	109.1	28.0	5
10 ppt	25 ppm	2	109.3	29.0	6
0 ppt	0 ppm	4	134.1	24.1	7
0 ppt	25 ppm	2	128.3	23.8	8

Figure Captions

Figure 1. Haemolymph osmolality upon acclimation to salinities ranging from 1 to 30 ppt. Means and standard deviations are plotted. The n for each mean is 5. The isosmotic line is plotted for reference.

Figure 2. The time course of adjustments in haemolymph osmolality upon a change in salinity from 20 ppt to 1 ppt. Means and standard deviation are plotted. The n for each mean is 3. The horizontal dashed lines show the osmolality of the 20 and 1 ppt waters.

Figure 3. The time course of adjustments in haemolymph osmolality upon a salinity change from 20 to 1 ppt in the presence of 25 ppm (A) and 50 ppm (B) alachlor. Means and standard deviation are plotted. The n for each mean was 3. The horizontal dashed lines show the osmolality of the 20 ppt and 1 ppt waters.

Figure 4. The weight specific oxygen uptake rate ($\dot{V}_{O2}$) at different oxygen tension in 10 ppt seawater. Data from 4 trials are plotted. The average animal weight was 0.433 g. The horizontal dashed line represents the average $\dot{V}_{O2}$ for oxygen tensions between 50 and 100 mm Hg. The vertical dashed line is the position of the critical oxygen tension (P_c).

Figure 5. The $\dot{V}_{O2}$ at different oxygen tensions in 10 ppt seawater and 10 ppm (A) or 25 ppm (B) alachlor. The figure is plotted in Figure 4, except only 2 trials were plotted in B. The average animal weight in A was 0.363 g and B was 0.34 g.

Figure 6. The $\dot{V}_{O2}$ at different oxygen tensions after a salinity change from 10 ppt to 0 ppt alone (A) for 24 h. The same experiment was repeated in B except that crabs were also exposed to 25 ppm alachlor. The figure is plotted as in Figure 4 except only 2 trials were plotted in B. The average animal weight in A was 0.455 g and 0.435 in B.

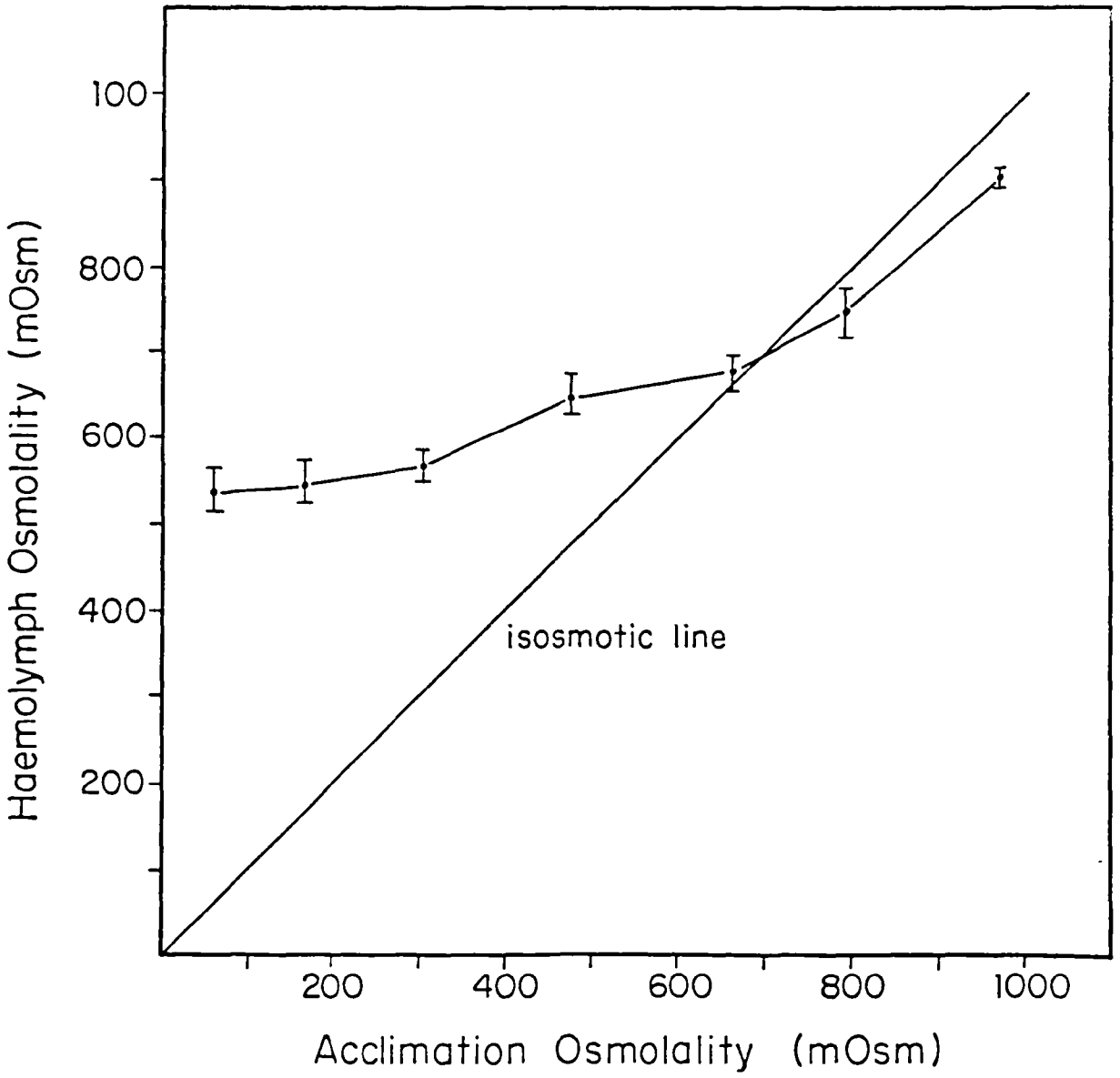
100
800
600
400
200
Haemolymph Osmolality (mOsm)
isosmotic line
200
400
600
800
1000
Acclimation Osmolality (mOsm)

Fig. 1

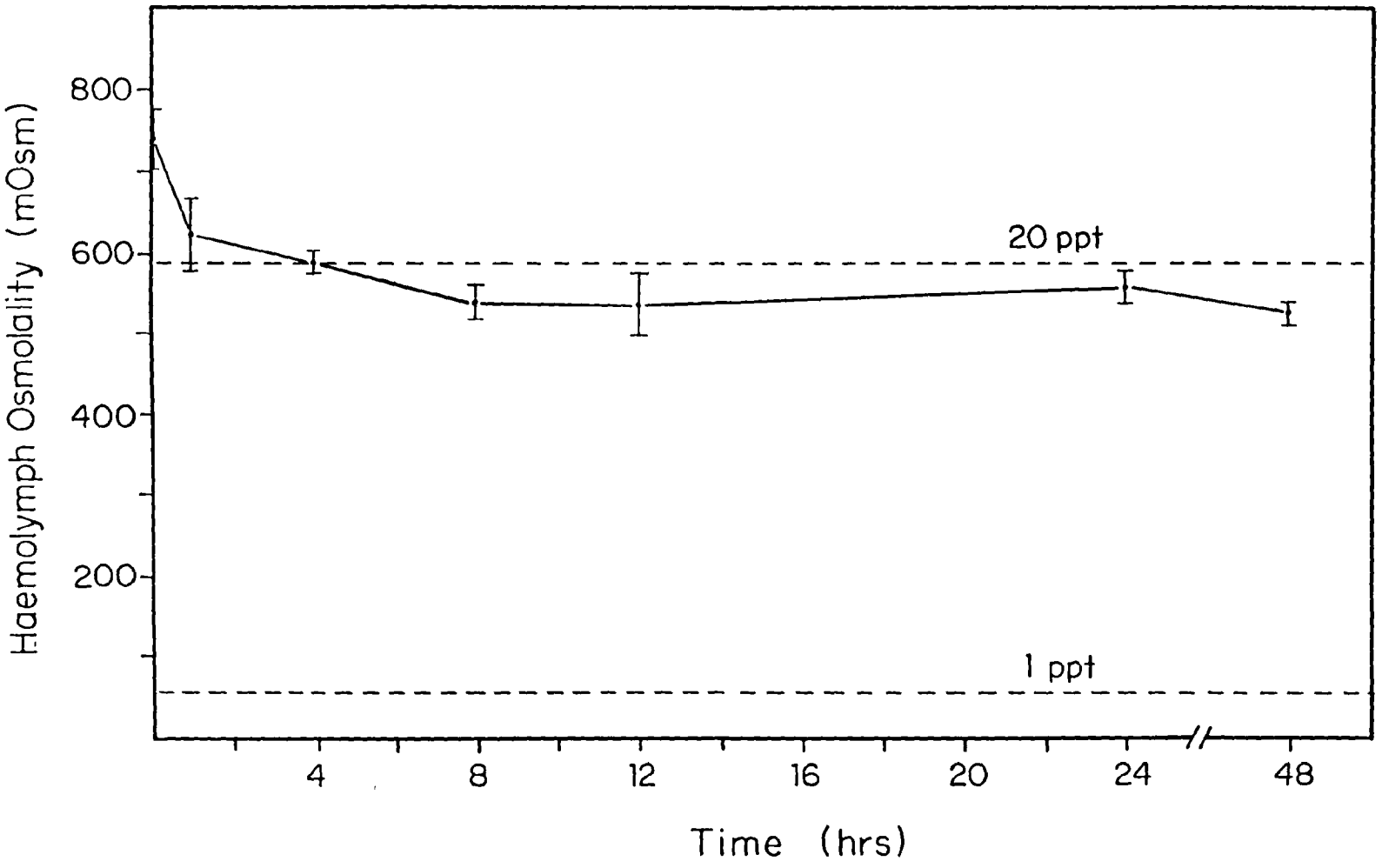
Haemolymph Osmolality (mOsm)
800
600
400
200
20 ppt
1 ppt
4
8
12
16
20
24
48
Time (hrs)

Fig. 2

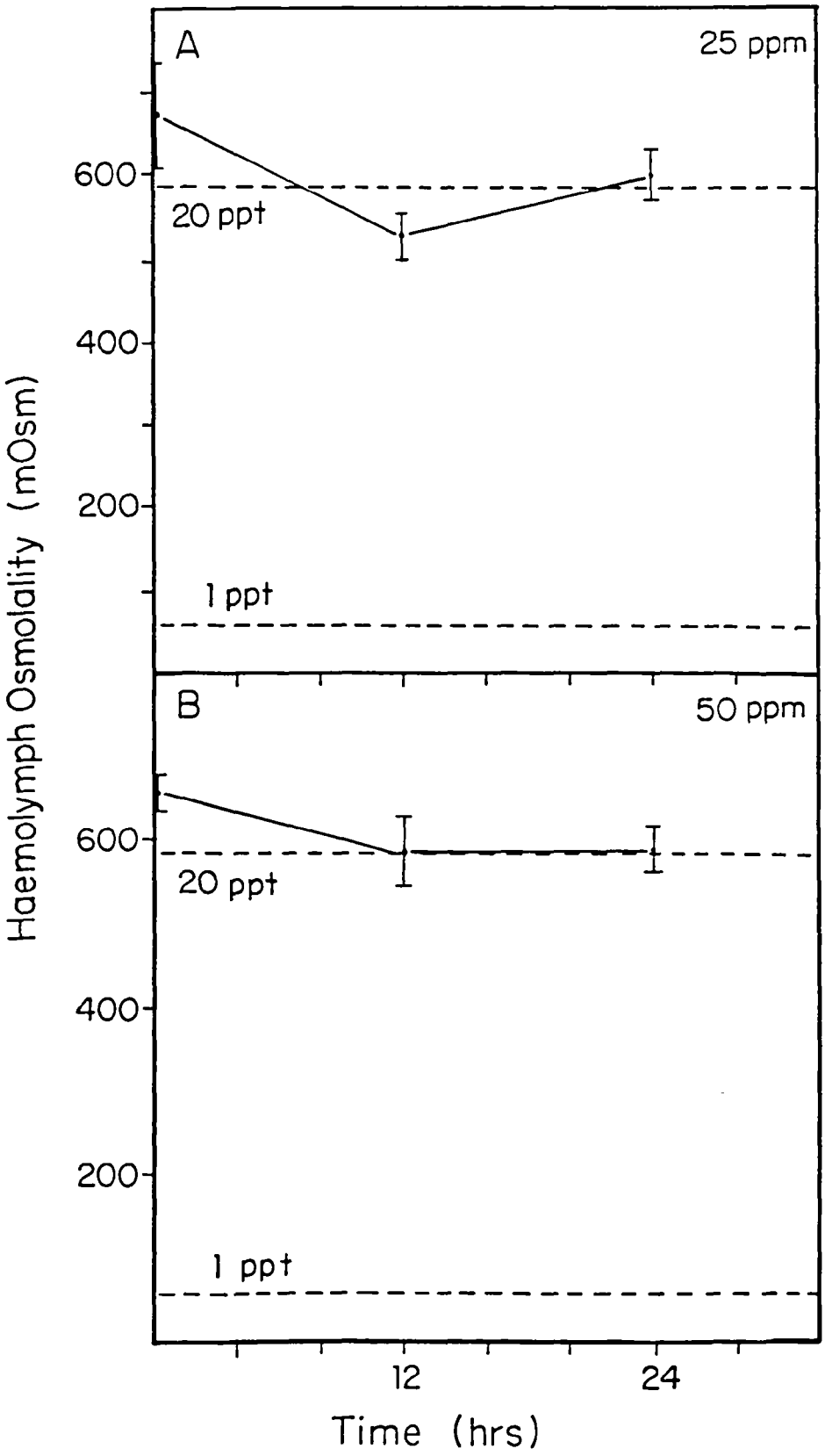
A
25 ppm
600
20 ppt
400
200
1 ppt
B
50 ppm
600
20 ppt
400
200
1 ppt
12
24
Time (hrs)
Haemolymph Osmolality (mOsm)

Fig.3

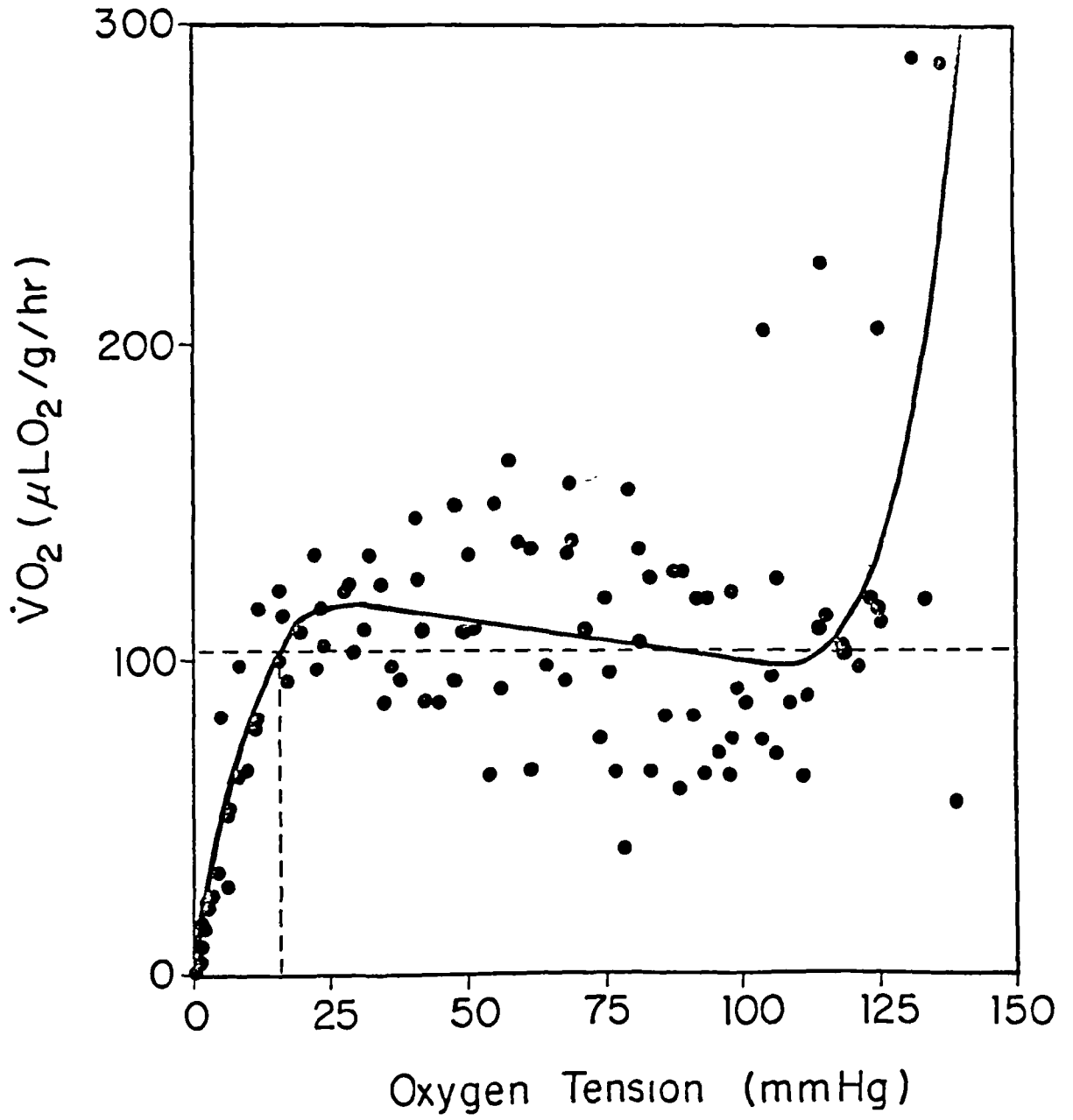
300
200
100
0
$\dot{V}O_2$ (μL O_2 /g/hr)
0
25
50
75
100
125
150
Oxygen Tension (mmHg)

Fig. 4

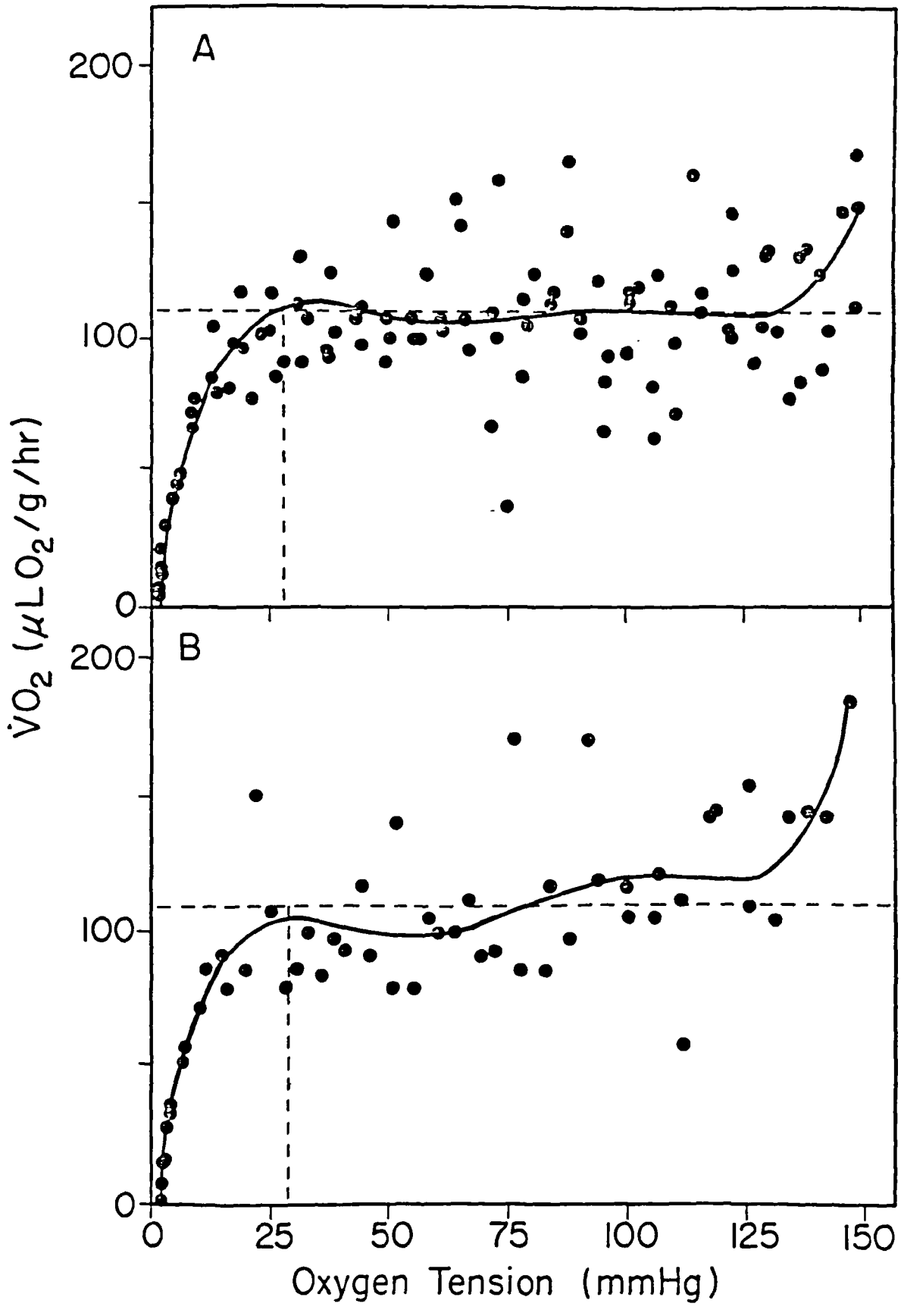
A
B
200
100
0
200
100
0
0
25
50
75
100
125
150
$\dot{V}O_2$ (μL O_2/g/hr)
Oxygen Tension (mmHg)

Fig. 5

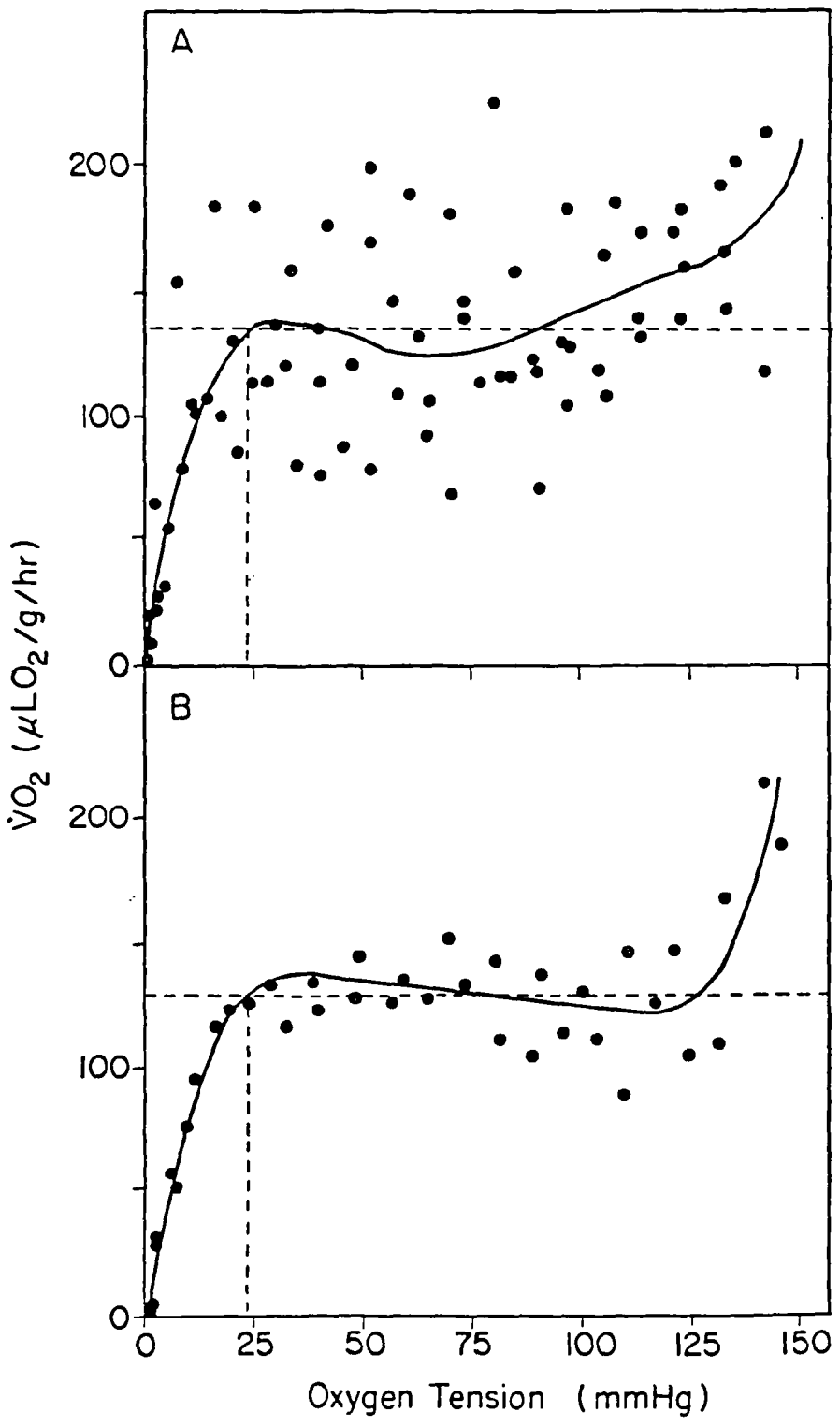

Fig. 6