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Ambient Toxicity and Chemical Characterization of Four Bayside creeks of the Eastern Shore

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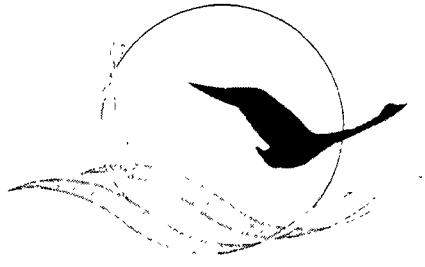

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Ambient Toxicity and Chemical Characterization of Four Bayside creeks of the Eastern Shore

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ABSTRACT

The purpose of this study was to characterize selected bayside creeks of the Virginia portion of the Delmarva Peninsula with respect to chemistry and toxicity. The peninsula is an area in which the primary land use is agriculture. When compared to urbanized and industrialized areas, one might expect fewer persistent impacts but with potentially severe intermittent impacts. To detect persistent impacts, the ambient toxicity methods applied in the Chesapeake Bay area during the past decade were used.

Severe intermittent impacts are generally undetected by these methods. Some agricultural practices and rain events can produce severe pulses of toxicity. To detect intermittent or pulsed impacts, *in situ* exposures with the grass shrimp, *Palaemonetes pugio*, were employed.

Four creeks were selected for examination. Three creeks (Hungar, The Gulf, and Old Plantation), all in Northampton County, were known to have plasticulture of tomatoes and other nightshade vegetables. The fourth creek (Onancock), in Accomac County, was selected as free of plasticulture, but including an urbanized community (Onancock).

The chemical characterization of water from was limited to measuring cadmium, cobalt, chromium, copper, mercury, nickel, lead, and zinc. At least two, and in two cases, five stations, were sampled in each creek. In no case were any metals present in concentrations exceeding water quality standards, acute or chronic. After observing water toxicity at all stations within two creeks, stored samples were analyzed for chlorinated compounds and tributyltin. All measurements were below the detection limits.

A more complete chemical characterization was made on sediment samples from two stations in each creek. Among the metals analyzed (cadmium, cobalt, chromium, copper, manganese, nickel, lead, and zinc), only nickel exceeded either of the benchmarks considered. Nickel showed exceedances of the Effects Range Low in two of eight samples (Similar concentrations were observed in three replicate samples from Carter's Creek, a reference site in the York River drainage.). Similarly, no semi-volatile organic analytes or chlorinated organic analytes were found to exceed sediment benchmarks, and most were non-detectable. Tributyltin, formerly used in antifoulant paints for boats, both recreation and work, was also sought in the sediment without a single instance of occurrence above the detection limit (1 ng/g). In contrast, TBT was detected in sediment from the Poropotank River, a tributary of the York River where concentrations of 1.2 and 1.6 ng/g were observed.

The *in situ* test was conducted for one month at one site in each creek, a site selected to be at risk from the primary land use for that creek. There was a single rain event that occurred in all four creeks, a rain of 9.5 to 50 mm over two days. There was a slight increase in mortality associated with this event in all creeks except Old Plantation. Other lesser rain events in three of the creeks which occurred later in the month-long deployment were not associated with mortality.

Water samples from Hungar Creek and The Gulf produced significant mortality in *Palaemonetes pugio* at all stations, but in a subsequent test with *Cyprinodon variegatus* larvae, no mortality was observed. Chemical analyses of the water in the first test did not reveal any material in concentrations to explain the mortality.

Sediment samples from five sites in each creek were all found non-toxic using a *Cyprinodon variegatus* embryo test or a *Leptocheirus plumulosus* amphipod test. This is consistent with the lack of elevated concentrations of any analyte in sediment from these sites.

Mulinia lateralis larvae were exposed to water from the creeks and to pore water extracted from sediment samples. The latter exposure has not to our knowledge been used previously. Only two stations had water that was toxic, site 4 in Onancock Creek and The Gulf. No potential causative agent was identified. Larvae exposed to pore water from seven sites, one or two in each creek, had reduced survival and increased percentage abnormal. Again, there is no evidence to implicate any particular causative agent.

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INTRODUCTION

Tidal creeks of bayside Eastern Shore are areas where toxic substances are potentially introduced only intermittently. A lack of data has not permitted a characterization of these areas with respect to toxicity potential, and the absence of heavy industry makes this data limitation understandable. However, there are potentially significant sources of contaminants in these tidal creeks, albeit intermittent. In this predominantly agricultural area, major practices and crops include corn-soy bean-wheat rotations, tomatoes and other vegetables under plasticulture, and conventional vegetable culture, especially potatoes and melons. A diverse array of pest control chemicals are used extensively in each case. Plasticulture in particular poses a significant risk because rainwater drains off the crops, and accumulates exclusively between rows because of the plastic mulch. Deliberate efforts are made to remove this excess water from fields. Finally various management schemes are practiced to modulate the concentration of crop protectants before water enters tidal creeks. The practices range from release over adjacent forested land with ground infiltration to retention ditches and ponds capable of holding back substantial amounts of water thereby providing time for the removal of toxic substances by a variety of processes.

In recent decades, a new aquaculture industry has developed on the Eastern Shore to produce hard clams. A number of hatcheries/nurseries have been established on various creeks, both seaside and bayside. Juvenile clams produced in these facilities are then planted onto shallow intertidal mud flats for grow-out. Proximity to the shorelines makes such grow-out areas susceptible to adverse effects from land run-off, but perhaps more vulnerable are hatcheries and nurseries which utilize water drawn from creeks as culture media. The early life stages in hatcheries and nurseries (embryos, larvae, and early juveniles) are generally more sensitive to adverse conditions and toxic materials than the late juvenile and mature clams found on intertidal mud flats.

In hatcheries, minimal water treatment involves particle filtration and temperature modification. By 1995, the industry had matured to the point that culture failure in hatcheries and nurseries from poor techniques and limited expertise was reduced. Water quality is now considered the principle cause of culture failure. Of possible water quality problems, toxic chemicals are of major concern, though certainly not the sole issue. In 1996, allegations were made against a major tomato producer using plasticulture as the cause of clam culture failures in a Gargathy Creek hatchery on the seaside of the Eastern Shore and there were suspicions that similar events might explain culture failures in other creeks, both seaside and bayside, notably in The Gulf. Luckenbach *et al.* (1996) and Deitrich *et al.* (1996) provided some support for the allegations regarding a possible role of runoff on clam hatchery/nursery failure in Gargathy Creek.

Various biological assay methods using estuarine organisms are used to detect toxicity in ambient water and sediment (Roberts & DeLisle, 1988; Hall *et al.*, 1991, 1992, 1994; Anonymous, 1997). Water and sediment samples collected at a point in time are evaluated for toxicity using standardized methods. Test endpoints typically examined include both mortality (or a surrogate) and growth or reproductive measures used in chronic tests. The latter endpoints are used in order to enhance test sensitivity. Multiple species are tested as a hedge against different sensitivities of various species to different chemicals.

These toxicity testing methods are accepted tools to take a snapshot of ambient toxicity but assume that toxicity is uniform over time. These methods do not take into account intermittent or pulsed inputs of toxic materials. Since water is sampled at a point in time, pulsed inputs can go undetected if sufficient dilution occurs before sampling occurs. Therefore the methods are inherently insensitive to short-lived adverse conditions.

A method using *Palaemonetes* adults exposed *in situ* was developed by Scott *et al.* (1987, 1990) for use in tidal creeks receiving agricultural run-off. In this procedure, the test animals are continuously exposed to tidal creek water in cages and therefore subject to the effects of pulsed releases of toxic materials. The endpoint in this test is mortality which limits test sensitivity, but techniques to implement more sensitive endpoints such as growth or reproduction to tests involving field exposure have not been developed for this species. This methodology was applied in bayside creeks of Virginia in 1996 (Luckenbach *et al.*, 1996), and proved valuable in detecting ephemeral toxicity events related to rainfall events.

The objective of the present study was to characterize selected bayside creeks of the Eastern Shore with respect to ambient toxicity, to perform a limited chemical characterization of water and sediment, and to expand the chemical characterization of samples for which toxicity was apparent. This approach to chemical characterization, while less comprehensive than the characterizations typical of the decade-old ambient toxicity program of the Chesapeake Bay program, was selected to avoid analyzing many samples with little likelihood of a significant chemical load.

MATERIALS AND METHODS

Study Sites

Four creeks were selected for study: Onancock, Hungar, The Gulf, and Old Plantation (Fig. 1). These creeks are all less than 5 miles long and at most 0.6 miles wide. These creeks are all meso- or polyhaline, and have little continuous freshwater input at the headwater. Therefore the creeks are rather uniformly saline throughout their length. All four streams have sandy bottoms at the mouth and for considerable distances upstream. These streams are shallow with depths generally less than 6 ft except Onancock Creek which has depths reaching 9-10 ft in a few locations.

Onancock Creek, the most northern creek, is the longest of the four, totaling 4.6 miles in length (Fig. 2). This is the only creek studied that lies in Accomac County. The city of Onancock is located near the headwater at the confluence of two small streams draining agricultural lands to the east. The city has a small sewage treatment plant discharging into the smaller of the two streams about 0.2 miles upstream of the confluence. From the confluence, the creek flows generally westward to discharge into the Bay. The shoreline is fringed with agriculture (corn, soy bean, wheat), woodland, private residences, and marsh land. This creek has the highest concentration of residential commercial activity, but has no significant tomato culture.

Hungar Creek is the northernmost creek studied in Northampton county (Fig. 3). It is ca 4.6 miles in length and has finer grained sediments over a larger area than any of the other creeks, although sediments are still predominantly sandy. There is no significant community along its shoreline, but there are residences in some areas. Land use is predominantly agricultural, with some small amount of tomato culture.

The Gulf, near Eastville, VA, in Northampton County has no major community along its shores, though the land extending south from the mouth along the Bay shore has a string of residences (Fig. 4). This creek, the smallest of the four, is ca 1.9 miles long. There is at least one major tomato farm along the southern bank. In this case the run off from the plasticulture fields is collected in a retention pond that appears to be a dammed tributary of the creek. Water from the pond is recirculated to the fields to provide irrigation. Whenever we have visited the pond, there has been minimal discharge, though during heavy rainfalls, release of water to the creek is likely. In some years, another tomato field is operated further upstream, but we lack any information about how runoff from that field is managed. In addition there is other conventional agriculture in this watershed, some woodland, as well as some residential development. Salt marshes are limited within this watershed.

Old Plantation Creek is the creek located farthest south in Northampton County (Fig. 5). It is about 3 miles long, and follows a north-south axis inshore of the creek mouth oriented on an east-west axis. The shoreline is variable, with wooded, marsh, open field and residential areas. There did not appear to be any plasticulture activity in the watershed.

Five sampling sites were selected in each creek, located approximately equidistant from creek mouth to creek head. These station locations are shown on Figures 1 to 4 along with important landscape features. All stations were quite shallow, with a maximum depth of ca 3 ft in most creeks and 9-10 ft in Onancock Creek. In addition, a site is indicated in each creek at which the *in situ* test was performed. These latter sites were selected deliberately to be near locations where pulsed inputs of contaminants were likely in the event of a significant rain event (>½") and with easy access by road.

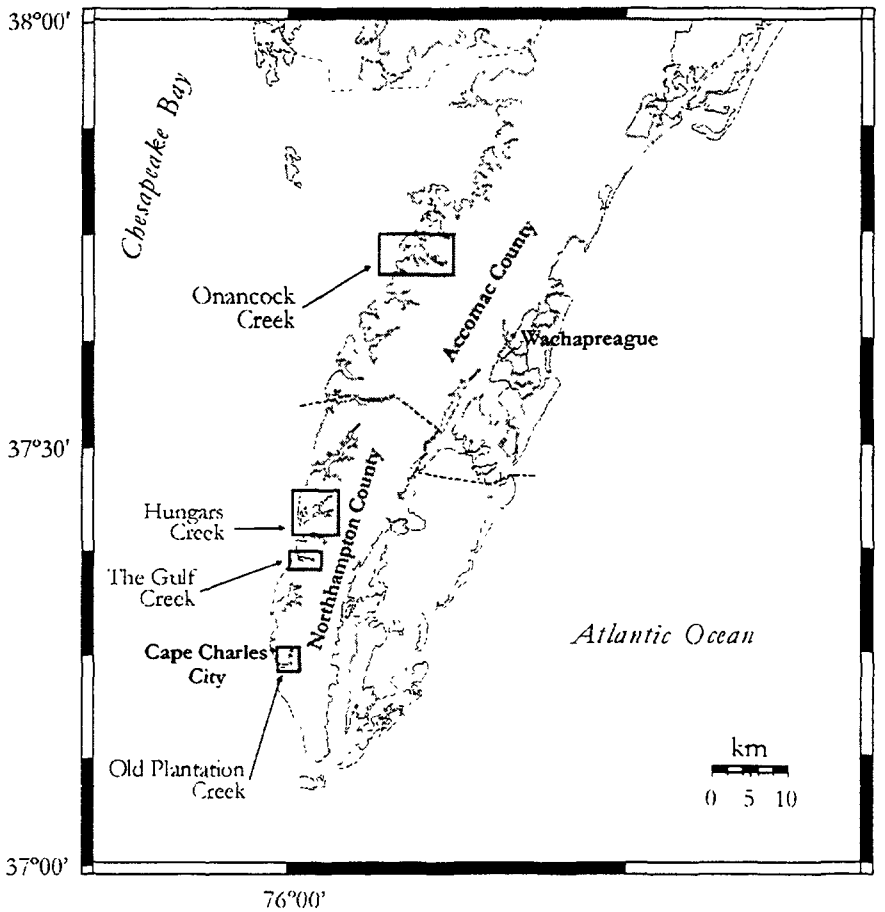

Figure 1 Map of Eastern Shore, Virginia showing some principal geopolitical features and locating the four creeks sampled in the present study.

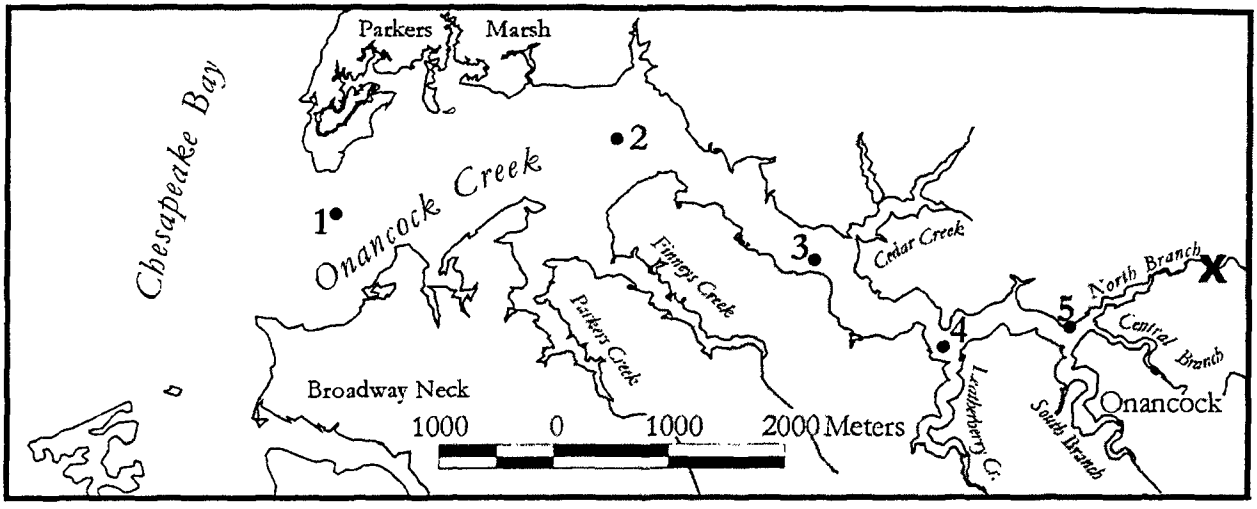

Figure 2 Onancock Creek with all stations located by solid circles. The site of the *in situ* test, located on the North Branch, is indicated by an 'x'

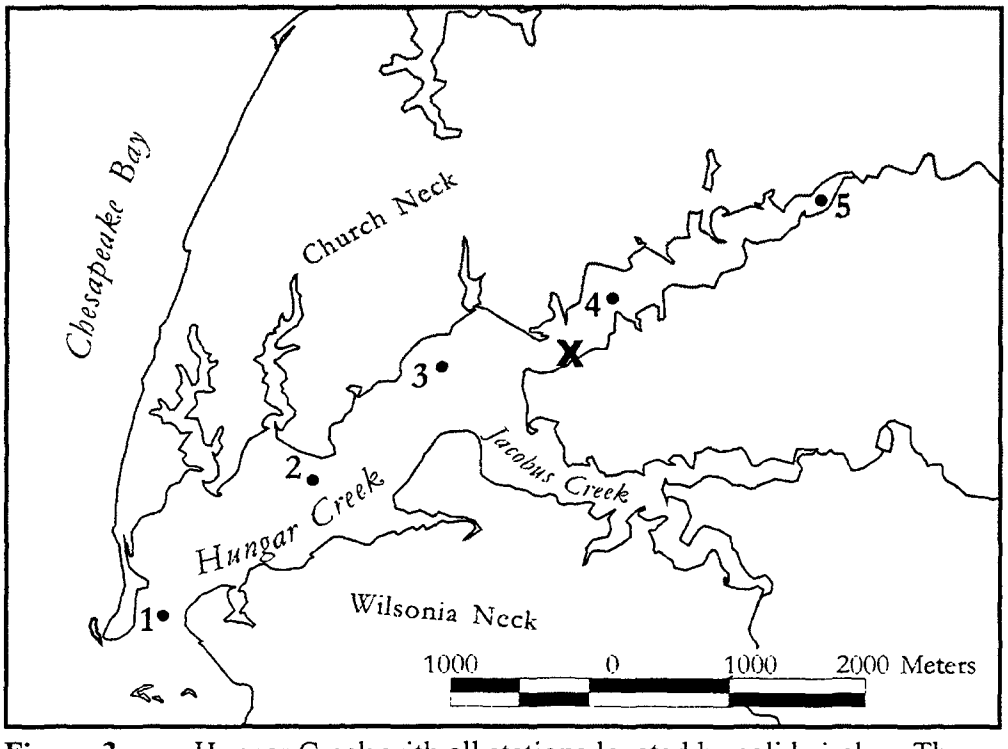

Figure 3 Hungar Creek with all stations located by solid circles. The site of the *in situ* test is located by an 'x'.

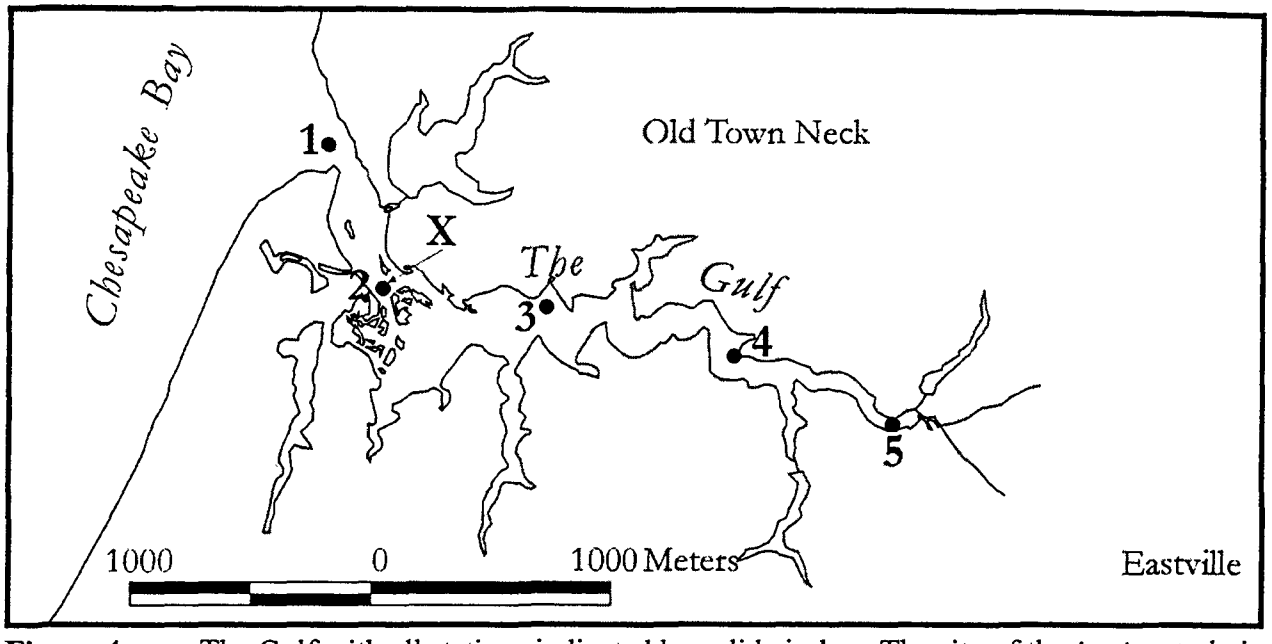

Figure 4 The Gulf with all stations indicated by solid circles. The site of the *in situ* study is coincident with Station 2, and is immediately downstream of the discharge from a retention pond serving two large tomato fields in which plasticulture is practiced.

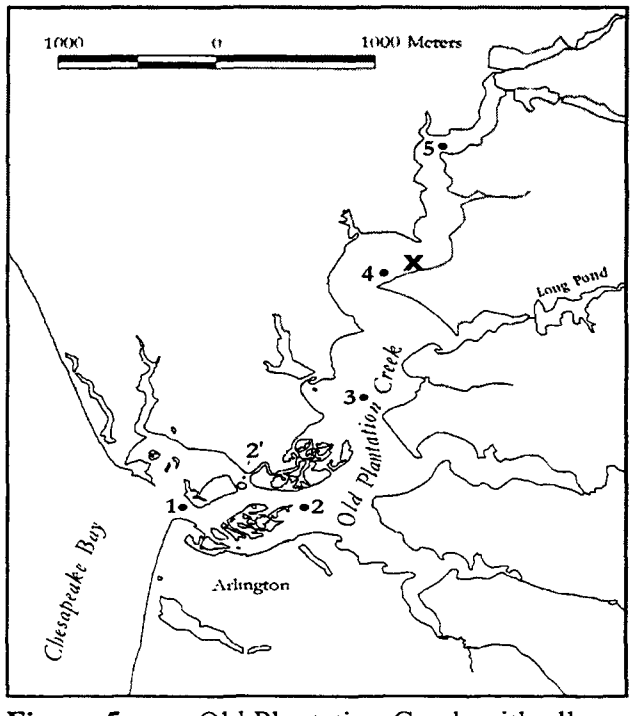

Figure 5 Old Plantation Creek with all stations located by solid circles. Station 2 was relocated by error to location 2' indicated by the open circle. The site of the *in situ* test is indicated by an 'x'.

Sampling of water and sediment

The eastern shore has been predominantly agricultural during this century coupled with boating activity in association with the various creeks fringing the peninsula. Therefore it was decided to make as comprehensive a toxicological characterization as possible and to limit the chemical characterization to a subset of stations and to those chemicals likely to be found in the matrix being examined as a result of known anthropogenic activities on the Eastern Shore. Samples were collected at all stations for the full suite of chemical analyses (exclusive of the sampling for *Mulinia lateralis* embryo tests), but only those from stations 2 and 4 in each creek were analyzed as part of the characterization effort.

Water samples were collected at a depth of 18 inches from the surface, which at many stations represented mid-depth. An 18 liter sample was collected for toxicity tests. At the same time, a 4 liter sample was collected for analysis of organic analytes and placed in a chemically clean amber glass bottle, chilled on ice during transport, and stored at 4°C on arrival at the Gloucester Point laboratory before extraction. Additionally, 1 liter samples were collected into high density polyethylene bottles for metals analysis. These samples were also placed on ice during transport, filtered and acidified for metals analysis. The filtered, acidified samples were stored at 4 °C pending analysis. Water samples were used in toxicity tests within 48 hr of collection.

Sediment samples were collected at each station using a stainless steel petit Ponar grab, with only the surface 1-2 cm of sediment retained for analysis. At each site, multiple grabs were taken to provide sufficient sediment for analysis of organic chemicals, metals, grain size, total organic carbon (TOC), pore water ammonia, AVS and toxicity to the selected test species. Sediments for organic analyses were placed in glass mason jars, kept on ice during transport, and frozen on arrival at the laboratory pending extraction. The bulk of the sediment was returned to the laboratory in 18 liter plastic pails with lids. On arrival at the laboratory, the samples were placed in a 4°C cold room. Subsampling for various analyses and processing for toxicity tests was initiated within 24 hours of arrival at the laboratory. Sediment was prepared for toxicity tests with amphipods by press sieving through a 500 μm mesh stainless steel screen to remove any resident amphipods or invertebrates that might prey on the amphipods. Additional sediment was prepared for tests with fish embryos by sieving through a 2.0 mm mesh stainless steel screen to remove large animals that might prey on fish embryos. The prepared sediments were returned to the cold room pending completion of preparations and initiation of each test. All tests with sediment were initiated within 7 days of collection.

Unscreened sediment samples were submitted to the VIMS Analytical Services Center for grain size analysis. Samples were also submitted to the Analytical Services Center for TOC analysis along with pore water samples for ammonia analysis. Two additional subsamples from each site were placed in whirl pac containers and chilled prior to analysis one for sediment metals and one for acid volatile sulfides (AVS).

Samples for ambient water toxicity tests were collected from Hungar Creek and The Gulf on 21 September 1998 and from Onancock and Old Plantation Creeks on 23 September 1998. These

samples were tested with *Palaemonetes pugio* larvae. Sampling was staggered primarily to accommodate the time needed to establish the toxicity tests with limited personnel. The full suite of tests with this species was accomplished on this schedule because sufficient larvae were produced daily for the tests in this time window and availability of ovigerous females later in the year was unlikely. The stations were resampled on 12 October 1998 and 14 October 1998 to provide ambient water for the tests with *Cyprinodon variegatus* larvae.

Samples for sediment toxicity tests were collected from The Gulf on 30 October 1998 for an initial test with both *C. variegatus* embryos and the amphipod *Leptocheirus plumulosus*. While we had considerable experience with the latter species, the test with fish embryos had not been performed previously. This experiment yielded inadequate data because of larval escape from the egg chambers (data are included in the appendix but not analyzed), and sampling of The Gulf was rescheduled. The egg baskets were refabricated with 400 μm mesh screen before additional tests were done.

Sediment samples were collected from Old Plantation Creek on 17 November 1998 and the sediment tests with both *C. variegatus* and *L. plumulosus* were performed. In this case the tests were successful with no larval escapement and with acceptable control survival. The sampling and testing schedule was interrupted by the holiday season and resumed in February 1999 with sediment collections from The Gulf and Onancock Creeks on 8-9 February 1999, and from Hungar Creek on 16 March 1999.

A major limitation of the sampling and toxicity testing scheme was the non-synoptic sampling necessitated by logistics. For the *Mulinia lateralis* tests, a synoptic sample set of water and sediment was accomplished on 26 April 1999, allowing both water and pore water tests to occur simultaneously with a single spawn of *Mulinia* involving 3-4 females and multiple males.

Analytical methods

Sediment characteristics

Grain Size

A weighed aliquot of sediment from each site was analyzed for grain size and percent total organic carbon (TOC) using standard gravimetric methods. All these analyses were performed by the VIMS Analytical Services Center. Sufficient sediment (typically 200 g) was centrifuged at 5000 rpm (7000 g) and the pore water decanted. The pore water sample was then analyzed for ammonia by the VIMS Analytical Services Center.

Acid volatile sulfide (AVS) in samples of sediment from each collection site was measured using the diffusion method described by Leonard, Cotter and Ankley (1996) who developed it as an efficient alternative to the purge-and-trap method of Di Toro et al. (1990, 1992). This diffusion method was based on earlier work of Brower and Murphy (1994) and Hsieh and Yang (1989).

Though it is common to measure the simultaneously extracted metals (SEM), we did not elect to do so. Sulfide was measured with a sulfide specific electrode (Orion Model 9616) connected to an Orion Expandable Ion Analyzer EA920. The electrode was calibrated daily using fresh sulfide stock solutions.

Metals

Water

Water samples were filtered through a 0.47 μm glass fiber filter and the filtrate containing dissolved metals was acidified (pH<2) and stored at 4°C pending analysis. A cobalt-coprecipitation method modified from Boyle and Edmond (1975) was used to extract metals from water samples, thereby reducing the background noise resulting from other salts in the samples and facilitating a twenty-fold concentration of analyte. A 100 g aliquot of each acidified sample water was amended with acetate buffer. Each sample received 4 ml of a chelator, (APDC or 1 pyrrolidiine carbodithioic acid ammonium salt) followed by solution 2 ml of $CoCl_2$ prepared according to Kraus & Moore(1953). After standing for a minimum of 30 minutes to allow the co-precipitate to form and scavenge other dissolved metals present in the sample, the precipitate containing the analyte metals samples is collected on a 0.47 μm polycarbonate filter. The filter was placed in precleaned LPE screw cap vials, acidified with 3N HCl and sonicated for 30 minutes in a warm water bath to dissolve the precipitate. Once dissolved, the resultant solution was diluted with deionized water and stored at room temperature until analyzed by atomic absorption spectrophotometry. For The Gulf and Hungar Creek samples, in which case water from all 5 stations was analyzed, three additional aliquots of water from Stations 1 and 5 were used for standard additions. These aliquots received 100, 200 or 500 μl spikes of a standard metals mixture containing cadmium, copper, nickel, zinc, chromium, lead, and iron. These spiked samples were then analyzed in the same manner as the unspiked samples. Water from two sites was used for spiked additions to assess whether a reliable curve could be produced using a single sample from a creek. The samples used for the spiked additions curves were selected to reflect our suspicion that upstream samples might differ those from the creek mouth stations. For Onancock and Old Plantation Creek samples, in which case only samples from stations 2 and 4 were analyzed, aliquots of all samples received spikes.

Mercury was determined by FIA cold vapor atomic absorption on aliquots of the original filtered samples. Zinc and cobalt were determined by direct flame atomic absorption. All remaining metals (cadmium, copper, chromium, nickel, and lead) were measured by flameless atomic absorption using the extracted metal sample. A Pd/Mg universal matrix modifier was used for graphite furnace analyses.

Sediment

Freeze dried sediments were microwave digested with concentrated nitric acid. The resulting digest was then analyzed by flame atomic absorption spectrometry or cold vapor atomic absorption spectrometry for mercury.

Organic Compounds

Ambient toxicity may not be related solely to "priority pollutants" as defined by EPA (Allred and Giesy, 1985). We therefore analyzed for a broader array of potentially toxic chemical agents. "Standard" protocols often overlook some organic compounds and their degradation products which in themselves may be more toxic than precursors. Samples were analyzed for select priority pollutants and specific pesticides analyzed in previous AMBTOX studies but we also used a semi-quantitative relative retention indices analytical approach to search for a broader array of chemicals (Greaves, et. al, 1991). Using this technique, it is possible to assign peak retention identities to unknown compounds that may be then identified by mass spectrometry when concentrations are sufficient. A listing of searchable compounds in the Aromatic Retention Indices (ARI), Halogenated Retention Indices (HRI), and Polar Retention Indices (POI) are presented in Appendix A, Tables A1-A9 . Performance of these methods was evaluated through the analysis of Standard Reference Materials (SRM) and matrix spikes.

Water Samples

Water samples were acidified with HCl, spiked with surrogate standards, and extracted three times with 100 ml of dichloromethane. The extract volume was reduced, the solvent exchanged to hexane and internal standards added. Analysis of extracted samples was performed on a Varian gas chromatograph equipped with an electrolytic conductivity detector (GC-ELCD). The instrument calibration was confirmed prior to sample analysis using four point calibration standards representing typical PCB and chlorinated pesticide analytes. The resulting chromatograms were analyzed using a halogen retention index (HRI) to identify tentatively any peaks found in the samples. Mass spectrometric analysis was conducted on representative samples. Chromatograms were searched to confirm the presence of the predetermined set of priority pollutants using retention time and EI spectra. Spectra were compared with computer searchable spectra published in the NIST Standard Reference Database, in-house library spectra generated from standards and spectra published in articles or books. Blank and fortified samples were analyzed in addition to environmental samples.

Sediment Samples

Sediment samples were analyzed by the VIMS protocol for toxic organic chemicals which is described in detail in the SOP (Greaves, et. al, 1991) . Briefly, sediments were freeze-dried, spiked with surrogate standards, and extracted with dichloromethane with an Accelerated Solvent Extractor (ASE). The resulting extracts were fractionated by GPC and silica gel, spiked with internal standards and analyzed for aromatic or heterocyclic compounds by capillary gas chromatography with flame ionization detection (GC/FID) or gas chromatography mass spectrometry (GC/MS) in the full scan electron ionization mode. Chlorinated hydrocarbons were analyzed by capillary gas chromatography with electrolytic conductivity detection (GC/ELCD). Blank samples, fortified samples and SRM 1941a were analyzed concurrently with environmental samples.

Tributyltin Analysis

Water and sediment samples were processed by an adaptation of the methodology published previously for butyltins in water samples, sediments and tissues (Unger et al, 1986, Unger, 1996). Samples were extracted with hexane/tropolone and derivatized with excess Grignard reagent (n-hexyl magnesium bromide). Remaining Grignard reagent was neutralized with 6 N HCL and the hexane layer removed and cleaned up by open column chromatography with Florisil®. Extracts were reduced in volume under dry nitrogen, spiked with tetrabutyltin internal standard and analyzed by gas chromatography with flame photometric detection.

Toxicity tests

In situ

In situ tests were accomplished between 5 August 1998 and 23 August 1998 using a modification of the method described in Luckenbach et al. (1996). At one site in each creek, three cages were deployed on 5 August, each cage containing 12 individually housed shrimp, for a total of 36 animals placed at each study site. Individual caging is necessary to prevent cannibalism that would otherwise be likely whenever a molting event occurred. The individual compartments consisted of mesh-covered tackle boxes (3 mm mesh) that allowed a relatively free exchange of water between the chambers and the water ambient at the station. The trays were deployed by suspending them at the surface from a PVC floatation ring. Every other day, the cages were examined for survival of shrimp, and the mesh cleaned by gentle brushing if necessary. On each observation day, air and water temperature, salinity and dissolved oxygen concentration were determined. pH was measured with a hand-held meter during the middle portion of the study period. The time of observation, tidal stage, wind direction and speed and percent cloud cover were also recorded. The first *in situ* exposure was terminated on August 13, and a new set of animals was deployed using the same procedures on 15 August, remaining in place until 23 August.

Dead shrimp decomposed rapidly under the prevailing conditions. A shrimp was recorded as dead if a dead body (whole or part) was found. If no body was found, the shrimp was classed as missing. A shrimp could become missing through escape (during observation), movement to an adjacent cell in the exposure tray, or death and decomposition between observation times. In the case of movement to an adjacent cell, the shrimp were left in the adjacent cell until the end of the exposure period rather than returned to the original cell. Only 16 animals were recorded as missing out of 288 animals deployed (4 stations, 36 per deployment, 2 deployments) (5.6%). Of these, 12 appeared in other chambers (4.2%) with only 4 either unobserved dead or escapees (1.4%).

Ambient Water tests

Palaemonetes larvae

Larvae of *Palaemonetes* were obtained from ovigerous female shrimp collected from Sarah Creek (at Sarah's End Lane off Route 216) and held in the laboratory in large tanks until needed. Seven to 8 days before a scheduled test, approximately 100 ovigers were selected, judged to be near hatch based on color of the egg mass and placed in hatching baskets in 10 gal aquaria of 15 psu filtered York River water. Sixteen hours later, the resultant larvae were harvested and placed in 4 liter glass jars in 15 psu water for culture. Each day, newly hatched *Artemia* nauplii were added to the culture jars as food after siphoning out any residual dead nauplii and debris from the jars. Larvae were used for tests after 7-8 days in culture. When the cultures were approximately 5 days old, the daily water change was done using 15 psu Hawaiian Marine Mix to acclimate the zoeae to the control water used in the test.

Water for a test was processed to adjust salinity and temperature beginning the day before the test (one day after collection). On the initial day of the test, water was placed in glass jars with 4 laboratory replicates of each treatment. Negative control samples consisted of artificial seawater at 15 psu prepared by dissolving Hawaiian Marine Mix in deionized water. Positive controls were cadmium chloride solutions prepared at the previously measured 96 hr LC50.

All jars were placed in a 25°C water bath over night preparatory to introduction of shrimp larvae. Larvae were distributed sequentially into small weigh pans, one larva at a time, until all pans contained 10 larvae. The contents of the pans were then placed into randomly chosen test containers, and the containers placed in the water bath according to a stratified random scheme, one replicate of each treatment per stratum.

On day 0 and day 8 of the test, the temperature, salinity, dissolved oxygen concentration, and pH were measured in water from one replicate of each treatment. On all other days, the temperature in a different replicate of each treatment was measured and recorded along with the minimum and maximum temperature of the water bath. Each day, the number of live larvae in all replicates and treatments was counted and any dead larvae removed. At the same time, a 50% water change was accomplished using water prepared the day previous and allowed to temperature equilibrate over night.

At the end of the exposure period, larvae were counted, rinsed with deionized water, and placed in tared aluminum weigh pans, one pan per treatment replicate. The larvae were dried at 103°C for 72 hours, then cooled in a desiccator, and weighed to the nearest 0.1 mg.

Cyprinodon larvae

Newly hatched fish larvae were purchased from Aquatic Biosystems (3800 Weicker Dr., Fort Collins, CO 80524) for delivery immediately before a test. On arrival in the laboratory, the fish were placed in large plastic pans of the shipping water and the salinity and temperature gradually

adjusted to 15 psu and 25°C respectively over a 24 hour period. Any dead or damaged larvae were removed.

Water was removed from the cold room a day prior to start of the test, the salinity adjusted to 15 psu, and distributed among the test beakers. Four laboratory replicates were established for each sample site, negative controls and positive controls. Negative controls consisted of artificial estuarine water prepared from Hawaiian Marine Mix and deionized tap water. Positive controls consisted of a cadmium chloride solution prepared at a concentration intended to be a previously determined LC50.

All beakers were placed in a 25°C water bath over night preparatory to introduction of fish larvae. Larvae were distributed sequentially into small weigh pans, one larva at a time, until all pans contained 10 larvae. The contents of the pans were then placed into randomly chosen test containers, and the containers placed in the water bath according to a stratified random scheme.

On day 0 and day 8 of the test, the temperature, salinity, dissolved oxygen concentration, and pH were measured in water from one replicate of each treatment. On all other days, the temperature was measured and recorded along with the minimum and maximum temperature of the water bath. Each day, the number of live larvae was counted and any dead larvae removed. At the same time, a 50% water change was accomplished using water prepared the day previous and allowed to temperature equilibrate over night.

At the end of the exposure period, larvae were counted, rinsed with deionized water, and placed in tared aluminum weigh pans, one pan per treatment replicate. The larvae were dried at 103°C for 72 hours, then cooled in a desiccator, and weighed to the nearest 0.1 mg.

Mulinia larvae

Adult *Mulinia lateralis* were procured from the Marine Biological Laboratory (Aquatic Resources Division, Woods Hole, MA 02543) and maintained in 10 gal aquaria with a 1-2 cm layer of sand on the bottom and about 8 gal estuarine water at 25 psu. Each day, 1.5 liters of resuspended *Isochrysis galbana* was added as food for the clams. Clams buried in the sediment and actively pumped to clear the algae in 3-4 hours. Clams that failed to burrow were removed and discarded.

When all water (and pore water) samples were ready for toxicity analysis, clams were removed to 8 inch glass fingerbowls and placed in a heated water bath to raise the temperature to ca 28°C. The temperature in the bowl was cycled up to 28°C, down to 12 °C and back to 28°C until one or more males released sperm. The males were isolated in separate bowls and allowed to continue sperm release. Small aliquots of sperm suspension were pipetted into the incurrent flow of remaining clams to facilitate female spawning. As females were identified, they were isolated in separate bowls to collect the eggs. When it was judged that sufficient eggs were available, eggs from several females were composited and a composite sperm sample added to accomplish fertilization. Once fertilized eggs were obtained, the eggs were washed on a 35 µm nylon screen

to remove excess sperm and resuspended and aliquots were counted. From the counts, the volume of egg suspension necessary for each test replicate was calculated.

Water (and pore water) samples (10 ml) tempered to 25 °C were distributed to each of three 20 ml glass scintillation vials (laboratory replicates), and the appropriate volume of egg suspension added to each to yield an egg density of 30 eggs/ml. In addition to field samples, negative control samples were tested (along with pore water obtained from reference site sediment collected from Carter's Creek and Poropotank River). Simultaneously, a cadmium chloride toxicity test was conducted using the same volume of test solution and embryos from the same spawn. All vials were incubated for 48 hours at 25°C. At that time, each vial was fixed with buffered formalin, and embryos were examined over several weeks to determine number of survivors and percent of survivors achieving the normal straight-hinge stage.

Ambient Sediment tests

***Cyprinodon* embryos**

Twenty-four hour old embryos were obtained from Aquatic Biosystems. These embryos were produced by spawning of fish stimulated with human gonadotrophic hormone. On receipt, fertile eggs were isolated from the embryos received and held overnight preparatory for a test. As will be seen in the results section, the percent hatch of these selected eggs in the control treatments was often in the 60-70% range and therefore did not meet the planned acceptability criterion for control hatch. However, the tests could not be repeated within the budget and time constraints. The results were consistent across laboratory replicates, an indication that the problem was a function of marginal egg condition, not technique. In no case did we observe overgrowth with fungi or other indications of a flawed exposure. In two instances, eggs not used in the test were maintained to measure % hatch when not exposed; in these cases, the % hatch was comparable to that for control sediment exposures.

For exposure tests, four laboratory replicates of each sediment treatment were prepared in 1 liter glass beakers and overlain with 1500 ml of artificial sea water at 15 psu salinity and 25°C. For each treatment replicate, embryos were placed in an egg basket with the bottom screen resting closely on the sediment to insure egg contact with sediment while not allowing burial. The egg baskets were constructed from a 4" PVC pipe section about 8" long. Two windows were cut in the sides of each basket and covered with nylon mesh. A piece of nylon mesh was held across the open end of the basket with a nylon pipe coupler. The initial set of baskets was prepared with mesh screen which proved too large to retain newly hatched larvae resulting in failure of the initial fish embryo test. Therefore, egg baskets were reconstructed with 400 μm mesh nylon.

On day 0 and day 8 of the test, the temperature, salinity, dissolved oxygen concentration, and pH were measured in water from one replicate of each treatment. On all other days, the temperature was measured and recorded along with the minimum and maximum temperature of the water bath. Throughout the tests, a slow stream of air bubbles was introduced into each vessel from a high-volume-low pressure laboratory air system to insure maintenance of dissolved oxygen levels

and to minimize accumulation of ammonia. Each day, after the egg baskets were removed to determine hatch and survival, a 50% water change was accomplished using water prepared the day previous and allowed to temperature equilibrate over night.

Daily during the test, the egg baskets were removed from each treatment, eggs (and hatched larvae) were removed with large-bore pipet, placed in a fingerbowl, and examined microscopically on a light table. Obviously non-developing embryos showing significant decay were removed. The number hatched on each day was recorded along with the survival of hatchlings. Some tests were terminated before the full 10-day exposure period based on a criterion that all viable eggs in the negative control replicates had hatched two days previously. Endpoints measured were percent hatch and post-hatch larval survival.

Leptocheirus 10-day test

Amphipods for the tests were produced in cultures maintained in the toxicology laboratory at VIMS. Twenty-four hours before a test, amphipods of the appropriate size were isolated from cultures by gradient screening, excluding animals collected on a 1.0 mm screen and those passing through a 0.5 mm screen. The procedure produces a more uniform sized test population than other approaches used to produce 7-day old animals.

The day before establishing a test, a 2-cm deep layer of sediment was placed into each container and overlain with 500 ml of 1 µm filtered York River estuarine water (adjusted to 15 psu with distilled water). Five laboratory replicates were prepared of each sediment sample. In addition to the field samples from the eastern shore creeks, sediments from two reference sites, one in the Poropotank River (corresponding to the control site used by AMRL) and one in the mouth of Carter's Creek (both tributaries of the York River) were prepared as reference samples. Reference sediments were subsampled for various analyses in the same manner and time as experimental site samples, but were not collected within the same time window as the experimental samples.

On day 0 and day 10 of the test, the temperature, salinity, dissolved oxygen concentration, and pH were measured in water from one replicate of each treatment. On all other days, the temperature was measured and recorded along with the minimum and maximum temperature of the water bath. Throughout the tests, a slow stream of air bubbles was introduced into each vessel from a high-volume-low pressure laboratory air system to insure maintenance of dissolved oxygen levels and to minimize accumulation of ammonia.

At the end of the exposure period, the amphipods were screened from the sediment, counted, rinsed in deionized water, and placed into tared aluminum weigh pans. The amphipods were dried at 103°C for 72 hours, then cooled in a desiccator, and weighed to the nearest 0.1 mg.

Mulinia embryo - pore water test

The pore water tests were performed simultaneously with the ambient water tests for this species

using the procedures described previously.

Reference Chemical Assays

For *Cyprinodon variegatus* larvae, *Palaemonetes pugio* zoeae, and *Leptocheirus plumulosus*, standard toxicity tests were performed with cadmium chloride as the reference toxicant, chosen for consistency with historical methods used in the ambient toxicity testing program. The basic method was that described in ASTM Designation E729. All treatments were tested in duplicate. The data for *C. variegatus* larvae were assumed to represent the status of the embryos in the absence of a widely accepted standard test applicable to the eggs. For the *M. lateralis*, the test method was that described in ASTM Designation E724, using the minimal test solution volume recommended, i.e. 10 ml in 20 ml scintillation vials. All treatments were tested in triplicate.

RESULTS

Sediment characterization

The Gulf, Hungar, and Old Plantation creeks are characterized by sandy substrates downstream, with varying amounts of silt/clay admixture at the upstream stations (Table 1). In contrast, Onancock Creek sediments were sandy (>93% sand) both downstream (stations 1 and 2) and upstream (stations 4 and 5), but silty in the middle (station 3: 8.6 % sand, 64% silt). Predictably, total organic carbon (TOC) at the sandy stations was low (<0.5 %), but variable at non-sandy stations; at the stations dominated by silt/clay (>90% silt/clay), the TOC was high (1.9-6.4%). Stations with intermediate grain size distributions have intermediate TOC concentrations. Acid-volatile sulfide concentrations showed no clear relationship to grain size distributions nor to TOC. Pore water ammonia concentrations were also highly variable, but were consistently above 5 mg/l in silty sediments, and ranged from <1 to 9 mg/l in sandy sediments.

Chemical characterization

Water

Ammonia and nitrite concentrations measured on ambient water samples collected during the sediment sampling events were low, between 0.010 and 0.026 mg/l for ammonia and 0.001 and 0.006 mg/l for nitrite (Table 2).

Water samples were analyzed for cadmium, chromium, copper, nickel, mercury, lead and zinc. The measurements are arrayed in Table 3 along with values for field or travel blanks and distilled water blanks. The method detection limit and limit of quantitation are included for each metal along with the US EPA chronic Water Quality Criterion (EPA, 1987) and the acute and chronic Virginia Water Quality Standards.

Table 1. Sediment characteristics at each station. Sediment from The Gulf (10/30/98) used for failed toxicity test, and included for comparative purposes only.

Station	Percent TOC	NH_4 (mg/kg)	Acid Volatile Sulfide	Percent Moisture	Percent Gravel	Percent Sand	Percent Silt	Percent Clay
Onancock 1	0.07	0.978	0.00		0.00	93.94	3.12	2.93
Onancock 2	0.04	1.409	0.00		0.00	95.89	2.15	1.96
Onancock 3	3.20	8.143	1.69		0.00	8.62	64.33	27.05
Onancock 4	0.26	3.163	2.30		0.00	93.66	4.05	2.29
Onancock 5	0.49	4.885	8.20		0.00	96.01	2.83	1.16
Hungar 1	0.08		0.00	17.0	0.00	97.80	0.80	1.40
Hungar 2	2.12		6.05	62.8	0.00	14.10	41.40	44.50
Hungar 3	1.92		8.20	55.8	0.00	23.10	37.90	39.00
Hungar 4	0.45		1.08	24.7	0.00	86.30	5.50	8.20
Hungar 5	4.34		2.80	84.4	0.00	2.30	40.70	57.00
The Gulf 1 (10/30/98)	0.04	6.00	0.00	16.6	0.00	99.00	0.22	0.74
The Gulf 2	0.06	3.01	0.00	16.9	1.35	97.10	0.34	1.22
The Gulf 3	0.77	4.26	1.35	33.8	0.00	81.90	9.45	8.63
The Gulf 4	2.79	6.56	2.75	61.7	0.00	2.91	48.32	48.76
The Gulf 5	3.81	7.98	1.16	71.9	0.60	13.09	49.20	37.12
The Gulf 1 (2/8/99)	0.04	2.24	0.02		0.00	97.59	1.24	1.17
The Gulf 2	0.02	1.62	0.00		0.00	98.16	0.92	0.92
The Gulf 3	0.14	6.43	4.35		0.00	88.73	7.54	3.73
The Gulf 4	4.21	6.13	4.80		0.00	2.86	65.47	31.67
The Gulf 5	6.38	5.17	10.45		0.05	7.26	37.77	54.92
Old Plantation 1	0.02	0.406	0.00	20.9	0.43	98.66	0.26	0.66
Old Plantation 2	0.05	0.981	0.18	19.7	0.00	98.55	0.48	0.96
Old Plantation 3	0.71	5.982	1.56	36.8	0.00	60.69	26.06	13.25
Old Plantation 4	0.66	5.573	0.51	30.9	0.36	80.26	10.59	8.78
Old Plantation 5	1.28	4.149	0.30	42.9	0.00	54.48	26.85	18.68

Table 2. Ammonia and nitrite concentrations in ambient water, selected sampling dates.

	mg NH_4/l	mg NO_2/l
Onancock 1	0.010	0.002
Onancock 2	0.014	0.002
Onancock 3	0.018	0.004
Onancock 4	0.022	0.004
Onancock 5	0.022	0.005
Hungar 1	0.011	0.001
Hungar 2	0.011	0.002
Hungar 3	0.012	0.002
Hungar 4	0.016	0.003
Hungar 5	0.025	0.003
The Gulf 1 (10/98)	0.026	0.001
The Gulf 2	0.023	0.001
The Gulf 3	0.014	0.001
The Gulf 4	0.011	0.002
The Gulf 5	0.014	0.003
The Gulf 1 (2/99)	0.015	0.001
The Gulf 2	0.015	0.001
The Gulf 3	0.014	0.002
The Gulf 4	0.020	0.003
The Gulf 5	0.014	0.005
Old Plantation 1	0.013	0.001
Old Plantation 2	0.012	0.001
Old Plantation 3	0.010	0.002
Old Plantation 4	0.012	0.003
Old Plantation 5	0.054	0.006

Table 3. Dissolved metal concentrations observed in water samples (in µg/l). Values are uncorrected for blank concentrations. All field blank concentrations as well as a distilled water blank are included in the table.

Sample	Cd	Co	Cr	Cu	Ni	Hg	Pb	Zn
Onancock 2	0.14	<0.99	<0.35	0.39	0.98	<0.50	<0.21	<20.2
Onancock 4	0.11	1.72	1.22	1.01	0.98	<0.50	0.29	<20.2
Onancock Field Blank	<0.11	<0.99	0.65	<0.10	<0.56	<0.50	<0.21	<20.2
Hungars 1	0.12	<0.99	0.58	0.49	1.08	<0.50	0.28	<20.2
Hungars 2	0.12	<0.99	0.66	0.45	1.10	<0.50	<0.21	<20.2
Hungars 3	0.11	1.95	0.53	0.42	1.11	<0.50	<0.21	<20.2
Hungars 4	0.11	<0.99	0.63	0.45	1.11	<0.50	<0.21	<20.2
Hungars 5	<0.11	<0.99	<0.35	0.42	1.33	<0.50	<0.21	<20.2
Hungars Field Blank	0.11	<0.99	0.68	<0.10	<0.56	<0.50	<0.21	<20.2
The Gulf 1	0.22	<0.99	0.57	0.52	0.95	<0.50	~	<20.2
The Gulf 2	0.27	<0.99	0.75	0.75	0.93	<0.50	<0.21	<20.2
The Gulf 3	0.17	<0.99	0.87	0.54	1.33	<0.50	<0.21	<20.2
The Gulf 4	0.45	<0.99	0.83	1.68	1.12	<0.50	<0.21	<20.2
The Gulf 5	0.24	<0.99	<0.35	0.21	1.57	<0.50	<0.21	<20.2
The Gulf Field Blank	0.13	<0.99	0.44	<0.10	<0.56	<0.50	<0.21	<20.2
Old Plantation 2	0.19	1.27	~	0.52	0.83	<0.50	0.60	<20.2
Old Plantation 4	0.11	1.00	1.38	0.56	0.94	<0.50	0.26	<20.2
Old Plantation Field Blank	0.17	<0.99	0.67	<0.10	<0.56	<0.50	<0.21	<20.2
Distilled Water Blank	0.13	<0.99	1.06	<0.10	<0.56	0.76	<0.21	<20.2
Method Detection Limit	0.11	0.99	0.35	0.10	0.56	0.50	0.21	20.15
Limit of Quantitation	0.38	3.33	1.16	0.35	1.86	0.79	0.71	67.15
US EPA Water Quality Criterion, Chronic (US EPA, 1987)	9.3	-	50	2.9	8.3	0.025	5.6	86
Virginia Water Quality Standard, Acute (VAC 25-260-140)	43	-	1100	5.9	75	2.1	240	95
Chronic	9.3	-	50	3.8	8.3	0.025	9.3	86

"-" means that there is no Water Quality Criterion or Water Quality Standard for comparison.

The field blanks exhibit substantial unexplained variability. For these reasons, no corrections have been made on the field measurements. Clearly, however, the measured concentrations of most metals were equal to or greater than the field blanks. If corrected for the field blanks, the concentrations would generally be below the method detection limit. Even if one assumes that the field blanks were compromised (in which case the sample concentrations are the maximum possible), uncorrected metal concentrations were never above the EPA chronic WQC for a saline water or the Virginia chronic WQS.

Water samples indicating toxicity were extracted and analyzed for chlorinated compounds. Overall, concentrations were very low with no compounds above detection limits (Tables 4). All sample chromatograms were similar to each other in pattern composition and do not contain elevated concentrations of known contaminants. Tabular results from fortified sample analysis (Appendix A) show recoveries for a wide spectrum of contaminants. Deionized water blanks, sample replicates, and a field blank, all had no quantifiable analytes. Mass spectrometric analysis was conducted on representative samples. Complete printouts of the HRI for these samples can be found in Appendix B.

Aliquots of water from 2 sites in all four creeks were analyzed for the organometallic antifouling agent, tributyltin (TBT) and it's degradation products, dibutyltin and monobutlytin. These are all sufficiently water soluble that if present in toxicologically significant amounts, they would be detectable. None of the analyzed samples contained butyltins above the detection limit of 1 ng/l and matrix spiked samples (5 ng/L) showed 100% recovery of TBT (Table 5).

Table 4. Analyses of selected chlorinated compounds in water from Hungar Creek and The Gulf that was used in toxicity test with *Palaemonetes pugio* in which substantial toxicity was observed.

GCMS analysis of a typical water samples confirms the results below

"-" indicates not detected

"<x xx" indicates below quantitation limit

Concentration (ug/L)

Date collected:	9/21/98	9/21/98	9/21/98	9/21/98	9/21/98	9/21/98	9/21/98	9/21/98	9/21/98	9/21/98	9/21/98	10/13/98	9/21/98	10/20/98
Detection limit: (ug/L)	0 003	0 005	0 005	0 004	0 005	0 003	0 006	0 005	0 005	0.005	0 006	0 003	0 01	0 004
Water Sample Location:	TG 1	TG 2a	TG 2b	TG 3	TG 4	TG 5	HC 1	HC 2	HC 3	HC 4	HC 5	Lab Blank #1	Field Blank HC 5	Lab Blank #2
Probable Compound Id														
Trifluralin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzenehexachloride, alpha	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hexachlorobenzene	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzenehexachloride, beta	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzenehexachloride, gamma-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PCB-26	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PCB-28,31	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alachlor	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PCB-47,75,48	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aldrin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Metolachlor	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlorpyrifos	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heptachlor epoxide	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlordane,trans-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlordane, cis-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDE,4,4'-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Endrin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDD,4,4'-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDT,4,4'-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methoxychlor	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Permethrin, cis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fenvalerate	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Selected Analytes Listed	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00

Table 5 Butyltin concentrations in water from all sites in two creeks, Hungar and The Gulf, exhibiting toxicity to shrimp larvae..

Water Samples
Detection Limit 1 ng/L
All concentrations as Cation in ng/L
Matrix Spike 5 ng/L

Sample	Date Collected	Date Analyzed	TBT	DBT	MBT
Hungar Creek Site 1	9/21/98	11/3/98	<1	<1	<1
Hungar Creek Site 2	9/21/98	11/3/98	<1	<1	<1
Hungar Creek Site 3	9/21/98	11/3/98	<1	<1	<1
Hungar Creek Site 4	9/21/98	11/3/98	<1	<1	<1
Hungar Creek Site 5	9/21/98	11/3/98	<1	<1	<1
The Gulf Site 1	9/21/98	11/3/98	<1	<1	<1
The Gulf Site 2	9/21/98	11/17/98	<1	<1	<1
The Gulf Site 3	9/21/98	11/17/98	<1	<1	<1
The Gulf Site 4	9/21/98	11/17/98	<1	<1	<1
The Gulf Site 4 Replicate	9/21/98	11/17/98	<1	<1	<1
The Gulf Site 5	9/21/98	11/17/98	<1	<1	<1
Matrix Spike	11/17/98	11/17/98	6	1	<1
Matrix Spike Duplicate	11/17/98	11/17/98	5	2	<1
Field Blank	9/21/98	11/3/98	<1	<1	<1
Lab Blank	11/3/98	11/3/98	<1	<1	<1
Lab Blank	11/17/98	11/17/98	<1	<1	<1

Sediment

Sediment samples from sites 2 and 4 in each creek were analyzed for cadmium, cobalt, chromium, copper, manganese, nickel, lead and zinc. Measured concentrations in most cases exceeded the sample detection limit (Table 6). In Onancock Creek, The Gulf, and Old Plantation Creek, the concentrations of all metals were higher at the upstream location (site 4) than the downstream site. In Hungar Creek, the opposite was true. When compared to concentrations in sediment from the references sites, the concentrations were generally lower for all metals at the eastern shore sites than at the reference sites.

Table 6. Metals concentrations in sediment samples from sites 2 and 4 in each study creek, replicate sediment samples from each reference site, with a comparison to Effects Range Low and Effects Range Median levels of Long et al. (1995). Values exceeding the ERL are denoted by single underline.

Sample Site	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
Onancock Site 2	0.01	0.13	<0.69	<0.49	12.01	0.60	1.41	2.83
Onancock Site 4	0.04	0.74	3.80	1.06	23.79	1.51	3.63	11.19
Hungar Site 2	0.46	6.75	19.00	17.39	192.05	22.15	21.15	104.70
Hungar Site 4	0.09	1.58	11.00	2.55	44.27	4.48	28.98	20.96
The Gulf Site 2	0.02	0.17	<0.70	<0.50	6.48	<0.30	1.23	1.83
The Gulf Site 4	0.61	6.21	38.57	25.35	155.16	23.39	31.13	114.94
Old Plantation Site 2	0.01	0.17	<0.70	<0.50	2.88	<0.30	1.33	2.43
Old Plantation Site 4	0.12	2.00	5.30	4.04	53.29	4.22	7.18	28.37
Poropotank, Rep 1	0.21	5.20	28.55	7.59	294.14	11.31	14.92	62.72
Poropotank, Rep 2	0.18	6.55	26.69	7.99	262.19	11.58	14.32	62.19
Carter's Creek, Rep 1	0.20	7.84	44.10	20.03	286.16	23.67	31.06	126.36
Carter's Creek, Rep 2	0.25	8.49	48.20	20.54	274.45	25.04	32.53	127.80
Carter's Creek, Rep 3	0.23	7.65	46.93	19.38	225.59	21.30	30.65	125.94
ERL	1.2		81	34	[730]	20.9	46.7	150
ERM	9.6		370	270	[1700]	51.6	218.0	410

[] values taken from Ingersoll, et al. (1996) which are based on data for the amphipod *Hyalella azteca* and the midge *Chironomus riparius*, both freshwater species rather than marine.

Only nickel exhibited concentrations that equaled the ERL. This occurred in sediment from two sites: Hungar 2 and The Gulf 4. Similar concentrations of nickel were observed at the Carter's Creek reference site, but not the Poropotank River reference site. There is virtually no other data pertaining to Carter's Creek. There is no obvious modern source of nickel at this site, but that does not preclude an historic source that is unknown.

Comparing the two reference sites, there was approximately twice as much chromium, nickel, lead, and zinc in sediments from Carter's Creek and three times as much copper. Concentrations

of all other metals were virtually identical.

As noted, the concentrations of metals equaled or exceeded the Effects Range Low only for nickel (Hungar Creek Site 2 [H2], The Gulf Site 4 [TG4] and Carters Creek [CC]). In no case was the measured concentration close to the ERM. These stations were also notable for being among the lowest in % sand and having nearly identical silt-clay concentrations (Table 1). However, other stations with similar sediment type did not have metals concentrations approaching the ERL. Regardless, no toxic effects are expected when concentrations are at the ERL.

All samples contained very low concentrations of organic compounds of interest. Many analytes were below reported detection limits which ranged from 1-6 ppb (depended on sample dry weight). Total semi-volatile compounds ranged from 83-807 ppb dry weight (Table 7). When present, individual polycyclic aromatic hydrocarbons (PAH) were below 60 ppb dry weight. Similarly, non-polar chlorinated compounds (including PCB) were low in all samples with a range of <1-38 ppb dry weight (Table 8). Concentrations of polar organic compounds (including pesticides) were also very low and ranged from < 1-3.6 ppb for totals (Table 8). Recoveries for surrogate standards in all samples as well as recovery of select target analytes in fortified samples and SRM 1941a are presented in Appendix A.

The Poropotank River sample contained 1.4 pbb TBT while the TBT concentration in sediment from all other locations sampled was below detection limit (1 ppb dry weight) (Table 9). Recovery of TBT in spiked sediment samples averaged greater than 90%.

Table 7 Concentrations of Selected Semi-volatile Organic Compounds in Sediment Samples at Creek Sites Selected for Chemical Characterization

"-" indicates not detected
"<x xx" indicates below quantitation limit

SET #1	Concentration (ng/g) - Dry Weight										
Date collected:	09-Feb-1999	09-Feb-1999	16-Mar-1999	16-Mar-1999	16-Mar-1999	08-Feb-1999	08-Feb-1999	17-Nov-1998	17-Nov-1998	03-Feb-1999	
Quantitation Limit:	2 07	2.62	4.24	4.92	2.84	3 27	5.58	2.17	2.22	3.96	1.13
Sediment Sample Location	ON 2-1	ON 4-1	HC 2-1	HC 2-1a	HC 4-1	TG 2-1	TG 4-1	OP 2-1	OP 4-1	Poropotank #1	Blank #1
Probable Compound											
Naphthalene	-	-	-	-	-	-	-	-	-	-	-
Naphthalene, 2-methyl-	-	-	-	-	-	-	-	-	-	-	-
Naphthalene, 1-methyl-	-	-	-	-	-	-	-	-	-	-	-
Biphenyl	-	-	-	-	-	-	-	-	-	-	-
Naphthalene, 2,6-dimethyl-	-	-	-	-	-	-	-	-	-	-	-
Acenaphthene	-	-	-	-	-	-	-	-	-	-	-
Fluorene	-	-	-	-	-	-	-	-	-	-	-
Phenanthrene	-	4.84	11.84	10.37	<2.84	-	15.51	-	5.76	5.58	-
Anthracene	-	<2.62	<5.11	<4.92	<2.84	-	<5 58	-	<2.22	<3.96	-
Phenanthrene,1-methyl-	-	<2.62	<5.11	<4.92	-	-	5.85	-	<2.22	-	-
Fluoranthene	<2.07	13.63	38.26	37.95	7.28	<3.27	56.19	-	18.58	19.00	-
Pyrene	-	11.80	31.55	29.92	4.53	-	42.54	-	13.81	15.45	-
Benz(a)anthracene	-	11.59	16.14	16.99	-	-	20.95	-	6.22	6.58	-
Chrysene	-	18.09	26.29	26.99	-	-	42.71	-	10.15	18.93	-
Benzo(e)pyrene	-	13 56	21.26	22.36	-	-	27.39	-	7.81	11.37	-
Benzo(a)pyrene	-	14.88	21.68	22.73	-	-	28.33	-	7.66	7.61	-
Perylene	-	6.73	30.76	31.72	3.87	-	29.03	-	7.75	40.28	-
Indeno(1,2,3-cd)pyrene	-	8.28	20.58	22.28	15.47	-	26.88	8.56	12.91	11.28	-
Dibenz(a,h)anthracene	-	<2.62	-	-	-	-	-	8.35	-	-	-
Benzo(ghi)perylene	-	8 68	17.98	18.71	-	-	23.97	-	6.73	6.36	-
Selected Analytes Listed (ng/g) =	0.00	112 08	236 32	240.02	31.15	0.00	324.28	16.90	97.37	142.42	0.00

Table 7 (cont.) Concentrations of Selected Semi-volatile Organic Compounds in Sediment Samples at Creek Sites Selected for Chemical Characterization

	Concentration (ng/g) - Dry Weight										
SET #2											
Date collected:	09-Feb-1999	09-Feb-1999	16-Mar-1999	16-Mar-1999	08-Feb-1999	08-Feb-1999	17-Nov-1998	17-Nov-1998	17-Nov-1998	03-Feb-1999	
Quantitation Limit:	2.16	1.78	4.24	2.18	3.09	5.36	2.64	2.84	1.84	3.82	2.74
Sediment Sample Location.	ON 2-2	ON 4-2	HC 2-2	HC 4-2	TG 2-2	TG 4-2	OP 2-2	OP 4-2	OP 4-2a	Poropotank #2	Blank #2
Probable Compound											
Naphthalene	-	-	-	-	-	-	-	-	-	-	-
Naphthalene, 2-methyl-	-	-	-	-	-	-	-	-	-	-	-
Naphthalene, 1-methyl-	-	-	-	-	-	-	-	-	-	-	-
Biphenyl	-	-	-	-	-	-	-	-	-	-	-
Naphthalene, 2,6-dimethyl-	-	-	-	-	-	-	-	-	-	-	-
Acenaphthene	-	-	-	-	-	-	-	-	-	-	-
Fluorene	-	-	-	-	-	-	-	-	-	-	-
Phenanthrene	-	2 90	10.51	3.50	-	14.68	-	<2.84	2.62	8.00	-
Anthracene	-	-	-	-	-	-	-	-	-	-	-
Phenanthrene,1-methyl-	-	-	-	-	-	-	-	-	-	-	-
Fluoranthene	-	10.28	38.13	10.25	-	55.55	-	8.27	9.16	32.56	-
Pyrene	-	9.43	30.02	8.21	-	43.07	-	7.53	7.53	30.26	-
Benz(a)anthracene	-	4 77	16.24	3.90	-	21.08	-	4.17	3.94	16.44	-
Chrysene	-	7 85	26 12	5.85	-	42.48	-	6.41	7.00	22.91	-
Benzo(e)pyrene	-	3.87	17.06	4.26	-	25.14	-	4.12	3.96	12.73	-
Benzo(a)pyrene	-	3.92	16.11	4.49	-	26.12	-	4.23	3.19	13.63	-
Perylene	-	1.83	24.92	5.39	-	26.07	-	5.84	4.67	42.69	-
Indeno(1,2,3-cd)pyrene	-	4.20	18.40	7.61	-	27.24	-	7.66	6.33	12.58	-
Dibenz(a,h)anthracene	-	-	-	-	-	-	-	-	-	-	-
Benzo(ghi)perylene	-	2.22	12.46	3.87	-	18.79	-	-	2.77	9.19	-
Selected Analytes Listed (ng/g) =	0 00	51.27	209.98	57.33	0.00	300.23	0 00	48.22	51.15	201.00	0.00

Table 8 Concentration of Selected Chlorinated Analytes in Sediment Samples at Creek Sites Selected for Chemical Characterization

Combined results from both
"-" indicates not detected
"<x xx" indicates below

SET #1

Date collected.	09-Feb-1999	09-Feb-1999	16-Mar-1999	16-Mar-1999	16-Mar-1999	08-Feb-1999	08-Feb-1999	17-Nov-1998	17-Nov-1998	03-Feb-1999	
Detection limit (ng/g)	0.006	0.01	0.015	0.015	0.011	0.007	0.019	0.007	0.01	0.014	0.011
Sediment Sample Location	ON 2-1	ON 4-1	HC 2-1	HC 2-1a	HC 4-1	TG 2-1	TG 4-1	OP 2-1	OP 4-1	Poropotank#1	Blank #1
Probable Compound Id											
Trifluralin	-	-	-	-	-	-	-	-	-	-	-
Benzenehexachloride, alpha	-	-	-	-	-	-	-	-	-	-	-
Hexachlorobenzene	-	0.252	-	-	-	-	-	-	-	-	-
Benzenehexachloride, beta	-	-	-	-	-	-	-	-	-	-	-
Benzenehexachloride, gamma-	-	-	-	-	-	-	0.075	-	-	-	-
PCB-26	-	-	-	-	-	-	-	-	-	-	-
PCB-28,31	-	-	-	-	-	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-	-	-	-	-	-
Alachlor	-	-	-	-	-	-	-	-	-	-	-
PCB-47,75,48	-	-	-	-	-	-	-	-	-	-	-
Aldrin	-	-	-	-	-	-	-	-	-	-	-
Metolachlor	-	-	-	-	-	-	-	-	-	-	-
Chlorpyrifos	-	-	-	-	-	-	-	-	-	-	-
Heptachlor epoxide	-	-	-	0.165	-	-	-	-	-	-	-
Chlordane,trans-	-	-	0.099	0.088	0.037	-	0.454	-	0.030	-	-
Chlordane, cis-	-	-	-	-	-	-	-	-	-	-	-
DDE,4,4'-	-	-	1.240	1.178	0.474	-	17.373	-	0.302	0.143	-
Dieldrin	-	-	-	0.180	-	-	-	-	0.216	-	-
Endrin	-	-	-	-	-	-	-	-	-	-	-
DDD,4,4'-	-	-	0.269	0.262	0.136	-	4.160	-	0.107	0.048	-
DDT,4,4'-	-	-	0.083	0.085	0.089	-	1.993	-	-	-	-
Methoxychlor	-	-	-	-	-	-	-	-	-	-	-
Permethrin, cis	-	-	-	-	-	-	-	-	-	-	-
Fenvalerate	-	-	-	-	-	-	-	-	-	-	-
Selected Analytes Listed	0.000	0.252	1.691	1.958	0.736	0.000	24.055	0.000	0.655	0.191	0.000

Table 8 (con't.) Concentration of Selected Chlorinated Analytes in Sediment Samples at Creek Sites Selected for Chemical Characterization

	Concentration										
SET #2											
Date collected.	09-Feb-1999	09-Feb-1999	16-Mar-1999	16-Mar-1999	08-Feb-1999	08-Feb-1999	17-Nov-1998	17-Nov-1998	17-Nov-1998	03-Feb-1999	
Detection limit (ng/g)	0.006	0.007	0.010	0.010	0.005	0.018	0.005	0.007	0.007	0.007	0.005
Sediment Sample Location.	ON 2-2	ON 4-2	HC 2-2	HC 4-2	TG 2-2	TG 4-2	OP 2-2	OP 4-2	OP 4-2a	Poropotank #2	Blank #2
Probable Compound Id											
Trifluralin	-	-	-	-	-	-	-	-	-	-	-
Benzenehexachloride, alpha	-	-	-	-	-	-	-	-	-	-	-
Hexachlorobenzene	-	-	-	-	-	-	-	-	-	-	-
Benzenehexachloride, beta	-	-	-	-	-	-	-	-	-	-	-
Benzenehexachloride, gamma-	-	-	-	-	-	-	-	-	-	-	-
PCB-26	-	-	-	-	-	-	-	-	-	-	-
PCB-28,31	-	-	-	-	-	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-	-	-	-	-	-
Alachlor	-	-	-	-	-	-	-	-	-	-	-
PCB-47,75,48	-	-	-	-	-	-	-	-	-	-	-
Aldrin	-	-	-	-	-	-	-	-	-	-	-
Metolachlor	-	-	-	-	-	-	-	-	-	-	-
Chlorpyrifos	-	-	-	-	-	-	-	-	-	-	-
Heptachlor epoxide	-	-	-	-	-	-	-	-	-	-	-
Chlordane,trans-	-	-	0.165	-	-	0.476	-	-	0.030	0.023	-
Chlordane, cis-	-	-	-	-	-	-	-	-	-	-	-
DDE,4,4'-	-	0 051	1.784	0.756	-	19.032	-	0.307	0.310	0.193	-
Dieldrin	-	-	-	-	-	-	-	-	-	-	-
Endrin	-	-	-	-	-	-	-	-	-	-	-
DDD,4,4'-	-	-	0.586	-	-	6.482	-	-	-	0.102	-
DDT,4,4'-	-	-	0.100	-	-	6.155	-	-	-	-	-
Methoxychlor	-	-	-	-	-	-	-	-	-	-	-
Permethrin, cis	-	-	-	-	-	-	-	-	-	-	-
Fenvalerate	-	-	-	-	-	-	-	-	-	-	-
Selected Analytes Listed	0 000	0.051	2 635	0.756	0.000	32.708	0.000	0.307	0.340	0.318	0.000

Table 9 Butyltin concentrations in sediments from two sites in each creek.

Detection Limit 1 ng/g
All concentrations as Cation in ng/g
Matrix Spike 13 ng/g

Sampling Site	Date Collected	Date Analyzed	TBT	DBT	MBT
Onancock Site 2	2/9/99	8/4/99	<1	<1	<1
Onancock Site 4	2/9/99	8/4/99	<1	<1	<1
Hungar Creek Site 2	3/16/99	8/4/99	<1	<1	<1
Hungar Creek Site 4	3/16/99	8/4/99	<1	<1	<1
The Gulf Site 2	2/8/99	8/4/99	<1	<1	<1
The Gulf Site 4	2/8/99	8/4/99	<1	<1	<1
Old Plantation Site 2	11/17/98	8/4/99	<1	<1	<1
Old Plantation Site 4	11/17/98	8/4/99	<1	<1	<1
Poropotank	2/3/99	8/4/99	1.6	<1	<1
Poropotank Replicate	2/3/99	8/10/99	1.2	<1	<1
The Gulf 4 Matrix Spike	2/8/99	8/10/99	11	<1	<1
The Gulf 4 Matrix Spike Duplicate	2/8/99	8/10/99	13	<1	<1
Blank	8/4/99	8/4/99	<1	<1	<1
Blank	8/10/99	8/10/99	<1	<1	<1

Toxicological characterization

in situ test results

During the field deployment period, there were two relatively small rain events, one on 11 August, and a second on 17 August (Table 10). The initial rainfall deposited the greatest amount on Hungar Creek, with 50 mm (~ 2") collected over a two day period, and smaller amounts of rainfall recorded on the other three creeks. The rainfall on the 17th was smaller and no rain fell at the sampling site on Hungar Creek from this storm (this does not preclude rain elsewhere within the watershed but this is unlikely given the small size of the creek). These differences in rainfall among the locations emphasize the variability in conditions over the Eastern Shore.

Table 10. Conditions during the *in situ* tests with the number of live shrimp and cumulative number dead. Thirty-six shrimp were deployed on 5 August and monitored for 8 days. A second group of 36 shrimp was deployed on 15 August and monitored for an additional 8 days.

Creek	Date	Air Temperature	Water Temperature	Salinity	Dissolved Oxygen	pH	Precipitaton	Number of Live Shrimp	Cumulative Number of Dead Shrimp
Onancock	8/5/98	25.5	25.6	17	8.2	ND	ND	36	
	8/7/98	29.5	26.7	16	5.5	ND	0	36	1
	8/9/98	36.0	28.0	16	8.6	ND	0	35	2
	8/11/98	27.0	27.4	15	10.6	8.1	9.5 mm	34	3
	8/13/98	29.0	27.7	18	5.9	6.76	0	34	3
	8/15/98	36.0	28.4	17	ND	8.04	0	36	
	8/17/98	32.0	29.0	17	7.9	7.26	26 mm	35	
	8/19/98	22.0	29.8	18	10.5	ND	4 mm	35	
	8/21/98	34.0	28.4	18	10.4	ND	0	35	
	8/23/98	29.0	28.8	20	11.2	ND	0	35	
Hungar	8/5/98	22.4	25.3	20	5.7	ND	ND	36	
	8/7/98	29.0	26.6	20	6.3	ND	0	35	1
	8/9/98	35.0	28.4	20	6.6	ND	0	34	1
	8/11/98	31.0	27.4	22	5.69	7.3	50 mm	32	4
	8/13/98	30.0	28.6	21	6.36	7.58	0	32	4
	8/15/98	34.0	28.6	20	6.7	7.57	0	36	
	8/17/98	33.0	29.5	20	5.6	7.32	0	37	1
	8/19/98	23.0	28.1	22	8.2	ND	0	37	1
	8/21/98	28.0	26.0	22	7.4	ND	0	34	1
	8/23/98	36 0	28.1	22	8.1	ND	0	34	1

Table 10 (cont.). Conditions during the *in situ* tests with the number of live shrimp and cumulative number dead. Thirty-six shrimp were deployed on 5 August and monitored for 8 days. A second group of 36 shrimp was deployed on 15 August and monitored for an additional 8 days.

Creek	Date	Air Temperature	Water Temperature	Salinity	Dissolved Oxygen	pH	Precipitaton	Number of Live Shrimp	Cumulative Number of Dead Shrimp
The Gulf	8/5/98	22.6	24.0	24	5.5	ND	ND	36	
	8/7/98	28.0	26.0	22	6 4	ND	0	34	1
	8/9/98	33.0	28.4	21	7.2	ND	0	34	1
	8/11/98	34.0	26.7	21	6.6	7.12	28	34	1
	8/13/98	30.0	28.1	21	6.95	7.7	0	32	3
	8/15/98	32.0	28.6	22	ND	7.91	0	36	
	8/17/98	33.0	31.4	21	7.8	7.66	2	36	
	8/19/98	24.0	28.1	21	7.5	ND	0	36	
	8/21/98	34.0	25.1	22	7.6	ND	0	36	
	8/23/98	36.0	27.7	21	ND	ND	0	35	1
Old Plantation	8/5/98	21.8	24.8	23	4.4	ND	ND	36	
	8/7/98	29.0	26.6	22	4.8	ND	0	37	
	8/9/98	30.0	29.3	20	5.6	ND	2	36	
	8/11/98	31.0	28.8	23	5.93	7.19	11	36	
	8/13/98	32.0	29.2	23	6.3	7.62	0	36	
	8/15/98	32.0	29.4	23	ND	7.78	0	36	
	8/17/98	35.0	32.4	21	9.2	7.65	10	36	
	8/19/98	25.0	28 7	23	9.2	ND	0	36	
	8/21/98	24.0	25 7	25	6.6	ND	0	36	
	8/23/98	33.0	27 7	32	7.0	ND	0	36	

Water temperature increased during the study period from 24 to over 30°C, with slight differences in temperature among the four creeks (Table 10). Salinity was consistent over time within each creek, but differed from creek to creek. The average salinity was 17.2 psu at the Onancock Creek site, 20.9 psu at the Hungar Creek site, 21.6 psu at The Gulf site, and 23.5 psu at the Old Plantation Creek site. Oxygen concentrations were always above 4 mg/l, and at times above saturation. pH was usually between 7 and 8. These conditions are all well within the tolerance limits for the test species.

During the initial 8-day exposure, survival was 91.7% in Onancock Creek, 88.9% in Hungar Creek, 91.7% in The Gulf, and 100% in Old Plantation Creek. The mortalities were somewhat

associated with the rain event on 11 August, but the magnitude of the mortalities were small compared to what has been seen in seaside creeks at other times (Luckenbach et al, 1996). During the second 8-day exposure period, single crabs died in Hungar Creek and The Gulf, and the deaths were not associated with a measurable rain event.

Toxicity of Water Samples

The water from The Gulf and Hungar Creeks (September 1998) showed apparent toxicity to *Palaemonetes pugio* larvae after 4-5 days of exposure (Table 11). During the remainder of the 8-day test, mortality occurred daily. The onset of mortality may reflect the time of a molt when decapods are considered particularly vulnerable, but no observations were made to corroborate this. Survival for the control group in artificial seawater was 92.5% (range 80% - 100%), well above the test criterion. Survival in water from all sites in both the Gulf and Hungar Creek was significantly different from that in the control water in a Dunnett's Test. Using a Kruskal-Wallis analysis, there were no significant differences in responses among the sites, but only The Gulf station 2 and Hungar stations 4 and 5 were significantly different from the control group. Survival in the positive control group exposed to the measured LC50 concentration of 0.71 mg Cd/l was 27.5% after 4 days (data not shown).

Table 11 Survival of *Palaemonetes pugio* larvae exposed to water from each of four Eastern Shore creeks. Each value represents the mean of four laboratory replicates. Significant differences from the control are designated with a '*'.

		Mean Survival percentages by day							
Station	Sampling Date	1	2	3	4	5	6	7	8
Onancock 1	23 Sept 98	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Onancock 2		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Onancock 3		100.0	100.0	100.0	100.0	97.5	97.5	95.0	95.0
Onancock 4		100.0	97.5	97.5	97.5	97.5	97.5	97.5	97.5
Onancock 5		100.0	100.0	100.0	100.0	100.0	100.0	97.5	97.5
Hungar 1	21 Sept 98	100.0	100.0	97.5	95.0	80.0	52.5	32.5	32.5
Hungar 2		100.0	100.0	100.0	97.5	92.5	82.5	55.0	32.5
Hungar 3		100.0	97.5	97.5	97.5	65.0	47.5	25.0	17.5
Hungar 4		100.0	100.0	97.5	87.5	52.5	5.0	0.0	0.0
Hungar 5		100.0	100.0	100.0	95.0	70.0	32.5	10.0	5.0
The Gulf 1	21 Sept 98	100.0	100.0	100.0	100.0	85.0	65.0	47.5	45.0
The Gulf 2		100.0	100.0	97.5	82.5	37.5	15.0	12.5	10.0
The Gulf 3		100.0	100.0	100.0	87.5	67.5	55.0	27.5	25
The Gulf 4		100.0	100.0	97.5	92.5	65.0	25.0	12.5	12.5
The Gulf 5		100.0	100.0	97.5	95.0	85.0	47.5	30.0	30.0
Old Plantation 1	23 Sept 98	100.0	100.0	100.0	100.0	100.0	100.0	97.5	97.5
Old Plantation 2		100.0	100.0	100.0	100.0	97.5	97.5	97.5	97.5
Old Plantation 3		100.0	100.0	100.0	100.0	100.0	97.5	97.5	97.5
Old Plantation 4		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Old Plantation 5		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Carter's Creek A	21 Sept 98	100.0	100.0	100.0	100.0	100.0	97.5	97.5	92.5
Carter's Creek B	23 Sept 98	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

In contrast, water from Onancock and Old Plantation creeks did not show apparent toxicity during the study. Since several treatments did not have any mortality, a valid ANOVA and Dunnett's Test cannot be performed. However, for all treatments, survival ranged from 95 to 100%. A Kruskal-Wallis analysis did not indicate any significant differences for water from either creek.

Although we did not determine growth (defined as the difference between final mean weight of survivors and mean initial weight of a sample from the same experimental population), a

comparison of final weights of surviving *P. pugio* (Table 12) reveals no significant differences among the various treatments involving sites in all four creeks using Dunnett's test. Since the larvae at the start of the test were of the same age ± <24 hr and maintained *en masse* with consistent feeding, the larvae were of quite consistent, though unmeasured, size at the start. Thus the final weights in all tests are a reasonable surrogate for growth.

Water samples from the same locations collected a month later (October 1998) did not produce significant mortality of *C. variegatus* larvae (Table 13). In Hungar Creek water, survival was lowest at site 4 (92.5% survival), and statistically different from all other sites and the control in a Kruskal-Wallis analysis, but this reflects the lack of variance in all other treatments in which survival was 100%. In the Gulf, survival ranged from 92.5% to 100%, and no statistically significant differences were detected with the Kruskal-Wallis test. Similarly, water from Onancock Creek and Old Plantation Creek produced survival rates of 92.5% to 100%.

As with decapod larvae, growth was not determined. Nevertheless, final weights of surviving *C. variegatus* (Table 14) were not significantly different from controls at any site. As in the decapod larval test, fish larvae were intially uniform in size, so final weights are a surrogate for growth.

Table 12 Weight / individual for *Palaemonetes pugio* larvae on day 8 following exposure to water from each of four Eastern Shore creeks. Each value represents the mean of four laboratory replicates. Significant differences from the control are designated with a '*'.

Station	Sampling Date	Mean weight	SD
Onancock 1	23 Sept 98	0.820	0.332
Onancock 2		0.830	0.063
Onancock 3		1.250	0.450
Onancock 4		1.090	0.431
Onancock 5		0.980	0.093
Hungar 1	21 Sept 98	2.408	0.742
Hungar 2		0.787	0.377
Hungar 3		1.990	0.000
Hungar 4		nd	nd
Hungar 5		0.600	0.100
The Gulf 1	21 Sept 98	1.541	0.589
The Gulf 2		0.833	0.000
The Gulf 3		1.629	0.260
The Gulf 4		1.267	0.275
The Gulf 5		0.824	0.183
Old Plantation 1	23 Sept 98	1.090	0.335
Old Plantation 2		0.930	0.089
Old Plantation 3		0.870	0.117
Old Plantation 4		0.990	0.298
Old Plantation 5		0.920	0.111
Carter's Creek A	21 Sept 98	1.520	0.807
Carter's Creek B	23 Sept 98	1.080	0.250

Table 13 Survival of *Cyprinodon variegatus* larvae exposed to water from each of four Eastern Shore creeks. Each value represents the mean of four laboratory replicates. Significant differences from the control are designated with a '*'.

		Mean Survival percentages by day							
Station	Sampling Date	1	2	3	4	5	6	7	8
Onancock 1	14 Oct 98	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Onancock 2		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Onancock 3		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Onancock 4		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Onancock 5		100.0	100.0	100.0	100.0	100.0	97.5	97.5	97.5
Hungar 1	12 Oct 98	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Hungar 2		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Hungar 3		97.5	97.5	95.0	95.0	92.5	92.5	92.5	92.5
Hungar 4		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Hungar 5		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
The Gulf 1	12 Oct 98	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
The Gulf 2		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
The Gulf 3		97.5	97.5	95.0	92.5	92.5	92.5	92.5	92.5
The Gulf 4		100.0	100.0	100.0	97.5	97.5	97.5	97.5	97.5
The Gulf 5		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Old Plantation 1	14 Oct 98	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Old Plantation 2		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Old Plantation 3		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Old Plantation 4		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Old Plantation 5		95.0	92.5	92.5	92.5	92.5	92.5	92.5	92.5
Hawaiian Marine Mix A	12 Oct 98	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Hawaiian Marine Mix B	14 Oct 98	100.0	100.0	100.0	100.0	100.0	100.0	97.5	97.5

Table 14 Weight / individual for *Cyprinodon variegatus* larvae on day 8 following exposure to water from each of four Eastern Shore creeks. Each value represents the mean of four laboratory replicates. Significant differences from the control are designated with a '*'.

Station	Sampling Date	Mean Weight	SD
Onancock 1	14 Oct 98	1.03	0.08
Onancock 2		0.95	0.09
Onancock 3		1.06	0.07
Onancock 4		1.22	0.11
Onancock 5		1.16	0.10
Hungar 1	12 Oct 98	1.13	0.20
Hungar 2		1.06	0.04
Hungar 3		1.00	0.10
Hungar 4		1.02	0.25
Hungar 5		1.09	0.17
The Gulf 1	12 Oct 98	1.02	0.12
The Gulf 2		1.13	0.11
The Gulf 3		1.12	0.06
The Gulf 4		1.00	0.10
The Gulf 5		0.92	0.14
Old Plantation 1	14 Oct 98	0.10	0.09
Old Plantation 2		0.98	0.05
Old Plantation 3		1.10	0.16
Old Plantation 4		1.15	0.11
Old Plantation 5		1.29	0.11
Hawaiian Marine Mix A	12 Oct 98	1.09	0.033
Hawaiian Marine Mix B	14 Oct 98	1.170	0.172

Water from the four creeks collected in March of 1999 generally did not adversely affect *Mulinia lateralis* embryos. The mean percent normal embryos was 94% or higher in all but two cases (Onancock Creek site 4 and The Gulf site 4), with a coefficient of variation less than 10% (Table 15). In stark contrast, water from Onancock Creek site 4 and The Gulf site 4 had virtually no survivors in any replicate, and no normal embryos. Thus, very acute effects were observed, but there is no information suggesting a possible causation.

Toxicity of Sediment Samples

Sediment from all creeks had no adverse effects on *L. plumulosus* (Table 16). The 10-day mean survival at every station was high, averaging 94 to 100% regardless of location. The range of survival for all replicates for all creeks was 80% to 100%, the same as for the reference sites.

In short tests such as these, with no food added, it is generally accepted that growth cannot be reliably measured. We did, however, determine final weight, considering that there would likely be some food material derived from the sediments that in a food limited exposure system, might result in some differences, albeit the interpretation of such differences would be compromised.

In general, final weights for *L. plumulosus* were higher than for the reference site (Carter's Creek) in every test. The lowest mean weight for amphipods exposed to sediment from any creek site was at least 1.4 times that for the reference site. In part for this reason, after the initial test in this series, a second reference sediment was used. This sediment was from the Poropotank River at a site corresponding to that from which AMRL has obtained control sediment in the past. Though the final weights of amphipods exposed to these sediments was slightly higher than that for those exposed to Carter's Creek sediment, the differences were not significant in any test.

The highest mean weights were a factor of 2.3 to 2.8 above the lowest mean weights within each creek. There no significant differences were detected among stations within a creek by Dunnett's test.

For *Cyprinodon variegatus* embryo tests, there were no significant adverse impacts on either percent hatch or percent fry survival (Table 17). Percent hatch for sediment exposed embryos ranged from 62.5 to 92.5% with a similar range of percent hatch for the reference site sediments. Percent survival of fry was high, usually above 83%, and always above 77.5%.

While percent hatch was high in nearly all treatments involving creek sediments, reference sediments resulted in lower than desired percent hatch. In the first test with sediments from Old Plantation Creek, percent hatch was 62.5%, well below the desired hatch of 80%. A small number of eggs not used in the test exhibited a similarly poor hatch rate, suggesting that egg quality was poor. However, since at least two test sediments produced a hatch rate above 80%, one can also question the quality of reference sediment. Examination of control results with *Leptocheirus plumulosus* performed simultaneously does not support a sediment quality concern.

Table 15 Percent normal *Mulinia lateralis* embryos when exposed to water from each of the creek sites. Significant differences from the control are designated with a '*'.

Treatment	Mean % Normal	Std Dev % Normal
Hawaiian Marine Mix	99.28	0.75
Eastern Shore Reference	97.57	1.86
Onancock 1	97.78	2.81
Onancock 2	98.60	1.35
Onancock 3	100.00	0.00
Onancock 4	0.00*	0.00
Onancock 5	99.24	0.69
Hungar 1	95.98	4.35
Hungar 2	99.57	0.75
Hungar 3	99.42	0.50
Hungar 4	96.18	0.22
Hungar 5	95.73	2.46
The Gulf 1	96.74	4.59
The Gulf 2	97.62	4.12
The Gulf 3	94.64	4.65
The Gulf 4	0.00*	0.00
The Gulf 5	94.66	2.30
Old Plantation 1	99.31	0.69
Old Plantation 2	99.56	0.76
Old Plantation 3	99.42	0.02
Old Plantation 4	99.58	0.37
Old Plantation 5	99.28	6.22

Table 16. Mean survival and weight / individual for *Leptocheirus plumulosus* on day 10 following exposure to sediment from each of four Eastern Shore creeks. Each value represents the mean of four laboratory replicates. Significant differences from the control are designated with a '*'.

Station	Sampling Date	% Survival	Mean Weight	SD
Onancock 1	9 Feb 99	100.0 ± 0.0	0.27	0.01
Onancock 2		99.0 ± 2.2	0.27	0.02
Onancock 3		96.0 ± 6.5	0.40	0.03
Onancock 4		100.0 ± 0.0	0.55	0.02
Onancock 5		100.0 ± 0.0	0.71	0.04
Hungar 1	16 Mar 99	99.0 ± 2.2	0.33	0.01
Hungar 2		97.0 ± 2.7	0.42	0.03
Hungar 3		100.0 ± 0.0	0.35	0.01
Hungar 4		96.0 ± 8.9	0.38	0.05
Hungar 5		98.0 ± 2.7	0.74	0.04
The Gulf 1	8 Feb 99	100.0 ± 0.0	0.39	0.05
The Gulf 2		99.9 ± 2.2	0.24	0.02
The Gulf 3		99.0 ± 2.2	0.52	0.01
The Gulf 4		94.0 ± 5.5	0.67	0.04
The Gulf 5		98.0 ± 2.7	0.47	0.04
Old Plantation 1	17 Nov 98	94.7 ± 8.7	0.29	0.15
Old Plantation 2		93.7 ± 5.9	0.67	0.21
Old Plantation 3		96.0 ± 6.0	0.50	0.16
Old Plantation 4		96.0 ± 6.0	0.55	0.24
Old Plantation 5		100.0 ± 0.0	0.51	0.06
Control A (CC)	17 Nov 98	94.7 ± 5.6	0.23	0.05
Control B (CC)	8 Feb 99	97.0 ± 4.5	0.17	0.02
Control B (PR)		98.0 ± 2.7	0.19	0.02
Control B' (CC)	9 Feb 99	95.0 ± 8.7	0.15	0.02
Control B' (PR)		99.0 ± 2.2	0.21	0.01
Control C (CC)	16 Mar 99	97.0 ± 4.0	0.16	0.01
Control C (PR)		97.0 ± 4.0	0.18	0.02

Table 17 Survival data for *Cyprinodon variegatus* embryos and larvae exposed to sediment from each of four Eastern Shore creeks. Each value represents the mean of four laboratory replicates.

Station	Sampling Date	% hatched	SD	% fry survival[1]	SD	% live Fish[2]	SD
Onancock 1	9 Feb 99	80.0	12.25	100.0	0.00	80.0	12.25
Onancock 2		75.0	8.66	100.0	0.00	75.0	8.66
Onancock 3		77.5	4.33	93.3	6.73	72.5	8.29
Onancock 4		70.0	14.14	100.0	0.00	70.0	14.14
Onancock 5		72.5	10.90	100.0	0.00	72.5	10.90
Hungar 1	16 Mar 99	90.0	7.07	96.9	5 41	87.5	10.90
Hungar 2		92.5	8.29	86.9	8.83	80.0	7.07
Hungar 3		85.0	11.18	77.5	22 78	65.0	18.03
Hungar 4		92.5	8.29	90.0	17.32	82.5	14.79
Hungar 5		82.5	10.90	93.8	10.83	77.5	14.79
The Gulf 1	8 Feb 99	77.5	4.33	87.1	0.77	67.5	4.33
The Gulf 2		77.5	14.79	93.3	6.73	72.5	16.39
The Gulf 3		80.0	7.07	94.4	9.63	75.0	5.00
The Gulf 4		62.5	8.29	96.4	6.19	60.0	7.07
The Gulf 5		72.5	10.90	100.0	0.00	72.5	10.90
Old Plantation 1	17 Nov 98	85.0	5 00	100.0	0.00	85.0	5.00
Old Plantation 2		90.0	10.00	100.0	0 00	90.0	10.00
Old Plantation 3		70.0	15.81	100.0	0.00	70.0	15.81
Old Plantation 4		67.5	4.33	92 3	7.78	62.5	8.29
Old Plantation 5		75.0	15 00	92.2	8 39	70.0	17.32
Control A (CC)	17 Nov 98	62.5	8 29	96.4	6 19	60.0	7.07
Control B (CC)	8-9 Feb 99	75.0	11.18	94.1	5 92	70.0	7 07
Control B (PR)		65.0	8.66	83.3	16.67	55.0	16 58
Control C (CC)	16 Mar 99	90.0	12.25	96.4	6.19	87.5	16.39
Control C (PR)		80.0	0 00	78 1	10.36	62.5	8.29

1 % Fry Survival = No $Fry_{10\,d}$/Cumulative No Eggs $Hatched_{10\,d}$ x 100

2 % Fish Survival = No $Fry_{10\,d}$/Initial No Eggs x 100

In the first test in 1999, fish embryos were examined on receipt, and only eggs judged fertile were used to set up the test. Again, percent hatch for the reference site sediment was below the desired 80%, and this was also true for a small sample of eggs held in water without sediment (no replication). As in the previous test, though percent hatch was inadequate, survival of fry was exceedingly good. In the final test of this series, percent hatch of fry was high, matching the results for the test creek sediments, and again fry survival was excellent.

Though percent hatch was not acceptable in two exposures, and therefore caution is necessary in interpreting the test creek responses, there is no evidence of an effect on hatchability or survival of fry.

The test of sediment pore water with *Mulinia lateralis* is not a standard procedure, but rather exploratory. From a strictly operational perspective, the test was relatively easy to perform, the exposure period was brief, but the processing of samples after exposure was extremely time consuming.

A reduced percentage of normal embryos was observed among larvae exposed to sediment pore water from 7 sites, with at least 1 site in each creek (Table 18). The sites producing adverse effects were Onancock Creek 1 and 2, Hungar Creek 1 and 5, The Gulf 1, and Old Plantation 2 and 3. While survival was reduced at these sites, survival was never less than 9%. The percent normal observed in pore water from these sites ranged from 11.81 % to 72.12 %. Pore water from all other creek sites, ranged from 89.9% to 99.3%. In many cases, the coefficient of variation was larger than in the test with overlying water, and in several cases equaled or exceeded 10%.

The artificial sea water control (Hawaiian Marine Mix) used for the concurrent analyses of overlying water using the same batch of larvae served as the control in this exposure series as well. A second control series in conjunction with the reference chemical test had nearly the same response with 98.5% normal. A pore water sample from outside the creeks was also examined as a reference material, but since there is no long experience with the testing of pore water, we cannot assert *a priori* that this pore water does not produce adverse effects. The percent normal for pore water from the reference site was 78% with a coefficient of variation of about 14%. This percent normal was not significantly different from that of the control in any Kruskal-Wallis test involving a series of treatments.

Table 18 Percent normal *Mulinia lateralis* embryos when exposed to pore water from sediment collected at each of the creek sites. Significant differences from the control are designated with a '*'.

Treatment	Mean % Normal	Std Dev % Normal
Hawaiian Marine Mix	99.28	0.75
Eastern Shore Reference	77.96	10.88
Onancock 1	0.00*	0.00
Onancock 2	72.12*	9.93
Onancock 3	97.27	2.37
Onancock 4	91.92	1.60
Onancock 5	92.96	2.13
Hungar 1	37.09*	24.16
Hungar 2	93.03	2.81
Hungar 3	95.35	0.02
Hungar 4	92.63	1.16
Hungar 5	48.53*	10.69
The Gulf 1	55.44*	38.92
The Gulf 2	96.17	1.65
The Gulf 3	97.21	0.69
The Gulf 4	91.71	11.06
The Gulf 5	89.91	10.67
Old Plantation 1	97.41	1.83
Old Plantation 2	11.81*	6.74
Old Plantation 3	53.26*	3.61
Old Plantation 4	97.97	1.36
Old Plantation 5	97.85	2.64

Reference Chemical Tests

Tests were performed with specimens of each species used in the laboratory toxicity tests to determine the 4-day LC50 for these populations of animals against a reference toxicant (Table 19). In three cases, the acute toxicity test was performed with animals from the same population used for the ambient toxicity tests of creek water, but prior to them. In the fourth case (*Mulinia lateralis*), the acute toxicity test was performed simultaneously with the ambient toxicity tests of creek water. Within each ambient toxicity test, a replicated treatment with the reference chemical at the estimated LC50 was included.

For *Palaemonetes pugio*, a single acute toxicity test was performed with larvae produced from one or more females in the same population that produced larvae for the tests of creek waters. The LC50 was 0.71 mg/l (95% confidence range 0.5-1.0 mg/l). Control survival was 100%.

Table 19 Lethal concentrations for Cadmium tests with each test species.

Species	Test	LC50 (LCL, UCL)
Palaemonetes pugio	1	0.71 (0.5, 1.0)
Cyprinodon variegatus	1	3.63 (2.42, 7.65)
	2	4.19[1] (2.23, 115.69)
Leptocheirus plumulosus	1	0.78 (0.56, 1.00)
	2	0.78 (0.43, 1.08)
	3	1.15 (0.85, 1.60)
Mulinia lateralis	1	0.074 (not determinable)

[1] This experiment, with a dose range of 0.47 to 15 mg/l, when analyzed with all data, yielded a slope of 0 because of lower than expected mortality at the higher concentrations. When only the data for the lower 4 exposures were analyzed, the reported results were obtained, but these are judged unreliable. They are presented only for completeness.

Two tests were performed with *Cyprinodon variegatus* starting with ≤24 hr old larvae. In the first test, the LC50 was 3.63 mg/l (95% confidence range 2.42-7.65 mg/l). Control survival was 100% and the response slope was significantly different from 0. However, since the mortality rate at the highest exposure concentration was lower than expected, the test was repeated and an additional exposure concentration was included on the upper end. In this test, the responses to the first 5 concentrations were generally similar to responses in the first test, and the response to the added high concentration not higher than that at lower concentrations. If one calculates an LC50 using the lowest four concentrations, the LC50 was 4.19 mg/l (95% confidence range 2.23-115.69 mg/l). The LC50 is withing the 95% fiduciary range of the first test, but the range in this test is greatly increased. What remains unexplained is the lack of dose-response relationship above the LC50. One might speculate that this results from a solubility issue, but in the absence of concentration measurements to confirm the exposures, this cannot be verified.

Three toxicity tests were performed with *Leptocheirus plumulosus*, in July 1998 and July and August 1999 (both 4 months or more after the final tests of field collected sediment). The LC50 was 1.15 mg/l in July 1998 and 0.78 in both tests in July and August 1999. Though similar, these concentrations were significantly different.

The acute test for *Mulinia lateralis* and cadmium was accomplished simultaneously with the ambient water and pore water exposures. The LC50 was 0.074 mg/l based on survivorship. Survival at 0.1 mg/l consisted of one abnormal individual. At 0.032 mg/l, the percent abnormal larvae averaged 11 (6-15%). The percent abnormal at lower concentrations and in the control ranged from 1.5 to 3.6% (0-5.7%). Clearly, the change in percent abnormal is not great even for the standard toxicant until the acutely lethal concentration was approached or exceeded. Confidence limits could not be calculated by the inverse regression method recommended in ASTM Designation E724 and described in Sokal and Rohlf (1969). This inability to produce confidence limits was a result of high variance in the data and the low number of degrees of freedom.

DISCUSSION

The eastern shore of Virginia, as a highly agricultural area with significant access to the Bay for fishing, has been considered an area with low risk of chemical contamination. In 1996, a year with extremely heavy rainfall, it became apparent that there was significant risk of pulsed inputs of selected contaminants, especially pesticides used in tomato culture. In modern tomato culture, raised beds are covered with plastic sheets that funnel runoff into valleys between rows which then drain rapidly off the field. Tomato fields differ in management of water leaving fields, ranging from diversion into wooded areas or green ways, retention ponds, and in rare instances directly into a tidal creek along the shoreline. This pattern of operation has been observed in South Carolina and elsewhere, where pulsed inputs and adverse effects have been observed (Scott et al. 1987, 1990).

In Virginia, the initial reports of adverse effects now attributed to this agricultural method occurred in the early 1990's, culminating in 1996 during the period of excessive rainfall. At that time, a clam hatchery located on Gargathy Creek about a mile downstream of a tomato field experienced catastrophic culture failures at all stages of clam development following rain events, and the owner was able to document direct diversion of field runoff into the creek. While this creek drains to the ocean, this type of tomato culture is practiced in areas draining into the Bay. A hatchery located at the mouth of The Gulf also experienced serious culture failure in 1996. A tomato culture operation is located a short distance upstream, with water from the fields being captured in a retention pond that in normal years is reported to have minimal release into the creek (water reuse for irrigation is practiced), but during 1996, there were times when the pond discharged significant amounts of water to the creek. In response to these events and allegations, a limited *in situ* experiment was initiated in an effort to demonstrate the effects of rainfall events on the grass shrimp, *Palaemonetes pugio* in the vicinity of tomato culture operations (Luckenbach, et al., 1996). A major conclusion was that there was significant mortality of shrimp in areas adjacent to some tomato fields during rain events whereas in areas without tomato fields, mortality was essential absent. A question that remained was whether there were residues of pesticides accumulated in the sediments in the creeks that could degrade the quality of the benthic community.

The Virginian portion of the Bay shore of the Delmarva Peninsula had not previously been the subject of any efforts to characterize water or sediment. This limited effort was initiated to provide a preliminary examination of these parameters and to attempt to demonstrate pulsed impacts through the use of the *in situ* test.

Chemical characterization of creek water was limited to selected analytes that are relatively soluble in water and likely to occur in this environment given the major human activities in the

area. These included metals and butyltin compounds at two selected sites in all creeks, and selected chlorinated compounds and butyltin compounds in Hungar Creek and The Gulf in water exhibiting toxicity in one test.

In no case, were any of the metals found at concentrations exceeding or even approaching water quality criteria or state water quality standards. The only possible exception to this among the metals analyzed is mercury for which the detection limit exceeded the chronic water quality criterion. In all other cases, the detection limit was substantially below the chronic water quality criteria. Chlorinated hydrocarbons and butyltins were not detected in any water samples from the two creeks even in samples of water in which toxicity was observed.

Sediment samples from two sites in each creek were also characterized chemically. The metals thought likely to be present in significant amounts were copper, used extensively in tomato culture and as an antifoulant in the boating industry, and zinc, also used in agriculture. Yet the only metal analyte observed to exceed a sediment quality criterion (the ERL), was nickel, found in excess at one site each in Hungar Creek and The Gulf. The greatest exceedance was only 12%, well short of the ERM. Similar exceedances were observed in sediment from one of the reference sediment sites, Carter's Creek.

Sediment samples were also examined for semi-volatile organic compounds chlorinated hydrocarbons, and butyltins. Low molecular weight semi-volatile compounds were not detected in any creek. High molecular weight compounds were found above the detection limit, but none in amounts approaching a sediment quality guideline (when available). Few chlorinated compounds were detected, and all were pesticides or derivatives of pesticides (hexachlorobenzene, heptachlor, chlordane, DDT, dieldrin, and endrin) many of which are no longer in use. Concentrations were in every case low and below sediment quality criteria. The butyltins were not detected despite the low detection limit (1 ng/g).

These observations are fundamentally consistent with past perceptions that the area is not heavily contaminated with industrial chemicals. The sediments do not seem to have accumulated agricultural chemicals to any significant degree despite many years of heavy use of copper in the potato industry prior to its present use in tomato culture.

From a biological perspective, these systems seem generally "clean" with periodic and isolated incidences of toxic response. In the *in situ* study, despite only relatively minor rain events in each creek, slight mortality coincident in time to these rain events was observed in three of the creeks (Onancock, Hungar, and The Gulf). These rain events were much smaller in scale than those during a previous study in the area in which higher mortalities were observed (Luckenbach, et al., 1996). This observation suggests that the concentrations of chemical contaminants were higher during the 1996 study than the present study, but no water samples were analyzed during a rain event in either study.

This approach to the study of pulsed events is extremely labor intensive, limiting the number of sites that can be studied, and the time interval of study. Once one commits to deploying such an experiment, observation of an effect is a function of the thoughtfulness in site selection and the

probability of a rain event of sufficient magnitude to produce a pulsed input of one or more contaminants. Without substantial correlated sampling of water during deployment and especially during rain events, one cannot through this approach demonstrate a correlation between the biological effect (death) and any of the possible contaminants. A major research need, therefore, is to develop a simpler approach to field study of pulsed events that can be rapidly and safely deployed immediately before an event coupled with means of sampling water for chemical characterization. Some efforts have been made to achieve these ends, especially in the sampling of water (Scott, et al. 1987, 1990), but these are expensive and not widely used.

Toxicity testing of ambient water and submerged surficial sediments has been used in the Bay system for over a decade to characterize conditions with respect to toxic materials (Hall, et al. 1991, 1992, 1994, 1997). Through the use of multiple species with differing sensitivities to classes of contaminants, one seeks to take a snapshot of ambient conditions. The snapshot of water conditions relates to a relatively recent time frame, whereas that of sediment may reflect an integration of contaminant inputs over a longer time scale. Taken together, one obtains a reasonable impression of ambient conditions. With care in test design, one can exclude effects of salinity, oxygen, temperature, ammonia, and other less defined parameters unrelated to chemical contaminants, and therefore it is reasonable to consider these tests to measure toxicity of contaminants. The tests for water, even with moderately frequent resampling for water replacement, are unlikely to capture the effect of pulsed inputs, but rather reflect average conditions.

The endpoints used are easily measured; death, animal size or growth, hatching of fish embryos, and normality of development in bivalve embryos. This list is a mix of common endpoints used for acute and chronic tests for which there is sufficient familiarity to understand their meaning and reliability. That is not to say that these are necessarily the most sensitive measures of effect, but they are cost effective.

In the present study, a toxicological effect was observed only with *P. pugio* in water collected in fall of 1998 from all stations in Hungar Creek and The Gulf. There is no evidence of toxic chemical presence in the suite of analytes examined. All general water quality parameters were well within normal limits, and ammonia concentration was low. Thus, the observed and rather acute effects cannot be explained. There are several possibilities for explanation, but no evidence for or against any of them. For example, there may be one or more chemicals not included in our analyte list that nevertheless occurred in high concentration. The semi-quantitative relative retention indices method (Greaves, et al., 1991), though capable of detecting a wide array of compounds, it still limited by virtue of the extraction procedure and the nature of the detectors used. Therefore analytes do go undetected. Alternatively, there may have been a disease in the shrimp larvae. The disease explanation seems unlikely since it would also have resulted in death among the animals exposed to control water, and that did not occur. Similarly, any type of laboratory error would most likely have affected the control as well as the experimental animals, and that did not occur. It would have been desirable to resample and retest these creeks with *P. pugio*, but larvae were not available.

There is the possibility that the observation reflects a pulsed input effect, albeit we cannot identify

a potential causative agent. To examine this question, an attempt was made to obtain rainfall information for the period preceding the sampling, but no such records have been found for the Eastern Shore. The nearest airport with a continuous record is the Norfolk airport, and records from there are not necessarily good surrogates for rainfall on the eastern shore.

Water taken from the same locations two weeks later and tested with *C. variegatus* revealed no similar toxic effects. This difference might reflect a change in ambient water quality or a difference in sensitivity between the test species. Toxicological effects were not observed with either species at any site in Onancock and Old Plantation Creeks.

A truly synoptic sampling of all sites was used for the test with the bivalve embryo of *M. lateralis*. In this test, a high degree of normal development was observed (mean of three laboratory replicates >94%) was observed at all but two stations, Onancock 4 and The Gulf 4. At these sites, no embryos survived. No explanation can be offered in either case, but clearly, laboratory error is highly unlikely since all three replicates exhibited no survival.

Sediment samples tested with the amphipod *L plumulosus* and the fish *C. variegatus* exhibited no toxic effects in survival or percent hatch of fish embryos. The amphipod tests met all test acceptability criteria. The fish embryo tests had hatchability of controls slightly below the acceptability criterion, but that appears to have been the result of poor batches of eggs obtained from the supplier. Despite that, there were no sites with lower hatchability than the reference sites; indeed most sites yielded substantially better hatchability than the reference sites.

This calls into question the quality of sediment from the reference sites. In the initial test with fish, only sediment from Carter's Creek, long used in this laboratory as a reference material for *L. plumulosus* tests, was included in the test. To evaluate whether the poor reference group performance was site specific, sediment was obtained from the Poropotank River at or near the site from which the Applied Marine Research Laboratory at Old Dominion University obtains control sediment and test animals (Joe Winfield, personal communication). In subsequent tests with sediment from both reference sites, the two sediments yielded comparable results, with sediment from the Poropotank River yielding slight lower percent hatch than Carter's Creek. As noted earlier, both creeks are characterized as chemically clean (for the analytes measured there are few or no exceedances of sediment quality criteria).

For these sediment tests, a suitable reference site with sandy substrate was not included to control for effects of grain size. In some sense, the sediments from Site 1 in every creek can be considered a clean reference site since all are overwashed by Bay water at least twice daily. The animal responses to these sediments in every case were better than the responses to reference sediments, and in many cases could not be improved upon.

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APPENDIX A

Table A-1 Location references and Hydrographic / meteorological conditions for sampling dates.
Table A-2 List of Aromatic Retention Indices (ARI)
Table A-3 List of Halogenated Retention Indices (HRI)
Table A-4 List of Polar Retention Indices (POI)
Table A-5 Summary Table for Water Samples with Surrogate Recoveries
Table A-6 Summary Table for Sediment Samples with Surrogate Recoveries
Table A-7 Fortified Water Sample Results with Recoveries
Table A-8 Fortified Sediment Sample Results with Recoveries
Table A-9 Standard Reference Material 1941a Results with Recoveries

Table A-1 Summary of field sampling sites, dates, and conditions.

Creek	Site	Latitude	Longitude	Collection Date	Air Temp	Water Temp	Salinity	Tide Stage	Notes
Chesapeake Bay	Reference Site	N 37 13.934'	W 76 01.212'	4/26/99	14	14	22	Flood	
Onancock Creek	1	N 37 43.234'	W 75 49.861'	09/23/98			19		
Onancock Creek	2	N 37 43.619'	W 75 48.123'	09/23/98			19		
Onancock Creek	3	N 37 43.121'	W 75 46.935'	09/23/98			19		
Onancock Creek	4	N 37 42.629'	W 75 46.315'	09/23/98			19		
Onancock Creek	5	N 37 42.635'	W 75 45.574'	09/23/98			18		
Onancock Creek	1			10/14/98			20		
Onancock Creek	2			10/14/98			20		
Onancock Creek	3			10/14/98			20		
Onancock Creek	4			10/14/98			19		
Onancock Creek	5			10/14/98			19		
Onancock Creek	1	N 37 43.245'	W 75 49.775'	2/9/99	6	6	20	Ebb	cloudy
Onancock Creek	2	N 37 43.649'	W 75 48.130'	2/9/99	7	7	20	Ebb	
Onancock Creek	3	N 37 43.127'	W 75 46.927'	2/9/99	7	7	19	Ebb	
Onancock Creek	4	N 37 42.673'	W 75 46.344'	2/9/99	9	8	19	Ebb	
Onancock Creek	5	N 37 42.712'	W 75 45.527'	2/9/99	14	8	18	Slack	
Onancock Creek	1	N 37 43.246'	W 75 49.926'	4/26/99	18	17	19	Flood	cloudy
Onancock Creek	2	N 37 43.667'	W 75 48.112'	4/26/99	21	19	18	Flood	cloudy
Onancock Creek	3	N 37 43.140'	W 75 46.844'	4/26/99	22	19	16	Flood	cloudy
Onancock Creek	4	N 37 42.623'	W 75 46.326'	4/26/99	24	19	16	Low Slack	cloudy
Onancock Creek	5	N 37 42.707'	W 75 45.525'	4/26/99	21	21	14	Flood	cloudy

Table A-1 (con't.) Summary of field sampling sites, dates, and conditions.

Creek	Site	Latitude	Longitude	Collection Date	Air Temp	Water Temp	Salinity	Tide Stage	Notes
Hungars Creek	1	N 37 24.268'	W 75 58.612'	09/21/98			24		
Hungars Creek	2	N 37 24.932'	W 75 58.009'	09/21/98			23		
Hungars Creek	3	N 37 25.532'	W 75 57.456'	09/21/98			22		
Hungars Creek	4	N 37 25.853'	W 75 56.783'	09/21/98			22		
Hungars Creek	5	N 37 26.466'	W 75 59.898'	09/21/98			21		
Hungars Creek	1			10/12/98			22		
Hungars Creek	2			10/12/98			22		
Hungars Creek	3			10/12/98			22		
Hungars Creek	4			10/12/98			22		
Hungars Creek	5			10/12/98			20		
Hungars Creek	1	N 37 24.210'	W 75 58.604'	3/16/99	8	6	22	Ebb	
Hungars Creek	2	N 37 24.907'	W 75 57.952'	3/16/99	7	7	20	Ebb	
Hungars Creek	3	N 37 25.485'	W 75 57.502'	3/16/99	8	6	20	Ebb	
Hungars Creek	4	N 37 25.840'	W 75 56.715'	3/16/99	7	7	16	Ebb	
Hungars Creek	5	N 37 26.489	W 75 55.951'	3/16/99	9	7	4	Ebb	
Hungars Creek	1	N 37 24.238'	W 75 58.573'	4/26/99	18	18	20	Ebb	
Hungars Creek	2	N 37 24.906'	W 75 58.021	4/26/99	18	19	20	Ebb	
Hungars Creek	3	N 37 25.545'	W 75 57.416'	4/26/99	18	18	20	Ebb	
Hungars Creek	4	N 37 25.837'	W 75 56.789'	4/26/99	16	19	20	Ebb	
Hungars Creek	5	N 37 26.493'	W 75 55.902'	4/26/99	17	19	15	Ebb	

Table A-1 (con't.) Summary of field sampling sites, dates, and conditions.

Creek	Site	Latitude	Longitude	Collection Date	Air Temp	Water Temp	Salinity	Tide Stage	Notes
The Gulf	1	N 37 22.163'	W 75 59.003'	09/21/98			24		
The Gulf	2	N 37 21.875'	W 75 58.980'	09/21/98			24		
The Gulf	3	N 37 21.789'	W 75 58.518'	09/21/98			23		
The Gulf	4	N 37 21.763'	W 75 58.004'	09/21/98			21		
The Gulf	5	N 37 21.639'	W 75 57.674'	09/21/98			18		
The Gulf	1			10/12/98			24		
The Gulf	2			10/12/98			24		
The Gulf	3			10/12/98			23		
The Gulf	4			10/12/98			22		
The Gulf	5			10/12/98			20		
The Gulf	1	N 37 22.159'	W 75 59.077'	10/30/98	18	16.7	24	Ebb	
The Gulf	2	N 37 21.874'	W 75 58.989'	10/30/98	18	16.9	22	Flood	
The Gulf	3	N 37 21.823'	W 75 58.549'	10/30/98	20.5	17.5	21	Flood	
The Gulf	4	N 37 21.758'	W 75 58.065'	10/30/98	19	18	20	Flood	
The Gulf	5	N 37 21.637	W 75 57.600'	10/30/98	17	18.2	19	Flood	
The Gulf	1	N 37°22.160'	W 75°59.095'	2/8/99	7	7	23	Flood	cloudy & windy
The Gulf	2	N 37 21.849'	W 75 58.976'	2/8/99	7	7	23	Flood	cloudy & windy
The Gulf	3	N 37 21.775	W 75 58.516'	2/8/99	6	7	23	Flood	cloudy & windy
The Gulf	4	N 37 21.736'	W 75 58.047'	2/8/99	5	8	21	Flood	cloudy & windy
The Gulf	5	N 37 21.657'	W 75 57.636'	2/8/99	5	8	20	Flood	cloudy & windy
The Gulf	1	N 37 22.175'	W 75 59.084'	4/26/99	16	17	22	Ebb	
The Gulf	2	N 37 21.875'	W 75 58. 961'	4/26/99	16	16	22	Ebb	
The Gulf	3	N 37 21.774'	W 75 58.527'	4/26/99	16	18	20	Ebb	
The Gulf	4	N 37 21.760'	W 75 58.040'	4/26/99	16	20	18	Ebb	
The Gulf	5	N 37 21.669'	W 75 57.630	4/26/99	17	17	11	High Slack	

Table A-1 (con't.) Summary of field sampling sites, dates, and conditions.

Creek	Site	Latitude	Longitude	Collection Date	Air Temp	Water Temp	Salinity	Tide Stage	Notes
Old Plantation Creek	1	N 37 13.961'	W 76 00.586'	09/23/38			25		
Old Plantation Creek	2	N 37 14.019'	W 76 00.342'	09/23/38			25		
Old Plantation Creek	3	N 37 14.394'	W 75 59.797'	09/23/38			24		
Old Plantation Creek	4	N 37 14.891'	W 75 59.765'	09/23/38			24		
Old Plantation Creek	5	N 37 15.412'	W 75 59.492'	09/23/38			22		
Old Plantation Creek	1			10/14/98			26		
Old Plantation Creek	2			10/14/98			26		
Old Plantation Creek	3			10/14/98			25		
Old Plantation Creek	4			10/14/98			24		
Old Plantation Creek	5			10/14/98			24		
Old Plantation Creek	1	N 37 13.992'	W 76 00.569'	11/17/98	17	14	27	Ebb	
Old Plantation Creek	2	N 37 14.040'	W 76 00.295'	11/17/98	17	15	27	Ebb	
Old Plantation Creek	3	N 37 14.367'	W 75 59.789'	11/17/98	17.5	15	26	Ebb	
Old Plantation Creek	4	N 37 14.898'	W 75 59.774	11/17/98	17	16	25	Ebb	
Old Plantation Creek	5	N 37 15.414	W 75 59.516'	11/17/98	18	17	21	Low Slack	
Old Plantation Creek	1	N 37 13.999'	W 76 00.545'	4/26/99	14	14	24	High Slack	
Old Plantation Creek	2	N 37 14.026'	W 76 00.300'	4/26/99	13	14	23	High Slack	
Old Plantation Creek	3	N 37 14.398	W 75 59.769'	4/26/99	13	15	23	High Slack	
Old Plantation Creek	4	N 37 14.900'	W 75 59.781'	4/26/99	13	16	22	High Slack	
Old Plantation Creek	5	N 37 15.402'	W 75 59.484'	4/26/99	13	17	21	High Slack	

Table A-2 List of Aromatic Retention Indices (ARI) with corresponding probable compound identity.

ARI	Probable Compound Id
	Naphthalene,d8-
1000	Naphthalene
1035	Benzothiophene
1520	Naphthalene,2-methyl-
1615	Naphthalene,1-methyl-
2000	Biphenyl
2030	Naphthalene,ethyl-
2050	Naphthalene,C2H5-
2065	Naphthalene,2,6-dimethyl-
2100	Naphthalene,C2H5-
2150	Naphthalene,C2H5-
2175	Acenaphthylene
2195	Benzene,hexamethyl-
2255	Acenaphthene,d10-
2265	Acenaphthene
2280	Biphenyl,4-methyl-
2300	Biphenyl,3-methyl-
2325	Naphthalene,C3H7-
2345	Dibenzofuran
2360	Naphthalene,C3H7-
2380	Bibenzyl
2395	Naphthalene,C3H7-
2455	Naphthalene,C3H7-
2482	Naphthalene,1,6,7-trimethyl-
2518	Fluorene
2565	Biphenyl,methyl-
2620	Dibenzofuran,methyl-
2810	Fluorene,methyl-
2935	Dibenzothiophene
3000	Phenanthrene
3030	Anthracene
3235	Dibenzothiophene,methyl-
3310	Dibenzothiophene,methyl-
3360	Phenanthrene,3-methyl-
3375	Phenanthrene,2-methyl-
3425	Cyclopenta(def)phenanthrene,4H-
3440	Phenanthrene,methyl-
3457	Phenanthrene,1-methyl-
3605	Naphthalene,phenyl-
3610	Naphthalene,2-phenyl-
3740	Phenanthrene,C2H5-
3790	Base-neutral,MW=178, C2H5
3860	Fluoranthene
4000	Pyrene
4035	Naphthalene,methyl-,phenyl-
4130	Naphthalene,methyl-,phenyl-
4245	Terphenyl ISTD)
4300	Benzo(a)fluorene
4335	Retene
4366	Benzo(b)fluorene
4440	Base-neutral,methyl-202
4725	Binaphthyl,1,1'- (Sstd)
4777	Benzo(b)naphtho(2,1-d)thiophene
4800	Benzo(ghi)fluoranthene
4811	Benzo(c)phenanthrene
4910	Benzonaphthothiophene
4971	Benz(a)anthracene
5000	Chrysene
5060	Chrysene,tetramethyloctahydro-
5130	Base-neutral,methyl-228
5239	Phthalic acid,di-(2-ethylhexyl) ester
5310	Base-neutral,methyl-228
5390	Phenanthrene,1-phenyl-
5430	Chrysene,trimethyltetrahydro-
5738	Benzo(b)fluoranthene
5762	Benzo(k)fluoranthene
5805	Benzo(j)fluoranthene
5909	Benzo(e)pyrene
5925	Benzo(a)pyrene,d12
5946	Benzo(a)pyrene
6000	Perylene
6050	Base-neutral,methyl-252
6568	Quaterphenyl,para-
6820	Indeno(1,2,3-cd)pyrene
6860	Dibenz(a,h)anthracene
7000	Benzo(ghi)perylene

Table A-3 List of Halogenated Retention Indices (HRI) with corresponding probable compound identity.

HRI	RRF	Probable Compound Id	HRI	RRF	Probable Compound Id
870	0.8	Benzene,tetrachloro-	2674	0.68	PCB-40
1000	0.22	2-Chloronaphthalene	2682	0.76	PCB-103
1405	0.27	PCB-1	2699	0.68	PCB-67,100
1460	1	Pentachlorobenzene (ISTD)	2701	0.935	Chlordane(1)
1620	0.27	PCB-2	2721	0.68	PCB-63
1643	0.27	PCB-3	2735	0.9	Heptachlor epoxide
1770	0.45	PCB-4,10	2736	0.68	PCB-74
1913	0.45	PCB-7,9	2758	0.68	PCB-70
1973	0.45	PCB-6	2762	0.68	PCB-66
1999	1.03	Benzenehexachloride,alpha-	2768	0.76	PCB-95
2009	0.45	PCB-8,5	2771	0.76	PCB-88
2038	1.06	Benzene,hexachloro-	2783	0.76	PCB-121
2048	0.89	Anisole,pentachloro-	2791	0.76	PCB-91
2086	0.58	PCB-19	2802	0.935	Chlordane(3)
2116	1.03	Benzenehexachloride,beta-	2827	0.974	Chlordane,trans-
2128	0.58	PCB-30 (Sstd)	2827	0.68	PCB-60,56
2141	1.03	Benzenehexachloride,gamma-	2836	0.76	PCB-92
2151	0.45	PCB-11	2846	0.63	DDE,2,4'-
2181	0.58	PCB-18	2846	0.76	PCB-84
2189	0.58	PCB-17,15	2852	0.974	Chlordane(5)
2227	0.58	PCB-24,27	2857	0.76	PCB-90,101
2240	1.03	Benzenehexachloride,delta-	2876	0.741	Endosulfan I
2261	0.58	PCB-16,32	2876	0.76	PCB-99
2300	0.58	PCB-34	2886	0.974	Chlordane,cis-
2315	0.58	PCB-29	2899	0.76	PCB-119
2338	0.58	PCB-26	2906	0.99	Nonachlor,trans-
2345	0.58	PCB-25	2918	0.76	PCB-83
2358	0.883	Chlordane(C)	2934	0.974	Chlordane(7)
2380	0.58	PCB-28,31	2936	0.76	PCB-97
2414	0.58	PCB-33,20	2956	0.76	PCB-87,115
2417	0.68	PCB-53	2968	0.63	DDE,4,4'-
2424	0.883	Chlordene,alpha-	2971	0.76	PCB-85
2438	0.935	Heptachlor	2976	0.79	Dieldrin
2444	0.58	PCB-22,51	2979	0.83	PCB-136
2464	0.68	PCB-45	2989	0.68	PCB-77
2495	0.68	PCB-46	2996	0.76	PCB-110
2499	0.68	PCB-69	3000	0.63	DDD,2,4'-
2519	0.68	PCB-52	3034	0.83	PCB-82,151
2534	0.68	PCB-49	3052	0.83	PCB-135
2546	0.68	PCB-47,75,48	3054	0.792	Endrin
2562	0.68	PCB-65 (Sstd)	3067	0.83	PCB-107
2575	0.829	Aldrin	3085	0.741	Endosulfan II
2578	0.883	Chlordene,gamma-	3077	0.83	PCB-149
2578	0.58	PCB-35	3091	0.76	PCB-118
2598	0.68	PCB-44	3111	0.83	PCB-134
2608	0.68	PCB-37,42,59	3114	0.712	DDD,4,4'-
2644	0.68	PCB-41,64	3123	0.76	PCB-122,131

Table A-3 (con't.) List of Halogenated Retention Indices (HRI) with corresponding probable compound identity.

HRI	RRF	Probable Compound Id	HRI	RRF	Probable Compound Id
3130	0.7	DDT,2,4'-			
3135	0.99	Nonachlor,cis-			
3143	0.83	PCB-146			
3149	0.792	Endrin aldehyde			
3162	0.83	PCB-153,132			
3163	0.883	Chlordane(K)			
3177	0.76	PCB-105			
3207	0.89	PCB-179,141			
3229	0.83	PCB-130			
3231	0.7913	Endosulfan sulfate			
3233	0.89	PCB-176,137			
3241	0.7	DDT,4,4'-			
3255	0.83	PCB-138,158			
3283	0.89	PCB-178,129			
3302	0.89	PCB-175			
3312	0.89	PCB-187			
3328	0.89	PCB-183			
3346	0.83	PCB-128			
3356	0.83	PCB-167			
3369	0.89	PCB-185			
3389	0.89	PCB-174			
3390	0.792	Endrin ketone			
3407	0.89	PCB-177			
3422	0.83	PCB-171,156			
3437	0.5	Dicofol			
3435	0.43	Methoxychlor			
3444	0.93	PCB-157,201,173			
3453	0.93	PCB-204 (Sstd)			
3463	0.89	PCB-172			
3483	0.89	PCB-180			
3492	0.411	Diphenyl ether,2,2',4,4'-tetrabromo-			
3519	0.93	PCB-193			
3543	1.1	Mirex			
3580	0.93	PCB-170,190			
3607	0.93	PCB-199			
3625	0.93	PCB-196,203			
3676	0.89	PCB-189			
3721	0.96	PCB-208,195			
3742	0.96	PCB-207			
3771	0.26	Permethrin,cis-			
3781	0.44	Diphenyl ether,2,2',4,4',6-pentabromo-			
3788	0.93	PCB-194			
3797	0.26	Permethrin,trans-			
3805	0.93	PCB-205			
3859	0.44	Diphenyl ether,2,2',4,4',5-pentabromo-			
3906	0.96	PCB-206			
4000	1	PCB-209			
5000	0.87	Dibenzodioxin,octachloro-			

Table A-4 List of Polar Retention Indices (POI) with corresponding probable compound identity.

POI	Probable Compound Id
3185	Benzoquinoline
3210	Carbazole,9H-
3460	Carbazole,9H-,methyl-
3495	Carbazole,9H-,methyl-
3900	Carbazole,9H-,C2H5-
3920	Base-neutral,aza-202
3980	Base-neutral,aza-202
5040	Benzocarbazole
5110	Benzanthrone/Benzofluorenone
5175	Benzocarbazole
5215	Benzocarbazole
5680	Base-neutral,aza-252
5755	Base-neutral,aza-252
5920	Benzacridine,C2H5-
5955	Cyclopenta(def)chrysen-4-one
6100	Benzacridine,C2H5-
6100	Benzacridine,C2H5-
6635	Dibenzocarbazole
6750	Dibenzocarbazole
6980	Dibenzocarbazole
7030	Indenylanthracenone
7090	Dibenzocarbazole
7145	Dibenzocarbazole
7175	Dibenzocarbazole
7245	Dibenzocarbazole,methyl-
7310	Dibenzocarbazole,methyl-
7350	Dibenzocarbazole,methyl-
7370	Dibenzocarbazole,methyl-

Table A-6 Summary Table for Sediment Samples with Surrogate Recoveries

Sample Identification	Total Concentration (ng/g) Semivolatile PAHs	PCBs/OCPs	Semipolar PAHs	Polar OCPs	Extraction Weight -dry (g)	Percent Total Solids	Sample Collection Date	Surrogate Recovery (%) 1,1' binaphthyl	PCB 30	PCB 65	PCB 204
Blank #1	12.80	0.00	0.00	0.41	40	-		92%	15%	43%	90%
Blank #2	2 85	0 21	4.15	0.00	50	-		90%	38%	68%	84%
OP 2-1	21 48	1.30	0 00	0.05	50	81.6	11-17-98	101%	25%	61%	95%
OP 2-2	2 82	0 12	0.00	0.00	50	78.5	11-17-98	96%	48%	77%	96%
OP 4-1	144.23	1.60	191.36	0.55	40	66.7	11-17-98	101%	43%	69%	89%
OP 4-2	96.61	0.87	30.84	0.52	45	68.4	11-17-98	97%	54%	81%	97%
OP 4-2a	120.52	0.95	32.56	0.38	45	68.4	11-17-98	95%	54%	77%	91%
ON 2-1	8 45	0.93	9.71	0.03	50	81.7	02-09-99	94%	37%	68%	94%
ON 2-2	3.00	0 00	5 73	0.00	50	80.4	02-09-99	114%	51%	74%	81%
ON 4-1	193 34	1 32	90.49	0.75	45	75.7	02-09-99	91%	40%	64%	97%
ON 4-2	93 36	0.57	0.00	0.00	45	74.1	02-09-99	93%	45%	72%	88%
HC 2-1	340.17	6.56	310.39	1.21	25	42.2	03-16-99	100%	38%	61%	89%
HC 2-1a	377 15	8 10	315.88	0.89	25	42.2	03-16-99	98%	36%	60%	84%
HC 2-2	390.44	9.27	106.20	0.44	25	33.8	03-16-99	97%	59%	79%	90%
HC 4-1	94 03	1 69	124.67	0.34	40	69.7	03-16-99	102%	39%	67%	88%
HC 4-2	88 32	2 07	26.45	0.00	40	64.3	03-16-99	95%	55%	78%	98%
TG 2-1	4 03	0.40	18.10	0.03	50	80.8	02-08-99	99%	18%	48%	91%
TG 2-2	5 76	0.07	0.00	0.00	50	81.7	02-08-99	93%	45%	71%	93%
TG 4-1	644 61	34.00	940.07	3.61	20	29.2	02-08-99	100%	52%	72%	93%
TG 4-2	653 66	42.78	250 28	1 88	20	28.6	02-08-99	99%	52%	73%	78%
Poropotank #1	272.42	3.37	407.70	0.00	35	46.5	02-03-99	101%	38%	64%	91%
Poropotank #2	395 63	3 74	88.84	1 07	35	53.1	02-03-99	92%	59%	78%	83%
SRM 1941a	9466 60	376.38	1272.51	13.14	7	-	-	99%	47%	72%	90%

Total Concentrations (ng/g) are Total Resolved Peaks (ng/g) from respective Retention Indices

Table A-7 Fortified Water Sample Results with Recoveries

Analyte Identification	Recovery (%)	
	Deionized Water	Water Sample HC
Organochlorine Pesticide		
1,2Dibromo-3-chloropropane	74%	38%
Hexachlorocyclopentadiene	37%	29%
Ethridiazole	116%	112%
Chloroneb	117%	153%
Propachlor	225%	256%
Trifluarin	71%	96%
Diallate	79%	100%
Diallate	86%	103%
Hexachlorobenzene	62%	90%
PCB 30 - surrogate	58%	79%
Pentachloronitrobenzene	96%	112%
Chlorothalonil	161%	188%
Alachlor	227%	244%
PCB 65 - surrogate	70%	88%
Metolachlor	161%	175%
Chloropropylate	61%	48%
DCPA	140%	153%
Isodrin	129%	175%
Captan	148%	103%
t-nonachlor	161%	192%
Perthane	189%	208%
Chlorobenzilate	141%	147%
Captafol	96%	38%
Dicofol	102%	42%
PCB 204 - surrogate	90%	90%
Mirex	112%	100%
?-permethrin	225%	144%
?-permethrin	241%	144%
PCB 209 - surrogate	79%	52%
Carbamate pesticide		
chloropropham	91%	
SWEP	76%	
PCB 30 surrogate	258%	
Linuron	92%	
PCB 65 surrogate	88%	
Barban	122%	
PCB 204 surrogate	88%	
Nitrogen/Phosphorous pesticide		
dichlorovos	35%	
Propachlor	66%	
Chloropropham	41%	
Trifluralin	33%	
Atrazine	54%	
propazin	49%	
PCB 30 surrogate	154%	
Terbacil	77%	
Alachlor	59%	
PCB 65 surrogate	100%	
Metolachlor	77%	
Cyanazine	64%	
Chlorpyrifos	52%	
Stirofos	48%	
Butachlor	75%	
Norflurazon	38%	
PCB 204 surrogate	39%	
Fenarimol	36%	

Table A-7 (con't) Fortified Water Sample Results with Recoveries

Analyte Identification	Recovery (%)	
	Deionized Water	Water Sample HC
Chlorinated Herbicide		
3,5 dichlorobenzoic acid	53%	
Dichlorprop	54%	
2,4,D	60%	
pentachlorophenol, silvex, chloramben	64%	
PCB 30 surrogate	61%	
2,4,5-T	70%	
2,4-DB	73%	
picloram	99%	
PCB 65 surrogate	69%	
DCPA	75%	
PCB 204 surrogate	69%	

Table A-8 Fortified Sediment Sample Results with Recoveries. Analytes in bold type are added standards. Sediments are from station ON 2-2. Both samples were fortified with PAH analytes at 20.0 ug and organochlorine pesticide analytes at 500.0 ng each prior to extraction.

Analyte	Recovery (%) ON 2-2	ON 2-2	Analyte	Recovery (%) ON 2-2	ON 2-2
Naphthalene	0%	7%	**Pentachlorobenzene (ISTD)**	100%	100%
Acenaphthylene	13%	36%	a-BHC	65%	63%
Acenaphthene	19%	37%	b-BHC	68%	46%
Fluorene	53%	55%	**PCB-30 (Sstd)**	40%	82%
Phenanthrene	93%	74%	g-BHC	92%	107%
Anthracene	88%	72%	d-BHC	76%	90%
Carbazole	99%	79%	Heptachlor	74%	72%
Fluoranthene	117%	91%	**PCB 65 (Sstd)**	60%	74%
Pyrene	119%	93%	Aldrin	77%	55%
p-terphenyl (ISTD)	100%	100%	Heptachlor Epoxide	64%	77%
binaphthyl, 1,1' (Sstd)	124%	97%	t-chlordane	92%	90%
Benz(a)anthracene	109%	89%	endosulfan I	45%	62%
Chrysene	110%	90%	c-chlordane	102%	96%
Benzo(b)fluoranthene	115%	83%	DDE,4,4'-	93%	97%
Benzo(k)fluoranthene	73%	60%	Dieldrin	75%	91%
Benzo(a)pyrene	85%	63%	Endrin	51%	65%
Indeno(1,2,3-cd)pyrene	92%	62%	endosulfan II	90%	96%
Dibenzo(a,h)anthrene	65%	44%	DDD,4,4'-	72%	76%
Benzo(ghi)perylene	79%	51%	Endrin aldehyde	36%	36%
			Endosulfan sulfate	8%	11%
			DDT,4,4'-	80%	84%
			Endrin ketone	59%	65%
			Methoxychlor	5%	3%
			PCB-204 (Sstd)	86%	91%

Table A-9 Recovery Results for Standard Reference Material 1941a. Recovery of SRM 1941a certified (bold type) and non-certified analytes following cleanup and fractionation

Compound Id	Recovery (%)	Compound Id	Recovery (%)
Naphthalene	4%	PCB 8	43%
Biphenyl	7%	**Hexachlorobenzene**	43%
Acenaphthylene	22%	PCB 18	122%
Acenaphthene	52%	PCB 28	43%
Fluorene	29%	**PCB 52**	70%
Dibenzothiophene	87%	**PCB 49**	41%
Phenanthrene	56%	**PCB 44**	66%
Anthracene	46%	**PCB 95**	132%
Phenanthrene,3-methyl-	80%	**2,4' DDE**	375%
Phenanthrene,2-methyl-	56%	**PCB 101**	77%
Cyclopenta(def)phenanthrene,4H-	130%	**PCB 99**	135%
Phenanthrene,methyl-	46%	**a-chlordane (cis)**	57%
Fluoranthene	71%	**t-nonachlor**	117%
Pyrene	71%	**PCB 87**	49%
Binaphthyl,1,1'- (Sstd)	99%	**4,4' DDE**	118%
Benzo(c)phenanthrene	219%	Dieldrin	35%
Benz(a)anthracene	82%	**PCB 110**	105%
Chrysene	134%	**PCB 149**	83%
Benzo(b)fluoranthene	83%	**PCB 118**	68%
Benzo(k)fluoranthene	108%	**4,4' DDD**	73%
Benzo(j)fluoranthene	26%	**PCB 153**	55%
Benzo(e)pyrene	70%	**PCB 105**	91%
Benzo(a)pyrene	53%	4,4' DDT	114%
Perylene	54%	**PCB 138, 163,164**	99%
Indeno(1,2,3-cd)pyrene	54%	PCB 187, 182	65%
Dibenz(a,h)anthracene	86%	PCB 183	121%
Benzo(ghi)perylene	44%	**PCB 128**	190%
		PCB 156	267%
		PCB 180	111%
		PCB 170, 190	80%
		PCB 194	72%
		PCB 206	52%
		PCB 209	73%

APPENDIX B

(Provided as electronic copy only)

Consists of data files for toxicity tests as Quattro Pro spreadsheets and for organic chemistry chromatograms as Excel spreadsheets.

All toxicity test data is included in a subdirectory of the disc so named. Each file name is descriptive of content: a two-letter species code, the experiment number, and the type of data included. Since some tests were accomplished simultaneously, some files refer the reader to another file that contains the appropriate data. Each experiment has multiple files to include field collection data, daily mortality data, water quality data during test, and additional measurements made. All sediment tests include sed in the title.

All organic chemical data is grouped in a series of subdirectories indicating the type of data included. Each subdirectory includes a series of files, one for each sample processed for that data type.