THE ELIZABETH RIVER MONITORING PROGRAM 2006-2007: ASSOCIATION BETWEEN MUMMICHOG LIVER HISTOPATHOLOGY AND SEDIMENT CHEMICAL CONTAMINATION

A Final Report Submitted to

Mr. Roger Everton
The Virginia Department of Environmental Quality

by

Wolfgang K. Vogelbein, Michael Unger & David Gauthier
Department of Environmental & Aquatic Animal Health
Virginia Institute of Marine Science
The College of William and Mary
Gloucester Point, VA 23062

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ABSTRACT

The Virginia Department of Environmental Quality has conducted an environmental monitoring program in the Elizabeth River, Virginia for several years. The overall aim of this monitoring program has been to develop an assessment of the rivers "health" and to develop methods that would allow DEQ to track the state of the watershed through implementation of a long-term monitoring program for water, sediment and biota. This report summarizes the results from an investigation of mummichog (*Fundulus heteroclitus*) liver pathology and sediment chemical analyses. Adult mummichogs were collected during fall 2006 from 16 study sites within the Elizabeth River. We attempted to obtain 60 fish per study site but were not able to obtain that many at several sites. The livers of these fish were analyzed for adverse health effects by routine histological methods. Replicate sediment samples were collected from the same study sites and analyzed by capillary gas chromatography with flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS) for select polycyclic aromatic hydrocarbons (PAH). Results indicate that proliferative liver lesion prevalence (pre-cancerous altered hepatocellular foci and liver neoplasms [cancer]) varied among the study sites, with highest prevalences occurring in fish from heavily industrialized sites in the eastern and southern branches. Sites with the highest liver lesion prevalences were heavily contaminated with PAH. Lowest lesion prevalences occurred in fish from the more mildly PAH contaminated residential portions of the river. The clear positive association between sediment PAH concentrations and occurrence of liver cancers and related proliferative and toxigenic lesions strongly suggests a cause and effect relationship between the two. A prior monitoring study (DEQ Final Report, 2003) suggested that mummichog liver lesion prevalences in the Elizabeth River had experienced a significant decline between 1998 and 2001at two of the most heavily contaminated sites (SBB1, SBD3). However, based on a ranking scheme devised in collaboration with the DEQ and the Elizabeth River Project, it was clear that environmental quality of the river had not changed drastically over that time period. This study indicates that mummichog liver lesion prevalences remain very high at certain heavily contaminated sites in the Elizabeth and that the two study sites showing declining liver lesion prevalence in 2001 are again showing high prevalences of hepatic neoplasms and altered hepatocellular foci. Analysis of four new study sites in the Money Point area of the southern branch of the river indicates high prevalence of altered hepatocellular foci in the central portion of that area (SBD7). The data presented in this report provide DEQ with valuable baseline data on a suite of specific adverse health impacts in an indigenous shallow water estuarine cyprinodontid fish species and the associaton of these proliferative liver lesions with sediment concentrations of PAHs.

Additional future monitoring of mummichogs and sediment chemical contamination is suggested (perhaps at 3 year intervals) to determine if these liver lesions, in association with specific sediment chemical analyses, can be used effectively to track environmental recovery following site remediation efforts now underway at select sites in the Elizabeth River (e.g., Money Point, Scuffeltown Creek, Atlantic Wood).

INTRODUCTION

The Virginia Department of Environmental Quality (DEQ) has initiated a long-term environmental monitoring program of the Elizabeth River in Virginia. The overall aim of this program is to develop an assessment of the rivers "health" and to track the state of the watershed by implementation of a long-term ambient monitoring program for water, sediment and biota (Barbachem et al., 1997). One specific goal of this program is to evaluate and document any potential adverse biological effects of chemical exposure in indigenous biota of the river and to track environmental recovery over time as pollutant inputs are reduced and site-specific remediation efforts are advanced.

Recent investigations by the Virginia Institute of Marine Science within the Elizabeth River indicate that the small, abundant and non-migratory mummichog (*Fundulus heteroclitus*) is an effective bio-indicator of adverse health effects attributable to pollutant exposure (Vogelbein et al., 1999). Histologic endpoints (i.e. cytotoxic, pre-cancerous, cancerous and other liver lesions) have been used as indicators of health impacts caused by chemical exposure in these indigenous fish. Further, long-term laboratory challenge studies recently completed at VIMS, indicate that pre-cancerous and cancerous liver alterations can be attributed directly to polycyclic aromatic hydrocarbon (PAH) exposure, with lesion prevalences exhibiting a clear positive correlation to total sediment PAH concentrations (Vogelbein unpublished) . Prior DEQ-funded efforts (see Final Reports: Vogelbein, 1998; Vogelbein and Zwerner, 1999; Vogelbein and Unger, 2003) have identified a number of locations within the River where mummichogs exhibit exceedingly high prevalences of liver cancer and related lesions.

The application of fish liver histopathology and local sediment chemistry into the Elizabeth River Monitoring Program has provided DEQ managers with sound data on direct negative health impacts attributable to local chronic toxicant exposure occurring in a native animal population. Because this fish is largely non-migratory, with a very restricted summer home range (Lotrich, 1975), we believe that it is an excellent integrator of contaminant exposure in localized restricted estuarine environments. Thus, the health of a given local fish population will reflect the "health" or quality of that populations' immediate environment. This type of approach has in recent years been adapted by the National Oceanographic and Atmospheric Administration's (NOAA) National Status and Trends Program and by the U. S. Environmentqal Protection Agency (EPA). NOAA is now vigorously pursuing the use of these types of data in litigation of select polluters.

Briefly, an initial feasibility study was conducted during the spring of 1998 in order to obtain preliminary data from a small number of study sites (8) within the river and to determine the applicability of fish histopathology data in a pollution-monitoring context. The goal of this early study was to evaluate the utility of these sites for inclusion in the longer-term program and to evaluate the use of tissue histopathology as a routine method for pollution effects monitoring. Based on very promising results of this preliminary investigation, a more in-depth study was conducted during Fall 1998. DEQ staff selected 12 study sites for collection and histopathologic

analysis. This investigation focused on three target tissues in native mummichog, the liver, kidney and gills. Findings indicated that clear associations between lesion prevalence and levels of chemical pollution occurred only in the liver tissues. Although a variety of pathological endpoints were observed in the gills and kidney, a clear association with habitat degradation could not be made. However, mummichog liver histopathology was found to be an effective indicator of adverse health impacts directly attributable to chemical exposure of the fish in this river system (Vogelbein and Zwerner, 1999). In order to evaluate temporal trends, a similar monitoring effort was conducted in 2001 that focused specifically on mummichog liver. This was done because the 1998 study had shown no clear associations between gill and kidney pathology and chemical contamination, it allowed us to control costs and thereby add wide spectrum sediment chemical analyses in close proximity to where the fish were actually being collected.

The monitoring study described here was conducted during Fall 2006 and aims to extend observations made during Fall 1998 and 2001 (Vogelbein and Zwerner, 1999; Vogelbein and Unger, 2003) on the adverse health effects of contaminant exposure in Elizabeth River mummichog. This effort extends the use of mummichog liver histopathology in the long-term pollution field monitoring program initiated in the Elizabeth River by the DEQ during 1998. Because previous experience indicated that liver pathology in mummichog is most significant during the fall of the year, we conducted the collection phase of this study during November – December, 2006. This collection effort was somewhat delayed (late November vs. late October) in comparison with the prior monitoring efforts because of a delay in obtaining the funding. The overall goal of this monitoring study was to again use mummichog liver histopathological endpoints within this heavily industrialized river system as a method to monitor the extent of adverse chemical contaminant effects. Specific objectives included: 1) a histopathological evaluation of the livers of mummichogs from 16 study sites, four of them new ones and 2) an evaluation of chemical contaminants, with a focus on selected polycyclic aromatic hydrocarbons (PAH) present in the immediate environment where the fish were collected.

The present monitoring study extends our spatial and temporal coverage of adverse health impacts in Elizabeth River mummichog as well as sediment PAH measurements. For this sampling effort we added 4 new study sites in the Money Point area of the southern branch as this area is scheduled for sediment remediation efforts and we urgently need some baseline information on adverse health impacts in this portion of the river.

MATERIALS & METHODS

Field samples of mummichogs from 16 study sites within the Elizabeth River were collected during mid to late November, 2006 (Table 1, Figure 1). Six standard metal minnowtraps were baited with whole frozen crushed blue crab and deployed for 30 - 60 minutes at each of the study sites. Traps were retrieved and the largest fish were culled from the sample. The remaining smaller fish were released back into the habitat from which they were trapped. Sixty large adult mummichogs were obtained at 10 of the 16 study sites. However, as the season

progressed and water temperature declined, it became increasingly difficult to catch fish. At certain study sites, the fish were also smaller than those collected earlier. Sample sizes for the other 6 study sites ranged from 11 to 52 fish, despite setting the traps at these sites numerous times. Fish were collected along the shore, as near to the original DEQ-designated stations (see Vogelbein and Zwerner, 1999) as possible, in all cases within several hundred meters of the exact coordinates for the given stations. Four study sites are noted in bold in Table 1 (SBD6, 7, 8, 9). These were sites in the Money Point portion of the Southern Branch that were newly included in the monitoring program for 2006. Specific GPS coordinates for all of the study sites are provided in Table 1. These coordinates represent the actual locations where fish and sediment samples for this study were collected. A total of 750 fish were collected over 5 days. All fish were successfully transported live to VIMS and were necropsied within 7 days of collection.

Sediments were collected on 7 March and 9 March, 2007 at the16 Elizabeth River study sites listed in Table 1 and illustrated in Figure 1. Specific coordinates for sediment collection sites differ slightly from those for fish and are provided in Table 2. As sediment chemical levels exhibit spatial patchiness, even within a given study site, three replicate composite samples were collected at each locality. To produce a composite sample, three surficial grab samples were obtained from each of the study sites with a Ponar grab and then homogenized in a stainless steel bucket. Sediment samples were collected in solvent washed glass jars and sealed with teflon-coated lids. They were transported to the laboratory on ice and frozen until analysis. Two composite samples were slated for PAH analysis and the third was kept as a back up to be analyzed if replicates were observed not to be in good agreement. Previous work has shown that variability at a station is relatively low when compared to differences across the station locations.

Fish Necropsy, Tissue Processing and Histologic Evaluation: Fish were dissected and livers were chemically preserved for >72 h in Z-Fix fixative (Anatech, Battle Creek). They were subsequently rinsed in 70% EtOH. Livers were sectioned with a single-edge razor blade into 6 slices, placed in a tissue embedding cassette and assigned a unique specimen identification number. In this way, each liver was simultaneously evaluated at 6 different levels. After cassetting, livers were processed for routine paraffin histology (Prophet et al., 1994). All liver samples were dehydrated through a graded ethanol series, cleared and infiltrated with paraffin in a Shandon Hypercenter tissue processor. Tissues were then embedded in paraffin on a Tissue Tech embedding center. Tissue blocks were sectioned at 5ìm on a rotary microtome (Olymbus Cut 4055) and stained with hematoxylin and eosin (H&E) in a Shandon Varistain 24-3 automatic slide stainer. Slides were coverslipped, oven-dried and evaluated histologically on an Olympus AX-70 photomicroscope. Liver lesion nomenclature for the current study follows that outlined elsewhere (Vogelbein et al., 1990, 1997, Vogelbein and Zwerner, 2000).

Sediment Chemical Analyses: Sediment samples were analyzed by the VIMS protocol for toxic organic chemicals (Greaves et al., 1991; see also Mulvey et al., 2002, Ownby et al., 2002, Mulvey et al., 2003). Briefly, sediments were freeze dried, spiked with surrogate standards, and extracted with dichloromethane by accelerated solvent extraction (ASE). The resulting extracts were fractionated by GPC and silica gel and analyzed for aromatic or heterocyclic compounds by

capillary gas chromatography spectrometry (GC/MS) in the full scan electron ionization mode. Aliquots of each environmental sediment sample were also analyzed for grain size and total organic carbon. Blank samples, duplicates and standard reference materials were analyzed along with environmental samples to assure data quality.

Statistics: Standard linear regression models are often inappropriate for the analysis of the relationship between binary (e.g. prevalence) or categorical (e.g hepatotoxicity score) outcomes and exposures (Woodward 2005). Consequently, logistic regression analysis was conducted to investigate the relationship between liver disease outcomes and the risk factors sex, weight (as a proxy for age), and mean sediment log PAH concentration for the site at which fish were collected. Logistic regression models were originally formulated with all variables and potential interaction terms, and the latter were eliminated if found to have a nonsignificant odds ratio (OR) (Kleinbaum et al. 2002). Logistic regression examines multiple risk factors simultaneously, and assigns an "odds ratio" to each variable that is corrected for the other variables. This corrected odds ratio indicates whether a particular exposure is significantly positively or negatively related to risk for an outcome. An odds ratio (abbreviated OR) greater than one indicates that the variable is a positive risk factor for the outcome, whereas an odds ratio of less than one indicates that a variable is negatively related to the outcome, or in some cases, protective against the outcome. An odds ratio that is not significantly different from one (i.e. 95% confidence interval includes one) indicates no relationship between the outcome and the variable of interest.

RESULTS

Fish Pathology: Fish collection data and meristics are summarized in Table 3 and Figure 2. Sixty adult mummichogs were obtained from each of ten of the 16 study sites, whereas fewer and generally smaller fish were obtained from the 6 remaining sites. Mean total lengths and weights varied from 67.37 - 89.7 mm and 3.67 - 9.44 g, with the largest fish collected at station SBD7 (new Money Point Central site). The smallest fish were obtained from EBB2 (Colona Shipyard), a highly industrialized site in the eastern branch with very little and highly marginal mummichog habitat. Sex ratios varied widely from site to site.

Results of mummichog liver pathology and parasitology from the 16 study sites are summarized in Table 4 and Figure 3. Highest proliferative liver lesion prevalences, including putatively pre-cancerous altered hepatocellular foci (AHF) and cancerous lesions such as hepatocellular adenoma and carcinoma (HN), were observed in fish from study sites in the Eastern Branch (EBB2) and Southern Branch (SBB1, SBD3, SBD6, SBD9) only. Elevated prevalence of AHF occurred at six study sites (SBB1, SBD3, EBB2, SBD7, SBD6, SBD9). Site SBD7 (Money Point central) exhibited a very high a prevalence (40%) of AHF but no hepatic neoplasms. All of these sites are located in the most industrialized portions of the river, with the Eastern Branch site located near a commercial ship yard and the Southern Branch sites located near three wood treatment plants that have traditionally used creosote to pressure treat timbers. None of the other study sites exhibited cancerous lesions. Most, however, exhibited low (background <5%) prevalences of AHF, with lowest lesion prevalences observed in fish from

the more residential stretches of the river (e.g., Western Branch, Lafayette River, southern-most portion of the Southern Branch). Parasitic infections of the liver were relatively rare in all of the fish with the exception that moderate to high levels of *Myxidium* sp. infections were observed in the bile ducts of fish from the two sites in the Eastern Branch (Table 4). Figure 4 illustrates proliferative liver lesion prevalences for 12 study sites investigated during fall 1998 and 2001. Although liver lesion trends were very similar for 1998 and 2001 and again mirror essentially the same pattern in 2006, lesion prevalences exhibited a substantial drop from 1998 to 2001 for several heavily contaminated sites (e.g., SBB1, SBD3). In contrast, site EBB2 exhibited a substantial increase in altered foci and hepatic neoplasms from 1998 to 2001. Liver lesion prevalence at sites SBB1 and SBD3 are again high in 2006 and very similar to those observed during 1998.

Logistic regression analysis showed Log PAH concentration to be a significant risk factor for development of altered foci (OR 3.50; 95% CI 4.66-2.63), and hepatic neoplasms (4.21; 7.13-2.48). Increasing log PAH concentration was a significant risk factor for stage-3 (6.16; 16.301-2.32), stage-2 (2.27; 3.59-1.44), and stage-1 (1.68; 2.4-1.31) hepatotoxicity indices (See Table 4). Sex and weight were found not to be significant risk factors for liver pathology, and interaction terms between all variables were nonsignificant (and thus not included in final models).

Sediment Chemical Contaminants: Concentrations of 18 selected polycyclic aromatic hydrocarbons (PAH) in sediments from the 16 Elizabeth River study sites are summarized in Table 5. Ten low molecular weight and 8 high molecular weight PAH are tabulated. Sum concentrations for the low and high molecular weight compounds and total PAH concentrations are tabulated. Sediment PAH concentrations across the 16 study sites varied by several orders of magnitude, with highest concentrations observed in the heavily industrialized portions of the southern and eastern branches of the river.

Sediment particle composition and total organic carbon varied considerably among the 16 Elizabeth River study sites (Figures 5, 6, Table 6). Whereas sediments at some study sites were very sandy (% sand >50: SBA2, SBD8, EBB1, SBD4, SBD2, SBD9, SBD6, SBD7), others were characterized by higher concentrations of finer sediment particles (% sand<50: WBB1, LFA1, LFB2, SBB2, EBB2, SBD3, SBB1). Percent total solids in sediments (sediment dry weight/sediment wet weight) and total organic carbon (TOC: organic C dry weight/sediment dry weight) also varied widely among study sites. Highest organic C levels were observed at study site SBB1. Table 5 illustrates percent total organic carbon (TOC) in sediment samples and sediment PAH concentrations that have been normalized to TOC by the formula:

$$[PAH_{normalized}] = [PAH] / (\%TOC/100)$$

Hydrophobic organic contaminants such as PAH are concentrated in organic matter normally associated with the fine grain particles in estuarine sediments. To facilitate a direct comparison of liver lesion prevalence between various station locations having very different sediment types and organic carbon content, we have normalized PAH concentrations relative to

the amount of organic carbon present in the sediment samples. Table 7 summarizes mummichog liver lesion data and mean sediment PAH concentration data (means of two replicate samples), both mean total PAH concentration as well as TOC- normalized PAH level. Figure 7 illustrates the association between mummichog proliferative liver lesions and sediment PAH concentration. Both total sediment PAH concentrations and TOC normalized PAH concentrations exhibited a positive relationship with mummichog liver pathology. Study sites where sediment PAH concentrations were the highest (e.g., in the industrialized portions of the southern and eastern branches) also exhibited the highest liver lesion prevalences. In contrast, mummichogs from the more residential portions of the river (e.g., western branch, Lafayette River, southernmost portion of the southern branch, eastern portion of the eastern branch) where PAH concentrations in sediment were substantially lower, exhibited much lower prevalences of liver lesions. Neoplasms were not observed in fish from these less contaminated sites. The spatial distributions of altered hepatocellular foci and hepatic neoplasms in Elizabeth River mummichogs and sediment PAH concentrations are illustrated in Figures 8, 9 and 10. Figure 8 summarizes overall spatial distribution of mummichog liver lesions in the river, whereas Figure 9 provides detail of liver lesion prevalence and sediment PAH concentrations at the newly investigated Money Point study sites. Figure 10 illustrates spatial distribution of sediment PAH concentrations for the 16 study sites, as uncorrected (Figure 10A) and as TOC-normalized values (Figure 10B). These data indicate that, based both on liver lesion data from 1998, 2001 and 2006 and sediment chemistry from 2001 and 2006, the heavily industrialized portions of the southern and eastern branches of the Elizabeth River are the most heavily impacted sites where adverse biological impacts linked to PAH exposure are most severe.

SUMMARY AND RECOMMENDATIONS

The goal of this investigation was to conduct biological effects and sediment chemical monitoring in the Elizabeth River, Virginia to update the available information from prior studies on the contaminant-associated health status of the mummichog, *Fundulus heteroclitus*. This effort is part of a long-term monitoring program established in 1998 in collaboration with Virginia DEQ and the Elizabeth River Project. Its primary objective was to document the spatial occurrence of toxicopathic (chemical contaminant-induced) liver disease, including precancerous and cancerous liver lesions in this small non-migratory estuarine cyprinodontid fish. A second objective was to document the relationship between occurrence of liver disease in mummichogs and sediment PAH concentrations in the immediate estuarine habitats in which these fish live.

Results of this study, conducted during Fall 2006, strongly support previous observations indicating that liver histopathology in mummichogs is an effective bioindicator of sediment chemical contamination in the Elizabeth River, Virginia (Vogelbein et al, 1990, 1997, Vogelbein 1998, Vogelbein and Zwerner, 1999, Vogelbein and Unger, 2003). When coupled with sediment chemical analyses, use of mummichog liver pathology represents an effective way to characterize environmental quality on a micro-scale within the highly industrialized Elizabeth River. This approach is possible because the mummichog is abundant throughout the system and is found in large, self-sustaining populations in habitats ranging from relatively uncontaminated to heavily

contaminated ones. Mummichogs are largely non-migratory exhibiting a restricted summer home range of 40-50 m (Lotrich, 1975). Thus, in the Elizabeth River they constitute stable, semi-isolated sub-populations that inter-mix minimally and that are resident year-round. They therefore act as "biological integrators" of local sediment contaminants, effectively reflecting the quality or "health" of their immediate environment. The Atlantic Wood (SSB1) and Scuffeltown Creek (SBB2) study sites effectively illustrate the fine level of spatial discrimination we obtain with this approach. Liver lesion prevalences and sediment PAH concentrations were very high at site SBB1 (AHF: 83.3%, HN: 38.3%; PAH_{total}: 383,186 ng/g dry wt), while they were low at site SBB2 (AHF: 5.0%, HN: 0%; PAH_{total}: 26,375 ng/g dry wt). These two study sites are directly across the river from one another and separated by < 500 m.

In a previous DEQ-funded monitoring study (Vogelbein and Zwerner, 1999), we examined liver, kidney and gill pathology as potential indicators of contaminant effects in Elizabeth River mummichogs. We demonstrated that the proliferative liver lesions (altered hepatocellular foci and hepatic neoplasms) and certain hepatotoxic lesions were indicative of environmental exposure to potent chemicals in localized habitats within the river. Although sediment chemical analyses were not conducted in that study, several of the sites investigated are known to be heavily contaminated with polycyclic aromatic hydrocarbons (PAH) of creosote origin (Vogelbein et al. 1990, 1997). Several of these compounds are potently carcinogenic. Fish from these sites exhibited extremely high prevalences of liver cancers and pre-cancers. In contrast, the gill and kidney lesions observed in that study were largely non-specific, potentially caused by a variety of possible insults, with no clear relationship to sediment chemical contaminant concentrations. They therefore could not be attributed directly to chemical exposure and we recommended that subsequent biological effects monitoring in the Elizabeth River focus on liver as the primary target organ and to reduce effort and cost.

Based on these findings, a follow-up study conducted in 2001 focused only on mummichog proliferative liver lesions but added chemical analyses of sediments from the 12 study sites where fish were collected (Vogelbein and Unger, 2003). Using this approach we obtained convincing evidence of a positive relationship between sediment chemical contaminant exposure and development of liver disease in Elizabeth River mummichogs. Similar field observations were reported in separate but related contemporary investigations (Ownby et al. 2002, Mulvey et al. 2002, 2003). Although these field studies documented a strong positive association between mummichog liver disease and contaminant exposure, this relationship must be interpreted as circumstantial as it does not constitute direct evidence of a cause and effect relationship.

First direct experimental evidence of the causative link between PAH exposure and development of liver cancer in mummichogs came from a laboratory study in which we exposed fish to creosote-contaminated sediment and a sediment/diet amended with eight PAH, six of them known or suspected mammalian carcinogens (Vogelbein and Unger, 2006). Mummichogs developed a high prevalence of altered hepatocellular foci and hepatic neoplasms following year-long laboratory exposure, strongly supporting the hypothesis that PAH are among the causative

agents of the proliferative liver lesions observed in wild Elizabeth River mummichogs.

As with our prior investigations, the present monitoring efforts demonstrates a clear positive association between sediment PAH concentrations and liver pathology in Elizabeth River mummichogs. As in the prior studies, liver lesion prevalence was highest in the most heavily industrialized and PAH-contaminated sites and lowest at those sites where sediment PAH concentrations were low. As in 1998 and 2001, highest lesion prevalence was observed at study sites SBD3, SBB1 and EBB2. These three sites exhibited sediment PAH concentrations more than an order of magnitude higher than any of the other study sites. Lowest lesion prevalence, in some cases near background levels, was observed in the more residential portions of the river where PAH concentrations remain relatively low.

Logistic regression analysis to investigate the relationship between mummichog liver disease and the risk factors sex, weight (as a proxy for age), and mean sediment log PAH concentration for the site at which fish were collected, indicated log PAH concentration to be a significant risk factor for development of altered foci (odds ratio 3.50; 95% CI 4.66-2.63), and hepatic neoplasms (4.21; 7.13-2.48). Increasing log PAH concentration was a significant risk factor for stage-3 (6.16; 16.301-2.32), stage-2 (2.27; 3.59-1.44), and stage-1 (1.68; 2.4-1.31) hepatotoxicity indices (See Table 4). Sex and weight were found not to be significant risk factors for liver disease, and interaction terms between all variables were not significant (and thus not included in final models). Logistic models were originally formulated with all variables and potential interaction terms, and the latter were eliminated if found to have a nonsignificant odds ratio (OR) (Kleinbaum et al., 2002). It should be noted that the OR described above represent OR for each log (1000-fold) increase in PAH concentration. Thus, the OR for altered foci can roughly be thought of as representing a 3.5-fold increased odds of being positive for altered foci for every 1000-fold (e.g ppb to ppm) increase in sediment PAH concentration.

An initially encouraging finding of the 2001 monitoring effort (Vogelbein and Unger, 2003) was that liver lesion prevalence at some sites had declined since the 1998 investigation. This was especially evident for the two most heavily contaminated sites, SBD3 and SBB1, where AHF and HN prevalence had declined substantially. However, lesion prevalences were elevated over those from 1998 for one heavily contaminated site (EBB2). The reasons for these changes have remained unclear. At the time it was thought possible that chemical contaminants at SBD3 and SBB1 were becoming less bioavailable with time. Site SBB1 (Atlantic Wood) is an EPA Superfund site that has not actively treated timbers with creosote during the last 15 years. SBD3, the site of a long abandoned wood treatment facility, has to our knowledge not received additional point source inputs for over 25 years. Both sites appeared to have had minimal disturbances in the past several years and we considered it possible that progressive burial of contaminants at these sites was contributing to the observed decline in mummichog liver lesion prevalences. In contrast, EBB2 has remained an active commercial shipyard with significant industrial activities during 2000 - 2006. We noted in our final report (Vogelbein and Unger, 2003) that, because of these activities, it was possible that sediment-bound chemical contaminants at EBB2 were likely to be intermittently disturbed and thus remain bioavailable to

the fish. We also noted this as conjecture and that only additional long-term monitoring of these fish would clarify if the observed downward trends in lesion prevalence were real (Vogelbein and Unger, 2003). Results of the present study suggest that declining lesion prevalence observed at these study sites during 2001 was probably not an indication of habitat quality improvements, as lesion prevalence was again highly elevated at these three sites during fall 2006. A possible alternate explanation for the striking decline in liver disease at two of the most heavily contaminated sites during 2001 is that this time period saw increasing research interest in the mummichog, with several research groups from VIMS, Duke University and EPA Naragansett conducting active investigations in the Elizabeth River. It is possible that the Atlantic Wood site (SBB1) in particular, was regularly sampled during that time period in efforts to obtain the largest and oldest fish. This may have artificially skewed population structure towards smaller (younger) and healthier individuals, resulting in the observed lower prevalence of liver disease in the 2001 study.

Four new study sites located near Money Point in the southern branch of the Elizabeth River were added to the monitoring program during Fall 2006. This site is thought to be heavily contaminated with PAH of creosote origin and is targeted for sediment remediation in the near future. Thus it was important to obtain baseline data on mummichog liver disease prior to initiation of remediation efforts. This location bordered the Eppinger and Russell wood treatment facility, which operated during the early to mid 1900's. Sediment chemical analysis identified the highest concentrations of PAH observed during 2006 at the Money Point North site (SBD6). High PAH concentrations also occurred at the Money Point Central site (SBD7). Pre-cancerous liver lesion prevalence was moderate to high at these two sites (SBD6: AHF 13.3%, HN 1.7%, SBD7: AHF 40%, HN 0%), however, hepatic neoplasms were rare. Sediment PAH concentrations at these sites were highest some distance off-shore and did not significantly overlap the near shore habitat of the fish. Thus it is likely that exposure of these populations is less than that of fish at, for example, the Atlantic Wood site where the near-shore marsh edge (optimal mummichog habitat) is severely contaminated with creosote. Alternatively it is possible that the composition of chemical contaminants at this new site is different from that of other heavily contaminates sites where mummichogs exhibit liver disease.

We were unable to catch adequate numbers of fish at four study sites. This was most likely caused by the late start of the sampling season (late Nov.). It included two sites that were only mildly contaminated and where historically, we have not seen liver disease in mummichogs (site WBB1: N=11; site SBD4: N=21). One study site (EBB2) was unusual in that PAH concentrations were lower than the historically high levels we have seen here. We also had considerable difficulty obtaining fish at this site (N=27) and those that we were able to collect were the smallest fish obtained in 2006 (mean total weight = 3.67g). Despite this, liver lesion prevalence remained high at this site (AHF: 37%; HN: 11%). The fourth study site where we obtained insufficient numbers of fish was SBA2. These fish were also some of the smallest obtained during 2006 (mean total weight: 3.98g). We saw no liver disease in these fish in 2006, whereas during 1998 the prevalence of AHF was > 20% at this site. The temporal difference in liver disease prevalence at this site is most readily attributed to the smaller size of fish obtained

during 2006. We have always targeted the larger and oldest fish for these efforts because liver carcinogenesis is a chronic disease process that advances over extended time periods, resulting in highest lesion prevalence in the largest (oldest) fish. It is thus of critical importance that factors other than just the sediment contaminant concentrations be considered in interpretation of liver lesion prevalence data. It is important to realize that spatial distribution of sediment PAH concentration is highly variable and can be patchy within a given study site. Thus, in some instances, replicate sediment samples may show tremendous within-site variability. In studies such as this one where the number of replicate samples is necessarily constrained by cost, it is possible that one or both samples are not representative of average sediment contaminant levels for a given study site. Alternatively, if the bulk of contamination is found in deeper portions of a particular site and contaminant distributions do not overlap habitat of the indicator species, then exposures and adverse biological effects are likely to be affected. Further, it is critical to take into account the biology, life history and behavior of the mummichog as well as the disease endpoints being investigated. All are likely playing a role in overall health status of the fish.

In addition to the hepatic proliferative lesions observed in the mummichog, we observed a suite of lesser, largely non-specific liver lesions. Some of these represent acute hepatotoxicity whereas others are compensatory proliferative and inflammatory changes. Although some of the hepatotoxic lesions appeared to exhibit a positive association with chemical exposure, both in prior studies and in the current one (see hepatotoxicity index (HTI)) and gross liver lesion index (GLI: Table 6), they have not been adequately investigated in the context of environmental health monitoring and require further characterization and quantification. A future goal is to develop computer-based morphometric methods for quantifying non-cancerous liver lesion severity with the aim of using them to characterize the more mildly to moderately contaminated sites where we do not expect to see development of liver cancers.

In 1998, the Elizabeth River Project in collaboration with partners including DEQ, other state agencies, academic institutions (VIMS, ODU) and citizens groups developed a set of criteria to rank environmental quality in the Elizabeth River. Criteria developed for ranking mummichog health were as follows:

RANK	DEFINITION	EXPLANATION
0	Insufficient/Inadequate Data	No fish or too few fish (< 60)
1	Not a Problem	Background liver lesions (AHF: <5%; HN: 0%)
2	Borderline	AHF:5-20%; HN: 0%
3	A Problem	AHF: 20-30%; HN: <5%
4	Severe Problem	AHF: >30%; HN: >5%

Based on these criteria, 16 study sites investigated in the present effort and 12 sites studied previously were scored and ranked as follows:

Study Site	1998	2001	2006
SBB1	4	4	4
SBD3	4	4	4
EBB2	3	4	4
SBD2	3	2	2
SBA2	3	2	1
SBD5	2	2	2
SBB2	2	3	2
SBD4	2	2	2
EBB1	1	2	1
LFB1	1	1	1
LFA1	1	1	1
WBB1	1	1	1
SBD6			**3**
SBD7			**4**
SBD8			**1**
SBD9			**3**

It is clear that the health of Elizabeth River mummichogs has not changed significantly over the past 10 years. Despite the fact that lesion prevalences were substantially lower in 2001 than previously at several study sites, the overall rankings have not improved. In fact, site quality appears to have declined slightly based on higher rankings for sites EBB2, SBB2, and EBB1 in 2001. At the same time however, rankings for sites SBD2 and SBA2 went down. These fluctuations may just be due to low sample sizes and high variance and may only reflect natural variation inherent in our sampling design (e.g., 60 adult fish may not be sufficient to obtain an accurate estimate of lesion prevalence). Alternatively it may suggest that the somewhat arbitrary values for lesion prevalence assigned to represent the different rank scores in the scheme above are not sufficiently representative of environmental quality at the sites. Or it may suggest that at certain sites contaminants may not be as bioavailable to the fish as they used to be. Similar minor fluctuations were again noted during 2006, with site rankings declining a step at several study sites. Only additional monitoring will clarify whether habitat quality is improving within the River.

This monitoring approach provides the DEQ with an effective tool to evaluate adverse biological effects associated with exposure to a class of chemical contaminants in an important

fish species indigenous to the Elizabeth River. We suggest that this approach will be effective in tracking environmental recovery following specific remediation and cleanup efforts within the Elizabeth River. Proposed sediment remediation efforts now in the planning stages for several Elizabeth River sites will require monitoring in order to measure their ultimate success at restoring diverse biological function and "health" to heavily impacted sites. Presumably, if remediation efforts at, for example, the Atlantic Wood (SBB1) and the Money Point (SBD6-9) sites effectively reduce sediment contaminant levels or bioavailablity, then we would expect that liver lesion prevalence in native mummichogs will decline over time. The current and prior studies provide the DEQ and ERP with critical temporal and spatial baseline information on a significant adverse health impact directly attributable to contaminant exposure. We recommend that mummichog histopathology coupled with sediment chemical analyses be continued in the Elizabeth River on an intermittent basis. We believe it is not necessary to conduct this type of monitoring annually. It is probably adequate and more cost-effective to conduct these types of evaluations every three years. We propose mummichog histopathology as an effective metric of success following planned sediment remediation in the Elizabeth River.

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TABLES AND FIGURES

Station Code	Station Name	Fish Collection Station Latitude	Longitude	# of Fish	Collection Dates
SBB2	South Branch, Scuffeltown Cr	36°48'28.31"N	76°16'59.78"W	60	11/15/06
SBB1	South Branch, Atlantic Wood	36°48'28.68"N	76°17'38.74"W	60	11/15/06
SBA2	South Branch, Crown Tank Farm	36°49'15.17"N	76°17'13.56"W	13	11/29/06
EBB2	East Branch, Colonna Ship Yard	36°50'11.85"N	76°16'24.35"W	27	11/28, 11/29/06
EBB1	East Branch, New Ford Plant	36°50'23.89"N	76°14'57.07"W	60	11/28/06
LFA1	Lafayette River, Lafayette Point	36°54'49.29"N	76°19'11.55"W	52	11/30/06
LFB2	Lafayette River, East Haven	36°53'28.83"N	76°16'54.80"W	50	11/30/06
WBB1	West Branch, PH	36°50'59.14"N	76°22'2.40"W	11	11/30/06
SBD5	South Branch, Paradise Cr	36°47'59.49"N	76°17'54.03"W	60	11/15/06
SBD3	South Branch, Refueling Station	36°47'35.94"N	76°17'27.21"W	60	11/15 & 11/28/06
SBD6	South Branch, Money Pt North	36°47'24.31"N	76°17'52.27"W	60	11/17 & 11/28/06
SBD7	South Branch, Money Pt Central	36°47'2.73" N	76°18'5.30"W	60	11/17/06
SBD9	South Branch, Money Pt Reference	36°47'27.43"N	76°18'22.74"W	60	11/17, 11/28/06
SBD8	South Branch, Money Pt South	36°46'42.18"N	76°18'12.89"W	60	11/17, 11/28, 11/29/06
SBD2	South Branch, Power Plant	36°45'50.67"N	76°17'50.66"W	60	11/28, 11/29/06
SBD4	South Branch, New Mill Cr	36°44'30.76"N	76°18'7.42"W	21	11/29/06

Table 1. Latitude and longitude coordinates for 16 Elizabeth River study sites where mummichog, *Fundulus heteroclitus*, were collected during fall 2006. * Stations in bold are new stations assigned a new code based on current DEQ convention.

Study Site	Ref Latitude		Ref Longitude		Actual Latitude		Actual Longitude	
	Deg	Min	Deg	Min	Deg	Min	Deg	Min
WBB1	36	50.986	76	22.040	36	50.986	76	22.040
LFB2	36	53.481	76	16.913	36	53.489	76	16.904
LFA1	36	54.822	76	19.193	36	54.845	76	19.160
EBB1	36	50.398	76	14.951	36	50.366	76	14.956
SBD4	36	44.513	76	18.124	36	44.482	76	18.108
SBD2	36	45.845	76	17.844	36	45.848	76	17.849
SBD8	36	46.703	76	18.215	36	46.694	76	18.233
SBD7	36	47.215	76	18.088	36	46.975	76	18.129
SBD9	36	47.457	76	18.379	36	47.449	76	18.345
SBD6	36	47.437	76	17.871	36	47.421	76	17.889
SBD3	36	47.610	76	17.454	36	47.636	76	17.452
SBD5	36	47.992	76	17.901	36	47.972	76	17.909
SBB2	36	48.474	76	17.014	36	48.497	76	16.985
SBB1	36	48.478	76	17.646	36	48.480	76	17.614
SBA2	36	49.253	76	17.226	36	49.228	76	17.238
EBB2	36	50.209	76	16.406	36	50.221	76	16.414

Table 2. Reference and actual latitude and longitude coordinates for 16 Elizabeth River study sites where sediment samples for chemical analyses were collected during 2007. * first four stations were sampled on 3/7/2007 and remaining stations were sampled on 3/9/2007. Stations in bold are new stations assigned a new code based on current DEQ convention.

Station Code	N	Collection Date	Females	Males	Mean TL (Mm)	Mean WT (g)
SBB2	60	11/15/06	21	39	85.8 (5.42)*	8.2 (1.79)
SBB1	60	11/15/06	32	28	80.4 (5.06)	6.5 (1.47)
SBA2	13	11/29/06	1	12	68.5 (6.44)	3.9 (1.11)
EBB2	27	11/28, 11/29/06	9	18	67.4 (7.61)	3.7 (1.52)
EBB1	60	11/28/06	27	33	75.7 (6.05)	5.7 (1.59)
LFA1	52	11/30/06	27	25	68.3 (8.06)	4.1 (1.83)
LFB2	50	11/30/06	32	18	72.6 (7.79)	4.9 (1.88)
WBB1	11	11/30/06	5	6	71.0 (3.41)	4.6 (0.79)
SBD5	60	11/15/06	32	28	86.4 (6.28)	8.3 (2.09)
SBD3	60	11/15 & 11/28/06	25	35	80.4 (6.43)	6.7 (1.75)
SBD6	60	11/17 & 11/28/06	27	33	80.9 (6.61)	6.9 (1.89)
SBD7	60	11/17/06	22	38	89.7 (6.35)	9.4 (2.36)
SBD9	60	11/17, 11/28/06	35	25	81.9 (9.19)	7.3 (2.40)
SBD8	60	11/17, 11/28, 11/29/06	29	31	87.9 (12.48)	9.2 (4.18)
SBD2	60	11/28, 11/29/06	40	20	77.3 (6.87)	5.6 (1.84)
SBD4	21	11/29/06	10	11	74.2 (6.09)	5.2 (1.48)

Table 3. Fish collection data and basic measurements for mummichog, *Fundulus heteroclitus*, collected from 16 study sites in the Elizabeth River, Virginia during Fall 2006. * std. dev. in parentheses.

Station Code	Altered Hepatocellular Foci (AHF)	Adenoma/ Carcinoma (HN)	GLI	HTI	Parasitic Infections (%)				
					Calyptospora sp.	*Myxidium* sp.	NE	CE	TR
SBB2	0.050	0	0.017	0.933	0.03	0	0.03	0	0
SBB1	0.833	0.383	1.083	1.55	0	0	0	0	0
SBA1	0	0	0	0	0	0	0.08	0	0
EBB2	0.37	0.11	0.11	0.63	0.04	0.3	0	0	0.04
EBB1	0.033	0	0.017	0.017	0.02	0.07	0.02	0	0.03
LFA1	0.038	0	0.269	0.019	0	0	0.02	0	0.38
LFB2	0.04	0	0.02	0.22	0.08	0	0	0	0
WBB1	0	0	0.364	0	0	0	0	0	0
SBD5	0.133	0	0.017	0.433	0	0.03	0	0.03	0
SBD3	0.467	0.1	0.133	0.783	0	0	0.02	0	0
SBD6	0.133	0.017	0.017	0.25	0.02	0	0.05	0	0.08
SBD7	0.4	0	0.133	0.433	0.02	0	0	0	0.4
SBD9	0.033	0.017	0.05	0.2	0.03	0.02	0	0	0.25
SBD8	0.033	0	0.033	0.15	0	0	0	0	0
SBD2	0.048	0	0	0.25	0	0	0	0	0.02
SBD4	0.0833	0	0.048	0.143	0	0.05	0.1	0	0.05

Table 4. Liver histopathology/parasitology summary data for mummichog, *Fundulus heteroclitus*, collected from 16 study sites in the Elizabeth River, Virginia during fall 2006. GLI: Gross Liver Lesion Index, HTI: Hepatotoxicity Index for fall 2006 expressed as proportions.

Compound	SBD4		SBD2		EBB1		WBB1		LFB2		LFA1	
	Rep-1	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*
Naphthalene	0	0	0	1	1	3	1	11	1	1	33	18
2-methyl naphthalene	0	0	2	3	3	3	3	12	2	3	84	17
1-methyl naphthalene	0	0	1	3	2	2	3	9	1	2	97	20
Biphenyl	0	0	1	0	2	2	2	5	2	1	43	0
2,6-dimethyl naphthalene	0	0	4	5	5	4	7	12	6	3	113	22
Acenaphthene	0	1	3	5	24	10	4	8	4	3	652	96
fluorene	1	1	11	15	36	14	10	11	11	5	791	141
Phenanthrene	6	36	92	165	444	139	65	67	99	58	9885	1746
Anthracene	5	8	138	36	113	53	27	23	32	28	1839	291
1-methyl phenanthrene	2	4	21	151	0	23	17	21	25	25	413	102
Fluoranthene	109	125	518	449	821	440	260	250	313	391	10288	2214
Pyrene	157	167	397	345	677	415	234	284	287	414	7485	1899
Benzo(a)anthracene	146	125	252	165	356	204	135	163	212	215	2276	558
Chrysene	132	120	320	237	398	243	147	166	163	294	2693	678
Benzo(e)pyrene	59	50	220	128	377	211	158	124	168	311	1090	698
Benzo(a)pyrene	81	67	193	167	446	253	144	107	160	257	1311	747
Perylene	23	27	143	159	140	58	71	84	106	151	473	282
Dibenzo(a,h)anthracene	9	9	29	73	59	26	22	16	71	129	186	75
Total Select PAHs	730	742	2345	2107	3904	2104	1307	1371	1664	2291	39751	9604
Sum Low MW PAHs	14	50	273	384	631	253	138	177	184	129	13949	2454
Sum High MW PAHs	716	692	2072	1723	3272	1850	1170	1194	1480	2162	25802	7150

Table 5. Concentrations of select polycyclic aromatic hydrocarbons (PAH) in sediment (ng/g dry weight) from 16 study sites sampled during spring 2007 in the Elizabeth River, Virginia. * replicates from these study sites labeled with an "a" represent analytical replicates and are an indication of "within sample" variance.

Compound	SBD8		SBD9			SBB2		SBD7			SBD5	
	Rep-1	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-2a*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-2a*	*Rep-1*	*Rep-2*
Naphthalene	4	12	2	19	27	34	266	17	860	646	36	76
2-methyl naphthalene	5	14	2	9	10	34	154	17	303	239	26	69
1-methyl naphthalene	6	13	3	0	9	23	87	10	323	232	18	49
Biphenyl	4	14	2	4	4	16	49	8	130	106	10	20
2,6-dimethyl naphthalene	10	33	5	7	8	43	161	30	319	248	29	72
Acenaphthene	24	101	23	27	32	49	405	39	2237	1625	35	76
fluorene	42	101	28	26	31	78	579	58	1069	791	44	88
Phenanthrene	519	1773	312	237	354	609	8442	390	6432	4166	261	589
Anthracene	162	389	145	254	338	451	1626	252	6117	4674	174	292
1-methyl phenanthrene	64	194	48	0	34	102	462	0	7219	3006	46	74
Fluoranthene	1417	3460	758	1684	1435	2712	8949	1435	99746	74386	1502	1360
Pyrene	1061	2562	712	2125	1301	2594	6837	960	70821	52693	1086	1040
Benzo(a)anthracene	561	1159	379	607	573	1002	2138	315	17323	15837	431	405
Chrysene	604	1498	488	1408	822	1827	2828	506	16357	15885	629	678
Benzo(e)pyrene	448	1136	337	1164	661	1919	1951	410	4561	5846	457	490
Benzo(a)pyrene	589	1467	466	1285	856	1724	2091	343	6827	8751	462	444
Perylene	237	388	102	336	208	537	1236	225	2184	2385	229	261
Dibenzo(a,h)anthracene	118	98	98	235	159	406	331	65	856	1051	96	49
Total Select PAHs	5874	14412	3910	9427	6861	14158	38592	5080	243682	192567	5568	6132
Sum Low MW PAHs	839	2644	570	584	847	1438	12231	821	25007	15733	678	1406
Sum High MW PAHs	5035	11768	3339	8843	6014	12720	26361	4260	218674	176835	4890	4727

Table 5 contd. Concentrations of select polycyclic aromatic hydrocarbons (PAH) in sediment (ng/g dry weight) from 16 study sites sampled during spring 2007 in the Elizabeth River, Virginia. * replicates from these study sites labeled with an "a" represent analytical replicates and are an indication of "within sample" variance.

Compound	SBD6			SBA2		SBD3		EBB2		SBB1	
	Rep-1	*Rep-1a*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*
Naphthalene	62	132	585	98	64	199	300	46	136	1915	386
2-methyl naphthalene	21	47	588	153	69	111	123	19	39	919	722
1-methyl naphthalene	19	40	4030	130	50	68	85	15	28	663	0
Biphenyl	11	22	943	52	28	47	39	12	18	311	0
2,6-dimethyl naphthalene	14	35	3965	206	70	84	65	16	26	665	1656
Acenaphthene	68	231	39748	255	98	620	498	83	132	4792	3534
fluorene	75	228	29784	302	164	636	384	97	193	3641	2534
Phenanthrene	749	2739	169945	4898	3284	4723	4295	1135	1650	25221	18025
Anthracene	701	1733	35336	671	657	16317	7500	932	2347	15962	10880
1-methyl phenanthrene	103	175	5417	226	349	577	327	91	77	2431	0
Fluoranthene	2597	4891	118395	5468	6801	33161	20599	5049	4760	106813	91665
Pyrene	3306	6007	87590	4166	5408	29729	26606	5105	4436	68550	53233
Benzo(a)anthracene	1650	1669	17088	1163	1761	6718	5577	2296	1924	25764	19507
Chrysene	2666	3807	20494	1453	2184	15159	13755	3489	2510	55658	46906
Benzo(e)pyrene	2109	3838	9339	737	1731	5518	9668	2533	2146	42165	36807
Benzo(a)pyrene	3149	5089	12751	888	2031	5929	9907	2811	2308	44071	39429
Perylene	1157	567	5573	353	1160	2463	4657	784	676	10000	23633
Dibenzo(a,h)anthracene	724	539	1043	86	243	418	907	519	358	3446	4473
Total Select PAHs	19181	31790	562613	21307	26152	122479	105293	25032	23764	412983	353389
Sum Low MW PAHs	1823	5382	290340	6991	4833	23382	13616	2446	4646	56518	37736
Sum High MW PAHs	17358	26407	272273	14315	21319	99097	91676	22586	19118	356465	315653

Table 5 contd. Concentrations of select polycyclic aromatic hydrocarbons (PAH) in sediment (ng/g dry weight) from 16 study sites sampled during spring 2007 in the Elizabeth River, Virginia. * replicates from these study sites labeled with an "a" represent analytical replicates and are an indication of "within sample" variance.

COMPOUND/TOC	SBD4		SBD2		EBB1		WBB1		LFB2		LFA1	
	Rep-1	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*
TOC %	0.996	0.360	1.707	1.556	1.319	1.176	2.085	2.207	2.123	2.632	2.483	1.843
TOC (%)/100	0.00996	0.00360	0.01707	0.01556	0.01319	0.01176	0.02085	0.02207	0.02123	0.02632	0.02483	0.01843
Naphthalene	0	0	28	85	101	222	51	479	40	41	1316	969
2-methyl naphthalene	0	0	95	163	222	264	135	540	101	97	3373	948
1-methyl naphthalene	0	0	80	207	147	187	123	419	66	79	3891	1095
Biphenyl	34	0	53	0	134	128	108	207	88	53	1722	0
2,6-dimethyl naphthalene	0	0	226	317	404	365	320	532	285	113	4537	1202
Acenaphthene	34	397	165	348	1846	871	179	341	169	109	26256	5205
fluorene	55	345	634	944	2758	1197	457	495	523	205	31877	7636
Phenanthrene	580	9944	5395	10603	33652	11800	3122	3022	4676	2202	398111	94757
Anthracene	517	2121	8100	2315	8603	4521	1313	1029	1527	1055	74045	15777
1-methyl phenanthrene	209	1219	1232	9678	0	1975	802	950	1176	952	16634	5542
Fluoranthene	10964	34746	30320	28828	62226	37455	12449	11329	14741	14865	414355	120105
Pyrene	15771	46521	23262	22196	51296	35318	11225	12858	13510	15740	301449	103058
Benzo(a)anthracene	14661	34842	14756	10622	26955	17379	6482	7381	10004	8157	91677	30285
Chrysene	13249	33446	18725	15213	30174	20622	7054	7530	7685	11178	108473	36791
Benzo(e)pyrene	5883	13859	12914	8203	28569	17903	7585	5599	7928	11813	43894	37870
Benzo(a)pyrene	8175	18644	11328	10744	33790	21524	6886	4859	7527	9760	52786	40527
Perylene	2268	7403	8356	10233	10592	4925	3384	3788	5000	5722	19052	15299
Dibenzo(a,h)anthracene	932	2637	1718	4722	4489	2216	1034	747	3323	4897	7475	4044
Total Select PAHs	73333	206125	137388	135422	295959	178871	62708	62106	78368	87040	1600924	521107
Sum Low MW PAHs	1430	14028	16009	24661	47867	21529	6609	8014	8650	4906	561763	133130
Sum High MW PAHs	71903	192097	121379	110761	248092	157343	56099	54092	69718	82134	1039161	387978

Table 6. Total percent organic carbon (TOC) and $TOC_{normalized}$ sediment PAH concentrations (ng/g carbon) for 16 study sites sampled during spring 2007 in the Elizabeth River. * replicates from these study sites labeled with an "a" represent analytical replicates and are an indication of "within sample" variance.

COMPOUND/TOC	SBD8		SBD9			SBB2		SBD7			SBD5	
	Rep-1	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-2a*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-2a*	*Rep-1*	*Rep-2*
TOC %	1.329	1.486	0.565	0.964	0.964	3.272	3.609	0.809	1.959	1.959	1.866	2.892
TOC (%)/100	0.01329	0.01486	0.00565	0.00964	0.00964	0.03272	0.03609	0.00809	0.01959	0.01959	0.01866	0.02892
Naphthalene	266	818	353	2011	2792	1051	7369	2043	43891	32962	1908	2640
2-methyl naphthalene	406	957	360	923	1087	1029	4258	2142	15471	12190	1403	2400
1-methyl naphthalene	424	842	538	0	897	704	2413	1290	16474	11866	950	1680
Biphenyl	298	948	310	390	449	479	1346	1003	6624	5403	518	683
2,6-dimethyl naphthalene	753	2252	900	719	834	1304	4470	3683	16267	12648	1579	2503
Acenaphthene	1808	6771	4124	2795	3314	1510	11219	4766	114188	82930	1878	2643
fluorene	3165	6794	4965	2705	3245	2376	16045	7217	54551	40394	2377	3041
Phenanthrene	39050	119344	55287	24609	36686	18601	233903	48167	328309	212639	13968	20358
Anthracene	12171	26146	25629	26394	35030	13778	45066	31109	312245	238614	9327	10094
1-methyl phenanthrene	4810	13061	8475	0	3536	3116	12809	8	368518	153453	2445	2561
Fluoranthene	106633	232844	134123	174691	148808	82884	247957	177339	5091678	3797148	80480	47028
Pyrene	79818	172417	126023	220390	134958	79280	189441	118646	3615149	2689772	58177	35964
Benzo(a)anthracene	42217	78004	67014	63006	59456	30614	59253	38995	884272	808412	23073	13994
Chrysene	45445	100807	86374	146017	85253	55834	78353	62565	834981	810857	33704	23448
Benzo(e)pyrene	33681	76420	59719	120729	68574	58661	54069	50658	232816	298424	24493	16934
Benzo(a)pyrene	44310	98688	82510	133268	88829	52675	57947	42453	348503	446725	24736	15353
Perylene	17855	26109	17974	34820	21564	16420	34249	27840	111479	121768	12250	9013
Dibenzo(a,h)anthracene	8906	6622	17272	24420	16459	12393	9160	8069	43673	53671	5121	1705
Total Select PAHs	442016	969843	691950	977886	711769	432709	1069326	627996	12439089	9829875	298387	212043
Sum Low MW PAHs	63151	177932	100940	60546	87869	43947	338898	101429	1276537	803099	36352	48603
Sum High MW PAHs	378865	791910	591009	917340	623900	388762	730429	526566	11162552	9026776	262035	163440

Table 6 contd. Total percent organic carbon (TOC) and $TOC_{normalized}$ sediment PAH concentrations (ng/g carbon) for 16 study sites sampled during spring 2007 in the Elizabeth River. * replicates from these study sites labeled with an "a" represent analytical replicates and are an indication of "within sample" variance.

COMPOUND/TOC	SBD6			SBA2		SBD3		EBB2		SBB1	
	Rep-1	*Rep-1a*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*	*Rep-1*	*Rep-2*
TOC %	0.855	0.855	1.232	2.187	5.106	2.943	3.463	2.252	2.801	6.657	7.085
TOC (%)/100	0.00855	0.00855	0.01232	0.02187	0.05106	0.02943	0.03463	0.02252	0.02801	0.06657	0.07085
Naphthalene	7240	15430	47446	4460	1262	6774	8655	2042	4842	28763	5445
2-methyl naphthalene	2453	5547	47692	7008	1354	3770	3548	830	1378	13801	10191
1-methyl naphthalene	2253	4642	327087	5951	974	2318	2468	666	1015	9954	0
Biphenyl	1272	2612	76575	2389	554	1613	1122	554	648	4666	0
2,6-dimethyl naphthalene	1665	4053	321848	9440	1377	2865	1880	722	945	9987	23370
Acenaphthene	7903	26979	3226291	11682	1910	21052	14379	3670	4727	71980	49880
fluorene	8785	26691	2417525	13788	3206	21603	11090	4305	6888	54691	35759
Phenanthrene	87640	320330	13794234	223946	64309	160479	124037	50402	58901	378864	254409
Anthracene	81947	202725	2868156	30691	12868	554430	216563	41366	83784	239783	153563
1-methyl phenanthrene	12038	20496	439728	10325	6841	19595	9453	4055	2738	36513	0
Fluoranthene	303738	572073	9609974	250039	133188	1126784	594844	224184	169949	1604520	1293787
Pyrene	386708	702532	7109589	190495	105914	1010172	768305	226684	158376	1029738	751347
Benzo(a)anthracene	192967	195216	1387011	53185	34486	228271	161049	101972	68699	387016	275323
Chrysene	311853	445267	1663466	66449	42774	515098	397189	154916	89600	836076	662044
Benzo(e)pyrene	246614	448904	758048	33698	33895	187511	279182	112483	76632	633391	519510
Benzo(a)pyrene	368246	595193	1035001	40597	39780	201473	286085	124840	82399	662017	556518
Perylene	135344	66316	452327	16160	22721	83707	134473	34817	24123	150216	333569
Dibenzo(a,h)anthracene	84726	63071	84653	3934	4768	14201	26182	23053	12767	51761	63132
Total Select PAHs	2243392	3718077	45666653	974239	512180	4161716	3040503	1111561	848411	6203738	4987849
Sum Low MW PAHs	213195	629505	23566584	319681	94654	794499	393193	108612	165865	849003	532618
Sum High MW PAHs	2030197	3088573	22100070	654558	417526	3367217	2647309	1002948	682545	5354735	4455231

Table 6 contd. Total percent organic carbon (TOC) and $TOC_{normalized}$ sediment PAH concentrations (ng/g carbon) for 16 study sites sampled during spring 2007 in the Elizabeth River. * replicates from these study sites labeled with an "a" represent analytical replicates and are an indication of "within sample" variance.

Station Code	Altered Hepatocellular Foci (AHF)	Adenoma/ Carcinoma (HN)	GLI	HTI	TOC[1]	Total PAH[2]	Total PAH TOC_{norm}[3]
SBD4	0.0833	0	0.048	0.143	0.678	736	139,729
SBD2	0.048	0	0	0.25	1.632	2226	136,405
EBB1	0.033	0	0.017	0.017	1.283	3004	237,415
WBB1	0	0	0.364	0	2.146	1339	62,407
LFB2	0.04	0	0.02	0.22	2.378	1978	82,704
LFA1	0.038	0	0.269	0.019	2.163	24,679	1,061,016
SBD8	0.033	0	0.033	0.15	1.408	10,143	705,929
SBD9	0.033	0.017	0.05	0.2	0.765	6667	834,918
SBB2	0.050	0	0.017	0.933	3.441	26,375	751,018
SBD7	0.4	0	0.133	0.433	1.384	124,381	6,533,542
SBD5	0.133	0	0.017	0.433	2.379	5850	255,215
SBD6	0.133	0.017	0.017	0.25	1.044	290,897	23,955,023
SBA2	0	0	0	0	3.647	23,729	743,210
SBD3	0.467	0.1	0.133	0.783	3.203	113,886	3,601,109
EBB2	0.37	0.11	0.11	0.63	2.527	24,398	979,986
SBB1	0.833	0.383	1.083	1.55	6.871	383,186	5,595,794

Table 7. Summary data (2006-07) for 16 Elizabeth River study sites including liver lesion prevalences, hepatic proliferative index (HPI), hepatotoxic index (HTI), mean % TOC, mean total PAH concentrations, and mean PAH concentrations normalized to TOC.

[1]TOC: Percent total organic carbon. Mean of two replicate samples.

[2]Total PAH: in ng/g dry sediment. Mean of two replicate samples.

[3]Total PAH TOC_{norm}: total sediment PAH normalized to TOC (in ng/g carbon). Mean of 2 replicate samples

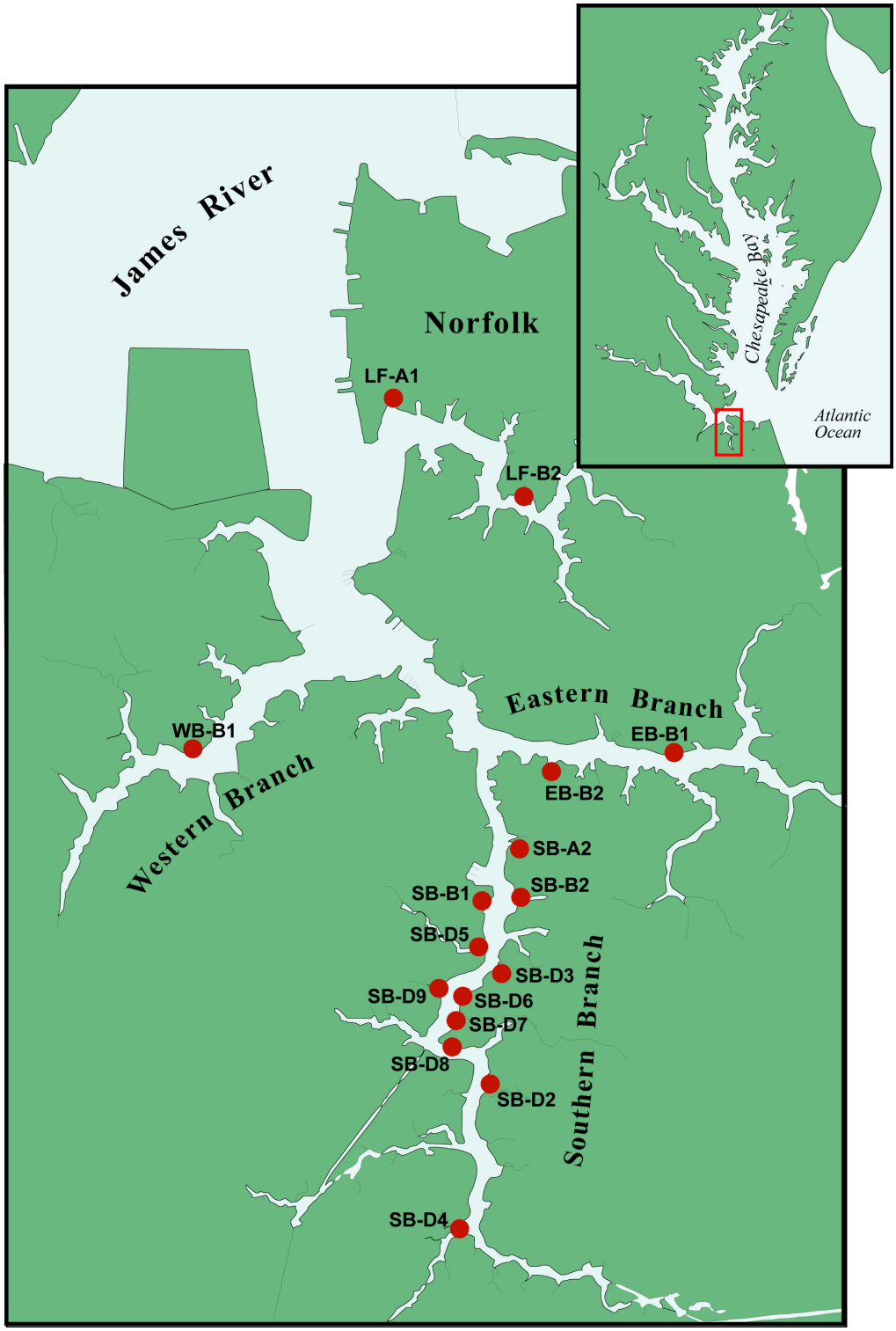
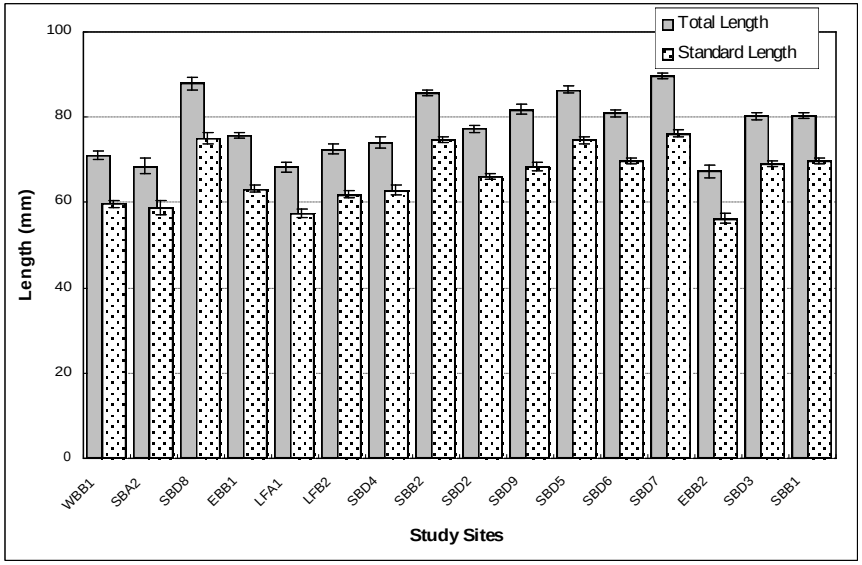
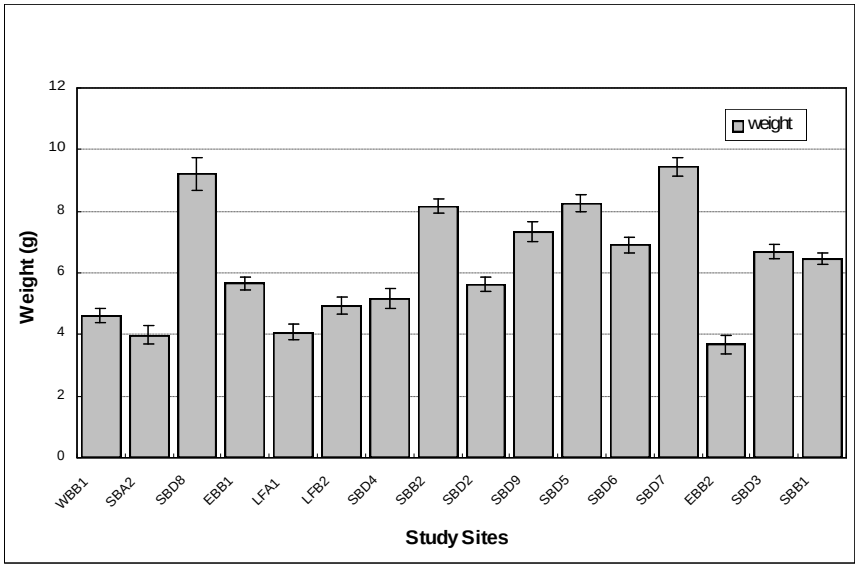


Figure 1. Station location map for collection of mummichog (*Fundulus heteroclitus*) and sediments at 16 study sites during 2006-07 in the Elizabeth River, VA.



A



B

Figure 2. Mean total and standard lengths (A) and mean weight (B) for mummichogs (*Fundulus heteroclitus*) collected during fall 2006 at 16 Elizabeth River, Virginia study sites. * error bars represent standard error of the mean.

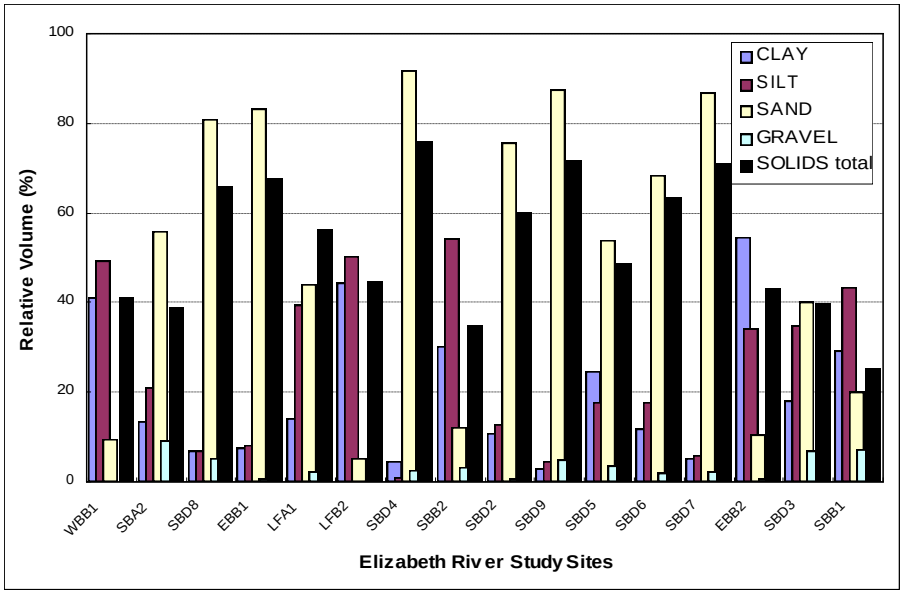


Figure 3. Grain size composition for sediments from 16 study sites in the Elizabeth River, Virginia collected in Spring 2007.

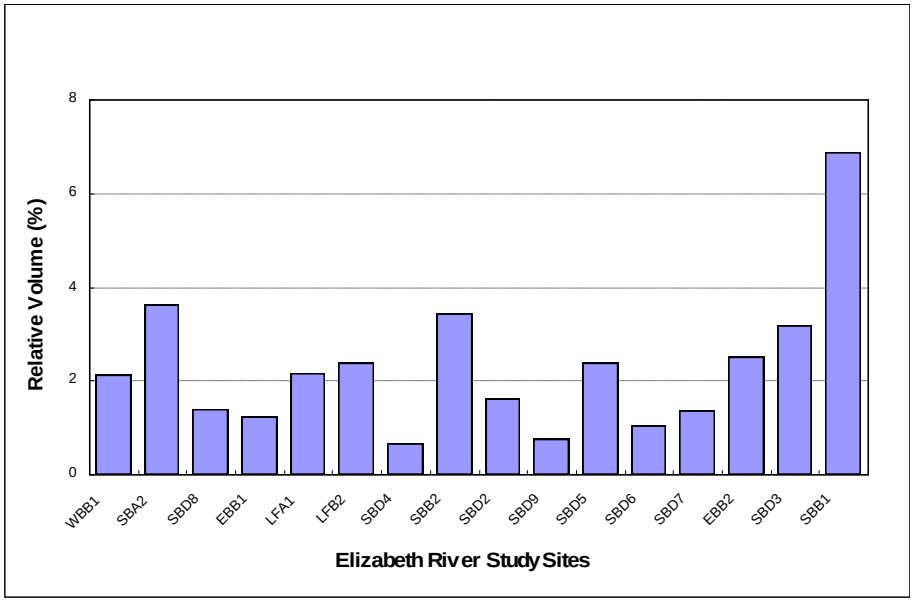


Figure 4. Total organic carbon in sediments from 16 study sites collected spring 2007 from the Elizabeth River, Virginia

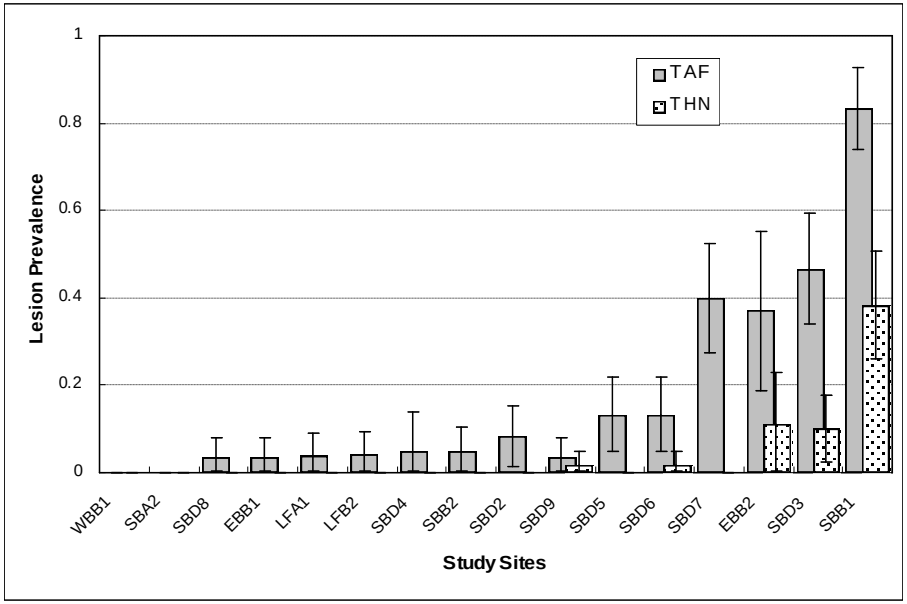


Figure 5: Liver lesions prevalences in mummichogs (*Fundulus heteroclitus*) collected during fall 2006 from 16 study sites in the Elizabeth River, Virginia. TAF: altered hepatocellular foci$_{total}$. THN: hepatic neoplasms$_{total}$.

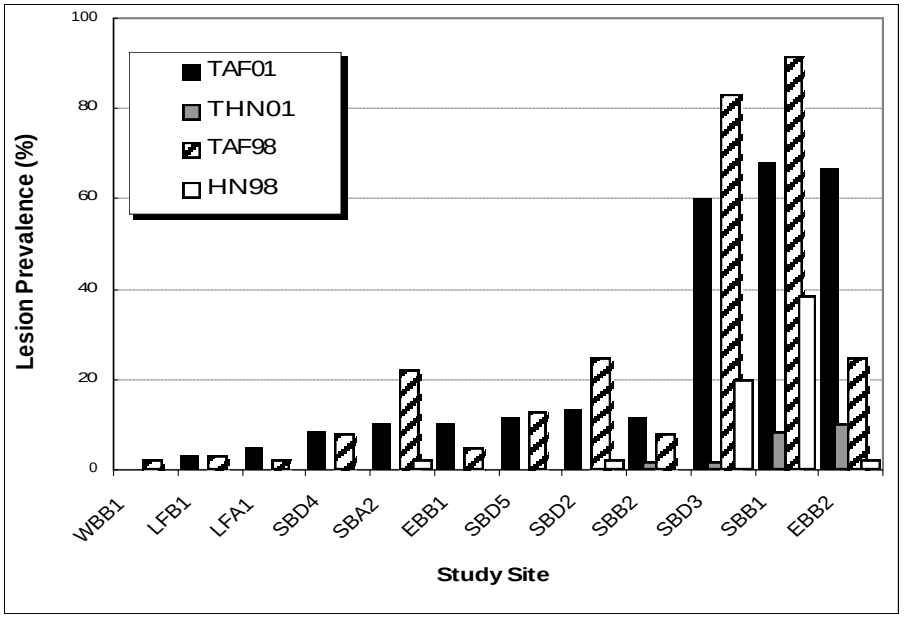
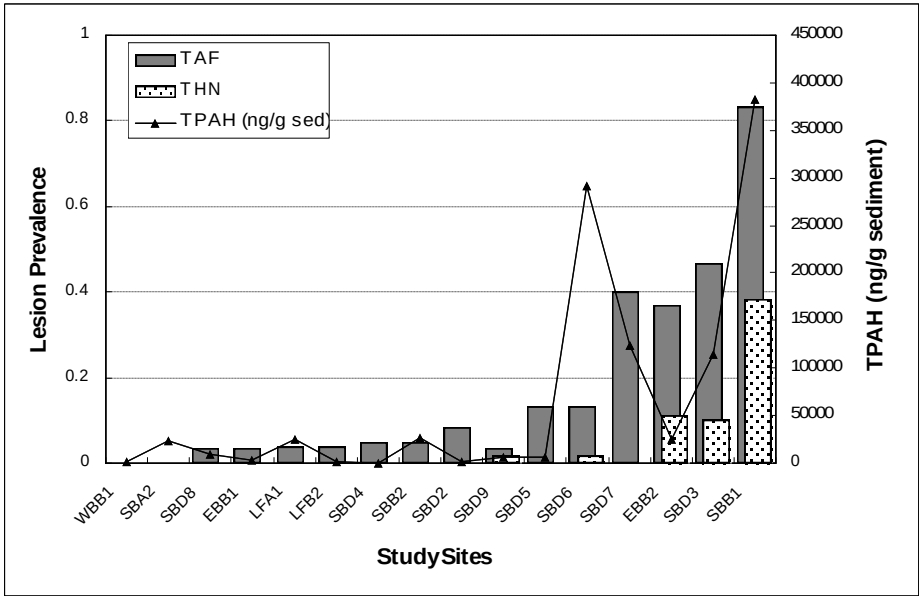
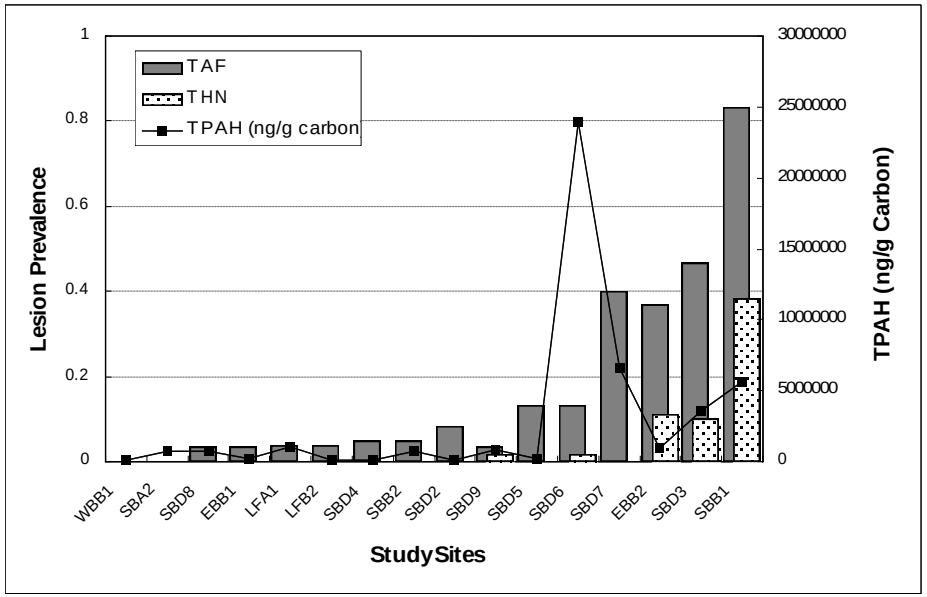


Figure 6. Liver lesion prevalences in mummichogs (*Fundulus heteroclitus*) collected during fall 1998 and 2001 from 12 study sites in the Elizabeth River, Virginia. TAF: altered hepatocellular foci$_{total}$. THN: hepatic neoplasms$_{total}$.



A



B

Figure 7. Mummichog proliferative liver lesion prevalence and total sediment PAH concentrations (A) and total organic carbon (TOC)-normalized sediment PAH concentrations for 16 Elizabeth River study sites investigated during 2006-07.

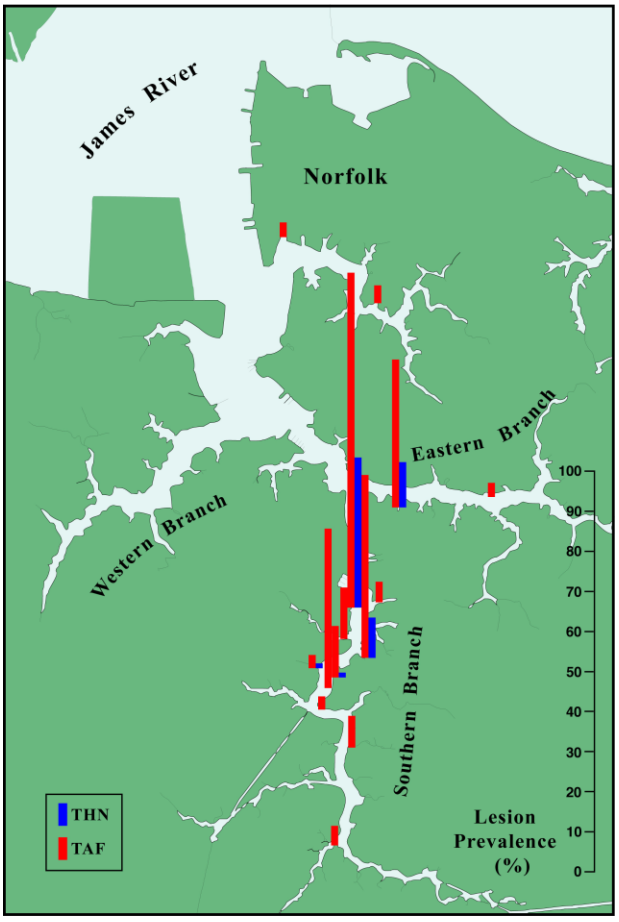


Figure 8. Spatial distribution of mummichog liver pathology in the Elizabeth River. Liver lesion prevalence in mummichogs collected during fall 2006.

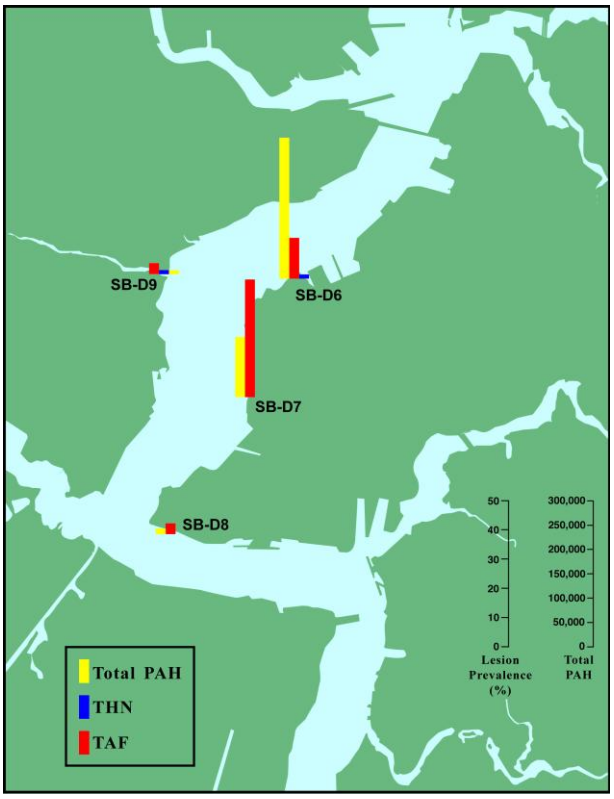
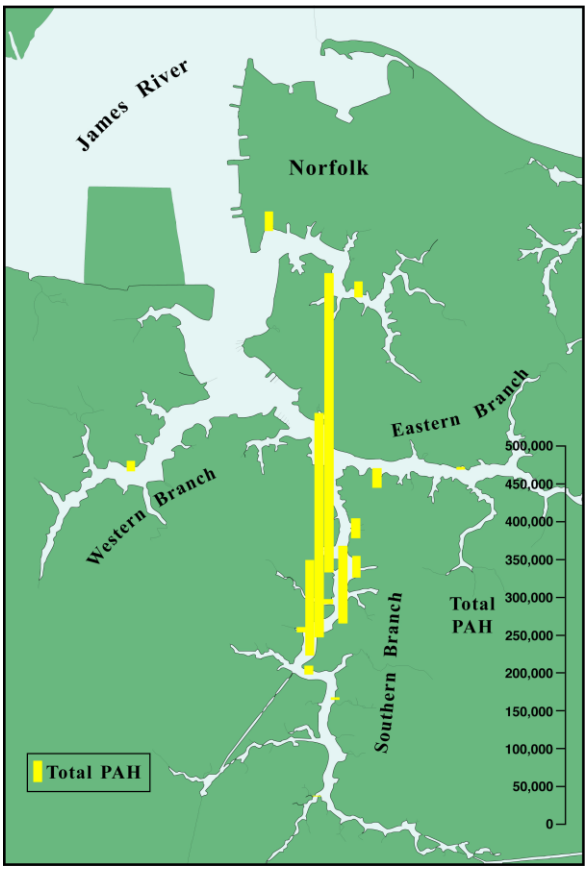
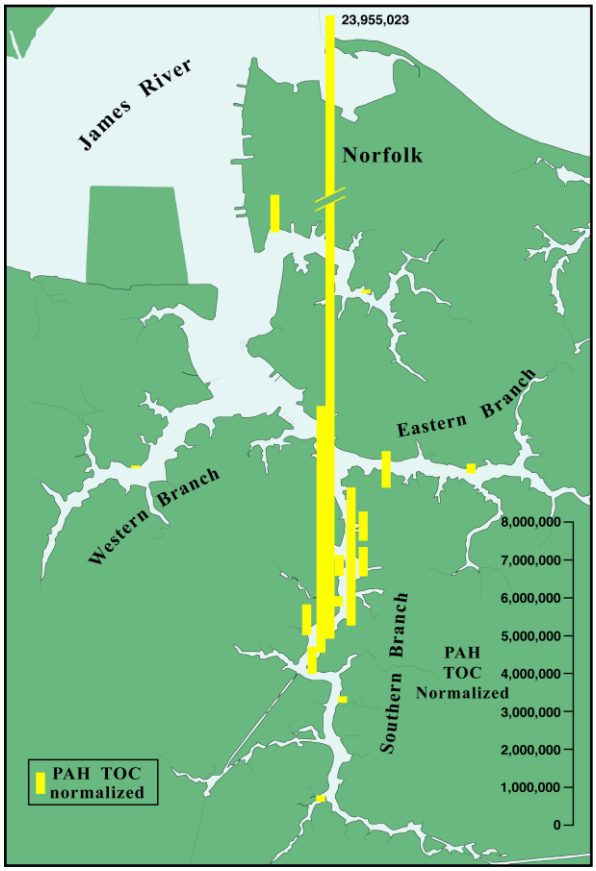


Figure 9. Liver lesion prevalence in mummichogs and sediment PAH concentrations collected during 2006 at the Money study sites. HN: Hepatic neoplasms. AHF: altered hepatocellular foci.



A



B

Figure 10. Spatial distribution of (A) total sediment PAH concentrations and (B) TOC-normalized PAH concentrations at 16 study sites in the Elizabeth River during fall 2006.