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Contaminant Accumulation in Hard and Soft Shell Blue Crabs from an Urban Subestuary

Robert F. Mothershead

College of William and Mary - Virginia Institute of Marine Science

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CONTAMINANT ACCUMULATION IN HARD AND SOFT SHELL
BLUE CRABS FROM AN URBAN SUBESTUARY

A Thesis
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

by
Robert F. Mothershead, II
1991
APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirement for the degree of Master of Arts

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Committee Chairman/Advisor

Approved, June 1991

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Peter Van Veld, Ph.D.
Dedication

This work is dedicated to my wife, Jody, and my parents, Robert and Peggy, in appreciation of the love and support they have all given me throughout my endeavors.
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ABSTRACT

Muscle and hepatopancreas of blue crabs (*Callinectes sapidus*), collected from the Elizabeth River, Virginia, contained residues of polycyclic aromatic hydrocarbon (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides. Analytical procedures involved dichloromethane extraction of lyophilized tissue, followed by molecular size and polarity-based purification of the extract. Capillary gas chromatography with flame ionization detection and electrolytic conductivity detection was used for quantitation and tentative identification of the xenobiotic compounds. Mass spectrometry was used for confirmation of these identities. Contaminant concentrations were greater in hepatopancreas than in muscle in crabs from every site examined. Hepatopancreas PAH burdens were as high as 11 mg/kg dry weight, with an accompanying muscle level of 3.1 mg/kg. The PAHs present consisted mainly of alkylated low molecular weight compounds (e.g., acenaphthenes and naphthalenes), except near a creosote-impacted site. At the latter location, unsubstituted, higher molecular weight PAHs (e.g., pyrene and chrysene) dominated. Organochlorines, which included PCBs, 4,4′-DDE, and chlordane were found in lower concentrations.

The effect of molting on xenobiotic accumulation in Crustacea was also examined. Molting is essential for crustacean growth and is integral to the reproductive process in some species. Molting and intermolt blue crabs were maintained in cages at the creosoted-impacted Elizabeth River site mentioned above. PAH concentration in muscle and hepatopancreas was measured for both molt groups. Newly molted blue crabs possessed statistically higher tissue burdens than intermolt crabs of three unsubstituted PAHs (cyclopenta(def)phenanthrene, fluoranthene, and pyrene) characteristic of the creosote-contaminated exposure site. Mean concentration of the three PAHs in hepatopancreas was 9560 µg/kg in newly molted crabs and 3360 µg/kg in intermolt crabs. Mean PAH concentration in muscle was 1380 µg/kg in new-molts and 498 µg/kg in intermolts. The elevated tissue burdens may be due to increased water uptake and shell permeability at ecdysis or decreased metabolism of PAH during the molt process. Newly molted blue crabs are regarded as a seafood delicacy and tissue burdens of xenobiotics, such as PAHs, may be of human health concern.
CONTAMINANT ACCUMULATION IN HARD AND SOFT SHELL BLUE CRABS
FROM AN URBAN SUBESTUARY
CHAPTER I
GENERAL INTRODUCTION

Part of mankind's legacy to our planet has been the introduction into the environment of xenobiotics, compounds that are foreign to living organisms. These compounds may be anthropogenic, such as polychlorinated biphenyls, or they may be combustion and distillation products of organic material, as in the case of polycyclic aromatic hydrocarbons. The marine environment has been notably contaminated with these organic pollutants, especially in coastal and inland waters associated with a large human population or heavy industrialization.

Polychlorinated biphenyls (PCBs) are synthetic compounds having low vapor pressures, high dielectric constants, low flammability and low water solubility. These properties make PCBs ideal for use in hydraulic fluids and lubricants, as insulators in capacitors and transformers, as additives in paints and varnishes, and as flame retardants (Eisenberg et al., 1980). PCBs were commercially produced as various mixtures of the 209 possible congeners.

Due to their hydrophobic nature, PCBs tend to accumulate in organic sediments and lipid-rich biological tissues. Bioaccumulation of PCBs in finfish, shellfish, and
crustaceans has been reported worldwide (Duke et al., 1970; Malins et al., 1987; Marcus and Mathews, 1987; Villeneuve et al., 1987). The tissue burdens are typically higher in the biota than in associated sediments. In the late 1960’s it was discovered that PCBs pose human health problems. They are thought to cause reproductive impairment and act as carcinogens and teratogens in humans and other biota (Clark et al., 1986; Helz and Huggett, 1987).

The United States restricted use of PCBs in 1971 to ‘closed systems’, such as capacitors and transformers. By this time, however, environmental contamination was widespread. The persistence of these pollutants today is due in part to their previous ubiquitous application and in part to their resistance to degradation. Certain patterns for degradation have been inferred; namely, that two adjacent unsubstituted carbon atoms are necessary. Also, compounds having two or more chlorine atoms in the ortho position are highly resistant (Oliver et al., 1989). Recent research has indicated possible dechlorination of highly chlorinated PCBs to mono- and dichlorobiphenyls by anaerobic bacteria in sediments, but substantial evidence for this route as a major means of PCB degradation is still lacking (Quensen et al., 1988).

Polycyclic aromatic hydrocarbons (PAHs) are composed of fused benzene rings. A major source of these compounds is the incomplete combustion of fossil fuels, but natural
pyrogenesis and diagenesis may also contribute PAHs (Neff, 1979). As with PCBs, the major sink in the aquatic regime of these hydrophobic organic molecules is in the sediment. PAHs accumulate in shellfish, crustaceans and, often, in lower concentrations in finfish (Wade et al., 1989; Speer et al., 1990). PAHs may be introduced into an area via atmospheric deposition, land runoff and spillage.

Some hazards of PAHs to terrestrial animals have been documented. Acute toxicity of parent compounds and chronic toxicity of metabolic products have been observed (Wislocki and Lu, 1988). In general, the low molecular weight (2-3 ring) PAHs exert primarily acute toxicity, while some higher molecular weight aromatics (4-7 rings) act as procarcinogens and promutagens (Neff, 1979).

In aquatic animals PAHs have been implicated in a number of toxic effects. Vascular lesions and significant mortalities observed in the American oyster, *Crassostrea virginica*, upon exposure to petroleum products have been attributed to PAHs (Gardner et al., 1975; Mahoney and Noyes, 1982). Acute mortalities, lesions, cataracts, fin erosion and hepatic neoplasms have been reported in finfish exposed to PAH-enriched sediment (Hargis et al., 1984; Vogelbein et al., 1990). Liver neoplasms developed in fish injected with benzo(a)pyrene, a PAH commonly found in the environment (Black et al., 1988). Although the implications are strong, the evidence to directly link PAHs with chronic toxic
effects in feral marine organisms is insufficient at this time.

In geographic locations where a dense human population is in proximity to the aquatic regime, the input of organic xenobiotics to the environment can be substantial. Heavy industrial activity, spillage, and accidents can contribute significantly to the pollutant levels. Such is the case in the Elizabeth River, a subestuary of the lower Chesapeake Bay. This tidally influenced river is bordered by three cities and a diversity of heavy industries (Figure 1). Two major creosote spills in the 1960's, along with other industrial inputs, have contributed substantial amounts of unsubstituted PAHs, such as fluoranthene and pyrene, to the southern branch (Bieri et al., 1986). The result is a sediment PAH load that is one of the highest recorded in the nation (Bieri et al., 1986). PCBs and chlorinated pesticides also have been detected in sediment from the eastern and southern branches of the river (Hale and Smith, 1988).

Xenobiotic contamination in the Elizabeth River has not been restricted to the sediment. Accumulation of PAHs in oysters and, recently, PAH metabolites in estuarine fish have been reported (Pittinger et al., 1985, 1987; Deshpande, 1989). PCB concentrations as high as three parts per million were found before 1971 in oysters from this subestuary for Aroclor 1242 and 1254, two PCB mixtures
containing 42% and 54% chlorine, respectively (Helz and Huggett, 1987).

To date no published literature has focused on the xenobiotic tissue burdens in the blue crab, *Callinectes sapidus* Rathbun, taken from the Elizabeth River. This crustacean is an extremely valuable fisheries resource for the lower Chesapeake Bay and its tributaries (Commercial Fisheries Statistics, 1988). While female blue crabs traverse the salinity regime of the Bay, the male of the species has a more limited migratory range (Van Engel, 1958). Blue crabs are bottom-dwelling scavengers. As such, they are susceptible to pollutant exposure from the sediment, water, and benthic biota. The high PAH levels reported for each of these ‘sources’ from the Elizabeth River could result in high tissue burdens in blue crabs. Detectable levels of PCBs and alkylated PAHs in the part per billion range were found in blue crabs sampled from the James River, the Rappahannock River, and Pocomoke Sound (Hale, 1988). In spite of the heavy industrialization and shipping traffic, commercial fishing for blue crabs continues in the Elizabeth River.

The elimination of xenobiotics from living organisms requires the transformation of lipophilic compounds to more water-soluble products. In aquatic biota, this transformation is believed to be initiated principally by cytochrome P450-mediated monooxygenases, often referred to
as the mixed function oxygenase (MFO) system. For aromatic compounds, such as PAHs, hydroxylation to phenols and diols is the major monooxygenase reaction. The MFO system has been most extensively studied in mammals, where it has been observed to be involved in metabolism of both endogenous and foreign compounds (Nelson and Strobel, 1987). Cytochrome P450 and monooxygenase activities have been reported in Crustacea (James, 1989b).

While most marine animals have the necessary components of the MFO system, there are differences in specific enzyme activities between vertebrates and invertebrates. Biotransformation of phenanthrene was found to be rapid for teleosts, but much slower for crustaceans, such as lobsters (Solbakken and Palmork, 1981). Molluscs also have a relatively low biotransformation capability (Stegeman, 1985). The rapid metabolism of PAHs in teleosts may explain their characteristically low tissue burdens of parent PAHs, relative to levels in invertebrate aquatic animals.

Crustaceans, such as blue crabs, can only grow by periodic molting of their hard exoskeleton. Intermolt, or stage C, Crustacea are those specimens with a completely rigid exoskeleton (Warner, 1977). Following this classification, stage D represents the premolt condition, stage E represents the ecdysial event and stage A represents the newly molted condition. During stages E and A, the specimen rapidly takes up water from the environment, which
serves to inflate the new integument prior to its hardening. Stage A blue crabs are commonly referred to as "soft shells," because of the poorly calcified exoskeleton. They are considered a seafood delicacy and support a significant commercial fishery in the lower Chesapeake Bay.

Molting is under hormonal control, in cooperation with the central nervous system (Barnes, 1987). The molting hormone, crustecdysone, is activated by hydroxylation of a precursor molecule, ecdysone (Chang and O'Connor, 1978). P450-mediated hydroxylation of ecdysone has been demonstrated in several tissues of spiny lobster (James and Shiverick, 1984). Interestingly, Singer and Lee (1977) found that the capability for P450-mediated xenobiotic biotransformation was depressed in blue crabs during stages E and A, when circulating crustecdysone titers are maximal.

Exposure of crustaceans to a variety of xenobiotics has been shown to affect molting success (Fingerman and Fingerman, 1979; Schimmel et al., 1979; Wang and Stickle, 1987). In general, there is a decrease in successful completion of the molt process or there are less frequent molts. Based on this type of evidence, Lee (1988) speculated that pollutant exposure may interfere with the monooxygenases involved in molting.

Data has been generated in the laboratory which suggests that molting may interfere with the tissue burdens of xenobiotics. Hale (1988) found reduced depuration of
total radiolabel associated with a PAH in newly molted blue crabs compared to intermolt specimens. Conklin and Rao (1978) measured a thirty-fold increase in uptake of pentachlorophenol in newly molted grass shrimp compared with similarly exposed intermolt shrimp.

Blue crabs are an integral part of the ecosystem and economy of Chesapeake Bay. Unfortunately, contaminants have been introduced into many of the blue crabs' habitats, such as the Elizabeth River in the southern portion of the bay. The major objectives of this study were:

1) To document the tissue burdens of PAHs and PCBs in feral male blue crabs from the Elizabeth River, Virginia.

2) To determine whether newly molted blue crabs contain higher tissue burdens of PAH than intermolt blue crabs upon exposure to the high environmental loading of select unsubstituted PAH found in the southern branch of the Elizabeth River.
CHAPTER II
XENOBIOTIC COMPOUNDS IN BLUE CRABS
FROM THE ELIZABETH RIVER

Introduction

The objectives of this part of the study were to determine the concentrations of organic xenobiotic compounds in muscle and hepatopancreas of blue crabs sampled from several sites along the Elizabeth River and to ascertain the PAH and PCB patterns for each tissue. Muscle was chosen for analysis, since it is the major tissue consumed by humans and constitutes a significant percentage of the organism’s body mass. The hepatopancreas was selected because it is the major lipid storage organ and predominant site of organic xenobiotic accumulation (Lee et al., 1976).

Materials and Methods

Adult male blue crabs (carapace width 100 to 150 mm) were collected in June 1989 from six sites within the Elizabeth River, as shown in Figure 1. Three of the six stations were located along the southern branch (SB1, SB2 and SB3) and single stations were located in the western branch (WB), eastern branch (EB) and mainstem of the river.
Figure 1 - Sampling stations in the Elizabeth, York, and Nansemond Rivers.
Two sites outside the Elizabeth River were sampled for comparison purposes. One station was in the Nansemond River (NR), a tributary of the James and adjacent to the Elizabeth. This station was sampled in June 1989. The second comparison site was located in the York River (YR), about 60 km from the mouth of the Elizabeth. Crabs from the York River were collected in September 1989.

Resected muscle and hepatopancreas tissues were analyzed separately. Each sample consisted of the pooled tissues from ten to fourteen crabs. The analytical procedure was based on that reported by Hale (1988), with minor modifications. Initially, each pooled sample was lyophilized and the percent water content determined. Internal standards, 1,1'-binaphthyl and decachlorobiphenyl, were then added to the tissue samples. These compounds were used for quantitation. Dried samples were soxhlet-extracted in glass thimbles for 48 h with dichloromethane (DCM). A blank, consisting of pre-extracted sand, accompanied each batch of samples through all analytical steps, as a check on possible laboratory contamination.

An aliquot of each sample extract was removed for gravimetric determination of DCM-extractable lipoidal material. An aliquot of the remaining extract, containing a maximum of 1 g of lipid, was injected onto a gel permeation chromatography (GPC) column (S-X8 Biobeads, BioRad Laboratories) to separate high molecular weight biogenic
compounds from the lower molecular weight xenobiotic compounds. The GPC fraction of interest was then solvent-exchanged to hexane and reduced in volume to 1 ml with high purity nitrogen in a temperature controlled water bath. The fraction was applied to an open chromatography column containing 10 g of activated silica gel (Bio-Sil A, 100-200 mesh, BioRad Laboratories). Aliphatic compounds were removed by eluting the column with 25 ml of hexane (S1 fraction). The remaining aromatic xenobiotic compounds were eluted from the column with 40 ml of a 4:1 (v/v) mixture of hexane:DCM (S2 fraction). This fraction was concentrated to 1 ml.

Quantitation of xenobiotic compounds was accomplished using high resolution capillary gas chromatography. A Varian 3700 gas chromatograph (GC), equipped with a 30 m DB5 fused-silica column (0.32 mm i.d., 0.25 μm film thickness, J and W Scientific) and a flame ionization detector (FID), was used for PAH analysis. The detector was maintained at 330°C and the injector at 310°C. The linear velocity of the helium carrier gas was 30 cm/s. Sample injection was made in the splitless mode at an initial column temperature of 75°C. After a 1 min hold, the column temperature was increased at 6°C/min to 310°C.

Organochlorines were analysed on a Varian 3300 GC equipped with an OI 4420 electrolytic conductivity detector (ELCD; OI Analytical, College Station, TX). The temperature
program began at 60°C and increased to 310°C at 4°C/min. Analysis of the S1 fraction indicated the presence of small quantities of PCBs. Therefore, the S1 and S2 fractions for each sample were recombined before organochlorine analysis, to insure accurate PCB quantitation. A Hewlett Packard 3350A Lab Automation System was used to collect and integrate peak areas. All concentration values were determined on a dry tissue weight basis.

The retention times of chromatographic peaks were compared to those of PCB and PAH standards, and retention indexes (RI) calculated. These RI were used for tentative compound identification. Confirmation of individual peak identities for representative samples was accomplished using a GC/MS. PAHs were identified with a Dupont 21-492B magnetic sector MS, operating in the electron ionization mode. An Extrel ELQ400-2 quadrupole MS employing negative chemical ionization (NCI), with methane as the moderator gas, was utilized for organochlorine identification. The methodology is outlined as a flow chart in Figure 2.

Accuracy and precision determinations of the analytical methods were made by adding known amounts of representative compounds to aliquots of sample tissue and measuring the recovery of each xenobiotic (Table 1). Recovery was greater than 70% for compounds less volatile than phenanthrene. More volatile compounds gave lower yields. Quantitation of compounds using 1,1′-binaphthyl as the internal standard
Figure 2 - Methodology flow chart.
RESECTED
HEPATOPANCREAS OR MUSCLE
(10-15 CRABS)

FREEZE-DRYER

SOXHLET-EXTRACTED
CH₂Cl₂, 48 HRS

GEL PERMEATION
CHROMATOGRAPHY

SILICA GEL COLUMN

S₁ FRACTION
(ALKANES)

S₂ FRACTION
(XENOBIOTICS)

GAS CHROMAT.
FID, ELCD

GC/MASS SPEC.
E.I., N.C.I.
Table 1. Recovery of Representative Aromatic Compounds Added to Sand and Blue Crab Tissues.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sand</th>
<th>Hepatopancreas (n=4)</th>
<th>Muscle (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,5-dimethyl-naphthalene</td>
<td>9.72</td>
<td>21.3 (2.38)</td>
<td>11.4 (1.45)</td>
</tr>
<tr>
<td>acenaphthene</td>
<td>9.08</td>
<td>28.2 (2.78)</td>
<td>16.5 (2.90)</td>
</tr>
<tr>
<td>dibenzofuran</td>
<td>11.8</td>
<td>22.3 (3.29)</td>
<td>14.5 (2.50)</td>
</tr>
<tr>
<td>fluorene</td>
<td>10.8</td>
<td>37.4 (4.24)</td>
<td>31.6 (5.05)</td>
</tr>
<tr>
<td>phenanthrene</td>
<td>57.0</td>
<td>86.7 (6.62)</td>
<td>69.5 (15.4)</td>
</tr>
<tr>
<td>fluoranthene</td>
<td>109</td>
<td>109 (10.1)</td>
<td>112 (19.5)</td>
</tr>
<tr>
<td>pyrene</td>
<td>81.1</td>
<td>108 (6.85)</td>
<td>106 (16.1)</td>
</tr>
<tr>
<td>chrysene</td>
<td>77.4</td>
<td>95.8 (17.1)</td>
<td>105 (10.1)</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>71.0</td>
<td>104 (28.6)</td>
<td>77.0 (15.5)</td>
</tr>
<tr>
<td>PCB #133</td>
<td>70.9</td>
<td>61.7 (11.8)</td>
<td>79.1 (15.1)</td>
</tr>
<tr>
<td>PCB #180</td>
<td>91.0</td>
<td>160 (38.4)</td>
<td>92.4 (14.8)</td>
</tr>
</tbody>
</table>
will result in conservative estimates for these more volatile xenobiotics. The coefficient of variation for the mean compound recovery, as a measure of precision, was approximately 20%.

The limits of detection for chlorinated and non-chlorinated hydrocarbons are different due to the inherent sensitivity differences between FID and ELCD. In general, for compounds quantified by GC/FID, the methodology described has detection limits of 5-10 μg/kg. For the organohalides using GC/ELCD, detection limits are improved by an order of magnitude.

Results

PAHs, PCBs, 4,4’-DDE, and chlordane mixtures were detected in muscle and hepatopancreas tissues from crabs collected at all study sites (Tables 2 and 3). The samples from the Elizabeth River typically had the highest overall tissue burdens. Blue crabs from the Nansemond River contained the same classes of xenobiotic compounds, at levels comparable to the least contaminated crabs from the Elizabeth River. York River crabs generally had the lowest tissue burdens.

Chromatograms from the FID analysis often contained a complex of chromatographically unresolved compounds (Fig. 3). This unresolved complex mixture (UCM) has been noted previously in blue crabs and oysters analyzed in this
Table 2. Xenobiotic Organic Compounds in Muscle of Blue Crabs  
(μg/kg, dry weight).

<table>
<thead>
<tr>
<th>Station</th>
<th>Resolved Aromatics</th>
<th>Total* Hydrocarbons</th>
<th>PCBs</th>
<th>4,4’-DDE</th>
<th>Total Chlordane</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>1500</td>
<td>3100</td>
<td>28</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>SB1</td>
<td>3100</td>
<td>4100</td>
<td>82</td>
<td>38</td>
<td>26</td>
</tr>
<tr>
<td>SB2</td>
<td>1900</td>
<td>2900</td>
<td>110</td>
<td>60</td>
<td>41</td>
</tr>
<tr>
<td>SB3</td>
<td>1200</td>
<td>2900</td>
<td>58</td>
<td>35</td>
<td>26</td>
</tr>
<tr>
<td>EB</td>
<td>690</td>
<td>1700</td>
<td>51</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>WB</td>
<td>1600</td>
<td>3200</td>
<td>32</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>NR</td>
<td>860</td>
<td>2200</td>
<td>29</td>
<td>17</td>
<td>11</td>
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<tr>
<td>YR</td>
<td>110</td>
<td>340</td>
<td>4</td>
<td>1.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* Includes Unresolved Complex Mixture
Table 3. Xenobiotic Organic Compounds in Hepatopancreas of Blue Crabs
(μg/kg, dry weight).

<table>
<thead>
<tr>
<th>Station</th>
<th>Resolved Aromatics</th>
<th>Total* Hydrocarbons</th>
<th>PCBs</th>
<th>4,4'-DDE</th>
<th>Total Chlordane</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>3800</td>
<td>8500</td>
<td>710</td>
<td>310</td>
<td>260</td>
</tr>
<tr>
<td>SB1</td>
<td>11000</td>
<td>11000</td>
<td>2100</td>
<td>350</td>
<td>350</td>
</tr>
<tr>
<td>SB2</td>
<td>4800</td>
<td>7700</td>
<td>1600</td>
<td>380</td>
<td>360</td>
</tr>
<tr>
<td>SB3</td>
<td>4700</td>
<td>9800</td>
<td>1700</td>
<td>460</td>
<td>420</td>
</tr>
<tr>
<td>EB</td>
<td>2900</td>
<td>5900</td>
<td>1500</td>
<td>280</td>
<td>340</td>
</tr>
<tr>
<td>WB</td>
<td>11000</td>
<td>24000</td>
<td>580</td>
<td>190</td>
<td>200</td>
</tr>
<tr>
<td>NR</td>
<td>1400</td>
<td>2600</td>
<td>640</td>
<td>190</td>
<td>110</td>
</tr>
<tr>
<td>YR</td>
<td>2300</td>
<td>8100</td>
<td>410</td>
<td>40</td>
<td>120</td>
</tr>
</tbody>
</table>

* Includes Unresolved Complex Mixture
Figure 3 - GC/FID chromatogram of muscle extract from blue crabs collected from the western branch of the Elizabeth River. UCM= Unresolved Complex Mixture; Phe= phenanthrene, IS= Internal Standard (1,1'-binaphthyl).
Majority of peaks are alkylated naphthalenes and acenaphthenes
laboratory (Huggett et al., 1984; Hale, 1988; Bender and Huggett, 1989). The UCM area was largest in samples with the greatest concentration of resolvable compounds. Elizabeth River crabs had a concentration range of resolved PAHs in muscle of 690-3100 μg/kg, while the hepatopancreas contained 2900-11000 μg/kg. The concentrations of the resolved compounds, as well as the sum of resolved and unresolved compounds, are presented in Tables 2 and 3 for both tissues. Hepatopancreas PAH burdens were generally two to four times greater than the muscle burdens from the same crabs.

Within the Elizabeth River, the highest PAH levels in hepatopancreas were detected at SB1 and WB. Muscle PAH levels were highest at SB1 and SB2. Two and three ring, alkylated PAHs, such as substituted naphthalenes and acenaphthenes, constituted the bulk of the aromatic hydrocarbons for all three rivers. The notable exception was found at SB1, where, in addition to the previously mentioned compounds, 4 and 5 ring unsubstituted PAHs (such as fluoranthene and chrysene) predominated. Figures 3 and 4 present GC/FID chromatograms of hepatopancreas samples from WB and SB1. The chromatogram from WB illustrates the general pattern that was observed while that of SB1 shows the enhanced prominence of the unsubstituted PAHs.

Organochlorine levels in muscle from Elizabeth River crabs were 28-110 μg/kg for PCBs, 18-60 μg/kg for DDE and
Figure 4 - GC/FID chromatogram of muscle extract from blue crabs collected at SB1 in the Elizabeth River. 
UCM= Unresolved Complex Mixture; 
Ace= acenaphthene, Phe= phenanthrene, 
Fluo= fluoranthene, Pyr= pyrene, Chry= chrysene, 
Bf= benzofluoranthene, IS= Internal Standard 
(1,1′-binaphthyl).
Majority of peaks are alkylated naphthalenes and acenaphthenes.
17-41 µg/kg for chlordanes. Hepatopancreas levels for these same crabs were 580-2100 µg/kg PCBs, 190-460 µg/kg DDE and 200-420 µg/kg chlordanes. Organochlorines were concentrated to a greater extent in the lipid-rich hepatopancreas than in muscle, with a maximum ratio of 20:1. York River crabs showed the greatest hepatopancreas:muscle distribution ratios for both PAHs and PCBs. The PCB congener patterns were similar between tissue types and stations. The major congeners were penta- to heptachlorobiphenyls (Fig. 5), constituents present in Aroclor 1260 (Fig. 6).

Chlorinated pesticide residues were also detected in tissues from all sites, with the highest concentrations in crabs collected from the southern branch of the Elizabeth River. Tissue burdens of DDE and chlordane were comparable between the Nansemond River and the least contaminated Elizabeth River station. Pesticide levels in the York River crabs were generally lower, with the exception of chlordane in hepatopancreas, than in samples from the Nansemond.

GC/ELCD chromatograms of sand blanks contained early eluting peaks at low concentrations, which did not match the retention times of any peaks in tissue samples. A portion of the sand stock was soxhlet-extracted and direct analysis of this extract produced the same chlorinated peak pattern found in the blanks. There was no contamination detectable in DCM allowed to reflux through a barren soxhlet-thimble apparatus for 48 h. This indicated that the contamination
Figure 5 - GC/ELCD chromatogram of hepatopancreas extract from blue crabs collected in the York River. PCB peak numbers are based on IUPAC nomenclature. * - constituents of technical chlordane formulation. IS= Internal Standard (decachlorobiphenyl).
Figure 6 - GC/ELCD chromatogram of Aroclor 1260. Numbers associated with peaks are based on IUPAC nomenclature.
originated in the sand and was not introduced later in the methodology. It is recommended that future method blanks do not include the sand matrix.

Gravimetric determination of the DCM-extractable lipid content of each sample collected in June yielded a mean of 5.43% (SD=1.54) of dry weight for muscle and 49.3% (SD=5.01) for hepatopancreas. By comparison, the York River crabs sampled in September had a dry-tissue lipid content of 4.85% in muscle and 19% in hepatopancreas. The mean water content was 80% for muscle and 75% for hepatopancreas.

Discussion

The highest tissue burdens of organic xenobiotic compounds were found in *C. sapidus* collected from the highly contaminated Elizabeth River. Heavy industrialization, high human population densities, and marinas all contribute to contamination of the river. Elevated concentrations of PAHs in sediment, associated with large creosote deposits derived from industrial spills in the southern branch, have been previously reported (Bieri et al., 1986; Hale and Smith, 1988. The alkylated naphthalenes and acenaphthenes predominating in blue crab tissues from eight of the nine study sites were of relatively low molecular weight, and probably represent contamination by fuel oils. Alkyl naphthalenes have been found to be the predominate aromatic compounds in blue crab tissues upon laboratory exposure to
the water-accommodated fraction of #2 fuel oil (Melzian and Lake, 1987). In contrast, the crabs collected from SB1 contained higher levels of unalkylated compounds such as pyrene, fluoranthene and chrysene. These PAHs were found to be major aromatic compounds in sediment from this site as well as in effluent from a nearby wood-preserving plant (Hale and Smith, 1988). The characteristic PAH composition in crabs from SB1, relative to other areas in the Elizabeth River, may reflect greater availability of the 4 and 5 ring PAHs at SB1 than at the other sites.

PAH tissue levels are a function of uptake and elimination processes. American lobsters collected by Uthe and Musial (1986) near a coal-coking plant had elevated PAH levels in muscle and hepatopancreas and it was reported that depuration of the higher molecular weight PAHs was slow. After holding specimens in a clean environment for 12 months, the authors observed that only 6% of the original chrysene concentration was lost from digestive glands. Laboratory studies with benzo(a)pyrene (BaP) dosed crustaceans have reported conflicting results. American lobsters exhibited slow elimination of radiolabelled BaP (James et al., 1989), while spiny lobster metabolized the compound rapidly (Little et al., 1985). Even among the same species, differing depuration rates have been reported. Hale (1988) observed rapid removal of radiolabelled material from hepatopancreas of juvenile blue crabs, whereas Lee and
coworkers (1976) found a relatively slower rate. Ambient water temperature has been suggested to play a role in depuration rates (Melzian and Lake, 1987).

While the present study did not examine rates of depuration, it is noteworthy that the PAH composition of SB1 crabs differed from other crabs in the southern branch. Although migration of male crabs is thought to be limited, they may be expected to traverse short distances. Blue crabs collected from other sites in the southern branch may well have spent time at SB1, and been exposed to these characteristic PAHs. Rapid elimination of these compounds upon leaving that site may explain the compositional differences found between crabs from SB1 and sites upstream and downstream. Further research considering depuration after chronic exposure is indicated.

Blue crabs are epibenthic omnivores and, therefore, may be exposed to pollutants through the sediments, water column and their food. Laboratory studies have demonstrated extensive accumulation of water and food-borne organic xenobiotic compounds in blue crabs (Lee et al., 1976; Melzian and Lake, 1987; Hale, 1988). Although the present study did not address rates of accumulation, earlier field work with oysters (Crassostrea virginica) transplanted to the southern branch of the Elizabeth determined the total resolved aromatic hydrocarbon level in soft tissues to be as high as 60,000 μg/kg (dry weight) after nine weeks of
exposure (Huggett, 1984). The lipid content of whole oysters is typically on the order of 5% of dry weight (Bender and Huggett, 1989), which is similar to muscle lipid content in the blue crabs examined in this study. The lower PAH levels detected in blue crab muscle (1200–3100 μg/kg) from this same area may be a result of differences in exposure routes or differential metabolic capability between the two species. In vivo studies with a variety of mollusks have found high levels of parent PAHs upon contaminant exposure, but levels of metabolites were low or absent (James, 1989a).

The data for the Nansemond River indicate tissue burdens of comparable magnitude to those of crabs from the eastern branch, western branch and mainstem of the Elizabeth River. The Nansemond River, like the Elizabeth River, is located within a densely populated area.

The low, but detectable, levels of xenobiotic compounds in York River crabs indicate the widespread distribution of these compounds in the environment. The York River, while much less industrialized than the Elizabeth, does have a power generating plant, an oil refinery and a naval weapons facility along its shores. A recent study examined sediment concentrations near the outfalls for the refinery and the power plant (Hale and Smith, 1988). A combination of alkyl substituted and unsubstituted PAHs was detected. The sediment PAH concentration near the power plant was 1100
μg/kg, while 4500 μg/kg was found in sediment near the refinery outfall. Oysters from this same site contained high molecular weight PAHs at a concentration of 1500 μg/kg and PCBs at 60 μg/kg. In addition to local point sources, aerial fluxes of PAHs and PCBs into the marine environment may contribute to the total pollutant load (Webber, 1983; Baker and Eisenreich, 1990).

Although some PAHs are known procarcinogens in mammals, there are no established safe levels for aquatic biota destined for human consumption. The FDA limit for edible tissue burdens of PCBs is 2000 μg/kg, wet weight. Muscle levels for the Elizabeth River crabs, expressed on a wet weight basis, ranged from 5.6 to 22 μg/kg, or two orders of magnitude lower than the FDA limit. Blue crab muscle collected in upper Chesapeake Bay in 1976 (Eisenberg et al., 1980) contained 50 μg/kg of PCBs, reported as Aroclor 1260. More recently, blue crabs from South Carolina coastal areas were reported to possess muscle concentrations of 90 μg/kg (Marcus and Renfrow, 1990). While muscle is the major tissue of human consumption, hepatopancreas may also be ingested. Converting the highest hepatopancreas burden in the Elizabeth, at SB1, to a wet weight basis yields 420 μg/kg. Therefore, PCB contamination of blue crabs from the Elizabeth River does not appear to present a human health risk at this time, based on currently accepted safe levels.

Penta-, hexa- and heptachlorobiphenyls were the major
congeners observed in crabs examined in the present study. The bioaccumulation potential of a PCB increases as the number of chlorines increases. This is due to increased lipophilicity and decreased in vivo degradation. It has been suggested that oxidative metabolism of PCBs requires two adjacent unsubstituted carbon atoms. Most highly chlorinated PCB congeners are recalcitrant to oxidative metabolism and are, therefore, difficult to excrete once sequestered in an organism (Gamble, 1986).

The PCB congener pattern was similar for all samples. The five most prevalent congeners were 153, 138, 180, 187 and 118 (IUPAC nomenclature). All are ortho-chlorinated PCB and together constitute approximately 9% of Aroclor 1254 and approximately 30% of Aroclor 1260 (Schulz et al., 1989). These congeners have been reported to be significant contributors to the total PCB content of fish samples from lakes in the United States and Canada (Maack and Sonzogni, 1988; Niimi and Oliver, 1989). McFarland and Clarke (1989) have identified the same five congeners as PCBs of highest environmental concern based on their potential toxicity, abundance and frequency of occurrence.

The lipophilic nature of the PAHs, PCBs and chlorinated pesticides also leads to higher tissue burdens for the lipid-rich hepatopancreas than for the lipid-poor muscle. This differential accumulation was evident at every site studied. Earlier research found similar tissue
distributions (Lee et al., 1976; Melzian and Lake, 1987; Hale, 1988). The hepatopancreas of decapod crustaceans, the equivalent of the liver in vertebrates, has been demonstrated to be an active site of PAH metabolism (Lee et al., 1982). Differences in recalcitrance and lipophilicity between the aromatic hydrocarbons and the organochlorines may account for the lower hepatopancreas:muscle ratios observed for PAHs than for PCBs.

PCBs, chlordanes and DDT are no longer commercially produced in the United States. Their earlier usage, coupled with their lipophilic nature and overall resistance to degradation, has resulted in widespread environmental contamination. PAHs, on the other hand, are continuously supplied to the environment through incomplete combustion of fossil fuels and organic matter, as well as spillage of fossil fuels and coal tar products. The bioaccumulation of these organic xenobiotic compounds in commercial species, such as the blue crab, can lead to human exposure and may induce health problems. Blue crabs collected from the Elizabeth River have substantial tissue burdens of PAHs, as well as detectable levels of organochlorines.
CHAPTER III
INFLUENCE OF ECDYSIS ON THE ACCUMULATION
OF PAH IN FIELD EXPOSED CRABS

Introduction
To examine further the relationship between molting and xenobiotic tissue burdens in crustaceans, a field study was conducted in which molting and intermolt blue crabs were exposed to environmental PAHs. The study was conducted at SB1 in the Elizabeth River, due to the high tissue burdens and characteristic composition of PAHs found in feral blue crabs from this site, as reported in Chapter II. Dominant compounds found in indigenous crabs, as well as in sediment (Bieri et al., 1986), include unsubstituted PAHs such as fluoranthene and pyrene.

Materials and Methods
Field Experiment
Mature male blue crabs (carapace width 126 to 151 mm) were obtained from a commercial crab shedding operation. All crabs had been collected from, and maintained in, relatively pristine waters. Stage C intermolt crabs and stage D premolts were used in the experiment. Stage D crabs
from the holding tanks at the processing facility were also collected for direct tissue analysis to determine background concentrations.

Premolt and intermolt crabs were paired and placed in wire mesh baskets. Individuals of each pair were separated by a wire screen. Twelve such pairs were then placed in a wooden box float, constructed to allow water to flow through. Upon ecdysis, the newly molted (Stage A) crab and the intermolt companion were removed from the baskets. The hepatopancreas, and muscle from the base of the swimming legs, were resected from each crab and individually placed in clean pre-weighed jars with solvent-rinsed foil between the jar and the lid. The hepatopancreas, or digestive organ, typically has been observed to contain the highest tissue burdens of xenobiotics (James and Little, 1984; Hale, 1988) and has been suggested as a major site of hydrocarbon metabolism in blue crabs (Lee et al., 1976). Muscle was also analyzed since it constitutes the bulk of the biomass of the crab and is the predominant tissue of human consumption.

At each sampling, the water temperature, salinity and dissolved oxygen content were recorded. Water samples were also collected adjacent to the float, in 4 liter amber glass bottles, approximately 20 cm below the water’s surface at the beginning, midway through, and at the conclusion of the experiment. The water samples were placed on ice for
transport to the laboratory, where they were stored at 4°C until analyzed. The experiment was terminated after the last molting crab had completed ecdysis, approximately three days.

Laboratory Analysis

The water samples were analyzed for basic, neutral, and acidic compounds within seven days of collection. Surrogate standards, decachlorobiphenyl and 1,1'-binaphthyl, were added to one liter of unfiltered water in a two liter separatory funnel. The pH was adjusted with 10M NaOH to pH > 11. The samples were extracted with three 60-mL aliquots of dichloromethane (DCM). Any emulsions that formed were broken by passage over solvent-extracted glass wool or freezing. The remaining aqueous fraction was acidified to pH < 2 with 9M H₂SO₄. The samples were then sequentially extracted with three 60-mL aliquots of DCM and the organic layers containing acidic compounds combined. All extracts were concentrated with a rotary evaporator and then transferred to 15-mL centrifuge tubes, along with DCM rinsings of the collection flasks. The samples were then concentrated under N₂ to 0.1 mL. Water samples were analyzed for PAHs by GC/FID and for organochlorines by GC/ELCD.

Tissue analysis was conducted as detailed in Chapter II, with minor modifications. Samples consisted of
individual crab tissue and not pooled tissue from several crabs. The packing for the GPC column also was changed from 100 g SX-8 to 70 g SX-3 Biobeads (BioRad Laboratories). This resin was found to provide better separation between biogenic lipids and xenobiotics. Dichloromethane continued to be used as the elution solvent. Proper elution volumes were checked with PAH and PCB standards, as well as with DCM-extracted lipids of blue crab hepatopancreas.

To insure high activation, silica gel was stored at 110°C until just prior to use. As a result, xenobiotics were found exclusively in S2 fractions for the initial samples analyzed in the present study. Thus, S1 and S2 fractions were not combined for GC/ELCD analysis, as carried out in the earlier study. Recoveries of surrogates in tissue and water extracts were determined by adding internal standards to the samples immediately prior to gas chromatography. P-terphenyl was added for FID chromatography and pentachlorobenzene was added for ELCD chromatograms.

Statistical Analysis

Data were tested for normality with the Kolmogorov-Smirnov goodness-of-fit test and for homoscedasticity with the variance ratio test. Since the crabs were paired, a paired Student's t-test was used to test for no differences between intermolt and ecdysial crabs
for each tissue (Zar, 1984). Since more than one t-test was used, the α level for individual tests was adjusted to give an experimentwise error rate of 0.05 (Sokal and Rohlf, 1981).

Results

Of the twelve premolt crabs present at the start of the experiment, nine molted successfully. These nine crabs and their intermolt companions were used for tissue burden analysis. The average increase in carapace width upon molting was 20% of the initial size. Salinity ranged from 16 to 19 g/kg throughout the experiment. Water temperature was between 19.5 and 22.0°C and dissolved oxygen fluctuated from 6.6 to 8.1 mg/L. There were no xenobiotics detected in the water samples collected from the site. The detection limit for water analysis was approximately 0.2 µg/L per compound.

Hepatopancreas was determined to be 75% (SD=1.7) and 62% (SD=7.8) water in stage A and C crabs, respectively. Muscle was found to be 81% (SD=1.4) water for stage A and 79% (SD=6.2) for stage C crabs. The mean DCM-extractable lipid content of hepatopancreas was 33% (SD=4.4) of the dry weight for stage A and 38% (SD=5.8) for stage C crabs. Muscle lipids measured 5.8% (SD=1.8) of dry weight in stage A and 5.3% (SD=1.2) in stage C crabs.

Seven major aromatic compounds were found in the
hepatopancreas and muscle of all the exposed crabs (Fig. 7A). Background crab tissues contained four of these compounds (acenaphthene, dibenzofuran, fluorene, and phenanthrene), while the higher molecular weight PAH (cyclopenta(def)phenanthrene, fluoranthene, and pyrene) were absent (Fig. 7B). Therefore, for comparison of uptake between the molting and intermolt crabs, these three PAH were used. The three compounds were quantitated on a dry weight basis and their sample sum used for the statistical analysis. These PAH are planar molecules consisting of three or four fused benzene rings.

The difference in the PAH burdens between stage A and stage C crab tissues was calculated for all nine pairs. A null hypothesis stating the mean difference as zero was used for the one-tailed paired Student's t-test. This null hypothesis was rejected for PAH burdens in both muscle and hepatopancreas (Table 4). Each test was performed at $\alpha = 0.025$, yielding an experimentwise error rate of 0.05. The hepatopancreas concentrations of the three PAH were generally an order of magnitude greater than in the accompanying muscle tissue.

Organochlorines in background and field-exposed crabs consisted of mainly polychlorinated biphenyls (PCBs) and p,p'-DDE. These compounds were present at similar levels in all the crabs. For example, the total tissue burdens of the five predominant PCB congeners (IUPAC #'s 118, 153, 138,
Figure 7 - (A) GC/FID chromatogram of the hepatopancreas extract from a newly molted blue crab maintained at the experimental field site. (B) GC/FID chromatogram of the hepatopancreas extract from a "background" crab.
Table 4. Total Tissue Burdens (μg/kg) in Blue Crabs of Three PAH* and
The Differences Between Stage A and Stage C Crab Pairs.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Background</th>
<th>Newly-Molted (Stage A)</th>
<th>Intermolt (Stage C)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>BQL</td>
<td>1380 (753)</td>
<td>498 (245)</td>
<td>885 (642)</td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>BQL</td>
<td>9560 (3730)</td>
<td>3360 (2800)</td>
<td>6200 (2060)</td>
</tr>
</tbody>
</table>

a - PAH consist of cyclopenta(def)phenanthrene, fluoranthene, and pyrene.
b - Values are below the quantitation limit of 5 μg/kg.
c - Mean with standard deviation in parentheses, n=9.
d - Statistically different from zero using paired Student’s t-test at α=0.025.
180, and 187) were 283 μg/kg (SD=271) in hepatopancreas of background crabs, 410 μg/kg (SD=363) in exposed stage A crabs and 377 μg/kg (SD=168) in exposed stage C crabs. This similarity is not surprising, based on the earlier analyses that found comparable organochlorine tissue burdens between indigenous crabs from the Elizabeth River and those from the less industrialized Nansemond and York rivers. The blue crabs analyzed in the present study were originally captured in the York River.

The limits of quantitation were 5 μg/kg for GC/FID and 0.5 μg/kg for GC/ELCD. The method recovery of 1,1'-binaphthyl was 95.5% (SD=9.40) and for decachlorobiphenyl was 79.7% (SD=27.7).

Discussion

The estuarine site chosen for this exposure study has been characterized previously as having high sediment concentrations of unsubstituted PAHs, particularly three to five ring compounds (Bieri et al., 1986). The abundance of these xenobiotics was linked to spills of creosote mixtures into the river from a nearby wood preserving facility. Three of the most abundant PAHs in the sediment were cyclopenta(def)phenanthrene, fluoranthene, and pyrene. These compounds were also found to be dominant in effluents from another wood preserving facility near the exposure site (Hale & Smith, 1988). Feral blue crabs collected from SB1
contained these same three PAHs at combined tissue burdens of 2,400 µg/kg in hepatopancreas and 340 µg/kg in muscle. These levels are very similar to those in stage C crabs from the present three day study and indicate rapid accumulation of these compounds upon exposure.

The deleterious effects that xenobiotic exposure has on crustacean molting have been the subject of previous work (Fingerman and Fingerman, 1979; Schimmel et al., 1979). Juvenile blue crabs, exposed to the water-soluble fraction of crude oil for 21 days, demonstrated reduced growth with molting, in a concentration dependent manner (Wang and Stickle, 1987). Molting in adult blue crabs typically results in an increase in shell width of 25% to 33% of the initial size (Van Engel, 1958). The average growth increment (20%) in the nine molting crabs from the present study was close to the normal range, so there appears to have been no drastic effect on growth during the short term exposure.

Ninety one percent of stage D premolt crabs have been reported to molt successfully when placed in floats similar to those used in the present study (Beaven and Truitt, 1939). The 75% molt success rate achieved with the current experiment does not substantially differ from this value. However, the use of crabs that are in the late premolt stage may assure high molt success. Karinen and Rice (1974) found that the closer Tanner crabs are to ecdysis when exposed to
crude oil, the higher the molt success.

The results of the present study indicate that molting in blue crabs affects the observed tissue burdens of certain PAHs. Newly molted (stage A) blue crabs had higher levels in both muscle and hepatopancreas than intermolts. This phenomenon has been reported previously by Conklin and Rao (1978), who measured a thirty-fold increase in uptake of pentachlorophenol in newly molted grass shrimp compared with similarly exposed intermolt shrimp. Two possible hypotheses may be provided to explain these results. The physiological mechanism of ecdysis involves increased uptake of ambient water, mainly through the gut (Warner, 1977). For water-borne xenobiotics, or those sorbed to suspended particles, this may lead to increased exposure and elevated accumulation in molting crustacea. At ecdysis, the new exoskeleton is more permeable and pollutant transport across the integument may also be enhanced. Lipophilic compounds may then partition into the tissues and accumulate in the lipid-rich hepatopancreas.

An alternate, and perhaps coexisting, explanation involves an interaction between molting and PAH metabolism. Hale (1988) found reduced depuration of total radiolabel associated with a PAH in newly molted blue crabs compared to intermolt specimens. P450-mediated aryl hydrocarbon hydroxylase (AHH) activity in blue crabs is depressed during stages E and A, when crustecdysone titers are highest
(Singer and Lee, 1977). O’Hara et al. (1985) found competitive P450 metabolism between cholesterol, a precursor to ecdysone, and PAHs in the shore crab, *Carcinus maenas*. In fish, levels of various P450’s, including the major PAH metabolizing form, were lowered following injection of steroids (Pajor et al., 1990). While ecdysone is not the steroid substrate for hepatopancreas microsomes in spiny lobster (*Panulirus argus*), it was hydroxylated by mitochondrial P450 proteins in the hepatopancreas and the green gland (James and Shiverick, 1984). The hepatopancreas of the crab, *Pachygrapsus crassipes*, is second only to the hemolymph in ecdysone content (Chang et al., 1976) and has the largest amount of total hydroxylase activity (Chang and O’Connor, 1978). Competition between PAHs and molting hormones need not be for specific enzyme active sites, but could be for cellular cofactors of the monooxygenase system, e.g., NADPH or necessary reductases. NADPH has been identified as the primary rate limiting cofactor in intact hepatocytes of mammals (Thurman and Kauffman, 1980). Whether elevated levels of ecdysone during molting are responsible for the increased concentrations of PAHs in newly molted crabs remains to be determined.

Whatever the mechanism for enhanced accumulation, the higher PAH levels in the ecdysial crabs are of concern. Soft shell blue crabs are a food source for aquatic organisms, such as fish and other crabs, as well as humans.
While this study concentrated on three specific PAHs, the observed phenomenon may be expected to occur with other xenobiotics at high environmental loadings. Many PAHs are considered proximate carcinogens in mammals (Wislocki and Lu, 1988) and fish (Metcalfe, 1989) and have been associated with pathological disorders in bottomfish (Baumann, 1989). Although such problems have not been described in blue crabs, the increased tissue burdens in newly molted crabs could yield negative effects for this species. Female blue crabs undergo a terminal molt, at which time they mate. The subsequent production of lipid-rich eggs may provide a reservoir for transfer of accumulated PAHs from other tissues, as observed to occur with the chlorinated pesticide Kepone (Roberts and Leggett, 1980). This could have deleterious effects on larval survival.
CHAPTER IV
CONCLUSIONS

Muscle and hepatopancreas from male blue crabs collected at sites in the Elizabeth River contained substantial levels of PAHs, as well as detectable levels of PCBs and other organochlorines. Hepatopancreas consistently possessed higher levels of all xenobiotics than muscle. Alkylated low molecular weight compounds formed the majority of PAHs in crabs from most sites, indicating fuel oil contamination. However, unsubstituted high molecular weight PAHs predominated in tissues of crabs collected from one site in the southern branch of the river. The presence of these compounds in crabs corresponds to their domination in sediment near this site and is believed to result from spillage of creosote (Bieri et al., 1986).

Newly molted blue crabs were found to have statistically higher tissue burdens of selected PAHs than intermolt crabs, after field exposure to the creosote-impacted area of the Elizabeth River. This phenomenon was observed in both muscle and hepatopancreas. Whether these observations are simply due to increased accumulation following the rapid water uptake characteristic of ecdysis or are the result of decreased metabolism of the PAHs is yet
to be elucidated. Future work into the mechanisms controlling this bioaccumulation may provide further insight.
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