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Comparison of Uptake and Disposition of Organic Pollutants in Intermolt and Ecdysial Blue Crabs, Callinectes sapidus

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Comparison of Uptake and Disposition of Organic Pollutants in Intermolt and Ecdysial Blue Crabs, *Callinectes sapidus*

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

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by

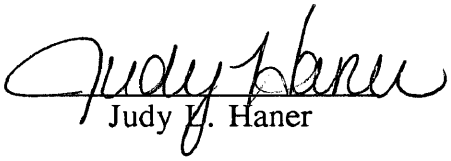
Judy L. Haner

1993

APPROVAL SHEET

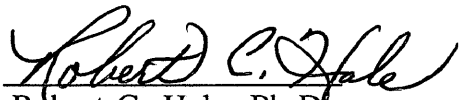
This thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Arts



Judy H. Haner

Approved: August 1993



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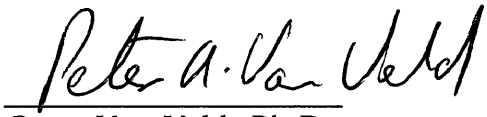

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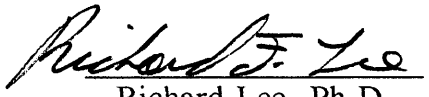

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DEDICATION

To my family for all of their love and support.

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ACKNOWLEDGEMENTS

A debt of gratitude goes to my major professor, Dr. Rob Hale, for his guidance and advice. His patience, understanding and encouragement were unsurpassed. A special thanks goes to my committee members: Dr. Romuald Lipcius for his invaluable assistance with statistics and ecology; Mr. Jacques Van Montfrans for his insight into crustacean biology; Dr. Pete Van Veld for his knowledge of methodology and bioconcentration; and Dr. Richard Lee for his copious knowledge of crustacean toxicology.

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ABSTRACT

Changes occurring during ecdysis may contribute to differences in uptake and concentration of pollutants in molting and non-molting blue crabs, *Callinectes sapidus*. Premolt and intermolt crabs were exposed to ^{3}H-BaP (0.01 ng/l) and ^{14}C-PCB-153 (21 ng/l) to examine the bioconcentration of xenobiotics during the molt cycle.

No differences in concentrations of BaP in the hepatopancreas were seen between premolt and intermolt crabs exposed for 24 or 48 hours. Intermolts concentrated more PCB-153 in the hepatopancreas, following 24 hours of exposure, than premolt crabs. However, no differences were seen in concentrations of PCB-153 in the hepatopancreas between premolt and intermolt crabs following a 48 hour exposure period.

BaP was more highly concentrated in the hepatopancreas than the gills for both premolt and intermolt crabs following a 24 hour exposure. PCB-153 was found at similar concentrations in the hepatopancreas and gills for both molting and non-molting crabs following a 24 hour exposure.

Metabolite analysis revealed that highest concentrations of BaP were present as water soluble metabolites in the hepatopancreas for both premolt and intermolt crabs. Conversely, PCB-153 in the hepatopancreas was found predominantly as parent compound.

Control and treated molting crabs were examined for growth differences following ecdysis. No differences were apparent between treated and control crabs for carapace width increase or weight gain.

Factors potentially affecting the accumulation of xenobiotics in crabs are numerous. Metabolic processes occurring at the tissue and cellular levels may determine the quantity of pollutants taken up from a waterborne source. These processes include, but are not limited to respiration, O_2 availability, biotransformation and translocation of compounds in the tissues of the organism.

COMPARISON OF UPTAKE AND DISPOSITION OF ORGANIC POLLUTANTS IN INTERMOLT AND ECDYSIAL BLUE CRABS, *CALLINECTES SAPIDUS*

INTRODUCTION

Xenobiotic compounds, or chemicals foreign to the body, may be introduced to the environment through many routes, including industrial and agricultural applications (Ahearn 1974). Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are two common classes of xenobiotics. PAHs are present in petroleum and creosote, and produced as a result of combustion of fossil fuels (Ahearn 1974). PCBs have been used mainly as additives in hydraulic and transformer fluids, lubricating oils, and as plasticizers. Production of PCBs has, in most cases, been halted (Philips 1986).

Contaminants in the atmosphere, terrestrial environment and water affect resident organisms. Depending on the compound and its concentration, effects may immediately manifest themselves in the exposed organism, or they may show a delayed effect, altering survival, growth or reproduction.

Aquatic organisms may be exposed to and accumulate xenobiotics through food, water or sediments (Lee et al. 1976, Marcus and Mathews 1987). Invertebrates, specifically crabs, can accumulate pollutants from contaminated food sources (Shchekaturina 1981). Observations also show bioconcentration, uptake through water, of toxicants by *Callinectes sapidus* (Roberts 1981, Lee et al. 1972).

The blue crab, *Callinectes sapidus*, is an important component of the Atlantic East and Gulf coasts of North America. It serves as scavenger, predator and prey (Van Engel 1958, Millikin and Williams 1984) in the fundamental food webs of the Chesapeake Bay ecosystem. The potential for uptake of xenobiotics from food, water

or sediments by blue crabs is high due to their diverse feeding habits and their interaction with the benthos (Van Engel 1958, Millikin and Williams 1984). The long-term growth and population-related effects of exposure to pollutants derived from sediments, food or water, have not yet been determined in this organism.

Blue crabs support a vital fishery, both as a hard-shelled and soft-shelled delicacy. Human consumption of crab tissues other than muscle, such as the hepatopancreas, is common (Mothershead and Hale 1992, Hale 1988). PAHs and PCBs are generally hydrophobic, lipophilic compounds, having relatively high bioconcentration factors (BCF). The BCF is a ratio of the concentration of a compound in the animal to the concentration in the water. (benzo(a)pyrene (BaP) in mysids = 7500; Landrum and Poore 1988) (total PCBs in decapod shrimp = 35,000; Verschueren 1983). High BCFs imply significant uptake from the water and association with lipophilic tissues. Contaminants which accumulate in lipophilic tissues, such as the hepatopancreas of crustaceans (Lee et al 1976), may remain within the lipid reserves of the organism for extended periods of time. Associated higher levels of PAHs and PCBs in certain tissues, such as the hepatopancreas, should be addressed when considering the health of consumers of *C. sapidus*.

Although not as lipid rich as the hepatopancreas, the gills may play a major role in uptake, metabolism and tissue distribution of dissolved pollutants (Barron et al. 1989, McKim and Goeden 1982). The gills serve as a primary point of intake for dissolved compounds. Enzymatic breakdown of some compounds may also occur in this organ (Barron et al. 1989). Translocation of some compounds from the gills, an

uptake organ, to the hepatopancreas, a storage organ, may occur.

Intermolt crabs may accumulate xenobiotics through ingestion of contaminated food or water, or through respiration, by passing water over the highly vascular gills. A primary mode of accumulation of xenobiotics during ecdysis may be through the uptake of dissolved compounds (Melzian and Lake 1986/87). During the ecdysial process, crabs do not eat; therefore, uptake of pollutants would be through water-borne sources only. During ecdysis, shedding of the hard exoskeleton exposes a larger, more permeable, surface. This membrane may allow enhanced accumulation of pollutants. While the new exoskeleton is soft, water is absorbed more readily (Johnson, 1980). In addition to the uptake through the permeable exoskeleton, accumulation also occurs due to uptake across the gills (Cantelmo 1976) and ingestion by drinking, after ecdysis (Johnson, 1980).

In a field study, Mothershead and Hale (1992) observed elevated concentrations of selected PAHs in muscle tissue and hepatopancreas of crabs progressing through ecdysis, when compared with intermolt crabs. Hale (1988) reported an overall lower uptake (35%) of the introduced ^{3}H-BaP by newly molted crabs when compared with intermolt crabs (65%). However, following 48 hours of depuration, the intermolt females had released 65% of the pollutants previously accumulated. Newly molted crabs released only 10% of the accumulated pollutants, resulting in increased retention of pollutants. A number of functional differences in the ecdysial process may contribute to the discrepancies in levels of pollutants between molting and non-molting crabs. Following ecdysis, respiration rates (deFur 1990) and

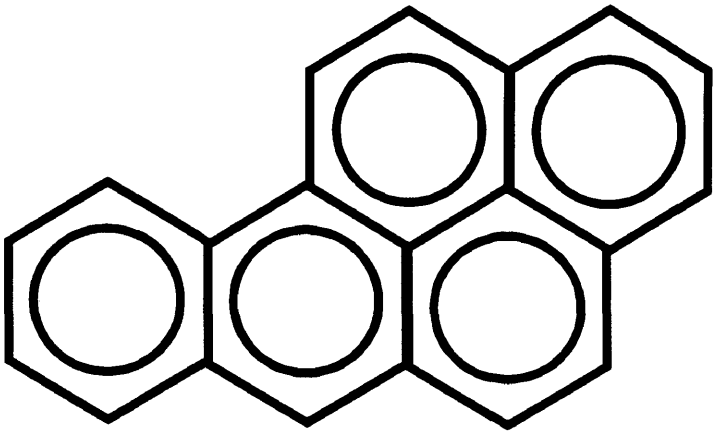
enzymatic activities (Lee and Singer 1982, Conklin and Rao 1982) decrease drastically, digestion processes cease and water uptake increases (Johnson 1980).

Blue crabs also have the potential to biotransform pollutants into other compounds, i.e. form metabolites, which may be more readily excreted. Some metabolites exhibit carcinogenic potential, in addition to acute toxicity (Buhler and Williams 1988). The monooxygenase (MO) system in blue crabs aids in contaminant degradation (Lee et al. 1976). The hepatopancreas is the major site of xenobiotic metabolism in *C. sapidus* (Lee et al. 1976, Johnston and Corbett 1986). The MO enzymes metabolize toxicants into more easily excreted organic soluble and water soluble compounds (Lee et al. 1976). While intermolt crabs have been shown to metabolize xenobiotics (Lee et al. 1976), the efficiency of the MO system during ecdysis will determine if pollutants are stored during this period, or if they are also biotransformed and excreted.

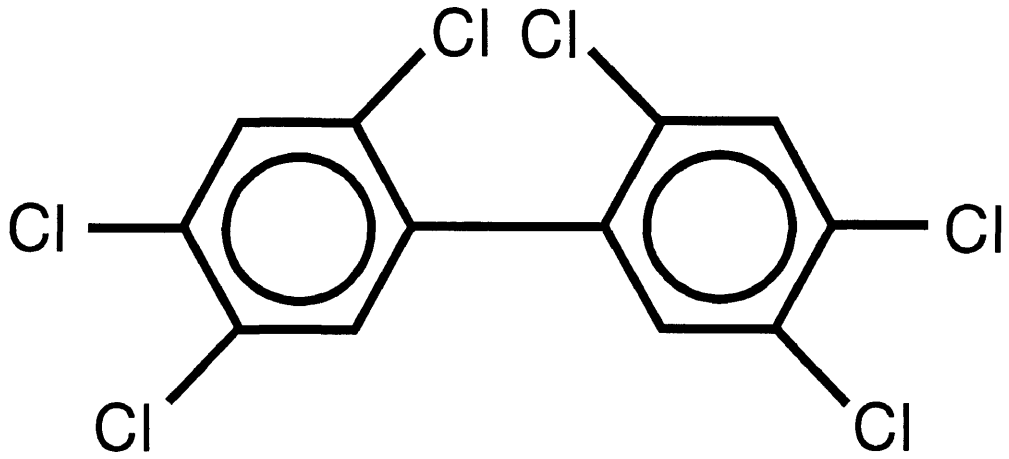
The major focus of this study was to examine the uptake in molting and non-molting crabs of two radiolabelled xenobiotics: benzo(a)pyrene and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) (Fig.1). BaP, a PAH, is efficiently metabolized by the blue crab (Lee et al. 1976, Lee 1989) to organic soluble and water soluble metabolites. Conversely, PCB-153 is not readily biotransformed due to its high degree and pattern of chlorination (Lee 1989, Bickel 1989, Hutzinger et al. 1972). Relative concentrations of these radiolabelled compounds will be examined in the crabs as a means of observing the bioconcentration process in molting and non-molting crabs.

Figure 1. Molecular structure and weight of 1, 3, 6,-^{3}H benzo(a)pyrene (BaP) and 2, 2', 4, 4', 5, 5'-^{14}C hexachlorobiphenyl (PCB-153).

1, 3, 6 - ^{3}H Benzo(a)pyrene (BaP)



2, 2', 4, 4', 5, 5'- ^{14}C Hexachlorobiphenyl (PCB-153)



The objectives of this study are to:

1. determine uptake of an easily metabolized PAH (BaP) and a poorly biotransformed PCB (PCB-153) in molting and non-molting crabs over 24 and 48 hour exposure periods;

2. compare BaP and PCB-153 concentrations in the gills, an uptake organ, and the hepatopancreas, a storage organ;

3. examine the proportions of radiolabelled material associated with parent compound, organic soluble metabolites and water soluble metabolites in the hepatopancreas of premolt and intermolt crabs;

4. examine possible growth related differences between treated and control premolts.

MATERIALS AND METHODS

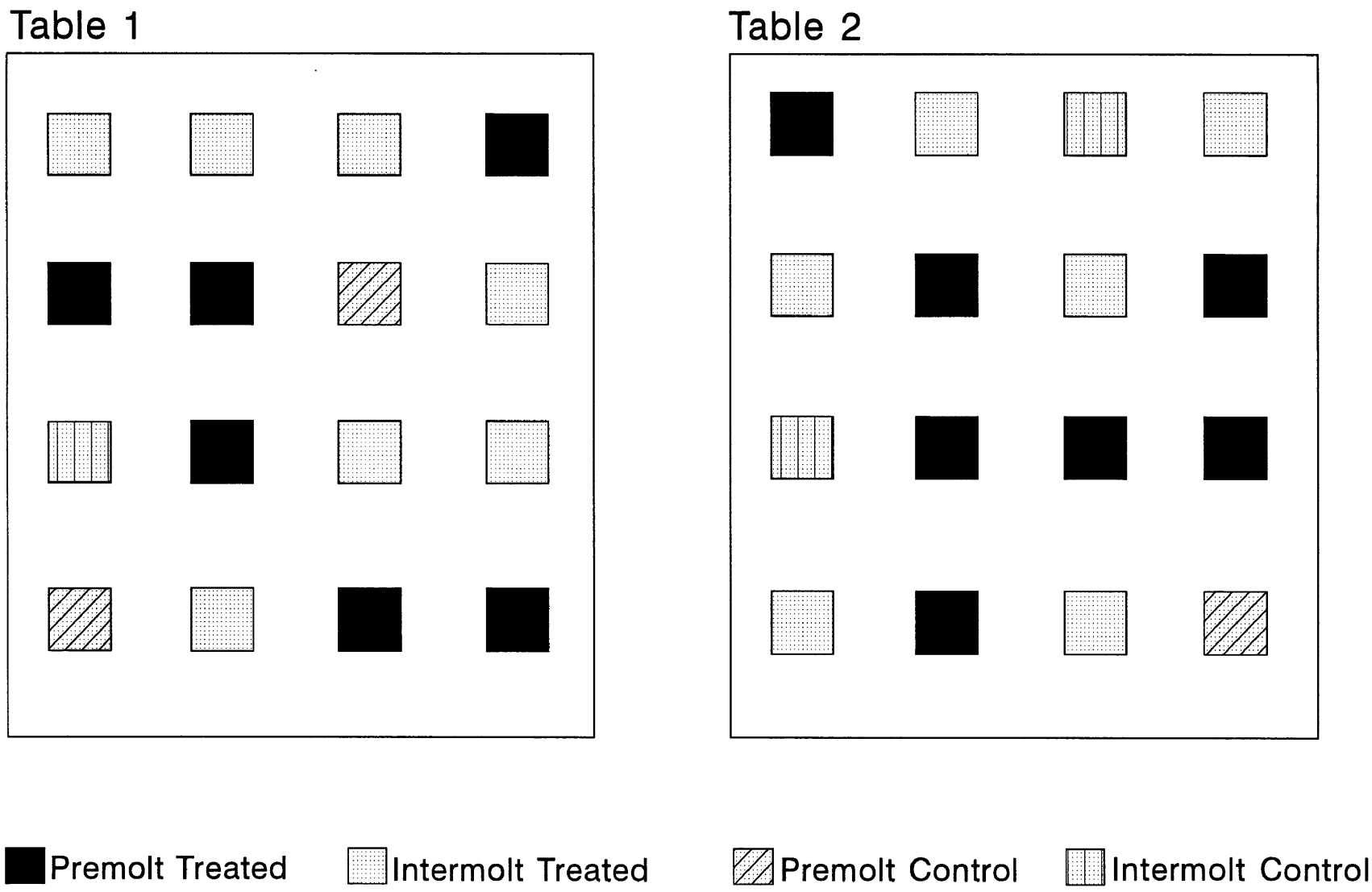
Juvenile female blue crabs, ranging in carapace width from 50 to 100 mm, were used in this experiment. Premolt crabs were obtained from local crab shedders, while intermolt crabs were collected using an otter trawl in the York River. Crabs were separated into tanks according to molt stage, intermolt (stage C) and late premolt (stage D) (Passano 1960). Only 'red sign' crabs (Oesterling 1984, Wescott 1984) were used in the experimental runs, as they should undergo ecdysis within 24 hours. During the period before ecdysis, crabs were fed frozen fish three times weekly.

The process of ecdysis for blue crabs of 40-120 mm carapace width is followed by a soft stage lasting approximately 2-6 hours and a paper-shell stage 4-18 hours in duration. Therefore, approximately 24 hours transpires from the initiation of ecdysis to a relatively hard-shell condition (Freeman et al. 1987). By designating 24 and 48 hour experimental time frames, the trials should encompass the soft-shell and early paper-shell stages.

All intermolt and premolt crabs were weighed, carapace width (spine to spine) measured, and placed in individual (Hurlbert 1984), aerated aquaria. Aquaria were aerated using glass Pasteur pipettes. Water temperature ranged from 20-22 °C and salinity ranged from 17-18 mg/l (parts per thousand). The aquaria were randomized (Hurlbert 1984) and statically dosed via the non-renewal method with radiolabelled ^{3}H-BaP (in toluene, specific activity 64 Ci mmol^{-1} - New England Nuclear) and ^{14}C-PCB 153 (in hexane, specific activity 12.6 mCi mmol^{-1} - Sigma) (Fig. 2).

Figure 2. Schematic of random sampling design.

Schematic of random sampling design.



Concentrations of BaP and PCB-153 were below their water solubilities, 3 ug/l (Verschueren 1983) and 0.9 ug/l (ppb) (Chiou et al. 1977), respectively. Trials were conducted under red light to minimize BaP photooxidation, and to reduce possible stress on crabs due to high intensity lights.

Premolt and intermolt crabs were exposed simultaneously for 24 or 48 hours. Further, additional crabs were exposed for 24 hours, placed in untreated water and allowed to depurate for 48 or 72 hours. These crabs were randomly selected for sacrifice following one of three treatment times: 24 hour exposure, 24 hour exposure and 48 hours of depuration or 24 hour exposure and 72 hours of depuration. Intermolt crabs may be able to biotransform and excrete BaP due to the activity of MO enzymes. Using this depuration study, treated premolts were analyzed before ecdysis, following ecdysis and following two depuration periods to define whether BaP was biotransformed by the premolt crabs and at what point this biotransformation begins.

Test crabs were observed every two hours to determine the time of ecdysis. Since premolt crabs did not molt simultaneously, knowledge of the time of ecdysis during exposure was important as it discloses the length of time the crab had a soft exoskeleton. This is crucial if uptake occurs through the soft exoskeleton, a permeable membrane (Johnson 1980), or if the MO system is repressed during or following ecdysis. A crab that molts early in the experiment will be soft to paper shell through most of the exposure, allowing more time for uptake through the semi-permeable membrane. Alternately, less uptake through the semi-permeable membrane may occur

if the crab molted late in the experiment. If the MO system is non-functional during or following ecdysis, elevated concentrations should be seen in molting crabs when compared to intermolt crabs.

Intermolt and premolt control crabs were maintained in untreated York River water to control for potential chemiluminescence, interference due to chemical reactions, and to establish baseline levels of ^{3}H and ^{14}C. The control premolt crabs also provided size increase data for comparisons between treated and untreated crabs, using measures of carapace width increase and weight gain. Both of these indices depend on the uptake of water by the crab. Studies have shown that crabs can detect pollutants in water, potentially altering normal uptake patterns of water and, hence, pollutants (Pearson et al. 1981, Pearson and Olla 1980, Pearson and Olla 1979). This may result in uptake differences between treated and untreated premolt crabs, and, consequently, size increase differences.

Exposure concentrations of BaP and PCB-153 were 0.01 ng/l (ppt - parts per trillion) and 20 ng/l (ppt) respectively. Ambient environmental concentrations of PAHs and PCBs in water are very low and, thus, are difficult to determine. Since these compounds are hydrophobic, they readily attach to particles in the water column. The factors affecting these compounds in water are such that a wide range of concentrations could be determined for the same area, each with its own justification, due to prevailing conditions and procedures for analysis. Thus, few reliable values are available in the literature on concentrations of xenobiotics in water. Concentrations of PAHs and PCBs for sediments are much more available.

Representative estuarine sediment values reported for BaP and PCBs are typically less than 100 ug/kg (ppb) (Marcus and Renfrow 1990, deFur and Smith 1987).

Following exposure, all crabs were weighed, measured (carapace width), sacrificed and their hepatopancreas and gills removed. The fresh hepatopancreas was divided into two portions. The first portion consisted of approximately 0.5 g of tissue (wet weight basis) to be used in the metabolite analysis. The remainder of the hepatopancreas was used in the determination of BaP and PCB-153 equivalents present. These values represent the total amount of radioactivity present in the tissues, irregardless of whether it is associated with parent compound, organic soluble metabolites or water soluble metabolites. Concentrations expressed are based on the molecular weight of the parent compound, i.e. BaP or PCB-153 'equivalents'.

Equivalents Analysis

The gill tissue and the appropriate portion of the hepatopancreas were freeze dried, weighed and ground to obtain a representative sample. A 10-20 mg subsample of each ground tissue (dry weight basis) from each crab was used for liquid scintillation analysis (Fig. 3) to determine the amounts of total radiolabelled compounds present. Scintigest solubilizer (Fisher Scientific) was added to digest the tissue, and Scintiverse I (Fisher Scientific) was used as the scintillant. Samples were counted for 10 minutes on a Beckman model LS 5000TD liquid scintillation counter (LSC) for both ^{3}H and ^{14}C labelled compounds (Fig. 3). Values obtained from the LSC were converted to concentrations using the following equation:

Figure 3. Flowchart of tissue analysis.


METHODS
Equivalents Analysis
Metabolite Analysis
hepatopancreas & gills
hepatopancreas
dissected & weighed
homogenize
2.0 ml water
freeze dried & weighed
2X
shake
w/1.0 ml ethyl acetate
ground
centrifuge
5 minutes
subsample 10-20 ug
remove ethyl acetate
digested
48 hrs., 60 F, in dark
200 ul ethyl acetate
counted, 3H & 14C
TLC plate
200 ul ethyl acetate
600 ul water
counted, 3H & 14C
add scintillant
24 hrs., in dark
eluted with benzene
count
3H & 14C
scraped
parent compound
counted, 3H & 14C
polar metabolites
counted, 3H & 14C

$$\text{Concentration} = (\text{dpm}/2.22 \times 10^6)(\text{molecular weight}/\text{specific activity})$$

where: 2.22×10^6 (constant) = dpm/Ci (Ci = Curies)

specific activity = Ci/mol

Thin-Layer Chromatography

Subsamples of fresh hepatopancreas from premolt and intermolt crabs, exposed for 24 and 48 hours, were removed and analyzed for parent compound, organic soluble and water soluble metabolites by thin-layer chromatography (TLC) (Fig. 3). Approximately 0.5 g of frozen hepatopancreas tissue were placed in a Potter-Elvehjem homogenizer. The tissue was homogenized with 2.0 ml of water and transferred to a centrifuge tube. Two ml of ethyl acetate (Burdick and Jackson, high purity) were then added to the homogenate. The mixture was shaken for one minute, and centrifuged at 2000 rpm for five minutes. The ethyl acetate portion was removed. Two additional ml of ethyl acetate were added to the homogenate, shaken, centrifuged for five more minutes and removed. A 600 ul aliquot of the water extract was analyzed for operationally defined water soluble metabolites using liquid scintillation counting. A 200 ul aliquot of the ethyl acetate extract was counted for combined parent compound and organic soluble metabolites. An additional 200 ul aliquot of the ethyl acetate extract was spotted onto a silica gel TLC plate (Whatman K6 Silica Gel Plates, 5 cm X 20 cm, 250 u thickness), eluted with benzene to separate parent compound and metabolites, scraped into scintillation vials and counted for radioactivity using LSC.

Due to analysis methodology, metabolite concentrations were determined on a wet weight basis, while all other analyses were determined on a dry weight basis.

Water samples of 1 ml each were analyzed from each tank at the beginning and end of each trial for BaP and PCB-153 equivalents, using liquid scintillation and Scintiverse I cocktail, to determine the consistency of the dosage between the tanks.

Pilot Study

Dissolved oxygen (D.O.) levels were measured in ten tanks containing either a premolt or an intermolt crab using a Y.S.I. D.O. probe in a pilot study to assure that adequate oxygen titers were maintained. The crabs ranged in size from 40-95 mm carapace width. deFur (1990) noted that oxygen uptake by crabs increases four fold from intermolt to ecdysis and declines rapidly following ecdysis. Oxygen values averaged 7-8 mg/l with aeration. The lowest D.O. concentration reached was 4.69 mg/l in a tank with a molting crab. No physical signs of distress were observed in this organism. Hypoxia ($\leq$ 2 mg/l) was not reached during the experimental period. Consequently, oxygen values were not measured during the exposure periods for the treatment trials. Monitoring this critical factor eliminated aquarium oxygen content as a confounding factor in the experiment. Hypoxic levels, however, may still be reached within the crab tissues.

RESULTS

Trial Run

A trial run was performed to determine variances in uptake for premolt and intermolt crabs for both BaP and PCB-153 equivalents. Five treated premolt and five treated intermolts, as well as two control premolts and two control intermolts were used in this experiment. These variances were used in a power test (Neter et al. 1990) to determine sample size. A sample size of 13 was determined for power at ß=0.95. Consequently, the 24 and 48 hour trials were conducted using 13 premolt and intermolt crabs each.

Significant differences were seen for both BaP equivalents (1-Way ANOVA, $F=49.31$, $df=1,8$, $P<0.001$) and PCB-153 equivalents (1-Way ANOVA, $F=6.61$, $df=1,8$, $P<0.03$) between the premolt and intermolt crabs. Intermolt crabs accumulated significantly higher concentrations of both BaP and PCB-153 than premolt crabs (Fig. 4).

Twenty-four and Forty-eight Hour Exposures

Concentrations of BaP and PCB-153 equivalents in the hepatopancreas of premolt and intermolt crabs were examined following both 24 and 48 hour exposure periods. Concentrations of both BaP and PCB-153 equivalents in the hepatopancreas were higher following the 48 hour exposure than after the 24 hour exposure (3-Way ANOVA, $F=8.53$, $df=1,96$, $P<0.005$). Intermolt crabs had significantly higher concentrations of BaP and PCB-153 equivalents (Fig. 5) in the hepatopancreas

Figure 4. Results from the 24 hour trial run. Concentrations of BaP and PCB-153 equivalents (dry weight basis) in the hepatopancreas of premolt and intermolt crabs. Significant differences between treatments at the 0.05 level are noted by an asterisk. Five individuals were used for each treatment.

Trial Run

Concentrations in the Hepatopancreas

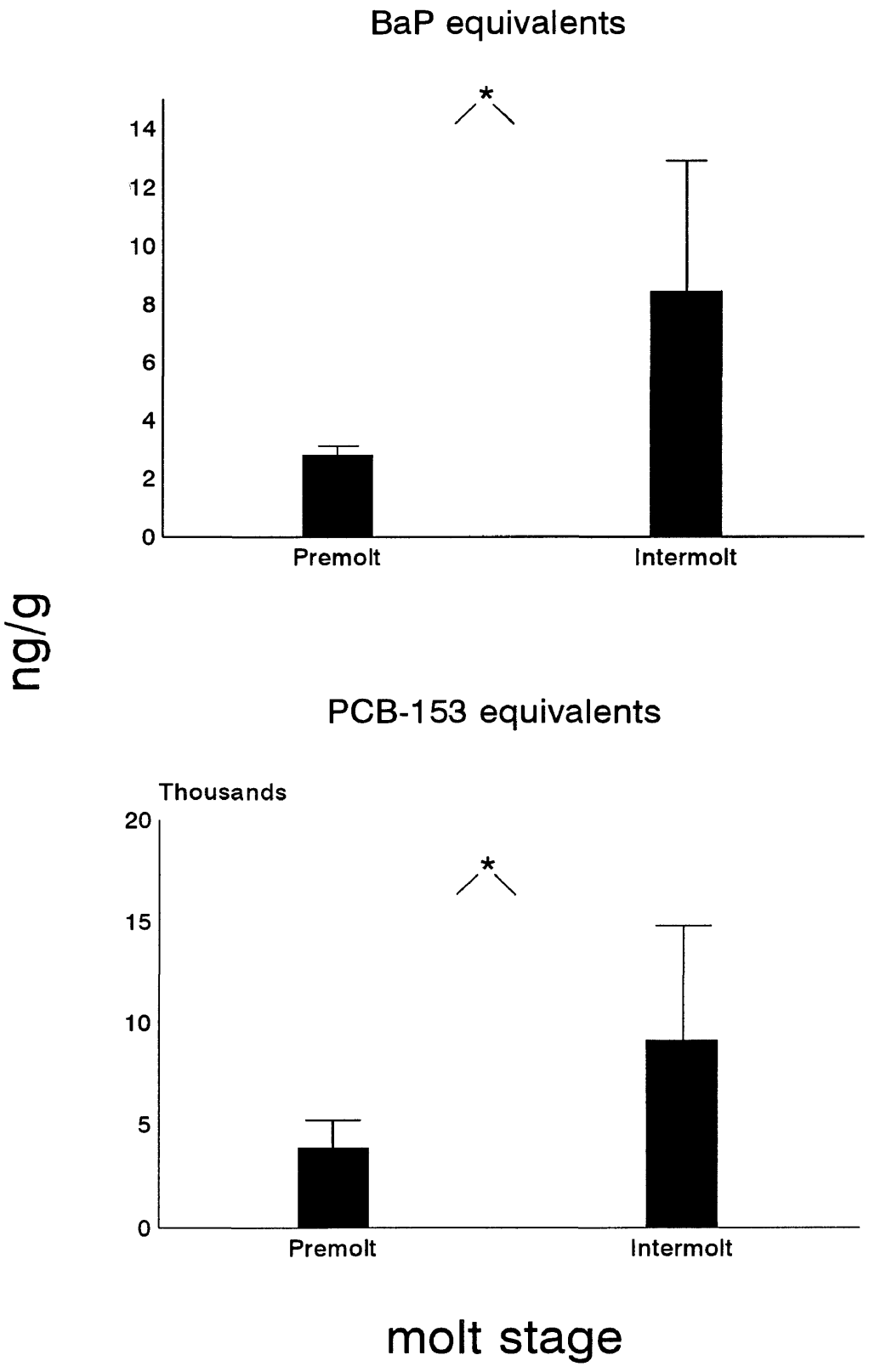
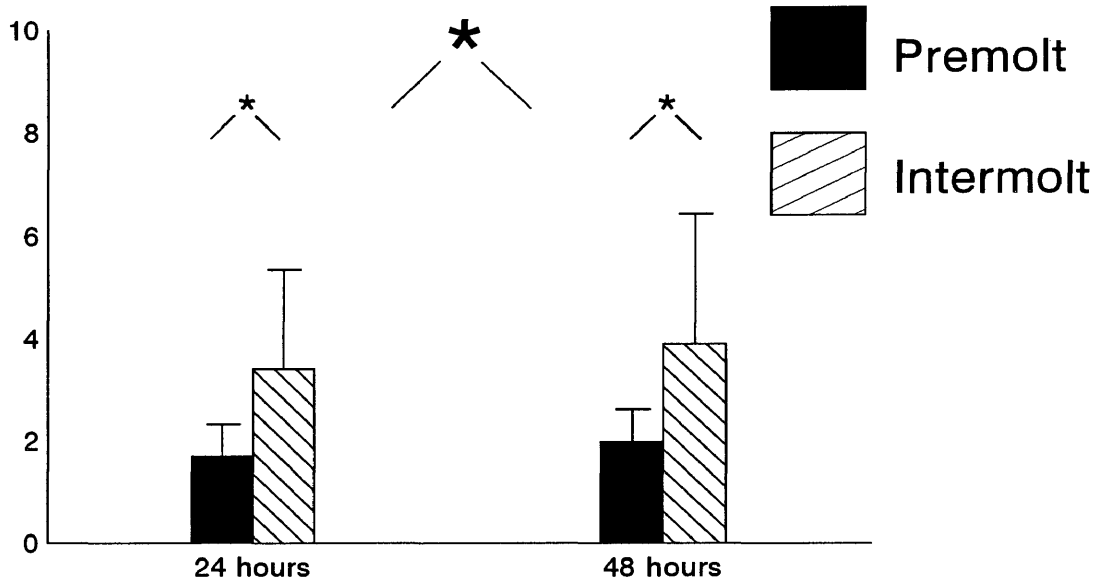


Figure 5. Concentrations of BaP and PCB-153 equivalents (dry weight basis) in the hepatopancreas of premolt and intermolt crabs following 24 and 48 hour exposure periods. Significant differences at the 0.05 level are noted by an asterisk. Thirteen individuals were used for each treatment.

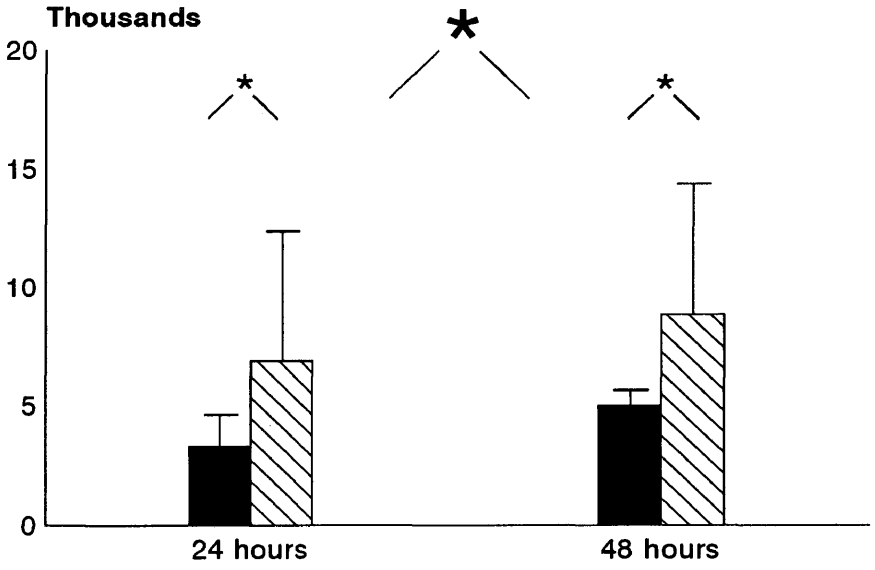
24 vs. 48 Hour Exposure

BaP equivalents in the hepatopancreas



ng/g

PCB-153 equivalents in the hepatopancreas



exposure period

(3-Way ANOVA, $F=20.42$, $df=1,96$, $P<0.001$) following both exposure periods than premolt crabs.

Gills

Gill tissues were examined for concentrations of BaP and PCB-153 equivalents in crabs exposed for a 24 hour period. Premolt and intermolt crabs accumulated similar concentrations of BaP equivalents ($ß<0.20$) (Fig. 6) in the gill tissues (Table 1). Additionally, concentrations of PCB-153 equivalents were not significantly different ($ß<0.20$) for premolt and intermolt crabs.

Hepatopancreas and Gill Tissues Following a 24 Hour Exposure

BaP equivalent concentrations were significantly higher in the hepatopancreas than in gill tissues (2-Way ANOVA, $F=22.92$, $df=1,48$, $P<0.001$) (Fig. 7). In contrast, no statistical differences between the hepatopancreas and gill tissue concentrations of PCB-153 equivalents were apparent for premolt ($ß=0.80$) or intermolt ($ß<0.20$) crabs (Fig. 7).

Metabolites

Metabolites in the hepatopancreas were analyzed following 24 and 48 hour exposure periods. In both experiments, concentration in the hepatopancreas of parent compound, organic soluble metabolites and water soluble metabolites did not differ between premolt and intermolt crabs for either BaP or PCB-153 (Table 2).

Figure 6. Concentrations of BaP and PCB-153 equivalents (dry weight basis) in the gills of premolt and intermolt crabs following a 24 hour exposure period. Thirteen individuals were used for each treatment.

24 Hour Exposure

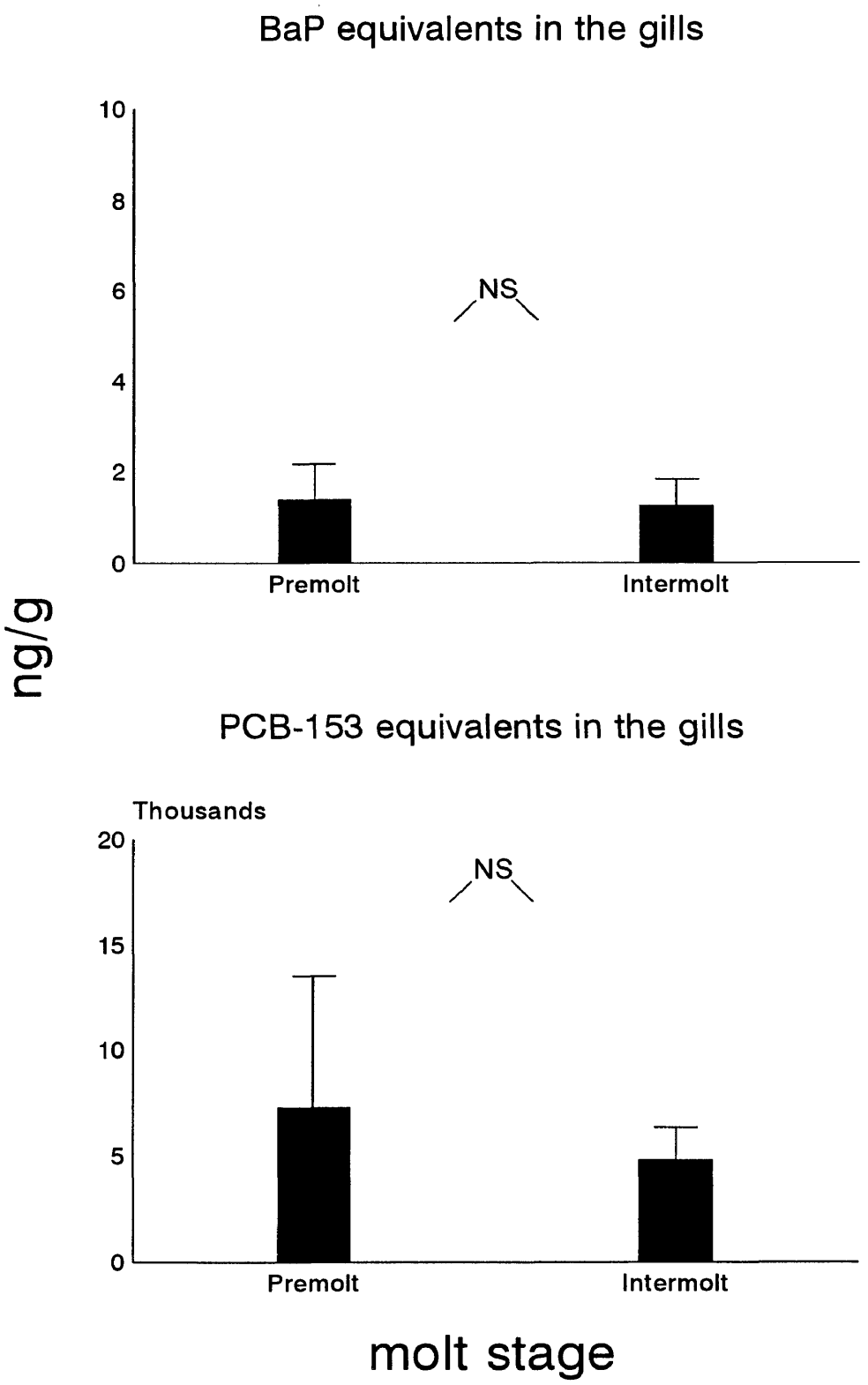


Table 1. Concentrations, ng/g dry weight, of BaP and PCB-153 in the hepatopancreas and gills following 24 and 48 hour exposure periods. Mean values are given, with standard deviations provided in parentheses.

Exposure time	Tissue	BaP			PCB-153		
		premolt		intermolt	premolt		intermolt
24 hours	hepatopancreas	1.6 (0.47)	*	3.3 (1.9)	3200 (2800)	*	6900 (6000)
	gills	1.6 (0.65)		1.7 (0.42)	7200 (5500)		4800 (1500)
48 hours	hepatopancreas	2.3 (0.46)	*	4.0 (2.4)	5000 (1600)	*	8900 (5900)

* denotes a significant difference between premolt and intermolt crabs (a=0.05)

Figure 7. Concentrations of BaP and PCB-153 equivalents (dry weight basis) in the hepatopancreas and gills of premolt and intermolt crabs following a 24 hour exposure period. Significant differences at the 0.05 level are noted by an asterisk. Thirteen individuals were used for each treatment.

Hepatopancreas vs. Gills

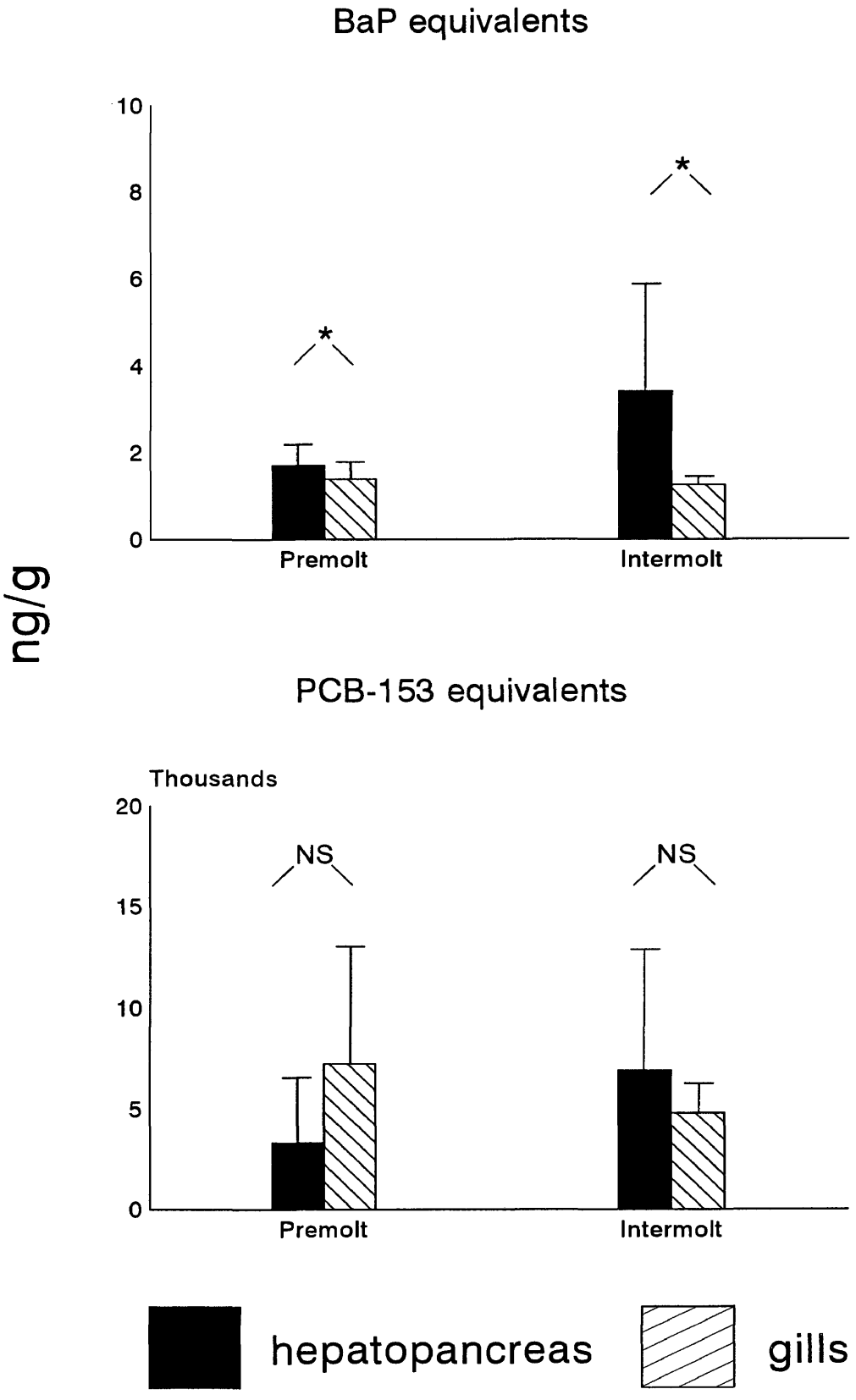


Table 2. Concentrations, ng/g wet weight, of parent compound, polar metabolites and water soluble conjugates in the hepatopancreas following 24 and 48 hour exposures. Mean values are given, with standard deviations provided in parentheses.

Exposure time	Compound	BaP		PCB-153	
		premolt	intermolt	premolt	intermolt
24 hours	parent compound	0.006 (0.006)	0.006 (0.006)	220 (150)	310 (140)
	OSM	0.007 (0.004)	0.011 (0.007)	38 (4.2)	46 (22)
	WSM	0.007 (0.004)	0.007 (0.002)	12 (2.7)	18 (2.4)
48 hours	parent compound	0.004 (0.004)	0.003 (0.005)	231 (66)	380 (150)
	OSM	0.014 (0.006)	0.018 (0.008)	47 (5.3)	55 (9.1)
	WSM	0.017 (0.010)	0.035 (0.025)	17 (1.9)	20 (3.2)

OSM = organic soluble metabolites
WSM = water soluble metabolites

Concentrations of BaP parent compound, organic soluble metabolites and water soluble metabolites in the hepatopancreas were similar following the 24 hour exposure (Fig. 8). However, following the 48 hour exposure, premolt crabs had higher concentrations of organic soluble metabolites (2-Way ANOVA, $F=18.94$, $df=1,38$, $P<0.001$) and water soluble metabolites (2-Way ANOVA, $F=56.94$, $df=1,38$, $P<0.001$) than parent compound. Intermolt crabs also had higher concentrations of organic soluble metabolites (2-Way ANOVA, $F=32.38$, $df=1,34$, $P<0.001$) and water soluble metabolites (2-Way ANOVA, $F=84.89$, $df=1,34$, $P<0.001$) than parent compound. This indicates metabolism of BaP by both molt stages (Fig. 8).

Concentrations of PCB-153 parent compound were significantly higher (3-Way ANOVA, $F=195.47$, $df=1,108$, $P<0.001$) than organic or water soluble metabolites for both premolt and intermolt crabs following both exposure periods (Fig. 9). Note that PCB-153 is a poorly metabolized compound (Lee 1989, Bickel 1989, Hutzinger et al. 1972), and that 'PCB-153 organic and water soluble metabolites' detected in this study are most likely due to procedural and experimenter carry over from incomplete separation during the extraction procedures.

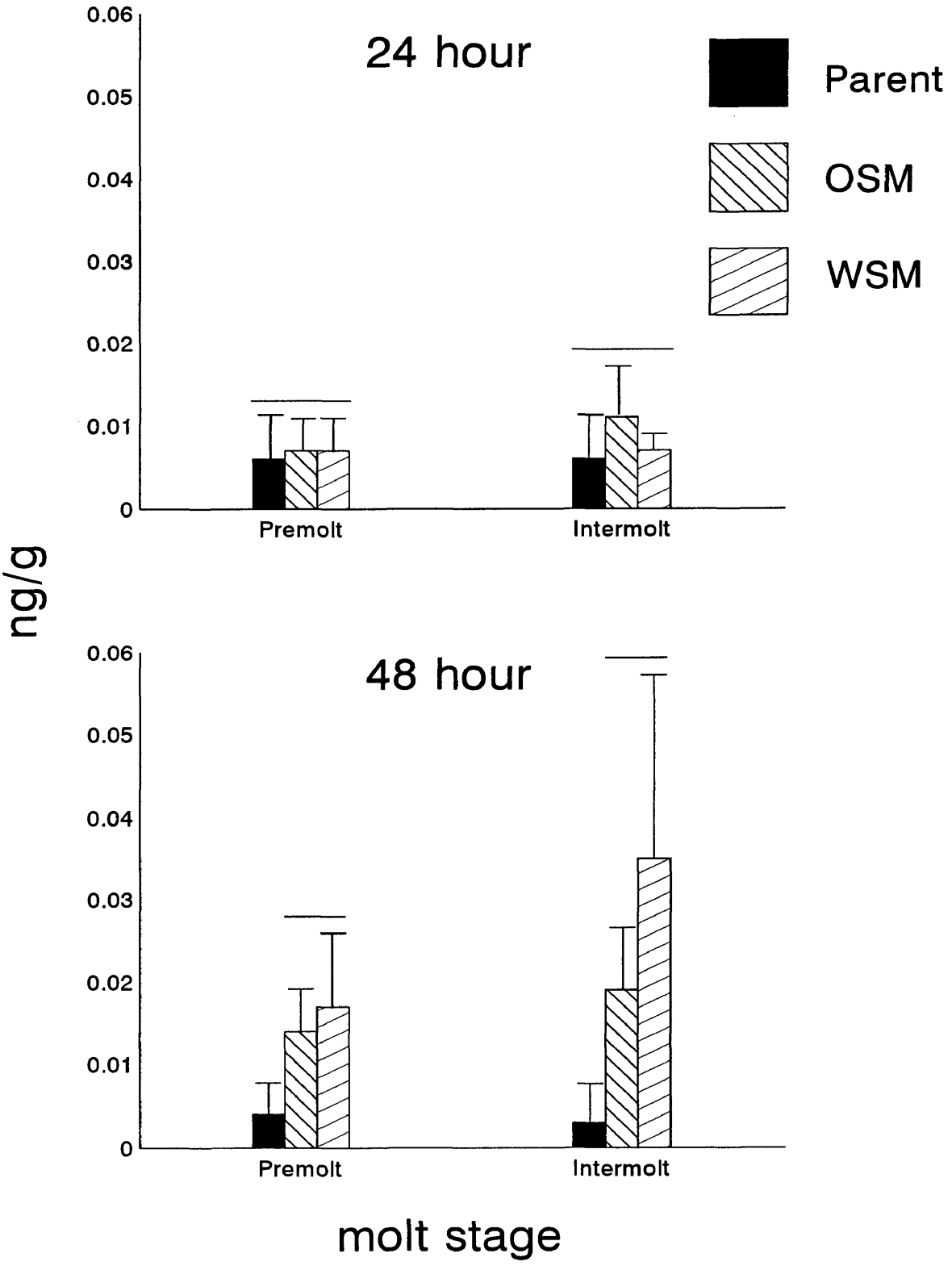
Depuration Study

Concentrations of BaP equivalents in the hepatopancreas of premolt ($ß=0.35$) or intermolt ($ß<0.20$) crabs did not differ following 24 exposure, 48 hour depuration and 72 hour depuration (Fig. 10). Concentrations of PCB-153 equivalents in the hepatopancreas of premolt crabs were significantly higher following 48 (1-Way

Figure 8. BaP parent compound, organic soluble metabolites and water soluble metabolites (wet weight basis) in the hepatopancreas of premolt and intermolt crabs following both a 24 and 48 hour exposure period. A solid bar denotes no statistical difference between fractions. Thirteen individuals were used for each treatment.

Parent Compound vs. Metabolites

BaP in the Hepatopancreas



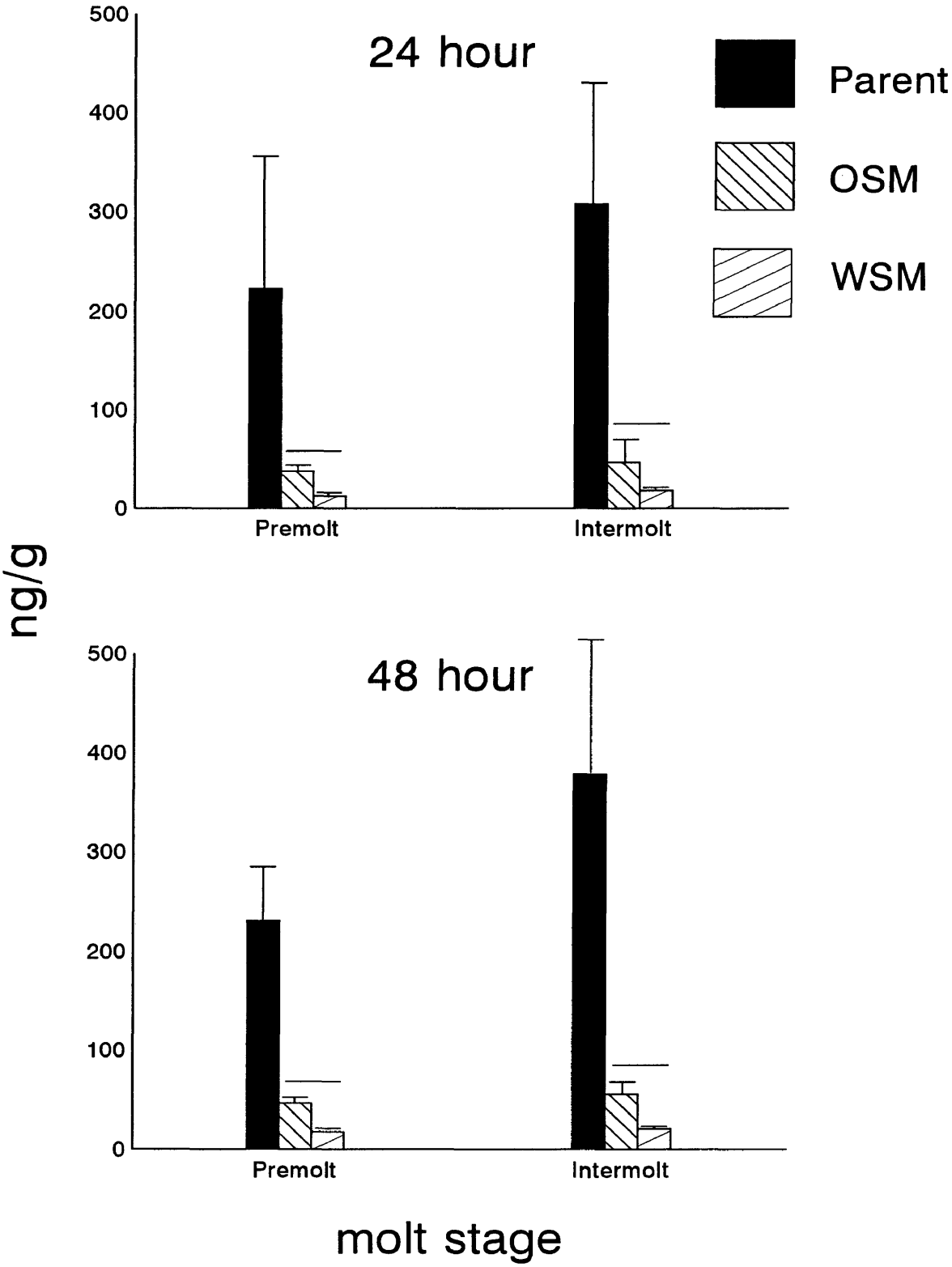
——— denotes no statistical significance (a=0.05)

* OSM = organic soluble metabolites
WSM = water soluble metabolites

Figure 9. PCB-153 parent compound, organic soluble metabolites and water soluble metabolites (wet weight basis) in the hepatopancreas of premolt and intermolt crabs following both a 24 and 48 hour exposure period. A solid bar denotes no statistical difference between fractions. Thirteen individuals were used for each treatment.

Parent Compound vs. Metabolites

PCB-153 in the Hepatopancreas



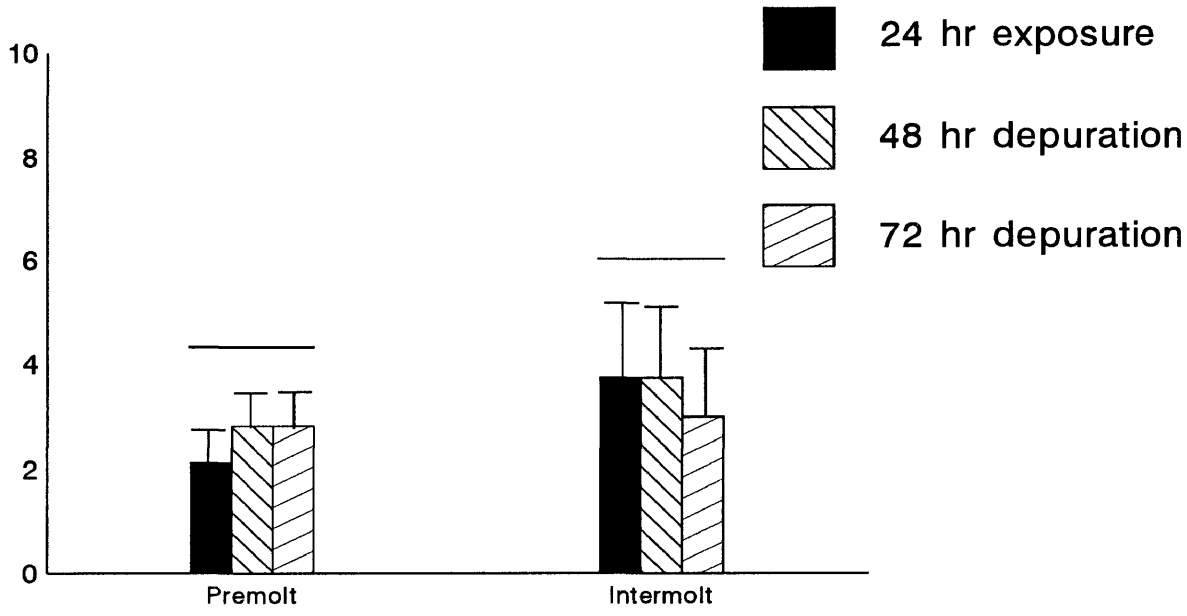
——— denotes no statistical significance (a=0.05)

* OSM = organic soluble metabolites
WSM = water soluble metabolites

Figure 10. Concentrations of BaP and PCB-153 equivalents (dry weight basis) in the hepatopancreas of premolt and intermolt crabs following three treatment times: 24 hours of exposure; 24 hours of exposure and 48 hours of depuration; or 24 hours of exposure and 72 hours of depuration. A solid bar denotes no statistical difference between fractions. Twenty-two premolt and twenty-three intermolt crabs were used in this study.

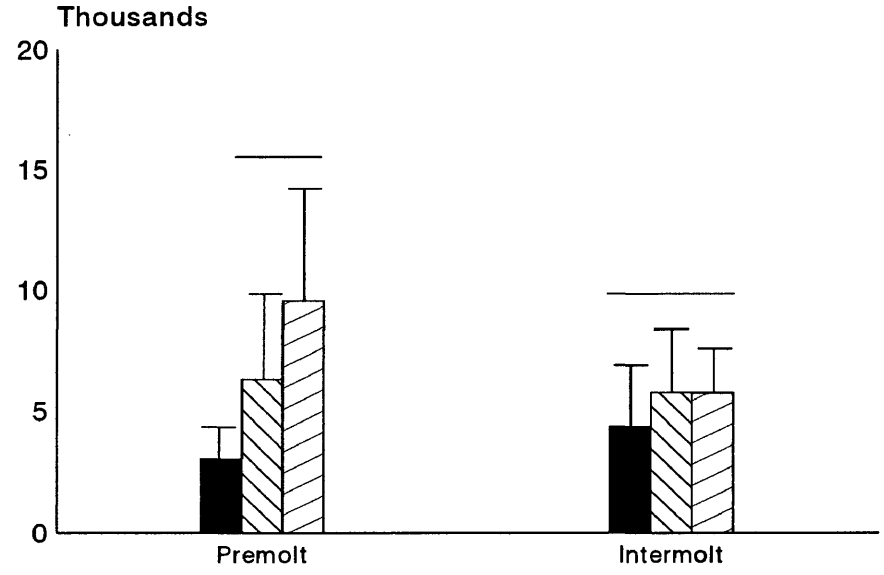
Depuration Run

BaP equivalents in the hepatopancreas



ng/g

PCB-153 equivalents in the hepatopancreas



——— denotes no statistical significance

F=16.52, df=1,13, P=0.002) hour depuration periods than after the 24 hour ANOVA, F=12.37, df=1,15, P=0.003) and 72 exposure period. Concentrations of PCB-153 (ß=0.40) equivalents in the hepatopancreas did not differ for intermolt crabs over the treatment times (Fig. 10).

Concentrations of BaP equivalents in the hepatopancreas of premolt and intermolt crabs did not differ following the 24 hour exposure (ß=0.93), 48 hours of depuration (ß<0.20) and 72 hours of depuration (ß<0.20) (Table 3). Concentrations of PCB-153 equivalents did not differ between premolt and intermolt crabs following a 24 hour exposure period (ß=0.43) and 48 hour depuration (ß=0.55). However, following 72 hours of depuration, premolt crabs had higher concentrations of PCB-153 equivalents in the hepatopancreas than intermolt crabs (1-Way ANOVA, F=6.48, df=1,13, P=0.02) (Table 3).

Time Series

Levels of BaP equivalents and PCB-153 equivalents in the hepatopancreas were examined for changes in concentrations due to time of exposure at soft state by simple regression. The time factor used corresponded to the amount of time exposed to the pollutants following ecdysis, in the soft shell state. Concentrations of BaP equivalents in the hepatopancreas of molting crabs did not increase as time of exposure following ecdysis increased for 24 or 48 hours of exposure (Fig. 11).

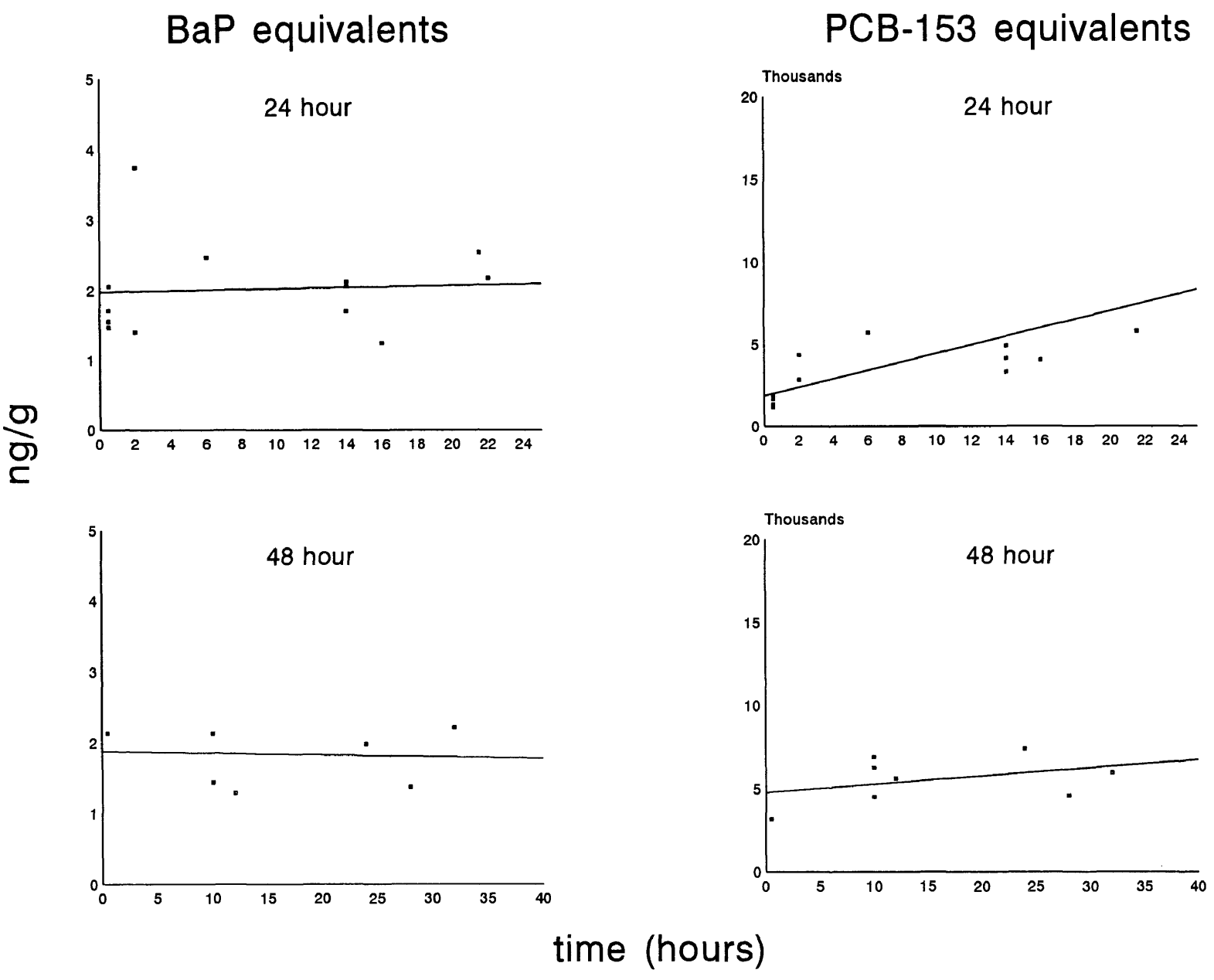
The concentration of PCB-153 equivalents did change, increasing, as time exposed following ecdysis increased for 24 hours of exposure (R-$sq.$ =63.9, F=17.68,

Table 3. Concentrations, ng/g dry weight, in the hepatopancreas of crabs exposed for 24 hours, then allowed to depurate for 48 or 72 hours. Mean values are given, with standard deviations provided in parentheses.

Time	BaP		PCB-153	
	premolt	intermolt	premolt	intermolt
24 hour exposure	2.1 (0.92)	3.7 (1.6)	3100 (1400)	4400 (2400)
48 hour depuration	2.8 (0.92)	3.7 (1.5)	6300 (3500)	5800 (2500)
72 hour depuration	2.8 (1.1)	3.0 (1.4)	9500 (4500)	5800 (1600)

Figure 11. Plot of simple regression showing relationship between number of hours a molting crab was exposed in the soft shell stage and concentrations of BaP and PCB-153 equivalents (dry weight basis) in the hepatopancreas. Twenty-seven individuals were used in each treatment.

Concentrations in the Hepatopancreas Premolt Crabs by Time



df=1,11, *P*=0.002) (Fig. 11). However, following 48 hours of exposure, the concentration of PCB-153 equivalents did not increase as time of exposure following ecdysis increased (Fig. 11).

Size Increase

All premolt crabs successfully completing ecdysis, treated and control, were examined for size increase differences. No significant differences were seen for size increase by carapace width (ß<0.30) or weight gain (ß<0.30) (Fig. 12).

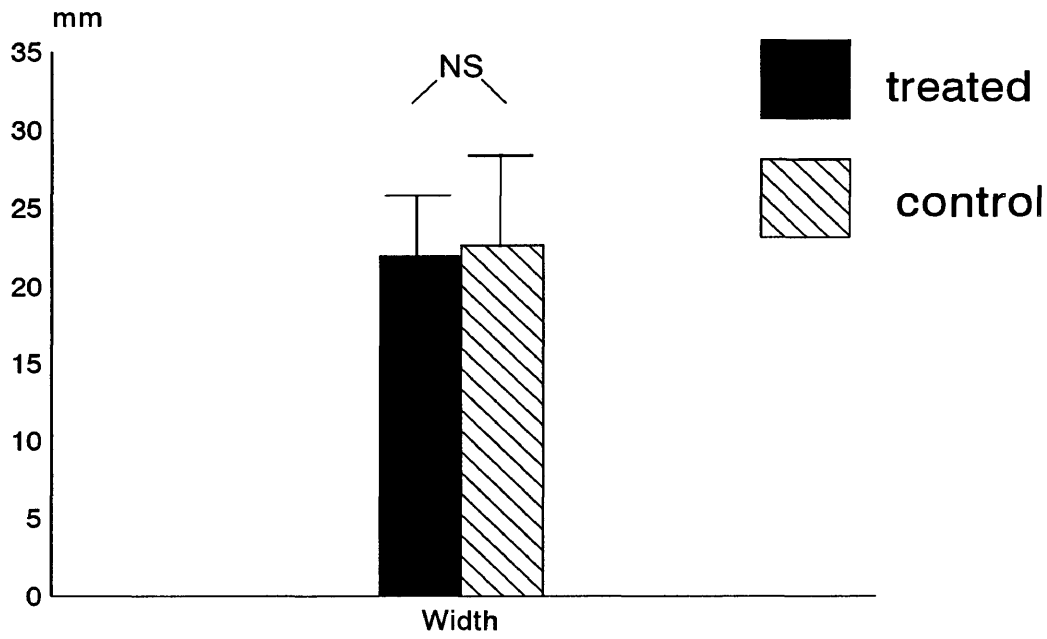
Water Analysis

Concentrations, ng/l, of BaP and PCB-153 equivalents were determined for all individual tanks for each experimental run. Mean values were calculated for both compounds during each trial. BaP (1-Way ANOVA, *F*=16.62, *df*=1,6, *P*=0.007) and PCB-153 (1-Way ANOVA, *F*=10.10, *df*=1,4, *P*=0.034) concentrations significantly decreased from the beginning to the end of each experimental period (Table 4). This suggests that some accumulation of these compounds by the crabs occurred. The concentrations calculated for BaP and PCB-153 in the water did not distinguish parent compound and metabolites. It is possible that some of the material measured in the water consisted of metabolites produced by the organisms, as well as photodegradation. Additionally, some adsorption of the compounds to the tank walls may have occurred.

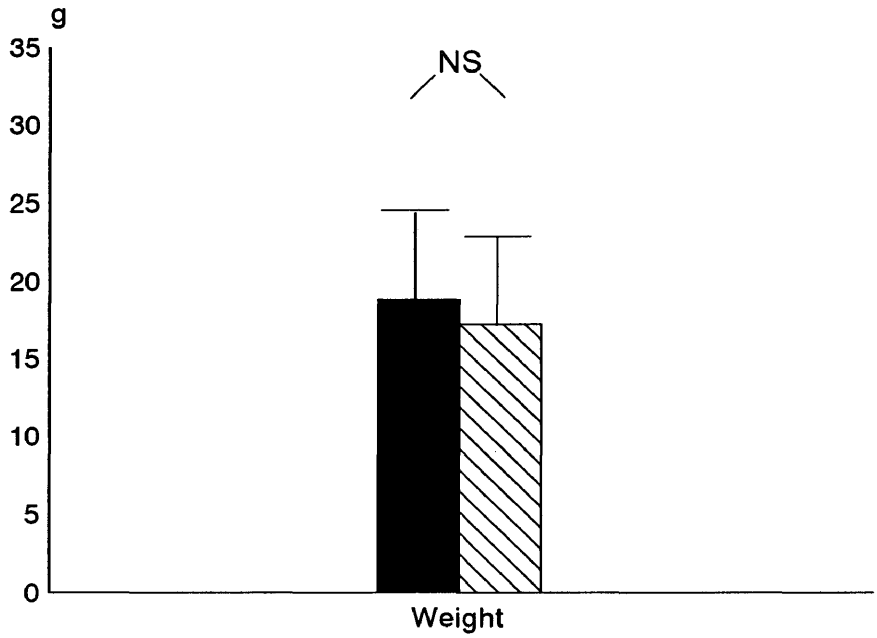
Nominal concentrations in the water were higher for both BaP and PCB-153

Figure 12. Size increase in molting crabs, using measures of width and weight. All premolts that successfully completed ecdysis, treated and control, for all experiments were used in this comparison. Thirty-two treated and eight control crabs were used in this study.

Size Increase in Molting Crabs
Width and Weight



increase in carapace width following ecdysis



increase in weight following ecdysis

Table 4. Mean concentrations, ng\l, of BaP and PCB-153 equivalents in treated water before and after each experimental run. Standard deviations are given in parentheses.

Date	BaP			PCB-153		
	before		after	before		after
trial run	0.011 (0.001)	*	0.008 (0.001)	13 (1.7)	*	6.4 (0.90)
24 hour exposure	0.008 (0.001)	*	0.004 (0.001)	29 (3.5)	*	11 (2.1)
48 hour exposure	0.013 (0.001)	*	0.004 (0.001)	42 (3.7)	*	11 (1.8)
depuration run	0.012 (0.001)	*	0.004 (0.001)	20 (4.4)	*	6.4 (1.4)

* denotes a significant difference between before and after concentrations (a=0.001)

than concentrations actually determined from the analysis. The nominal BaP concentration for the trials was approximately 0.03 ug/l, while the measured concentrations were much lower at a mean of 0.011 ug/l. The nominal PCB-153 concentration was 27 ug/l for the trials, while the measured concentrations averaged 30 ug/l. The decrease observed from nominal to actual concentrations may be due to the purity of the compound, photodegradation and adsorption of the compound to the walls of the tank.

Mortality

A total of 132 crabs were used in this study; 55 each of treated premolt and intermolt crabs and 11 each of control premolt and intermolt crabs. Mortalities occurred during the test periods. Three treated premolt crabs, one control premolt crab and one intermolt treated crab expired during the experimental runs. The mortality rate for premolt crabs was 6.1%, 5.4% for treated premolts; for intermolt crabs 1.5% total mortality, 1.8% for treated intermolts. Mortality for treated crabs was 3.6%, untreated crabs 4.5%. Total mortality during the experiment was 3.8%.

DISCUSSION

Several past studies have shown that crabs accumulate pollutants from water (Lee 1993, Mothershead and Hale 1992, Hale 1988, Marcus and Mathews 1987, Johnston and Corbett 1986, Cantelmo et al. 1981, Lee et al. 1976). These pollutants are transferred from uptake organs, e.g. gills, gut and integument, to lipid rich organs, such as the hepatopancreas (Lee et al. 1976) and ovary (Lee 1993), where they are metabolized and released or stored in the organism. Relative concentrations of pollutants in uptake and storage organs can aid in understanding mechanisms within the organism that control the concentration, biotransformation and removal of these pollutants.

In these previous studies, an increase in exposure time to these pollutants generally resulted in an increase in tissue concentrations of xenobiotics in the organism (Lee et al. 1976). Lee et al. (1976) showed that a doubling of exposure time resulted in an almost doubling of concentrations of pollutants in the tissues. In this experiment, it was not always the case that an increase in exposure time resulted in an increase in tissue burdens. While the metabolizable BaP in the hepatopancreas remained relatively constant with increasing exposure time, the poorly metabolizable PCB-153 increased significantly with time for molting crabs. This is suggestive of a problem with either uptake or tissue transfer in crabs progressing through ecdysis. Since it is known that uptake of water increases during ecdysis (Johnson 1980), we are left with a possible tissue transfer problem.

Lee et al. (1976) demonstrated that higher concentrations of BaP in hard crabs were present in the hepatopancreas, when compared with the gills. Data from this study support those previous results. However, in examining PCB-153 in the tissues of hard crabs, I found no significant differences between concentrations in the hepatopancreas and the gills, although the trend was to higher concentrations in the hepatopancreas. In fact, in molting crabs, although not statistically significant, concentrations in the gills tended to be higher than those in the hepatopancreas. This, again, suggests some problematic factor involving tissue transfer. BaP, a metabolizable compound, appears to be efficiently transferred from the gills to the hepatopancreas, while the poorly metabolized PCB-153 appears to be translocated at a slower rate.

Activity of monooxygenase enzymes, or metabolism, has been suggested to be inversely correlated to levels of ecdysone (Lee and Singer 1982). Levels of ecdysone in the green gland are lowest during the intermolt stage of the molt cycle. Concentrations increase as ecdysis approaches. Highest levels of ecdysone in the green gland are reached following ecdysis while the crab is soft (Lee and Singer 1982, Conklin and Rao 1982). Levels of ecdysone decrease dramatically following the soft stage, while monooxygenase enzyme activity increases rapidly. The presence of these enzymes may account for the similar values of BaP metabolites seen in both premolt and intermolt crabs. Most molting crabs had time to harden to a paper shell stage before the end of the experiment, initiating enzyme activity during the study and metabolizing BaP.

The exposure concentrations of 0.01 ng/l for BaP and 20.64 ng/l for PCB-153 used in this study are environmentally relevant. Studies examining concentrations of pollutants in tissues of blue crabs collected from areas in the Chesapeake Bay showed mean levels of PAHs in the hepatopancreas to be 220 ug/kg (Hale 1988). Mothershead and Hale (1992) exposed molting and non-molting crabs in suspended cages in the Elizabeth River, Virginia, one of the most polluted rivers in the world. They found no detectable xenobiotics in the water analysis at field sites (detection limit = 0.2 ug/l). However, total tissue concentrations of PAH revealed 1,380 ug/kg in crabs recently completing ecdysis, while concentrations of 498 ug/kg were observed in intermolt crabs (Mothershead and Hale 1992). Concentrations observed in tissues of crabs in the current study were lower than those found in the field exposure experiments. The field exposure experiments were conducted using adult female blue crabs, while my experiment used juvenile female blue crabs. Maturational changes may account for some of the discrepancies observed, since concentrations of pollutants in the water determined for both experiments were low.

Barron et al. (1989) demonstrated that metabolism in fish occurs not only in the hepatopancreas, but also to a lesser degree in the gills. Since BaP can be metabolized by the organism, biotransformation may be occurring in the gills, with subsequent transfer of some metabolites and parent BaP to the hepatopancreas. Being poorly metabolized, it may take longer to transfer PCB-153 to the hepatopancreas. In both premolt and intermolt crabs, there were no apparent differences in PCB-153 equivalent concentrations between the hepatopancreas and gills, whereas BaP

concentrations were significantly higher in the hepatopancreas in both cases.

Lee et al. (1976), examining uptake and depuration of xenobiotic compounds in blue crabs, found that concentrations in the hepatopancreas decreased by 50% following 48 hours in untreated water. Although past studies have shown 48-72 hours to be adequate depuration periods for blue crabs (Hale 1988, Lee et al. 1976), concentrations of BaP and PCB-153 equivalents in the hepatopancreas of premolt and intermolt crabs in this study did not decrease during the depuration period. Hale (1988) found concentrations of ^{3}H-BaP to decrease by 65% in intermolt adult female crabs, while concentrations in newly molted females only decreased by 10%. Alternately, in this study, concentrations of BaP remained fairly constant throughout exposure and depuration for intermolt and molting crabs. In fact, concentrations of PCB-153 equivalents in the hepatopancreas, although not statistically significant, appeared to increase for intermolt crabs to the 48 hour depuration. Concentrations of PCB-153 in molting crabs appeared to increase in the hepatopancreas during the 48 hour depuration period and again after the 72 hour depuration period.

Cantelmo et al. (1981) observed size increase differences between treated and untreated crabs using benzene in water at a concentrations of 1 mg/l. They reported that treated crabs absorbed significantly less water during ecdysis than untreated crabs. Additionally, treated crabs exhibited a smaller size increase versus untreated crabs and cumulative growth over multiple molts decreased in treated crabs relative to untreated crabs. Differences in carapace width and weight were not observed at the BaP and PCB-153 concentrations used in this experiment.

In conclusion, differences in uptake, metabolism and depuration patterns of BaP and PCB-153 between molting and intermolt crabs were observed. Results obtained in this study agreed with aspects of some previous works, while contrasting with others. Many of the previous studies incorporated pollutants in much higher concentrations than used in this study (Landrum et al. 1992, Lee 1989, Sheridan 1975). Metabolic processes associated with uptake, biotransformation and depuration may differ when concentrations are very low or very high, possibly accounting for some of the discrepancies seen between this and previous reports.

Additional work is needed to determine the mechanisms responsible for these differences. Studies would be useful to establish whether metabolism is occurring in the gills of blue crabs, the extent of this biotransformation and the changes occurring in metabolism before, during and after ecdysis. This research should also incorporate longer exposure periods, encompassing time before ecdysis, ecdysis itself and the period following ecdysis. Depuration periods should be extended to ensure adequate time for possible removal of xenobiotics from the organism, as well as to examine mechanisms of depuration in molting crabs. Concentrations of pollutants in these organisms may pose serious consequences to predators of the blue crab. Additionally, since soft crabs constitute a major fishery in Chesapeake Bay, enhanced concentration of pollutants during ecdysis may, in turn, affect human consumers.

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