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Polynuclear Aromatic Hydrocarbons and Cellular Proliferative Disorders in Bivalve Mollusos from Oregon Estuaries

Oregon State Univ., Corvallis

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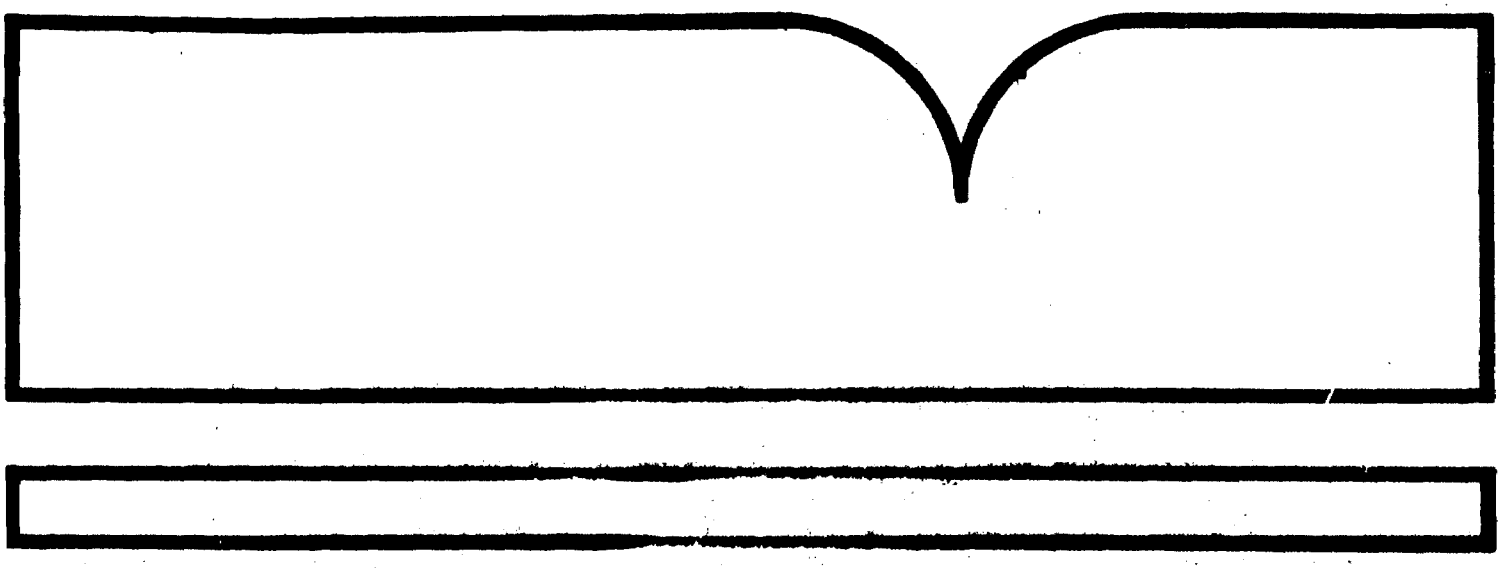

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POLYNUCLEAR AROMATIC HYDROCARBONS AND CELLULAR PROLIFERATIVE DISORDERS IN BIVALVE MOLLUSCS FROM OREGON ESTUARIES

by

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16. ABSTRACT

Indigenous populations of economically important bivalve molluscs were used as monitors for detecting and quantifying environmental PNAH, including 11 compounds classified as carcinogens, 11 EPA Priority Pollutants and 11 Toxic Pollutants. Baseline levels of PNAH were determined during a two-year period for mussels (M. edulis), clams (M. arenaria and T. capax) and oysters (C. gigas) from different sites, ranging from relatively pristine to moderately polluted, in Yaquina, Coos and Tillamook Bays, Oregon. Total concentrations of 15 unsubstituted PNAH were 30 to 60 µg/kg in shellfish from uncontaminated waters to greater than 1000 µg/kg in those from sites classified as contaminated. A major effort was made to determine and evaluate certain relationships between PNAH and their concentrations in shellfish. Studies were conducted to: determine the effects of depuration of PNAH concentrations; identify seasonal differences in PNAH concentrations; and measure BAP uptake and elimination. Preliminary studies indicated that mussels may possess a limited ability to metabolize BAP. Multiple regression and multiple correction techniques were used to identify and evaluate interrelationships between PNAH. Certain relationships may be useful for predictive purposes in evaluating environmental PNAH. The data from these studies indicate that it may be possible to identify and measure significant variables to assess total PNAH.

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FOREWORD

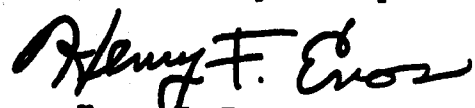
The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The EPA Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

the effects of toxic organic pollutants on individual species and communities of organisms;

the effects of toxic organics on ecosystem processes and components;

the significance of chemical carcinogens in the estuarine and marine environments.

Considerable interest has focused recently on the fate and possible effects of carcinogens and mutagens in the aquatic environment which usually is the ultimate receptacle for pollutants. This report describes the fate and some possible long-term effects of polycyclic aromatic hydrocarbons in the marine estuarine environment and biota. These data may serve to alert us to the role of certain carcinogens in the environment generally.



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ABSTRACT

The research project involved utilizing indigenous populations of economically-important bivalve molluscs as monitors for detecting and quantifying environmental PNAH, including 11 compounds classified as carcinogens, 11 EPA Priority Pollutants and 11 Toxic Pollutants.

Baseline levels of PNAH were determined during a two-year period for mussels (*M. edulis*), clams (*M. arenaria* and *T. capax*) and oysters (*C. gigas*) from different sites, ranging from relatively pristine to moderately polluted, in Yaquina, Coos and Tillamook Bays, Oregon. Total concentrations of 15 unsubstituted PNAH were 30-60 µg/kg in shellfish from uncontaminated waters to greater than 1000 µg/kg in those from sites classified as contaminated.

A major effort was made to determine and evaluate certain relationships between PNAH and their concentrations in shellfish. Studies were conducted to: determine the effects of depuration on PNAH concentrations; identify seasonal differences in PNAH concentrations; and measure BAP uptake and elimination. Preliminary studies indicated that mussels may possess a limited ability to metabolize BAP.

Multiple regression and multiple correlation techniques were used to identify and evaluate interrelationships between PNAH. Certain relationships may be useful for predictive purposes in evaluating environmental PNAH. The data from these studies indicate that it may be possible to identify site-specific, significant variables (individual PNAH) after a suitable period of sampling and to subsequently measure only those key variables for an adequate assessment of total PNAH. Combined with other approaches, this may result in considerable cost reductions for long-term biological monitoring programs.

Cellular proliferative disorders, resembling neoplastic conditions in vertebrates, were found in mussels with the greatest PNAH concentrations. Further studies will be necessary to determine the significance of this correlation.

This report was submitted in fulfillment of Contract No. R806224020 by Oregon State University, Corvallis, Oregon, under the sponsorship of the U. S. Environmental Protection Agency. This report covers the period October 1, 1978 to November 30, 1980.

CONTENTS

FIGURES

TABLES

TABLES (CONTINUED)

ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

^3H	-- tritiated compound
HPLC	-- high performance liquid chromatography
LOG S	-- water solubility (moles/L)
LOG S_s	-- solubility in seawater (moles/L)
LOG C_o	-- PNAH concentration
MW	-- molecular weight
PNAH	-- polynuclear aromatic hydrocarbon

METRIC MEASUREMENTS

g	-- grams	nmoles	-- nanomoles
kg	-- kilograms	pmoles	-- picomoles
L	-- liter	µg	-- micrograms
mg	-- milligram	µm	-- microns

PNAH

PHEN	-- phenanthrene	BBF	-- benzo(b)fluoranthene
FLUOR	-- fluoranthene	BKF	-- benzo(k)fluoranthene
PYR	-- pyrene	BEP	-- benzo(e)pyrene
BCP	-- benzo(c)phenanthrene	DBACA	-- dibenz(a,c)anthracene
TRI	-- triphenylene	BAP	-- benzo(a)pyrene
BAA	-- benzo(a)anthracene	DBAHA	-- dibenz(a,h)anthracene
CHRY	-- chrysene	BGHIP	-- benzo(g,h,i)perylene
BJF	-- benzo(j)fluoranthene	IP	-- indeno(1,2,3-c,d)pyrene
		COR	-- coronene

SAMPLE SITES (BAY, SPECIES)

Y1M	-- (Yaquina, *Mytilus edulis*)	C11G	-- (Coos, *Tresus capax*)
Y2M	-- (Yaquina, *Mytilus edulis*)	T1M	-- (Tillamook, *Mytilus edulis*)
Y1YO	-- (Yaquina, *Crassostrea gigas*)	TSS	-- (Tillamook, *Mya arenaria*)
C3S	-- (Coos, *Mya arenaria*)	TBC	-- (Tillamook, *Crassostrea gigas*)
CSS	-- (Coos, *Mya arenaria*)		

ACKNOWLEDGEMENTS

Mr. Randy Schaffer was responsible for developing the analytical methods used to measure PNAH and managing the sampling program; his assistance was invaluable and is gratefully acknowledged. The following individuals were directly associated with the project and their contributions were of great value: Dr. David LaTouche, Ms. Susan Hemingway and Mr. Keith King.

I am grateful for the assistance and support of the following: Drs. John Byrne, Robert Krauss, David Willis and Virgil Freed provided generous support which permitted acquisition of HPLC analytical capabilities; Dr. Bruce Dunn of the University of British Columbia shared his expertise on analytical methods; Dr. Donald Buhler permitted the use of his facilities and equipment; Dr. Cary Chiou provided valuable information about solubility relationships; Ms. Marilyn Henderson and Ms. Lori Mix provided valuable technical assistance; Ms. Leslie Mix helped transform climatological data; Mr. Paul Heikkala and Mr. Dale Snow shared valuable insights about sampling sites and shellfish populations, and Ms. Karla Russell typed the final report.

I am deeply indebted to Dr. John Couch for his support and wise counsel during the project period.

The organization and analysis of the data base associated with this investigation was carried out in part using the PROPHET system, a unique national resource sponsored by the NIH.

SECTION 1

INTRODUCTION

There has been much recent interest expressed about the presence of organic chemical carcinogens and mutagens in coastal estuaries and the possible effects of these compounds on indigenous organisms that inhabit these productive environments. Foremost among these chemicals, polynuclear aromatic hydrocarbons (PNAH) are ubiquitous in the marine environment and may present the greatest carcinogenic hazard (Kraybill, 1976).

Scientists from several diverse disciplines have advocated using bivalve molluscs to serve as biomonitors for the detection and quantification of environmental contaminants, including carcinogens (e.g. Couch et al., 1974; DiSalvo et al., 1975; Dunn and Stich, 1975; Goldberg, 1975; Mix et al., 1977). Bivalve shellfish have received the widest support for such an approach because they are permanent inhabitants of a specific environment and tend to concentrate toxic substances in their tissues.

An additional factor in utilizing bivalve molluscs in studies of environmental contaminants is related to the discovery that many species, from several different geographic locations, have been reported to have cellular, perhaps neoplastic, proliferative disorders (Mix et al., 1979a). Environmental pollutants were implicated as potential causative agents in several of those reports although no cause-effect relationships have yet been established.

Field studies to determine PNAH concentrations in marine organisms have only recently been initiated despite earlier suggestions that extensive investigations of the marine environment, including chemical identification, monitoring and surveillance, and identification of fish and shellfish tumors may play an important role in the epidemiology of cancer (Kraybill, 1976). Of the PNAH, benzo(a)pyrene (BAP) has been the most extensively studied carcinogen in the marine environment. There have been a number of reports of BAP concentrations in tissues of indigenous shellfish (Dunn and Stich, 1976a, b; Dunn and Young, 1976; Mix et al., 1977; Pancirov and Brown, 1977; Joe et al., 1979; Mix, 1979; Mix and Schaffer, 1979; Risebrough et al., 1980).

Excepting BAP, which has often been used to indicate the presence of other PNAH, there is relatively little information available on the presence or quantities of unsubstituted PNAH in tissues of aquatic organisms. Such information, based on the use of advanced analytical methods capable of measuring ng quantities, is needed. Considerable emphasis should be placed on determining the composition of PNAH assemblages in the tissues of organisms from both pristine and impacted areas in order to gain an understanding of the sources and fluxes of PNAH through the aquatic ecosystem (Neff, 1979).

Associations between high tissue concentrations of PNAH, and other carcinogens, and the appearance of cellular proliferative disorders in shellfish populations should also be identified and carefully investigated. The existence of such associations would suggest the necessity of additional studies to evaluate more fully potential cause and effect relationships.

A relatively simple rationale can be offered for conducting studies on PNAH in marine ecosystems. The environmental levels of these compounds can be expected to increase in the biologically productive coastal regions. Such contamination is inevitable because of increased ship traffic, escalating recreational demands and expanding industrialization. Yet, there is a scarcity of dependable qualitative information on PNAH in the inhabitants of marine ecosystems. Finally, little is known about the effects of chronic, low-level contamination with environmental PNAH on shellfish or the potential public health hazard associated with their consumption.

The present studies were designed to accomplish the following:

1. Develop state-of-the-art methods for identifying and quantifying unsubstituted PNAH in tissues of marine organisms;

2. To measure baseline levels of PNAH in bivalve molluscs from Oregon bays;

3. To evaluate critically the practicality of utilizing indigenous populations of bivalve molluscs for PNAH monitoring and surveillance studies;

4. To identify and evaluate the quantitative relationships between individual PNAH in tissues of bivalve molluscs;

5. To determine the prevalence of cellular proliferative disorders in bivalve molluscs that inhabit environments with significantly different degrees of PNAH contamination;

6. To determine rates of BAP uptake and elimination in bay mussel *(Mytilus edulis)* populations under ambient (field) conditions;

7. To identify tissue sinks or storage compartments for BAP in *M. edulis* from PNAH-contaminated sites;

8. To determine if there is any association between PNAH body burdens and the prevalence of cellular proliferative disorders in affected *M. edulis* populations;

9. To conduct preliminary studies to determine whether or not *M. edulis* is capable of metabolizing BAP.

SECTIONS 2 AND 3

CONCLUSIONS AND RECOMMENDATIONS

An analytical method, utilizing HPLC, was developed and used to identify and quantify 15 unsubstituted PNAH including: phenanthrene, fluoranthene, pyrene, benzo(c)phenanthrene, triphenylene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenz(a,c)anthracene, benzo(a)-pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene, indeno(1,2,3-c,d)pyrene and coronene. The method resolved most members of the benzpyrene group. Perylene was not identified because it did not absorb UV light at the wavelength used and benzo(j)fluoranthene and benzo(e)pyrene could not be separated. Additonal efforts will be required to completely resolve and identify B(e)P, B(j)F and perylene.

Baseline levels of PNAH in indigenous bivalve molluscs used as biomonitors reflected the degree of human onshore activity at the various sample sites and, presumably, the level of water contamination. PNAH concentrations in shellfish from relatively pristine areas ranged from 30-60 µg/kg while those from industrialized areas contained 500-1500 µg/kg. The data collected during the present study indicate that bivalve molluscs make excellent monitors for detecting and measuring PNAH in estuaries. Future efforts should be directed towards fully defining the sampling protocols to be used in monitoring studies and to identify and evaluate endogenous and exogenous factors that may influence PNAH concentrations under ambient (field) conditions. The latter should include studies of potential sources and measurements of PNAH in water.

Identification and evaluation of quantitative and qualitative relationships between individual PNAH and between PNAH and their concentrations in bivalve molluscs indicate that a significant potential exists for developing predictive models for PNAH in aqueous environments and their concentrations in certain seafood products. Some of the relevant findings and conclusions, based on thorough statistical analyses of the data from these studies are summarized below.

1. Quantities of a single PNAH present in shellfish cannot be used to predict total PNAH.

2. For each site, different independent variables (individual PNAH) were identified and used to predict total PNAH in bivalve molluscs. It may be possible to identify site-specific independent variables after a suitable sampling period and to subsequently measure only those variables for an adequate assessment of total PNAH. Complete analyses could perhaps be made periodically to confirm the continuing validity of the established relationship; deviations may indicate new sources of contamination. Such an approach

may result in considerable cost reduction for long-term monitoring programs.

3. Benzo(a)pyrene was not a significant variable for predicting total PNAH at any site. Thus, the concept that BAP can be used as an index of PNAH contamination was not supported by the results of this study. From this and other studies, it seems that the use of BAP for making decisions about the quantities and presence or absence of other PNAH should be abandoned or modified.

Data from studies of BAP in *M. edulis* suggest that the depuration rate for this compound was exponential with a half-life of 8-10 days while uptake was linear. Routine depuration procedures in which shellfish are placed in clean seawater for 24 hours, would have little effect in reducing BAP concentrations. Gametogenesis and/or incorporation of BAP, and presumably other lipophilic PNAH, into the gonad, do not appear to be directly responsible for seasonal increases of BAP in mussels during winter-spring. BAP storage occurred primarily in the somatic tissues compared to the gonad, even during the spring spawning period. It is tentatively concluded, based on incomplete, preliminary studies, that 7,10-^{14}C-BAP was metabolized by microsomal extracts from the visceral mass of *M. edulis*. Phenolic metabolites were the only measurable BAP metabolites present. More complete studies, utilizing advanced methods, will be required to fully evaluate the metabolic capabilities of *M. edulis* for altering BAP and other PNAH.

Different populations of shellfish were examined histologically for the presence of cellular proliferative disorders. Clams from Coos Bay and mussels from Tillamook Bay were not found with the large abnormal cells that characterize the conditions. The disorder was present in a significant number (mean prevalence = 10%) of Yaquina Bay mussels with the highest concentration of PNAH measured in this study while it rarely appeared in a second population at a "clean" site across the bay. The correlation between the degree of PNAH contamination and the prevalence of the cellular disorders may be significant, but no cause-effect relationship has been established. It remains to be determined if carcinogenic metabolites can be formed by this species. If bivalves are not subject to PNAH-induced carcinogenesis, and the presence of atypical cells is related to a neoplastic process, then other causative agents must be responsible. Assuming the condition is analogous to neoplasia, it seems evident that this disorder in *M. edulis* has great potential for serving as a model for studying cancer-like diseases in an invertebrate. The cells have many characteristics in common with malignant conditions in mammals and affected mussels can be obtained easily and on a regular seasonal basis by procedures developed during this study. Considerable future efforts should be directed towards further characterizing the cells, attempting to establish culture techniques suitable for maintaining and growing the cells, and identifying the causal agent(s).

4. While it was established that quantitative predictions about total PNAH could not be made on the basis of individual PNAH measurements, the results of this research suggest that certain qualitative relationships existed which may permit first approximations of individual PNAH concentrations. In general, there were no significant differences between individual PNAH with 4 rings, or between those with 5, 6 or 7 rings. Phenanthrene, a

3-ring compound, differed significantly from other PNAH. Thus, detection of a certain quantity of PYR, for example, suggested that a similar quantity of BCP, TRI and BAA was present; measurement of an individual PNAH concentration for any 5-, 6- or 7-ring PNAH indicated that approximately the same concentration would be found for any other unsubstituted PNAH with 5-7 rings.

It would be productive to conduct these kinds of analyses for PNAH data collected during future studies and from other, established biological monitoring programs. Confirmation of the relationships identified during this research may eventually lead to a simplified monitoring approach and result in considerable cost reductions.

Statistical analyses revealed an empirical relationship between individual PNAH concentrations and their respective solubilities. The concentrations in shellfish were greater for the PNAH isomer which had the higher solubility in water. This finding contrasts with the observation that organic/water (e.g. octanol/water) partition coefficients show an inverse relation to water solubility. Because the concentration in the organic phase (shellfish, in this study), C_o, is equal to the product of the partition coefficient (K) and concentration in water (C_w), the data suggest that the ratio of the PNAH concentrations in water would have to be generally greater than the ratio of their reciprocal partition coefficients or their water solubilities. Direct measurements of PNAH concentrations in seawater will be necessary to confirm whether the uptake of PNAH's by shellfish can be represented by a simple partition process.

SECTION 4

MATERIALS AND METHODS

SAMPLE SITES, SPECIES UTILIZED AND SAMPLING PROTOCOL

The Oregon coast is characterized by the presence of generally small bays and estuaries (Fig. 1). With the exception of Coos Bay and, to a lesser degree, Yaquina Bay, none can be characterized as being industrialized. To measure PNAH concentrations in species of interest, shellfish were collected from sites in Coos, Yaquina and Tillamook Bays, the three largest bays in Oregon. Those three bays were selected because they satisfied criteria adopted during previous studies (Mix, 1979). They each have major commercial and recreational shellfisheries with abundant populations of those species to be utilized in the present study. It was also considered desirable that each bay reflect different degrees of industrialization and human onshore and watershed habitation.

Mussels and clams from Yaquina and Coos Bays were also examined histologically to determine whether or not cellular proliferative disorders occurred in their populations and, if so, to calculate the prevalence of the condition for a 6 month - 1 year period.

Figure 1 identifies the specific sites from which samples were obtained for measuring PNAH concentrations and identifying cellular disorders. Table 1 lists the species sampled from each site and the periods during which samples were collected.

TABLE 1. INFORMATION ON SAMPLE SITES, SPECIES UTILIZED AND SAMPLING FREQUENCY

BAY	SITE	SPECIES COLLECTED	PERIOD OF SAMPLING	PURPOSE (NUMBER)
Coos	C3S	*Mya arenaria*	Bimonthly, 1978-80	PNAH (10+10)
	CSS	*M. arenaria*	Bimonthly, 1978-79	PNAH (20) histology (50)
	CIIG	*Tresus capax*	Bimonthly, 1978-80	PNAH (5)
Yaquina	Y1M	*Mytilus edulis*	Bimonthly, 1978-80	PNAH (30-40) histology (50)
	Y2M	*M. edulis*	Bimonthly, 1978-80	PNAH (30-40) histology (50)
	Y14O	*Crassostrea gigas*	Quarterly, 1976-80	PNAH (20)
Tillamook	T1M	*M. edulis*	Twice, 1979	PNAH (30-50)
	TSS	*M. arenaria*	Quarterly, 1979	PNAH (20)
	TBC	*C. gigas*	Thrice, 1979	PNAH (20)

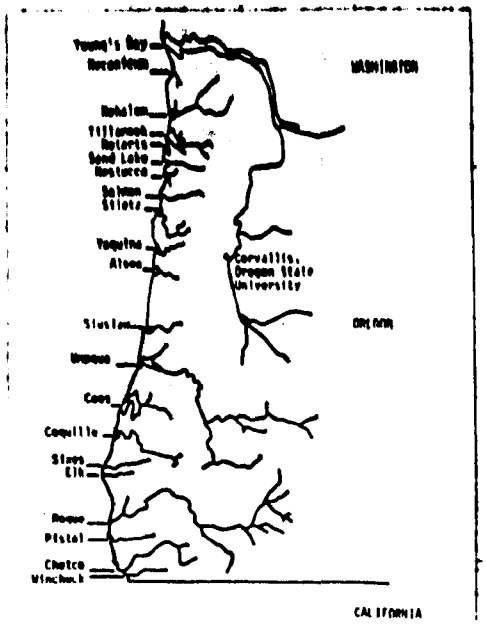

Figure 1. Oregon bays and estuaries.

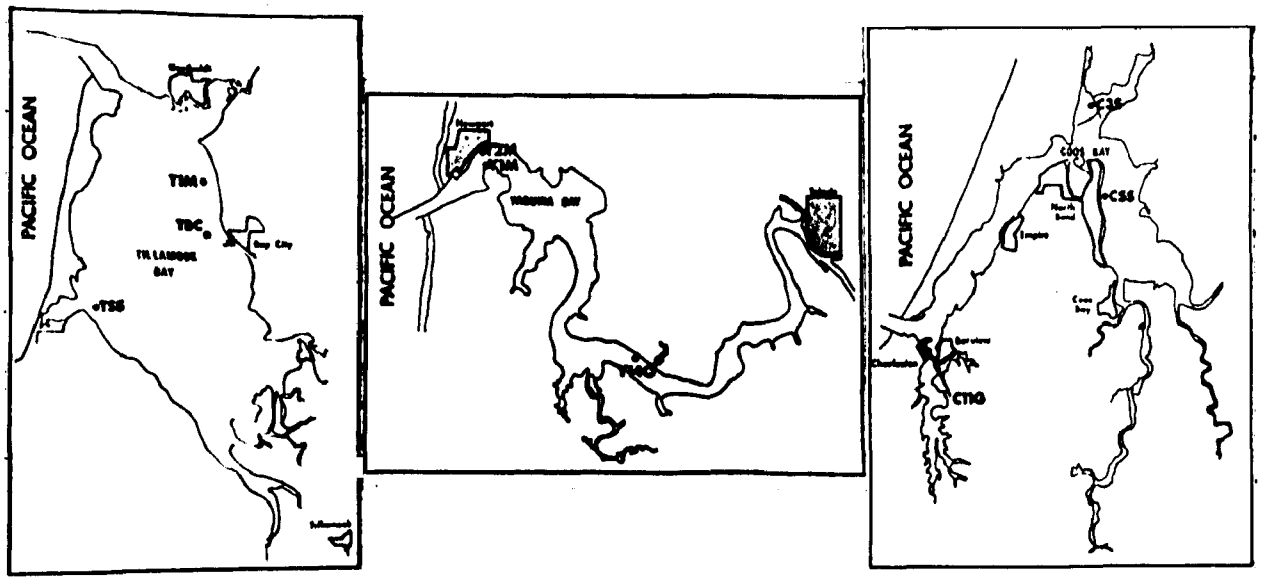

Figure 2. Sample sites in Tillamook, Yaquina and Coos Bays, Oregon.

The rationale for using more than one species has been discussed elsewhere (Mix, 1979). Briefly, utilization of a single shellfish species, such as *M. edulis*, for monitoring estuaries has certain limitations. A single species is rarely found to inhabit an entire estuary, particularly if, as in Oregon bays, they are freshwater dominated during major periods of the year. Thus, *M. arenaria* are useful as monitors of upbay sites, where much of the industry is located, since they are usually the only economically-important bivalve mollusc that thrives in these areas of low salinity. Mussels are used to monitor the lower bays where salinities are higher. While it is recognized that these 2 species occupy entirely different habitats, and direct comparisons in PNAH concentrations have limited value, they have both been found to be suitable as biomonitors for detecting the presence of PNAH in estuaries.

The specific sites in each bay were selected because they harbored substantial populations of the species of interest and each had unique features relative to anthropogenic impact. Information about each site is included below. *Y1M* consisted of a number of weathered pilings that formerly supported an old railroad trestle. From previous studies (Mix, 1979) it was known that mussels from this site had very low concentrations of BAP and a low prevalence of the cellular proliferative disorders. The site no longer exists since the pilings were removed during the summer of 1980. Mussels from *Y2M* were removed from pilings and ladders situated along the Newport bayfront. The creosoted pilings support cold storage facilities and fish processing plants. The earlier studies had shown mussels from this site had higher levels of BAP and cellular proliferative disorders. Oysters were collected from the shucking tables of a commercial oyster grower at *Y140*. The *C. gigas* were grown in trays suspended from pilings at this site. Softshell clams were collected from Coos Bay at *CSS*, an open, muddy area adjacent to timber products industries along Highway 101, and *C3S*, a mixed sandy mud flat on North Slough adjacent to Highway 101, which is removed from human onshore activities. Gaper clams were collected from *CIIG*, a small mixed sand-mud flat at Charleston adjacent to the Coast Guard station. Mussels from Tillamook Bay were collected from rocks located near the entrance, across from Kincheloe Point, clams from sand and mud flats near Pitcher Point and oysters from commercial growing grounds near Bay City.

COLLECTION AND PREPARATION OF SHELLFISH SAMPLES

Clams from the three bays were dug during low, approximately zero tides while mussels were collected during the entire ebb tide period, depending on location. Oysters were obtained from commercial growers and simply removed from the shucking tables. Immediately after collection, samples from each site were placed in labeled plastic bags, put on ice and transported back to Corvallis. Animals were then removed from their shell and the pooled sample from each site was then weighed. Each pooled sample was then stored at -20°C until it was processed for PNAH analysis.

To determine the effects of a depuration period on PNAH concentration in clams, 10 *M. arenaria* from the *C3S* sample were placed in a plastic bucket filled with salt water that contained a small quantity of cornmeal. The

bucket was placed in a cooler maintained at 10°C and the clams allowed to depurate for 24 hours. The depurated clams were then processed in the usual way. This procedure took place each time clams were sampled after June, 1979. Mussels to be examined histologically were placed in Davidson's fixative (3:3:2:1:1 - 95% ethanol:sea-water:formalin:glycerol:acetic acid added just before use), processed in the usual way, sectioned at 6µm, and stained with hematoxylin and eosin.

PNAH ANALYTICAL PROCEDURE

One of the major objectives of this study was to develop standard procedures that could be used confidently for measuring PNAH concentrations in bivalve shellfish. These methods, as developed and modified by Mr. Randy Schaffer, will be described in considerable detail.

Reagents and Standards

The following reagents were obtained from J. T. Baker (Phillipsburg, NJ): High performance liquid chromatography (HPLC) grade acetonitrile; photrex grade dimethyl sulfoxide (DMSO); reagent grade 2,2,4-tri-methyl pentane (TMP), potassium hydroxide and benzene. Ethanol (USP) was obtained from Central Solvents (Oregon Liquor Control Commission) and redistilled before use. Water for the HPLC was prepared in a Milli-Q System (Millapore Corp., Bedford, MA).

The following PNAH standards were purchased as dry powders: fluorane, phenanthrene, fluoranthene, pyrene, triphenylene, benzo(a)anthracene, chrysene, benzo(e)pyrene, dibenz(a,c,)anthracene, benzo(a)pyrene, dibenz(a,h)-anthracene, benzo(g,h,i)perylene and coronene, from RFR Corp. (Hope, RI); indeno(1,2,3-c,d)pyrene from Analabs (North Haven, CT); and benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(b)fluoranthene and benzo(c)phenanthrene from Dr. J. E. Meeker (U.S. EPA, Research Triangle Park, NC). Stock solutions of all standards were made with DMSO to prevent evaporation.

Apparatus

A Spectra-Physics Model SP8000 High Performance Liquid Chomatograph, equipped with a Schoeffel Model 770 variable wavelength UV detector and a Schoeffel Model 970 variable wavelength fluorescence detector connected in series, was used throughout the study. Chromatography conditions are summarized in Table 2.

Sample Preparation

Shellfish samples were prepared according to the methods of Dunn (1976) Approximately 30-40 g of pooled tissue was saponified in ethanol/KOH. The resulting supernatant was extracted with TMP and the organic phase passed through a column of partially deactivated florisil. The PNAH were eluted from the column with benzene and the eluate was extracted with DMSO. The resultant TMP fraction was then passed through a Sephadex LH-20 column (Pharmacia Fine Chemicals, Inc., Piscataway, NJ); tritiated BAP was added to determine BAP recovery. The end product was brought up to 100µl in DMSO.

PNAH Analysis

The HPLC analyses were conducted according to procedures described in Table 2.

TABLE 2. APPARATUS, OPERATING CONDITIONS AND PROCEDURES FOR PNAH ANALYSES.

LIQUID CHROMATOGRAPHY: Spectra-Physics 8000 with data system; Valco injector, model CV-6-U1+Pa-N60, with 10µl loop.

COLUMNS: Perkin Elmer HC-ODS-PAH number 258-0082, 0.26 x 25cm connected in series with a 0.32 x 10cm guard column slurry packed with Vydac 201 TP.

MOBILE PHASE: Acetonitrile(MeCN)/water gradient, constant flow mode 0.8 ml/min at 20°C.

Time(min)	% MeCN	% H_2O
0	60	40
4	60	40
22	100	0
45	100	0
55	20	80
65	20	80
70	60	40
95	60	40

DETECTOR 1: Schoeffel Model 770 variable wavelength UV detector, 296 nm range 0.02 and 254 range 0.02.

DETECTOR 2: Schoeffel Model 970 variable wavelength fluorescence detector, 326 nm excitation > 412 nm emission cutoff-type filter; range 0.1, sensitivity 580.

Identification and Quantification

Four methods were used to identify individual PNAH. (1) A preliminary identity was assigned to each sample peak by comparing its retention time to that of a known standard. Each sample was chromatographed at two UV wavelengths, 296 nm and 254 nm, and one fluorescent wavelength combination, 326 excitation greater than 412 nm emission. Precise identification of PNAH was then possible by calculating ratios of response at different wavelengths; (2) UV peak area at 254 nm/UV peak area at 296 nm, (3) UV peak area at 254 nm/fluorescent peak area, and (4) UV peak area at 296 nm/fluorescent peak area.

Individual PNAH were quantified by use of an internal data system in the HPLC; essentially, a comparison is made between a calibration standard run and an analytical (sample) run. A concentration factor, calculated in the calibration mode, was used to determine a concentration for the sample peak nearest the retention time of the calibration peak. Concentrations were

determined using a program designed for an HP-85 computer.

A portion of the sample was analyzed for ^{3}H by liquid scintillation counting in order to determine BAP recovery. That recovery figure was used to convert all PNAH quantities to 100%.

BENZO(A)PYRENE UPTAKE AND ELIMINATION

An appropriate number of *M. edulis* from *Y1M* and *Y2M* were collected and placed in fiberglass mesh bags designed to hold one sample of 20-30 mussels. Individual bags were then placed inside 46 x 92 cm nylon mesh drawstring bags and suspended at the +2.0 foot tide level.

For the uptake study, 10 samples were collected from *Y1M* and transferred to the more contaminated site, *Y2M*. An equal number of *Y2M* mussels were collected and hung next to the transferred *Y1M* mussels to serve as a control to measure background fluctuations in BAP concentrations. For the depuration study, 10 samples of BAP-contaminated mussels were collected from *Y2M* and transferred to *Y1M*. An equal number of *Y1M* mussels were collected and hung near the transferred *Y2M* mussels to serve as a control. Animals were sampled biweekly or weekly and analyzed for BAP using Dunn's techniques (Dunn, 1976; Mix and Schaffer, 1979).

TISSUE STORAGE SITES OF BENZO(A)PYRENE IN *M. EDULIS*

An initial effort was directed towards resolving the question of whether or not the gonad is a major tissue repository for BAP.

Site *Y2M* was selected for this study since it was known to harbor mussels with significant BAP concentrations (Mix and Schaffer, 1979). High BAP concentrations were necessary for detection when small gonad samples (<5g pooled net weight) were analyzed. Prior to initiating the study, 12 samples, consisting of at least 30 mussels, were collected from the pilings at *Y2M*. Each sample was placed in fiberglass mesh bags and then collected in a large, nylon mesh bag and suspended at the same location and tide level from which they were collected. Mussels were acclimated for two weeks before sampling began; samples were collected at approximately 10 day intervals from 9 Jan 79 to 25 May 79 when spawning had been completed.

Mussels in each sample were separated into gonadal tissue and somatic tissue fractions. Each tissue fraction was blotted dry on paper towels, placed in plastic bags, labeled, and held at -10°C until analyzed. Concentrations of BAP were determined by Dunn's TLC methods (Dunn, 1976).

BENZO(A)PYRENE METABOLISM BY *M. EDULIS*

Only studies of a preliminary nature were conducted during the present period. The basic techniques and protocol followed those developed by Anderson (1978) for American oysters (*Crassostrea virginica*). The method

involves use of a sensitive radioisotopic system that permits quantification of alkali-soluble and water-soluble BAP metabolites produced by digestive gland microsomes. High performance liquid chromatography was used to identify the metabolites.

STATISTICAL ANALYSES

The data gathered during this research were subjected to extensive statistical analyses using the PROPHET system. PROPHET is a National Computer Resource of the Division of Research Resources, National Institute of Health. It consists of two PDP-10 computers, located at ADP Network Services, Inc., Waltham, Mass. and appropriate software developed by Bolt, Beranak and Newman, Inc., Cambridge, Mass. HP-25C and HP-41C programmable calculators were also used for certain procedures. The information below is summarized from the PROPHET manuals.

After the basic measures were computed, it was general practice to pursue a course for drawing conclusions or making decisions about the data under study. The general method was to assume a testable hypothesis about a population and then use the data in an appropriate test of the hypothesis.

The appropriateness of any statistical test rests on certain underlying theoretical assumptions about the sample and the population. Parametric procedures were used when it could be determined that samples were obtained from populations whose distributions were normal. Parametric tests were always used where appropriate since they tend to be more efficient in detecting departures from the null hypothesis. For certain samples, where the assumption of normality was clearly violated, nonparametric procedures were used.

SECTION 5

RESULTS AND DISCUSSION

IDENTIFICATION OF PNAH IN BIVALVE MOLLUSCS

Table 3 includes a list of unsubstituted PNAH identified and quantified in indigenous populations of bivalve molluscs during this study and provides a summary of their activities according to several classification systems. There is not yet universal agreement about the carcinogenic potential of several PNAH, yet, except for coronene, all of the PNAH identified during the present study have obvious environmental importance.

The identification of this large group of individual PNAH in indigenous organisms represents a significant advance in studying levels of PNAH in marine organisms. The presence of PNAH in water, aquatic sediments and organisms has been recognized for more than 20 years (Neff, 1979). Yet, dependable high resolution techniques, such as those utilized in this study, have only recently been developed and a more precise information base can now be expected to develop.

Table 4 summarizes available information on certain physical and chemical characteristics of PNAH. Complete data on PNAH solubilities will be required for a full understanding of the behavior of these compounds in marine ecosystems.

BASELINE DATA ON PNAH CONCENTRATIONS IN CLAMS FROM COOS BAY

While there have been numerous reports of PNAH concentrations, particularly BAP, in mussels there is little similar information available for clam species. Tables 5-7 contain data on PNAH concentrations in 3 clam populations from Coos Bay, Oregon, sampled during a 2 year period.

Gaper clams, *T. capax*, are large (10-15 cm), suspension-feeding, bay clams that are found in sand or sand-mud bottoms, 25-70 cm below the surface. They are heavily dug by recreational fishermen and fried or minced for incorporation into chowder. PNAH concentrations in *T. capax* from Charleston were the lowest of any clams from Coos Bay, ranging from 31.4-109.7 µg/kg. It is apparent from examining Table 5, that PNAH concentrations fell into two narrow ranges, 37-51 µg/kg and 104-110 µg/kg. Regression analyses (day number vs. total PNAH) indicated that there were no seasonal differences, nor was there any apparent relationship between concentration and reproductive cycle. The differences in concentration may be due to variation in the samples analyzed, or higher concentrations may have been caused by local

TABLE 3. POLYNUCLEAR AROMATIC HYDROCARBONS ANALYZED IN SHELLFISH FROM OREGON ESTUARIES.

PNAH	CLASSIFICATION				
	EPA[1]	NAS[2]	NIOSH[3]	IARC[4]	IARC[5]
Phenanthrene (PHEN)	PP	-			
Fluoranthene (FLUOR)	PP,TP	-			
Pyrene (PYR)	PP	-	NEO		
Benzo(c)phenanthrene (BCP)		+++	CAR		
Triphenylene (TRI)					
Benzo(a)anthracene (BAA)	PP,TP	+	CAR	C	C
Chrysene (CHRY)	PP,TP	±	CAR		
Benzo(b)fluoranthene (BBF)	PP,TP	++	CAR	C	C
Benzo(k)fluroanthene (BKF)	PP,TP	-	CAR,NEO		
Dibenz(a,c)anthracene (DBACA)	TP	+	CAR		
Benzo(a)pyrene (BAP)	PP,TP	+++	CAR,NEO	C	C
Dibenz(a,h)anthracene (DBAHA)	PP,TP	+++	CAR,NEO	C	C
Benzo(g,h,i)perylene (BGHIP)	PP	-			
Indeno(1,2,3-c,d)pyrene (IP)	PP,TP	+	CAR	C	C
Coronene (COR)		-			

[1]EPA classification: PP-Priority pollutant; TP-Toxic pollutant (Keith and Telliard, 1979)

[2]NAS classification: - not carcinogenic, ± uncertain or weakly carcinogenic, + carcinogenic, ++, +++, ++++ strongly carcinogenic (NAS, 1972)

[3]NIOSH classification: CAR-carcinogenic effects in animals, NEO-neoplastic effects in animals (Christensen et al., 1975)

[4]IARC classification: C - evidence of carcinogenicity in experimental animals (IARC, 1972)

[5]IARC classification: C - sufficient evidence of carcinogenicity in experimental animals (IARC, 1979)

TABLE 4. SOME PHYSICAL AND CHEMICAL CHARACTERISTICS OF PNAH.

PNAH	NUMBER OF RINGS	MOLECULAR WEIGHT	WATER SOLUBILITY, S, (mg/L) M&S[1]	(-LogS)[3]	MAY[2]	(-LogS)[3]	-LogSs, 5‰ M&S	-LogSs, 5‰ MAY	-LogSs, 15‰ M&S	-LogSs, 15‰ MAY	-LogSs, 30‰ M&S	-LogSs, 30‰ MAY
PHEN	3	178.2	1.29	(5.14)	1.002	(5.25)	5.14	5.25	5.21	5.32	5.29	5.39
FLUOR	4	202.3	0.26	(5.89)	0.206	(5.99)	5.92	6.02	5.97	6.08	6.06	6.17
PYR	4	202.3	0.135	(6.18)	0.132	(6.19)	6.20	6.21	6.25	6.26	6.32	6.33
BCP	4	228.3										
TRI	4	228.3	0.043	(6.73)	0.0066	(7.54)	6.74	7.56	6.78	7.59	6.83	7.65
BAA	4	228.3	0.014	(7.21)	0.0094	(7.39)	7.25	7.42	7.30	7.48	7.40	7.57
CHRY	4	228.3	0.002	(8.06)	0.0018	(8.10)	8.09	8.13	8.15	8.19	8.23	8.28
BJF	5	252.3										
BBF	5	252.3										
BKF	5	252.3										
BEP	5	252.3										
DBACA	5	278.3	0.0055	(8.75)								
BAP	5	252.3	0.0038	(7.82)								
DBAHA	5	278.3										
BGHIP	6	276.3	0.0026	(9.03)								
IP	6	276.3										
COR	7	300.3	0.00014	(9.33)								

[1] MacKay and Shiu, 1977.

[2] May, 1980

[3] Log S, in moles/L, determined from the formula, $\text{LogS} = \frac{S \times 10^{-3} g/L}{MW}$

[4] Log S_S, solubility in saltwater, determined for various salinities by the formula, $\text{Log}\frac{S}{S_S} = K_S C_S$, where S and S_S are the concentrations of the solute in freshwater and saltwater, respectively; K_S is the Setschenow constant for the PNAH and C_S is the molar salt concentration for a specific salinity (after May, 1980).

TABLE 5. PNAH CONCENTRATIONS (μg/kg)[1] IN *T. CAPAX* FROM COOS BAY, OREGON, SITE C11G.

DATE SAMPLED	PHEN	FLUOR	PYR	BCP	TRI	BAA	CHRY	BBF
9/29/78	25.9	20.3	11.5	9.9	8.9	10.3	9.8	4.3
12/2/78	20.3	21.2	12.5	10.9	9.0	9.3	10.2	4.9
2/9/79	16.1	8.3	2.0	4.3	4.6	8.3	2.4	0.9
4/18/79	14.4	7.0	2.1	4.0	4.0	6.4	2.1	0.8
6/23/79	11.0	6.3	2.1	3.5	2.1	5.3	2.0	1.0
8/13/79	23.1	20.0	11.5	9.8	9.0	9.6	9.8	4.7
10/6/79	8.3	5.3	2.0	2.5	1.8	4.1	1.9	1.0
2/22/80	24.4	20.8	12.5	10.4	9.1	10.0	10.0	4.7
4/25/80	9.5	6.3	3.0	1.8	2.0	6.5	3.1	0.9
6/14/80	10.5	7.3	4.1	2.7	3.0	7.1	3.5	1.1
$\bar{X}$	16.4	10.0	6.3	6.0	5.4	7.7	5.5	2.4
(s.d.)[1]	(6.6)	(12.3)	(4.9)	(3.8)	(3.3)	(2.1)	(3.9)	(1.9)

DATE SAMPLED	BKF	DBACA	BAP	DBAHA	BGHIP	IP	COR	TOTAL PNAH
9/29/78	3.9	1.4	1.0	0.9	0.3	0.2	0.3	108.2
12/2/78	3.0	1.2	1.1	1.0	0.3	0.2	0.3	105.6
2/9/79	0.8	0.6	1.0	0.8	0.3	0.3	0.3	51.0
4/18/79	0.6	0.9	1.0	0.8	0.4	0.1	0.2	44.8
6/23/79	0.5	1.0	1.3	0.6	0.6	0.3	0.1	37.7
8/13/79	3.1	1.4	1.1	1.0	0.3	0.2	0.4	104.9
10/6/79	0.4	0.9	1.2	0.8	0.6	0.4	0.2	31.4
2/22/80	3.2	1.4	1.2	1.0	0.3	0.3	0.4	109.7
4/25/80	0.4	0.9	1.0	1.0	0.6	0.3	0.2	37.5
6/14/80	0.5	0.9	1.0	1.5	0.3	0.4	0.3	44.2
$\bar{X}$	1.6	1.1	1.1	0.9	0.4	0.3	0.3	67.5
(s.d.)	(1.5)	(0.3)	(0.1)	(0.2)	(0.1)	(0.1)	(0.1)	(34.5)

[1]one standard deviation

events related to boat or fish processing plant activities.

Two populations of soft-shell clams (*M. arenaria*) were studied during a two year period. Total PNAH concentrations in clams from CSS were much greater than those from C3S (Table 6). The two sites represent considerably different environments. Site C3S is in a relatively pristine section in North Slough with little evident impact from human onshore activities. Clams from CSS inhabited a soft mud flat located adjacent to the industrial waterfront complex at Coos Bay. Potential sources of PNAH at the latter site include, but are not limited to, timber products industries, fuel storage facilities, boats and pilings. It is noted that clams from C3S are dug by recreational fishermen while those from CSS are not because of access problems and difficulties in digging in soft mud. A paired t statistic was used to test the null hypotheses, H_0: $\mu_{xi} = \mu_{yi}$ where x = concentrations from C3S clams, y = concentrations from CSS clams and i represents individual, and total, PNAH. For 10 PNAH and total PNAH, the H was rejected; calculated values were greater than t .01.5 = 4.03. However, there were no significant differences in the mean concentrations of DBACA, DBAHA, BGHIP, IP or COR during the one-year period. There were no large differences in PNAH concentrations during the 6 bimonthly sampling periods. However, since all samples consisted of 20 pooled clams, no statistical analyses could be done in individual animals. Only by obtaining additional samples during each collection date would it be possible to use statistical tests to identify significant seasonal differences. The present results suggest that such an effort would not be warranted since the differences, if they existed, were small and the additional costs would be substantial. It is concluded that the means and standard deviations for individual and total PNAH represent an accurate baseline for concentrations of those compounds in *M. arenaria* from two geographical locations in Coos Bay, Oregon.

To determine the effects of depuration on PNAH concentrations in *M. arenaria*, samples from C3S were divided, beginning with the 6/23/79 collection. One group ("D") was placed in seawater containing corn meal and allowed to depurate for 24 hours; the control group (N) was simply placed in a refrigerator; both were maintained at the same temperature. After 24 hours, all animals were shucked and analyzed. Table 7 provides a summary of the data from the depuration study. A paired t statistic was used to determine if there were significant differences in PNAH concentrations between clams that had been depurated and those that had not. For all 3 and 4 ring PNAH, there were significant differences between the clams that had been depurated and those that had not; depurated clams had significantly lower concentrations of individual 3 and 4 ring PNAH and total PNAH. In contrast, concentrations of all 5, 6 and 7 ring compounds, which included most of the carcinogenic PNAH, were not statistically different in the two groups.

While it is not yet possible to fully explain these results, differences in water solubility of the PNAH may at least partially account for the observed differences between depurated and non-depurated clams. In general, the lower molecular weight (MW) PNAH are more soluble in water than those with greater MW (May, 1980). Previous depuration studies in shellfish (e.g. Stegeman and Teal, 1973) have produced results which suggest that PNAH are stored in two compartments. One of the compartments, perhaps fluid and/or

TABLE 6. PNAH CONCENTRATIONS (µg/kg) IN *M. ARENARIA* FROM COOS BAY, OREGON

SITE C3S

DATE SAMPLED	PHEN	FLUOR	PYR	BCP	TRI	BAA	CHRY	BBF
9/30/78	13.9	10.8	5.4	2.9	6.3	2.8	8.9	1.4
12/1/78	17.4	9.6	6.3	3.0	5.6	2.7	8.7	1.9
2/9/79	9.4	7.7	5.3	3.0	4.8	2.0	6.3	0.9
4/18/79	10.5	12.0	6.8	3.1	5.9	2.9	7.9	1.0
6/23/79	9.9	8.7	6.0	3.0	5.5	2.7	7.9	1.3
8/13/79	---	14.2	7.9	3.6	19.5	4.5	5.9	2.3
$\bar{X}$	12.2	10.5	6.3	3.1	7.9	2.9	7.6	1.5
(s.d.)	(3.4)	(2.4)	(1.0)	(0.2)	(5.7)	(0.8)	(1.2)	(0.5)

DATE SAMPLED	BKF	DBACA	BAP	DBAHA	BGHIP	IP	COR	TOTAL
9/30/78	3.2	2.8	3.3	5.4	6.4	5.8	1.5	80.8
12/1/78	3.0	2.1	3.1	5.0	6.0	5.5	1.4	81.3
2/9/79	2.0	1.0	2.3	1.5	3.2	4.3	0.9	54.6
4/18/79	3.1	2.1	3.4	4.7	6.0	6.4	1.5	77.3
6/23/79	3.4	2.1	3.3	4.9	4.6	7.5	1.6	72.4
8/13/79	1.1	12.8	5.5	6.7	6.4	---	1.0	91.4
$\bar{X}$	2.6	3.8	3.5	4.7	5.4	5.9	1.3	76.3
(s.d.)	(0.9)	(4.4)	(1.0)	(1.7)	(1.3)	(1.2)	(0.3)	(12.3)

SITE CSS

DATE SAMPLED	PHEN	FLUOR	PYR	BCP	TRI	BAA	CHRY	BBF
9/30/78	158.4	121.2	58.6	65.4	41.8	29.5	28.6	13.2
12/1/78	144.4	111.2	47.3	51.5	38.6	25.3	21.5	10.5
2/9/79	152.4	119.6	49.8	58.4	40.3	27.5	24.1	11.6
4/18/79	149.7	89.9	63.9	49.0	44.3	65.4	25.6	11.0
6/23/79	161.3	103.7	98.8	54.5	49.4	71.5	38.9	11.4
8/13/79	162.3	118.5	51.2	52.8	45.4	31.0	24.7	14.8
$\bar{X}$	154.8	110.7	61.6	55.3	43.3	41.7	27.2	12.1
(s.d.)	(7.1)	(12.1)	(19.1)	(5.9)	(3.9)	(20.9)	(6.2)	(1.6)

DATE SAMPLED	BKF	DBACA	BAP	DBAHA	BGHIP	IP	COR	TOTAL
9/30/78	10.9	9.9	8.5	6.4	3.4	2.0	1.4	559.2
12/1/78	8.8	8.3	7.4	5.2	2.1	1.4	1.0	484.5
2/9/79	9.9	9.5	8.0	5.3	2.0	1.3	1.0	520.7
4/18/79	8.5	6.0	9.4	8.3	7.0	7.9	6.0	551.9
6/23/79	8.8	9.1	11.4	9.5	8.0	8.7	5.4	650.4
8/13/79	10.4	8.3	7.6	6.6	3.0	1.9	1.2	539.7
$\bar{X}$	9.6	8.5	8.7	6.9	4.2	3.9	2.7	551.1
(s.d.)	(1.0)	(1.4)	(1.5)	(1.7)	(2.6)	(3.4)	(2.4)	(55.5)

TABLE 7. PNAH CONCENTRATIONS (µg/kg) IN *M. ARENARIA* FROM COOS BAY, OREGON, SITE C3S, BEFORE (N) AND AFTER DEPURATION (D) FOR 24 HOURS.

DATE	PHENANTHRENE			FLUORANTHENE			PYRENE		
SAMPLED	N	D	%[1]	N	D	%	N	D	%
6/23/79	9.9	7.5	-24.2	8.7	5.3	-39.1	6.0	1.0	-83.3
8/13/79	---	---	---	14.2	0.8	-94.4	7.9	0.8	-89.9
10/6/79	12.5	9.5	-24.0	9.8	7.8	-20.4	6.3	3.0	-52.4
2/22/80	13.5	5.4	-60.0	9.8	1.4	-85.7	5.4	1.4	-74.1
4/25/80	11.5	6.4	-44.4	9.6	7.7	-19.8	5.5	1.3	-76.4
6/14/80	10.1	5.3	-47.5	5.4	0.9	-83.3	4.7	1.1	-76.6
9/12/80	8.7	5.1	-41.4	4.8	1.3	-72.9	3.7	2.0	-46.0
$\bar{X}$	11.0	6.5	-40.2	8.9	3.6	-59.4	5.6	1.5	-72.2
(s.d.)	(1.8)	(1.7)	(14.0)	(3.1)	(3.2)	(32.1)	(1.3)	(0.8)	(16.1)
t value[2]	t = 5.39			t = 2.74			t = 6.59		
sig. level	.01			.05			.01		

DATE	BENZO(C)PHENANTHRENE			TRIPHENYLENE			BENZO(A)ANTHRACENE		
SAMPLED	N	D	%	N	D	%	N	D	%
6/23/79	3.0	0.9	-90.0	5.5	0.4	-92.7	2.7	1.4	-48.2
8/13/79	3.6	0.4	-88.9	19.5	3.1	-84.1	4.5	1.2	-73.3
10/6/79	3.0	0.9	-70.0	4.9	2.4	-51.0	2.0	1.0	-50.0
2/22/80	3.0	0.6	-80.0	5.4	0.9	-83.3	2.4	1.4	-41.7
4/25/80	3.5	0.8	-77.1	5.6	1.4	-75.0	2.3	1.0	-56.5
6/14/80	2.7	0.6	-77.8	9.5	3.5	-63.1	2.0	0.9	-55.0
9/12/80	3.1	0.8	-74.2	6.4	3.8	-40.6	2.2	0.5	-77.3
$\bar{X}$	3.1	0.7	-76.9	8.1	2.2	-70.0	2.6	1.1	-57.4
(s.d.)	(0.3)	(0.2)	(6.5)	(5.2)	(1.3)	(19.1)	(0.9)	(0.3)	(13.2)
t value	t = 15.6			t = 3.25			t = 4.94		
sig. level	.01			.05			.01		

DATE	CHRYSENE			BENZO(B)FLUORANTHENE			BENZO(K)FLUORANTHENE		
SAMPLED	N	D	%	N	D	%	N	D	%
6/23/79	7.9	1.3	-83.5	1.3	1.5	15.4	3.4	3.0	-11.8
8/13/79	5.9	---	----	2.3	2.1	-8.7	1.1	1.1	0.0
10/6/79	5.4	3.3	-38.9	1.4	1.1	-21.4	3.0	3.1	3.3
2/22/80	6.9	5.8	-15.9	1.1	1.1	0.0	3.0	2.8	-6.7
4/25/80	5.8	2.9	-50.0	1.2	1.0	-16.7	4.0	3.9	-2.5
6/14/80	2.7	1.1	-59.3	1.4	1.3	-7.1	3.0	3.0	0.0
9/12/80	2.8	1.0	-64.3	1.9	1.6	-15.8	3.1	2.9	-6.4
$\bar{X}$	5.3	2.6	-52.0	1.5	1.4	-7.8	2.9	2.8	-3.4
(s.d.)	(2.0)	(1.9)	(23.0)	(0.4)	(0.4)	(12.4)	(0.9)	(0.8)	(4.8)
t value	t = 3.27			t = 1.89			t = 1.80		
sig. level	.05			nsd			nsd		

[1]Determined by: $\frac{N-D}{N} \times 100$

[2]Paired t-statistic, to test Ho: $\mu_B = \mu_D$ for all dates sampled.

TABLE 7. (continued)

DATE SAMPLED	DIBENZ(A,C)ANTHRACENE N	D	%	BENZO(A)PYRENE N	D	%	DIBENZ(A,H)ANTHRACENE N	D	%
6/23/79	2.1	2.1	0.0	3.3	3.3	0.0	4.9	5.0	2.0
8/13/79	12.8	2.7	-78.9	5.5	2.4	-56.4	6.7	---	---
10/6/79	2.3	2.4	4.4	2.0	2.1	5.0	3.9	3.9	0.0
2/22/80	2.5	2.4	-4.0	3.2	3.1	-3.1	5.0	5.3	6.0
4/25/80	3.6	3.0	-16.7	2.9	2.7	-6.9	5.1	5.0	-2.0
6/14/80	2.0	1.9	-5.0	2.5	2.6	4.0	4.7	4.9	4.3
9/12/80	2.8	2.1	-25.0	2.5	2.3	-8.0	4.1	3.0	-26.8
$\bar{X}$	4.0	2.4	-17.9	3.1	2.6	-9.3	4.9	4.5	-2.8
(s.d.)	(3.9)	(0.4)	(28.7)	(1.1)	(0.4)	(21.3)	(0.9)	(0.9)	(12.1)
t value	t = 1.16			t = 1.11			t = -0.48		
sig. level	nsd			nsd			nsd		

DATE SAMPLED	BENZO(G,H,I)PERYLENE N	D	%	INDENO(1,2,3-C,D)PYRENE N	D	%	CORONENE N	D	%
6/23/79	4.6	5.0	8.7	7.5	6.5	-13.3	1.6	1.4	-12.5
8/13/79	6.4	9.6	33.3	---	---	---	1.0	2.1	110.0
10/6/79	5.0	5.8	16.0	4.0	4.0	0.0	2.0	1.7	-15.0
2/22/80	5.1	6.0	17.6	6.0	3.3	-45.0	2.4	1.9	-20.8
4/25/80	5.0	4.6	-8.0	5.8	5.7	-1.7	1.9	1.9	0.0
6/14/80	3.0	2.4	-20.0	3.1	2.9	-6.4	1.1	1.1	0.0
9/12/80	3.6	2.8	-22.2	4.8	3.1	-35.4	1.6	1.0	-37.0
$\bar{X}$	4.7	5.2	3.6	5.2	4.2	-17.0	1.7	1.6	-3.5
(s.d.)	(1.1)	(2.4)	(20.9)	(1.6)	(1.5)	(18.8)	(0.5)	(0.4)	(48.6)
t value	t = -0.97			t = 2.16			t = 0.33		
sig. level	nsd			nsd			nsd		

	TOTAL PNAH N	D	%
6/23/79	72.4	45.6	-37.0
8/13/79	78.8	26.3	-70.0
10/6/79	67.5	52.0	-23.0
2/22/80	74.7	42.8	-42.7
4/25/80	73.3	49.3	-32.7
6/14/80	57.9	33.5	-42.1
9/12/80	56.1	33.3	-40.6
$\bar{X}$	68.7	40.4	-41.2
(s.d.)	(8.7)	(9.5)	(14.5)
t value	t = 6.36		
sig. level	.01		

hemolymph, is characterized by the rapid turnover of PNAH while the other, more stable, compartment is characterized by slow turnover. Thus, more soluble PNAH, the 3 and 4 ring members, may be associated primarily with the fluid compartment in *M. arenaria* and were eliminated by a measurable degree during a 24 hour depuration period. The 5, 6 and 7 ring PNAH which have low aqueous solubilities may have been present primarily in tissues and were not released in significant quantities. Clearance of the gut contents may have also been associated with the decrease in PNAH concentrations. However, if the organic contents of the gut contained substantial quantities of PNAH, seasonal differences in rates of decrease, or percent change, would perhaps be anticipated since *M. arenaria* do not pump actively during the winter months. No such seasonal differences were evident for this study and regression analyses (day number vs. PNAH concentrations) confirmed that the amounts of PNAH decreases in depurated clams were independent of season ($F = 0.0 < F_{.05(7,15)} = 3.52$). Additional studies will be necessary to identify the underlying mechanisms responsible for the observed differences discussed above.

There are essentially no reports of PNAH concentrations in clams collected from their native environment; thus, comparisons of results with those other studies are not possible. Joe et al. (1979) reported on BAA, BAP, FLUOR, PYR, BBF and TRI concentrations in a single *M. arenaria* collected from an oil spill site. The total value was approximately 5X the mean concentration of the same PNAH for soft shell clams sampled from C3S (136 µg/kg vs. 29.8) but only half that for CSS clams (265.9 µg/kg). No conclusions are warranted from this comparison because of the unacceptable sample size in that study.

PNAH CONCENTRATIONS IN *M. EDULIS* FROM YAQUINA BAY

Baseline Concentrations of PNAH

Mussels were sampled monthly or bimonthly from two separate populations in Yaquina Bay. *M. edulis* from Y1M were collected and analyzed from 10/2/78 until 6/17/80, when the site was destroyed during construction of a marina. Since the population at Y2M was smaller, sampling did not begin until January, 1979, to insure that PNAH measurements could be made for an entire year (1979). Mussels were also collected and analyzed for the first 4 months of 1980 before their number was reduced to a level where it was no longer considered possible to obtain random samples from a normal population.

Tables 8A and 8B include data on PNAH concentrations in those two populations. It is evident that there were substantial differences in PNAH levels between the two populations. The average total concentration in Y1M mussels was 283.6 µg/kg compared to 986.2 µg/kg in mussels from Y2M. Individual PNAH measurements also reflected these differences. Paired t-tests were used to determine if there were significant differences in individual PNAH and total PNAH concentrations between the two sites for each sample collected on the same day (10/2/78, 4/20/79, 5/21/80, 6/2/80 and 6/17/80 data for Y1M were not included in any of the comparative tests). For all tests, calculated $t > t_{.05,11} = 2.2$; therefore, H_0: $\mu_{iY1M} = \mu_{iY2M}$ was rejected; concentrations of each individual PNAH (i) and total PNAH were obviously

TABLE 8A. PNAH CONCENTRATIONS (µg/kg) IN *M. EDULIS* FROM YAQUINA BAY, OREGON; SITE Y1M.

DATE SAMPLED	PHEN	FLUOR	PYR	BCP	TRI	BAA	CHRY	BBF
10/2/78	207.8	90.9	20.9	52.0	46.4	15.2	---[1]	1.6
1/24/79	135.3	62.1	24.6	28.9	30.8	49.6	---	4.0
2/13/79	146.8	64.8	45.6	38.2	37.0	48.9	---	5.1
3/21/79	84.8	40.3	23.6	18.7	20.2	32.3	---	2.5
4/20/79	178.4	66.8	17.2	48.2	40.9	14.0	---	1.5
5/31/79	100.8	46.4	28.8	23.9	24.5	39.2	---	3.2
7/2/79	165.4	59.8	17.0	46.4	42.1	7.0	---	0.2
7/30/79	45.9	10.7	25.7	24.9	22.9	7.7	---	0.8
10/8/79	140.6	67.5	15.4	33.1	30.6	10.4	---	1.2
11/14/79	76.4	42.2	20.5	13.1	18.0	35.7	---	2.9
1/14/80	103.2	46.9	28.2	22.6	23.0	37.5	---	3.0
2/11/80	86.4	39.4	24.6	19.6	21.4	33.1	---	2.7
3/10/80	92.4	42.4	24.9	20.0	25.5	37.8	---	3.0
4/23/80	100.4	42.5	27.0	21.3	27.3	40.2	---	2.6
5/21/80	89.5	40.4	25.5	21.0	24.3	34.4	---	2.6
6/2/80	87.5	39.7	25.1	20.0	20.5	33.8	---	2.6
6/17/80	88.4	39.1	25.4	20.5	21.1	32.6	---	2.3
$\bar{X}$	113.4	49.5	24.7	27.8	28.0	30.0	---	2.5
(s.d.)	(42.0)	(17.6)	(6.7)	(11.6)	(8.6)	(13.7)	---	(1.2)

DATE SAMPLED	BKF	DBACA	BAP	DBAHA	BGHIP	IP	COR	TOTAL
10/2/78	nd[2]	nd	1.9	1.1	0.3	0.0	0.8	438.9
1/24/79	3.4	2.6	1.2	2.8	0.6	0.3	0.9	347.1
2/13/79	4.2	3.1	1.4	3.6	0.9	0.4	1.2	401.2
3/21/79	2.0	1.5	0.8	1.8	0.4	0.2	0.6	229.7
4/20/79	nd	nd	1.7	1.0	0.2	0.4	0.6	370.9
5/31/79	2.7	2.2	0.9	2.3	0.6	0.2	0.7	276.4
7/2/79	nd	nd	1.6	1.0	0.2	0.4	0.6	341.7
7/30/79	nd	nd	1.0	0.5	0.2	0.3	0.4	141.0
10/8/79	nd	nd	1.2	0.8	0.3	0.4	0.6	302.1
11/14/79	nd	nd	2.7	3.2	0.4	0.5	0.9	216.5
1/14/80	2.9	2.1	0.9	2.1	0.5	0.2	0.7	273.8
2/11/80	2.1	1.5	1.1	2.5	0.5	0.2	0.5	235.6
3/10/80	2.0	1.6	1.3	2.7	0.4	0.3	0.7	255.0
4/23/80	2.2	1.6	1.6	2.9	0.4	0.4	0.6	271.0
5/21/80	2.2	1.4	1.2	2.4	0.4	0.3	0.5	246.0
6/2/80	2.0	1.4	1.2	2.4	0.4	0.3	0.5	237.4
6/17/80	2.1	1.3	1.1	2.2	0.4	0.2	0.5	237.2
$\bar{X}$	2.5	1.8	1.3	2.1	0.4	0.3	0.7	283.6
(s.d.)	(0.7)	(0.6)	(0.5)	(0.9)	(0.2)	(0.1)	(0.2)	(75.1)

[1]not identified

[2]below lines of detection

TABLE 8B. PNAH CONCENTRATIONS (µg/kg) IN *M. EDULIS* FROM YAQUINA BAY, OREGON; SITE Y2M.

DATE SAMPLED	PHEN	FLUOR	PYR	BCP	TRI	BAA	CHRY	BBF
1/24/79	284.3	242.2	142.3	107.3	123.9	154.3	137.6	22.4
2/13/79	256.6	213.7	131.9	105.2	119.8	141.2	102.5	23.6
3/21/79	237.5	198.7	122.4	104.3	120.6	103.5	98.7	24.4
5/31/79	205.4	175.3	111.2	94.6	109.4	91.3	87.6	20.1
7/2/79	202.2	162.1	99.4	91.2	95.5	87.6	65.5	17.6
7/30/79	186.4	131.3	81.2	76.9	71.3	62.4	51.2	14.4
10/8/79	173.3	127.5	75.7	68.5	70.3	58.3	47.6	10.4
11/14/79	191.3	146.4	92.3	81.3	92.5	60.4	61.2	12.3
1/14/80	206.4	183.2	105.4	96.4	112.6	92.6	98.4	17.3
2/11/80	231.3	193.2	117.6	100.4	122.4	98.4	96.5	18.0
3/10/80	222.5	199.4	120.6	105.3	125.5	99.4	100.8	19.2
4/23/80	198.4	185.3	111.1	97.5	102.4	81.5	87.4	16.3
$\bar{X}$	216.3	179.9	109.3	94.1	105.5	94.2	86.2	18.0
(s.d.)	(31.8)	(33.8)	(19.8)	(12.5)	(19.6)	(29.6)	(25.8)	(4.3)

DATE SAMPLED	BKF	DBACA	BAP	DBAHA	BGHIP	IP	COR	TOTAL
1/24/79	22.5	14.3	31.5	12.4	10.4	9.4	8.9	1323.7
2/13/79	21.6	14.2	29.5	10.5	11.0	9.0	6.6	1196.9
3/21/79	20.4	13.9	33.1	11.5	10.9	8.5	7.6	1116.0
5/13/79	18.0	10.3	24.4	9.4	8.4	5.4	4.3	975.1
7/2/79	14.3	9.8	20.4	7.6	5.4	3.2	2.0	883.8
7/30/79	9.6	7.4	18.7	5.3	3.2	1.0	1.0	721.3
10/8/79	8.5	6.3	17.0	4.9	2.4	1.0	1.1	672.8
11/14/79	9.5	8.3	24.5	6.0	4.3	1.1	2.5	793.8
1/14/80	20.2	12.3	28.5	11.5	9.9	8.8	6.5	1010.0
2/11/80	21.7	13.5	30.8	11.9	10.3	7.5	5.8	1079.3
3/10/80	22.5	13.7	31.6	12.6	11.4	7.6	5.4	1097.5
4/23/80	19.8	10.3	24.6	11.3	9.9	5.5	3.2	964.5
$\bar{X}$	17.4	11.2	26.2	9.6	8.1	5.7	4.6	986.2
(s.d.)	(5.4)	(2.8)	(5.4)	(2.9)	(3.3)	(3.3)	(2.6)	193.8

greater in Y2M mussels. These findings agree with results of an earlier study (Mix and Schaffer, 1979) in which it was shown that concentrations of BAP were significantly greater in mussels from Y2M during a two year period. It is also relevant that the mean BAP concentrations did not differ significantly at either site between 1976-78 (Y1M - 1.9 µg/kg; Y2M - 25.6 µg/kg) and 1978-80 (Y1M = 1.3 µg/kg; Y2M = 26.2 µg/kg) as indicated by a two-sample t-test (calculated $t < t_{.05,28} = 2.05$). This suggests that PNAH contamination has neither increased nor decreased during the 4-year period and that there is good agreement between the two analytical procedures used during each study. It is clear that there were substantial differences in the concentrations of individual PNAH in mussels (and clams) examined during these studies. In general the smaller, more water soluble compounds were concentrated to one or two orders of magnitude above the larger, less water soluble compounds. Thus, the more toxic 3 and 4 ring PNAH were present in much greater concentrations than the carcinogenic 5 and 6 ring PNAH. These observations will be analyzed more completely in a subsequent section of this report.

For a more complete analysis of the data it was necessary to transform each piece of concentration data into a percent value. This procedure permitted additional comparisons in PNAH concentrations in mussels from Y1M and Y2M and also provided data for evaluating seasonal differences. Percent values were determined for each data point in Tables 8A and 8B according to the following formula: concentration PNAHi (where i indicates a specific PNAH such as phenanthrene, pyrene, etc.) for sample date j (where j indicates a single sample date) ÷ sum of PNAHi for all j x 100 = percent value. For example, the PHEN concentration in Y2M mussels on 1/24/79 was 284.3 µg/kg. Thus, for PHEN on 1/24/79, 284.3 µg/kg ÷ 2595.6 µg/kg x 100 = 10.95%. Total percent values for each PNAH add to 100. For comparisons between Y1M and Y2M, only data from common sample dates were used. For evaluating seasonal variations, the mean percent for Y1M was considered to be 5.88% (100% ÷ 17 sample dates) and for Y2M, 8.33% (100% ÷ 12 sample dates).

The transformed data (% values) were used in a statistical test to determine if there were differences in the average percent contributions of individual PNAH that were related to sampling date. A one-way analysis of variance was used to test H_0: % mean values for all individual PNAH, for eacn sampling date, were equal. For Y1M, $F = 4.5 > F_{.05,16,221} = 1.69$ and for Y2M, $F = 38.0 > F_{.05,11,156} = 1.85$. Therefore, the null hypotheses were rejected at $P < .01$; there were differences in the percent PNAH concentrations that were related to sampling date or season.

Figure 3A and 3B describe the seasonal fluctuations in PNAH percent concentrations. The means and standard deviations were calculated by summing the % contribution for each PNAH for a specific day of year ("season") and dividing by the number of total PNAH (n = 14 for Y1M and 15 for Y2M; see Tables 8A and 8B). There were no general seasonal trends observed in mussels from Y1M. Only two values deviated measurably from the mean during the 20 month sampling period; mean PNAH concentrations were significantly higher during February, 1979 and lower during July-August, 1979. Mussels from Y2M showed a clear pattern that is interpreted as being seasonal. Highest concentrations were present during the late winter-early spring after which they declined to low concentrations during the summer and fall.

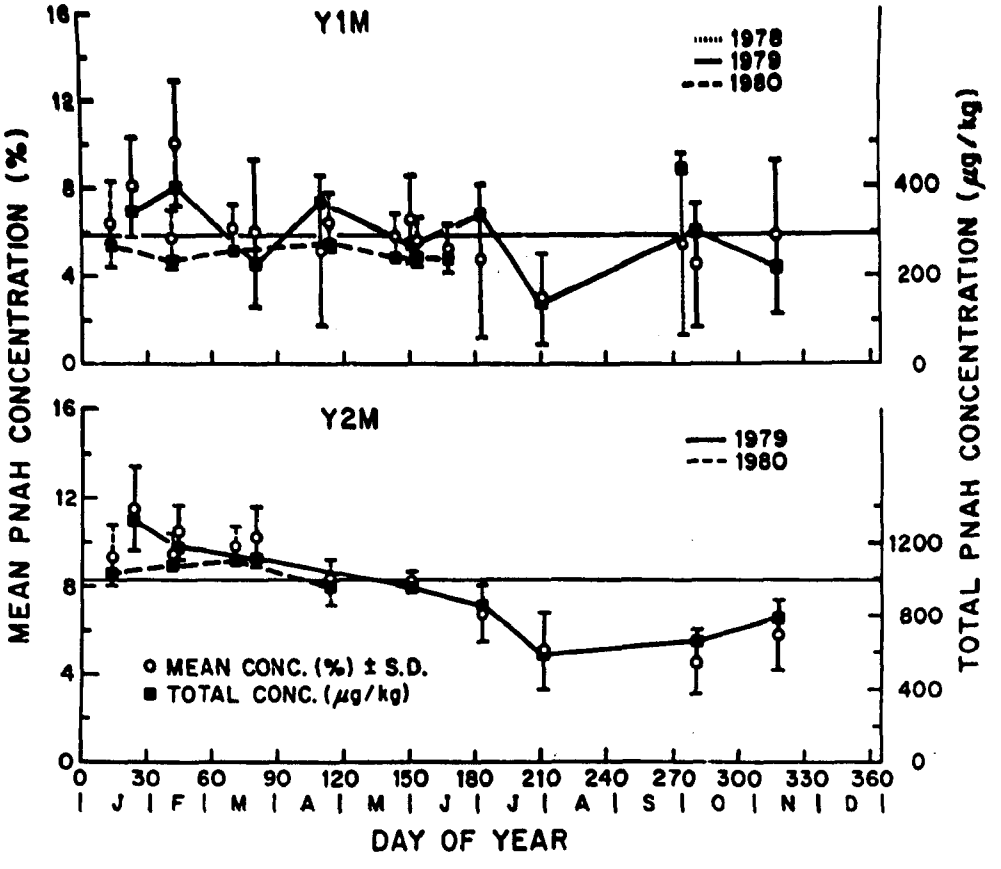

Figure 3. A. Seasonal differences in PNAH concentrations in *M. edulis* from Y1M, 1978-80. B. Seasonal differences in PNAH concentrations in *M. edulis* from Y2M, 1979-80. The left Y-axis indicates the mean which was derived by converting each concentration value (right Y-axis) to a percentage of the total PNAH for the entire period. Total PNAH concentrations are shown on the right Y-axis and correspond precisely to the mean percent value. The bars represent ± one standard deviation for total PNAH concentrations.

Excepting BAP, there are limited data available on PNAH concentrations in *M. edulis*. Dunn and Young (1976) found BAP levels at or near 0 in *M. edulis* and *M. californianus* collected from relatively pristine areas of Southern California. Elevated levels, up to 108 µg/kg were typical of mussels sampled on or near creosoted pilings. Dunn and Stich (1976a) reported BAP concentrations in mussels from various Vancouver sites that ranged from 2-8 and 10-35 µg/kg depending on location. Some values were as high as 215 µg/kg in mussels sampled from creosoted pilings. They observed seasonal variations with a tendency towards lower levels during the summer. Finally, Risebrough et al. (1980) measured concentrations of 3 PNAH in *M. edulis* from San Francisco Bay. They reported levels of 2-6, 8-26 and 4-7 µg/g for phenanthrene, fluoranthene and BAP, respectively, in mussels from metal and cement pilings. For mussels from wooden pilings, they found concentrations of 1,000, 4,200 and 2,000 µg/kg for those 3 PNAH. The latter concentrations are 1-3 orders of magnitude greater than those

reported for *M. edulis* collected from wooden pilings in Yaquina Bay and Vancouver Harbor (Mix, 1979; Mix and Schaffer, 1979; Dunn and Stich, 1976a). The reason for these considerable differences is not yet clear.

BAP Uptake and Elimination

The results of the BAP uptake and elimination studies are summarized in Table 9 and Figure 4. The control mussels maintained at Y1M showed no significant changes in BAP concentration during the experimental period (n = 50 days). The Y2M mussels that initially contained 23 µg/kg BAP reached background, Y1M, levels by approximately 15-20 days. The rate of BAP release was exponential and best described by the following formula, using 4 parameters: $Y = 23.7^{-0.12X} + 4.52^{-0.13X}$ ($R^2 = .96$; significance level = .005). By inspection of Figure 4, it can be determined that the BAP half-life, or time required for depuration of 50% of the BAP initially present, was 8-10 days. These results suggest that levels of available BAP in the immediate environment at Y1M remained low and stable during the experimental period.

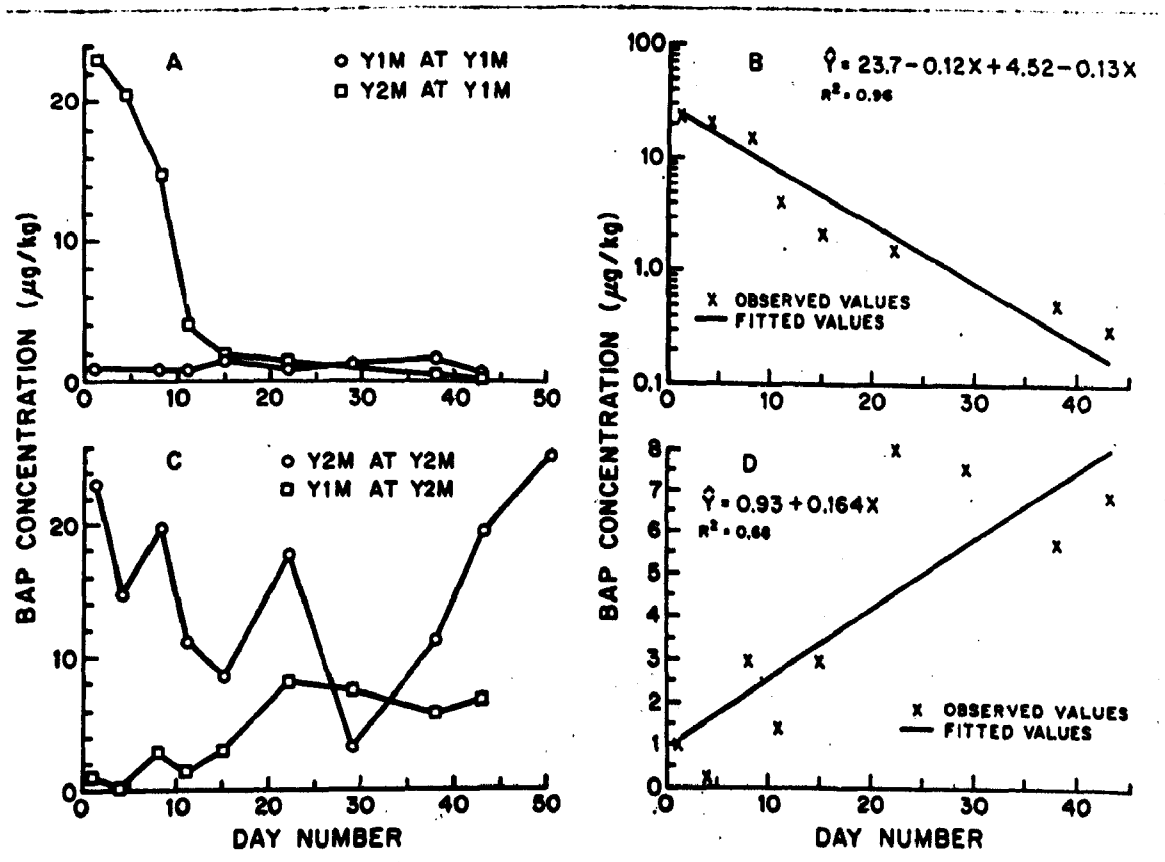

Figure 4. Rates of benzo(a)pyrene uptake and elimination. A. Decrease in BAP concentrations in contaminated mussels moved from Y2M and allowed to depurate at Y1M. B. Rate of BAP elimination determined by linear regression analysis. C. Uptake of BAP by uncontaminated Y1M mussels moved to Y2M. D. Rate of BAP uptake determined by linear regression analysis.

TABLE 9. BENZO(A)PYRENE UPTAKE AND ELIMINATION IN *M. EDULIS* MAINTAINED UNDER AMBIENT (FIELD) CONDITIONS IN YAQUINA BAY.

		BAP CONCENTRATION (µg/kg)			
SAMPLE DATE	DAY NUMBER	Y1 at Y1[1]	Y2 at Y1[2]	Y2 at Y2[1]	Y1 at Y2[3]
10/16/78	1	1.0	23.0	23.0	1.0
10/19/78	4	*	20.4	14.7	0.2
10/24/78	8	0.9	14.8	19.7	2.9
10/27/78	11	0.8	4.0	11.1	1.4
10/31/78	15	1.5	2.0	8.5	2.9
11/7/78	22	0.9	1.5	17.6	8.0
11/14/78	29	1.3	*	3.2	7.5
11/23/78	38	1.6	0.5	11.1	5.7
11/28/78	43	0.6	0.3	19.4	6.8
12/5/78	50	*	*	25.3	*

[1]control

[2]designed to measure BAP elimination

[3]designed to measure BAP uptake

*no measurement made

BAP uptake by Y1M mussels transferred to Y2M was linear where $Y = 0.93 + .164X$ ($R^2 = .68$; significance level = .006). As indicated by the data, BAP levels in Y2M mussels maintained at that site seemed to fluctuate widely. Those variations were not correlated with changes in salinity and/or temperature. There are at least two potential explanations for this pattern. First, sample variance within the population may have been substantial enough to account for the observed fluctuations, although this did not appear to be the case in other samples. Second, since it is thought that BAP, and other lipophilic PNAH, are isolated in two compartments within the mussel (see subsequent discussion), the fluctuations may reflect real changes in the short-lived compartment. That may occur if available BAP levels in the environment changed rapidly during the experimental period. Such differences may have been reflected by rapid changes in the short-lived compartment but not the long-lived compartment which would be assumed to be saturated. The BAP uptake pattern of Y1M mussels would also be consistent with this hypothesis; in those mussels, the long-lived compartment was not yet saturated. Thus, since tissues in those animals had not yet equilibrated with the levels of environmental BAP, they would not be expected to reflect rapid changes in BAP levels in the environment. It must be emphasized that this is all speculative, but is compatible with existing theories of PNAH uptake and storage in bivalve molluscs (Stegeman and Teal, 1973).

Despite the variable pattern of BAP concentration in Y2M mussels, there seemed to be a general decrease during the first phase followed by a period when BAP levels increased. Such a pattern is consistent with our earlier finding that BAP concentrations increased during the early winter in *M. edulis* from Yaquina Bay (Mix and Schaffer, 1979).

It is interesting to note that BAP levels in both mussel populations maintained at Y2M increased and/or fluctuated at a time of year when they were not engaged in gametogenesis. This may indicate that increased BAP and PNAH concentrations observed during the winter are related primarily to increased levels in the environment and not necessarily to metabolic processes associated with gametogenesis.

Our results on BAP uptake and elimination under ambient conditions are similar to those obtained by others (Stegeman and Teal, 1973; Fossato, 1975; Dunn and Stich, 1976b; Fossato and Canzonier, 1976). In common with all those studies, we observed a biphasic mode of release which was initially rapid, then decreased quickly and was constant. The VAP half-life in the present study, in which the ambient temperature ranged from 15°C on day 1 to 9°C at termination, was 8-10 days. This compares with 16 days in *M. edulis* maintained at 7-9°C (Dunn and Stich, 1976b) and 18 days for *C. virginica* maintained at 12°C. The differences may be attributed to lower water temperatures in the latter studies, although it has been reported that depuration in *M. edulis* are independent of temperatures between 7-26°C (Fossato, 1975).

It has been hypothesized previoulsy that biphasic depuration is indicative of PNAH storage in two compartments within shellfish (Stegeman and Teal, 1973). One of the compartments, perhaps fluid and/or hemolymph, is characterized by the rapid turnover of PNAH while the other, more stable, compartment is characterised by low turnover; the latter compartment evidently contains pentane-extractable lipids. The exact location of either compartment has not yet been established by definitive experiments. However, results of other studies seem to suggest that the digestive gland (hepatopancreas) is a primary candidate (Lee et al., 1972; Dunn and Stich, 1976b; Couch et al., 1979). The underlying CT and gonad may also serve as storage sites since it has been demonstrated that C^{14}-BAP (Couch et al., 1979) is possibly transported from digestive tubules to those tissues in *C. virginica* Perhaps spawning would be expected to account for sudden decreases in PNAH concentrations during the spring if the gonad is a major sink in shellfish. The next study attempted to determine if this occurred in *M. edulis*

Tissue Storage Sites for BAP

The purpose of this study were to measure the concentration of BAP in somatic and gonadal tissues of *M. edulis* and determine if changes in levels in these tissues could account for seasonal differences in BAP concentrations; the high January-February concentrations were of special interest. Mussels from Y2M were used because higher BAP levels insured that this PNAH could be quantified in small amounts of tissue. Table 10 contains the quantitative data from the 6 month study. Figure 5 illustrates the seasonal variation, in percent, in weights of somatic and gonadal tissue and the

amount of BAP associated with each of these tissues.

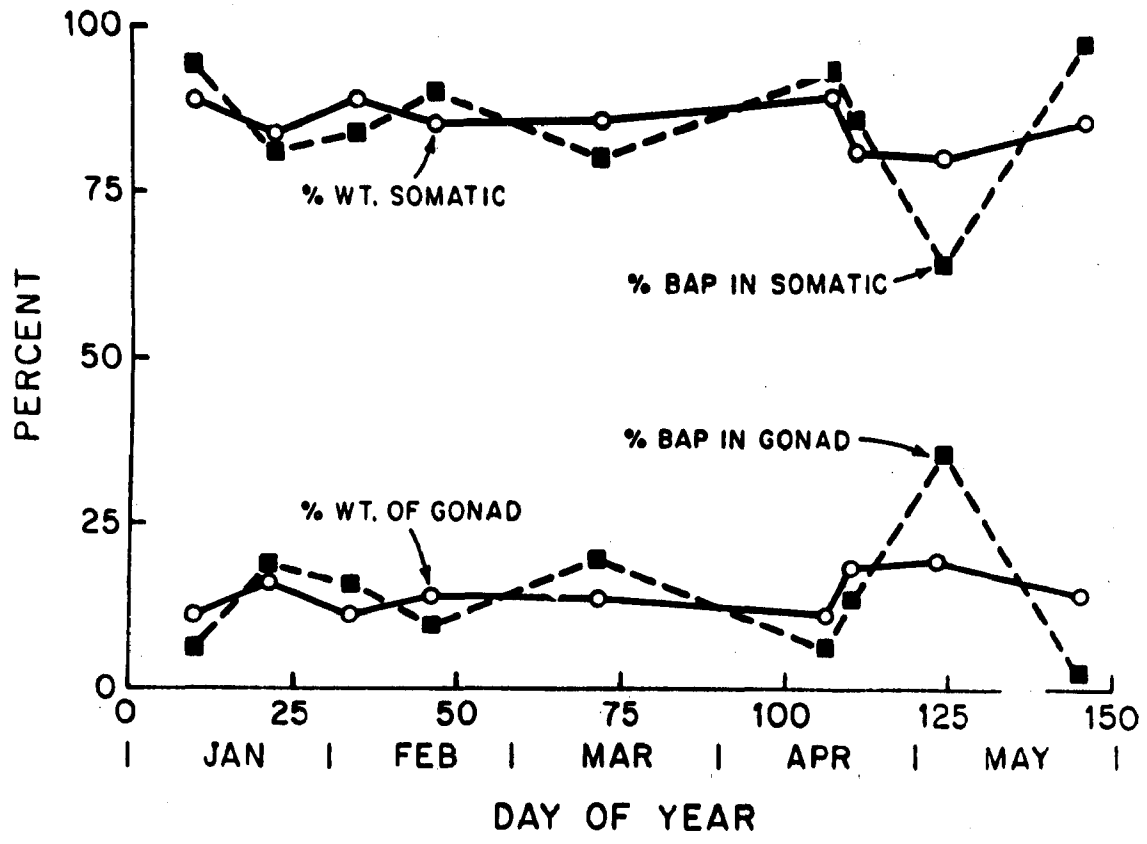

Figure 5. Benzo(a)pyrene concentrations in gonadal and somatic tissues. BAP concentrations in the 2 tissue groups are expressed as a percentage of total PNAH in whole animals. The weights of gonad and somatic fractions are also expressed as a percentage of total weight.

The results of this study suggest that gametogenesis and/or incorporation of BAP and presumably other lipophilic PNAH, were not directly responsible for the seasonal increases in BAP during January-February, 1979. There were no measurable increases in either gonad weight or the % BAP contributed by the gonad (Fig. 5). It is evident that BAP storage occurred primarily in the somatic tissues compared to the gonad, even during the spring spawning season. This is in agreement with Lee et al. (1972) and DiSalvo et al. (1975) who reported that somatic tissues, especially the hepatopancreas, contained higher concentrations of aromatic hydrocarbons than the gonad. Examination of Figure 5 reveals that during later April-early May, there was a slight increase in the weight of the gonad and a single period, May 3, when a more substantial amount of BAP was contained within the gonad than at any other time during the study. This peak was followed by a sharp decrease in % BAP in the gonad. Corresponding inverse changes occurred in the somatic

TABLE 10. BENZO(A)PYRENE CONCENTRATIONS IN THE GONAD AND SOMATIC TISSUES OF *M. EDULIS* FROM YAQUINA BAY, OREGON.

	GONAD		SOMATIC		WHOLE MUSSEL (GONAD + SOMATIC)				
	(A)	(B)	(C)	(D)	(E)				
DATE SAMPLED[1]	WEIGHT (GRAMS)	BAP CONC. (µg/kg)	WEIGHT (GRAMS)	BAP CONC. (µg/kg)	BAP CONC. (µg/kg)[2]	%WEIGHT GONAD[3]	% BAP GONAD[4]	% WEIGHT SOMATIC[5]	% BAP SOMATIC
1/9/79	4.8	31.4	37.4	70.4	66.0	11.4	5.4	88.6	94.6
1/22/79	5.1	50.7	26.2	43.9	45.0	16.3	18.4	83.7	81.6
2/2/79	5.0	42.3	37.6	29.2	30.7	11.7	16.2	88.3	83.8
2/15/79	4.4	13.5	26.6	20.9	19.8	14.2	9.6	85.8	90.4
3/13/79	3.8	39.5	24.3	25.3	27.2	13.5	19.6	86.5	80.4
4/16/79	4.2	21.8	33.8	38.7	36.8	11.0	6.5	89.0	93.5
4/20/79	5.6	30.4	25.0	44.9	42.2	18.3	13.2	81.7	86.8
5/3/79	5.0	30.2	20.2	13.3	16.6	19.8	36.0	80.2	64.0
5/25/79	2.9	4.2	17.9	22.6	20.0	13.9	2.9	86.1	97.1

[1]samples collected on 3/1/79, 3/21/79 and 4/3/79 were excluded because of analytical error.

[2]determined by the formula, (A)(B)+(C)(D)/A+C

[3]determined by the formula, A/A+C

[4]determined by the formula, [(A)(B)/A+C]E x 100

[5]determined by the formula, C/A+C

tissues during the same time. It has been suggested that spawning may constitute a potential release mechanism for aromatic hydrocarbons contained within the ova of *M. edulis* (DiSalvo et al., 1975). Perhaps the April-May changes may have been associated with spawning of the Y2M mussel population although this process normally occurs during February-April in Yaquina *M. edulis*. If so, spawning, and the concomitant loss of PNAH in gametes, may account for the lowered late spring early summer levels. Additional field studies will be necessary to more fully evaluate the relationship between seasonal concentrations of PNAH and fluxes involving somatic and gonadal tissues.

BAP Metabolism in *M. edulis*

The finding that BAP is concentrated, at least intially, in the digestive tissues may be significant since microsomal fractions from those tissues in *C. virginica* and perhaps *M. edulis* are capable of metabolizing BAP (Anderson, 1978). The enzymes responsible for this conversion and the metabolites produced have not yet been identified completely. A related point is that BAP depuration in bivalve molluscs may not be an entirely passive process. Metabolic alteration, conjugation and subsequent excretion may play a minor or major role in the release of these compounds from shellfish.

Only preliminary studies on BAP metabolism in *M. edulis* have been conducted during this investigation; the first results must be considered tentative. Briefly, it was found that 7,10-^{14}C-BAP was metabolized by microsomal extracts from the visceral mass of *M. edulis* from both Y1M and Y2M. Phenolic metabolites, identified by HPLC methods as 3-hydroxybenzo(a)pyrene (3-OH) and 9-hydroxybenzo(a)pyrene (9-OH), were the only measurable BAP metabolites present. The rates of formation were low, ranging from 27.3-76.7 x 10^{-12} pmoles BAP/min/mg microsomes.

These preliminary results are important since this species has been reported to be incapable of metabolizing PNAH (Lee et al., 1972; Vandermeulen and Penrose, 1978; Payne and May, 1979). More complete studies utilizing advanced methods will be required to fully evaluate the metabolic capabilities of *M. edulis* for altering BAP and other PNAH.

BASELINE DATA ON PNAH CONCENTRATIONS IN OTHER BIVALVE MOLLUSCS

PNAH concentrations were measured in oysters (*C. gigas*) from Yaquina Bay (Table 11) to provide some comparison with PNAH levels in *M. edulis* and because they are utilized as a food source. PNAH concentrations were also measured in *M. edulis*, *M. arenaria* and *C. gigas* from Tillamook Bay, Oregon, to compare levels in indigenous shellfish from a relatively pristine bay with those inhabiting more developed bays (Coos, Yaquina) (Table 12). It is clear that Pacific oysters from Yaquina Bay contained much lower concentrations of PNAH than mussels from the same bay. The oysters were sampled at a site approximately 7-8 km upbay from *M. edulis* at a site far removed from the downbay mussel sites where there is a considerable amount of industrialization and numerous potential point sources (e.g. marinas, fish processing plants, condominiums). The simplest explanation for the differences is that

TABLE 11. PNAH CONCENTRATIONS (µg/kg) IN *C. GIGAS* FROM YAQUINA BAY, OREGON; SITE Y140.

DATE SAMPLED	PHEN	FLUOR	PYR	BCP	TRI	BAA	CHRY	BBF
12/16/76	6.4	7.6	4.4	5.3	2.0	4.3	3.2	1.5
2/17/77	6.7	8.0	4.4	5.8	2.4	4.6	4.0	1.3
8/29/77	5.4	6.8	3.2	4.7	2.0	4.3	3.9	1.1
12/9/77	4.9	5.6	3.0	4.4	2.0	4.2	3.0	1.0
4/28/78	7.3	6.5	3.6	4.9	3.1	5.1	4.1	0.9
1/24/79	5.4	4.3	3.0	3.5	2.0	5.1	3.8	0.8
3/21/79	5.9	4.8	3.1	3.6	2.2	5.6	4.0	1.0
4/20/79	6.1	5.3	4.0	4.1	2.8	5.9	4.2	1.2
5/31/79	7.1	6.3	4.5	4.3	2.9	5.9	4.3	1.3
6/28/79	7.3	6.6	4.8	4.2	3.0	5.8	4.4	1.4
7/30/79	7.1	6.2	4.6	4.3	3.0	5.6	3.2	1.2
11/14/79	5.3	4.4	3.0	3.4	2.1	5.0	3.6	1.0
1/14/80	6.2	4.5	3.2	3.6	2.4	5.4	3.8	1.2
3/10/80	6.4	4.7	3.5	3.7	2.8	5.7	4.0	1.2
$\bar{X}$	6.2	5.8	3.7	4.3	2.5	5.2	3.8	1.2
(s.d.)	(0.8)	(1.2)	(0.7)	(0.7)	(0.4)	(0.6)	(0.4)	(0.2)

DATE SAMPLED	BKF	DBACA	BAP	DBAHA	BGHIP	IP	COR	TOTAL
12/16/76	1.6	1.2	1.6	0.5	0.3	0.3	0.4	40.6
2/17/77	1.7	1.4	1.7	0.6	0.4	0.3	0.4	43.7
8/29/77	1.4	1.3	1.5	0.4	0.3	0.3	0.4	37.0
12/9/77	1.1	1.1	1.0	0.3	0.3	0.2	0.3	32.4
4/28/78	1.2	1.4	1.3	0.4	0.3	0.3	0.2	40.8
1/24/79	0.9	0.9	1.1	0.3	0.3	0.2	0.2	31.8
3/21/79	1.0	1.0	1.2	0.3	0.3	0.3	0.3	34.6
4/20/79	1.2	1.2	1.3	0.4	0.4	0.3	0.4	38.8
5/31/79	1.2	1.3	1.5	0.5	0.3	0.3	0.4	42.1
6/28/79	1.3	1.4	1.6	0.4	0.3	0.3	0.4	43.2
7/30/79	1.1	1.2	1.4	0.5	0.3	0.3	0.4	40.4
11/14/79	0.8	0.6	1.0	0.3	0.3	0.3	0.3	31.4
1/14/80	1.1	1.0	1.3	0.4	0.4	0.3	0.5	35.3
3/10/80	1.2	1.3	1.4	0.5	0.4	0.3	0.5	37.6
$\bar{X}$	1.2	1.2	1.4	0.4	0.3	0.3	0.4	37.8
(s.d.)	(0.2)	(0.2)	(0.2)	(0.1)	(0.0)	(0.0)	(0.1)	(4.2)

TABLE 12. PNAH CONCENTRATIONS (µg/kg) IN BIVALVE MOLLUSKS FROM TILLAMOOK BAY, OREGON.

DATE SAMPLED	SPECIES	PHEN	FLUOR	PYR	BCP	TRI	BAA	CHRY	BBF
1/24/79	*M. edulis*	15.5	10.4	8.8	5.3	3.5	2.6	4.4	1.4
4/9/79	*M. edulis*	12.4	8.5	6.0	4.3	3.0	2.4	3.1	0.9
1/24/79	*M. arenaria*	17.4	15.1	12.1	3.2	5.4	2.0	1.4	1.0
4/19/79	*M. arenaria*	9.3	5.0	3.0	2.0	4.2	1.9	1.0	0.8
6/14/79	*M. arenaria*	9.0	5.3	4.1	2.3	4.6	1.9	1.1	1.0
8/23/79	*M. arenaria*	10.0	6.3	3.0	2.9	4.7	1.8	1.5	1.1
1/24/79	*C. gigas*	3.1	6.5	3.3	4.8	4.6	2.4	3.8	1.3
4/19/79	*C. gigas*	10.9	7.1	3.6	5.1	4.2	2.6	3.8	1.0
6/14/79	*C. gigas*	10.1	5.4	4.1	3.6	4.9	2.2	3.7	1.1

DATE SAMPLED	SPECIES	BKF	DBACA	BAP	DBAHA	BGHIP	IP	COR	TOTAL
1/24/79	*M. edulis*	1.0	0.6	0.4	0.3	0.2	0.2	0.2	54.8
4/9/79	*M. edulis*	0.6	0.4	0.2	0.2	0.1	0.1	0.1	42.3
1/24/79	*M. arenaria*	0.3	1.4	0.5	0.7	0.9	0.0	0.1	61.5
4/19/79	*M. arenaria*	0.0	1.7	0.4	0.6	0.8	0.0	0.0	30.7
6/14/79	*M. arenaria*	0.0	1.1	0.6	0.8	0.9	0.1	0.0	32.8
8/23/79	*M. arenaria*	0.1	0.9	0.5	0.7	1.0	0.1	0.0	34.6
1/24/79	*C. gigas*	1.6	1.0	1.0	1.0	1.1	0.9	0.6	37.0
4/19/79	*C. gigas*	1.6	0.8	1.1	0.9	1.1	0.9	0.5	45.2
6/14/79	*C. gigas*	1.3	1.6	1.1	0.6	1.1	0.3	0.6	41.7

water at the downbay mussel sites was more contaminated than the water at the more pristine area where oysters are grown in Yaquina Bay. It should be noted that oysters are grown in trays suspended from creosoted boards attached to creosoted pilings. Differences in feeding mechanisms would not appear to be a factor since mussels, oysters and softshell clams are all filter feeders.

There were no apparent seasonal differences in PNAH concentrations in oysters from Yaquina Bay such as occurred in *M. edulis*. That may be due to differences in metabolic capabilities, seasonal availability of PNAH at the different sites or reproductive patterns. *C. gigas* mature sexually but do not spawn in Yaquina Bay and, thus, there would be no release of PNAH associated with gametes during spawning that would result in summer and fall decreases. That may or may not occur in *M. edulis*. If oysters function metabolically in a way similar to *M. edulis* during the winter, these data would suggest that runoff from the upper watershed may not contribute in a major way to PNAH burdens in oysters and mussels or account for seasonal differences in the latter.

The levels of PNAH in *C. gigas* from Yaquina Bay were generally comparable to those reported for *C. virginica* from Galveston Bay for a smaller number individual PNAH (Fazio, 1971). No BAP was detected in oysters in that study. Cahnmann and Kuratsuna (1957) reported considerably higher levels, 700-1300 µg/kg total PNAH, in *C. virginica* from a polluted harbor area in the vicinity of Norfolk, Virginia. Murray et al. (1980) detected only 0.07-0.14 ppb PNAH in *C. virginica* from Galveston Bay while Bravo et al. (1978) reported that *C. virginica* from 10 stations along the Mexican coast had total mean PNAH concentrations of 2080-9160 µg/kg. The latter values included both unsubstituted and substituted PNAH but the levels have been questioned as being nearly 2 or 3 orders of magnitude too high for shellfish from a relatively pristine area (Neff, 1979). Since at least some of the samples were collected near an oil producing area, some of the oysters may have been contaminated by wastes which reached the lagoons (Bravo et al., 1978).

The data in Tables 11 and 12, while not as complete as that from similar studies on *M. edulis* from Yaquina Bay (Tables 8A and 8B) and *M. arenaria* from Coos Bay (Tables 6 and 7), provide some statistical basis for evaluating differences between species and sites and formulating tentative conclusions about PNAH concentrations in bivalve molluscs. One-way analyses of variance or t-tests were used to compare differences between populations. The limited number of samples precludes precise interpretation; the results are considered indicative but not definitive. Statistical analyses revealed the following relationships:

1. The mean concentration of total PNAH in *C. gigas* from Yaquina Bay did not differ during the 5 separate years, 1976-1980 ($F = 0.2 < F_{.01,4,9} = 14.5$), i.e. the mean did not differ significantly for any single year.

2. The mean concentration of total PNAH in *C. gigas* from Yaquina Bay differed significantly for *M. edulis* from Y1M ($t = 12.2 > t_{.01,29} = 2.7$) and Y2M ($t = 18.4 > t_{.01,24} = 2.8$) for all sampling periods.

3. The mean concentration for total PNAH did not differ significantly in *C. gigas* from Yaquina Bay and Tillamook Bay ($t = 1.3 < t_{.01,18} = 2.95$) for all samples.

4. The mean concentrations of total PNAH did not differ significantly in *M. edulis*, *M. arenaria* or *C. gigas* from Tillamook Bay ($F = 0.4 < F_{.01,2,6} = 19.3$) for all samples.

5. The mean concentrations of total PNAH differed significantly in *M. arenaria* from Tillamook Bay and Coos Bay (for C3S, $t = 4.28 > t_{.01,8} = 3.36$; for CSS, $t = 17.68 > t_{.01,8} = 3.36$).

The lowest PNAH concentrations measured in this research were recorded in mussels, clams and oysters from Tillamook Bay, the most undeveloped of the three Oregon bays. These data support the view that PNAH concentrations in shellfish monitors will reflect the degree of industrialization, human onshore habitation and/or relative pollution levels (Goldberg, 1975; Dunn and Stich, 1975; Mix et al., 1977; Risebrough et al., 1980). PNAH concentrations in the three bivalve species from Tillamook Bay did not differ even though they came from sites separated by several kilometers, and ranged from upbay to downbay locations. One interpretation of this finding is that all filter-feeding bivalve molluscs will contain baseline concentrations of PNAH in well-mixed estuaries that can be considered as background. Deviations above these levels may then be indicative of the relative degree of pollution. Table 13 categorizes the PNAH concentrations measured in shellfish monitors during this study with observations about the degree of contamination at the various sample sites.

TABLE 13. RANGE OF PNAH CONCENTRATIONS IN SHELLFISH MONITORS FROM VARIOUS SITES IN THREE OREGON BAYS.

BAY	SITE	SPECIES	PNAH CONC. (µg/kg)	DEGREE OF INDUSTRIALIZATION
Tillamook	T1M	*M. edulis*	40-60	relatively pristine
Tillamook	TSS	*M. arenaria*	30-60	relatively pristine
Tillamook	TBC	*C. gigas*	35-45	relatively pristine
Yaquina	Y140	*C. gigas*	30-45	relatively pristine
Coos	C3S	*M. arenaria*	70-90	relatively pristine; near highway
Coos	C11G	*T. capax*	30-110	light; nearby marinas, fish processing plants
Coos	CSS	*M. arenaria*	480-650	heavy; shipping docks, wood products industry, marinas
Yaquina	Y1M	*M. edulis*	140-440	light; shipping docks
Yaquina	Y2M	*M. edulis*	675-1325	heavy; marinas, fish processing plants, recreational developments

The values in Table 13 indicate that shellfish in relatively pristine areas of the three bays have a baseline PNAH load of approximately 50 µg/kg. Increased concentrations occurred in direct relation to the degree of indus-

trialization and human onshore activity.

It would be beneficial to have data relating PNAH measurements in shellfish monitors with ambient water concentrations. However, costs and statistical considerations preclude obtaining this sort of information on a routine basis. Perhaps future efforts can be directed towards relating PNAH concentrations in water and shellfish monitors, solubilities, octanol-water partition coefficients and bioaccumulation factors. Ultimately, such information could perhaps be incorporated into predictive models that could be used for predictions about the environmental behavior of PNAH (e.g. review by Kenaga and Goring, 1980).

CELLULAR PROLIFERATIVE DISORDERS IN BIVALVE MOLLUSCS FROM OREGON BAYS

Mussels from Y1M and Y2M were examined histologically for the presence of abnormal cells during the fall and winter of 1979-80 and *M. arenaria* during a four quarter period during 1978-79 from Coos Bay (Table 14). In addition, 50 mussels were examined during February and May, 1980 from Tillamook Bay.

TABLE 14. THE PREVALENCE OF CELLULAR PROLIFERATIVE DISORDERS IN *M. EDULIS* FROM YAQUINA BAY AND *M. ARENARIA* FROM COOS BAY, OREGON.

	YAQUINA BAY			COOS BAY	
DATE	Y1M(%)	Y2M(%)	DATE	CSS(%)	C3S(%)
9/10/79	0/47 (0.0)	2/47 (4.3)	9/30/78	0/48 (0.0)	0/46 (0.0)
10/8/79	0/43 (0.0)	5/46(10.9)	12/1/78	0/46 (0.0)	0/42 (0.0)
11/14/79	1/49 (2.0)	4/44 (9.1)	2/19/79	0/47/(0.0)	0/47 (0.0)
12/17/79	1/54 (1.8)	-- --	4/18/79	0/46 (0.0)	0/41 (0.0)
1/14/80	0/49 (0.0)	5/45(11.1)			
2/11/80	-- --	6/49(12.2)	TOTAL	0/187(0.0)	0/176(0.0)
3/10/80	0/50 (0.0)	-- --			
TOTAL	2/292(0.7)	22/231(9.5)			

No clams from Coos Bay or mussels from Tillamook Bay were found with the large, abnormal cells that characterize one type of cellular proliferative disorders in shellfish from Oregon and elsewhere (Mix et al., 1979a). The condition has appeared in a significant number of mussels from Y2M during a 4-year period from 1976-1980 while it occurred rarely in mussels from Y1M (Mix, 1979; Mix et al., 1979b). The correlation between the degree of PNAH contamination and the prevalence of the condition at Y2M is obvious but no cause-effect relationship has been established.

Although there have been numerous reports of apparent correlations between the appearance of abnormal cells in shellfish inhabiting oil-contaminated environments, there have been no published reports of cancer induction in bivalve molluscs by exposure to, or injection with, PNAH (Couch et al., 1979). Significant questions about the effects of PNAH on bivalve molluscs and the

metabolic capabilities these species have for altering PNAH remain unanswered. There have been numerous reports that bivalves cannot metabolize PNAH, yet the evidence presented is by no means definitive (Stegeman and Teal, 1973) and recent results indicate that at least some species can metabolize BAP (Anderson, 1978). It remains to be determined if carcinogenic metabolites can be formed by these species. If bivalves are not subject to PNAH-induced carcinogenesis, and the cellular abnormalities are related to a neoplastic process, then other causative agents must be responsible.

Figure 6 shows the prevalence-season relationship of these conditions in *M. edulis* from Y2M over a 5-year period, 1976-1981; the prevalence data was from Mix (1979) and the present report. In addition, one sample was taken in 1981; the prevalence was 20.0% on 1/16/81 (50 of 250 mussels were positive). For the 5 year period, 192/1911 mussels were diagnosed histologically and found positive for the condition; the average prevalence was 10.05%. Examination of Figure 6 reveals the seasonal occurrence of the disorders. There was a consistent pattern characterized by highest prevalences during the later winter followed by a period of decline to lowest prevalences during the summer and fall after which there was a subsequent increase. The data were subjected to curve-fitting analyses and it was determined that the following quadratic equation is quite good (R^2 = .71) for describing the seasonal-prevalence relationship ($\hat{Y}$ = prevalence; X = day of year):

$$\hat{Y} = 18.70 - 0.14X + 0.0001X^2$$

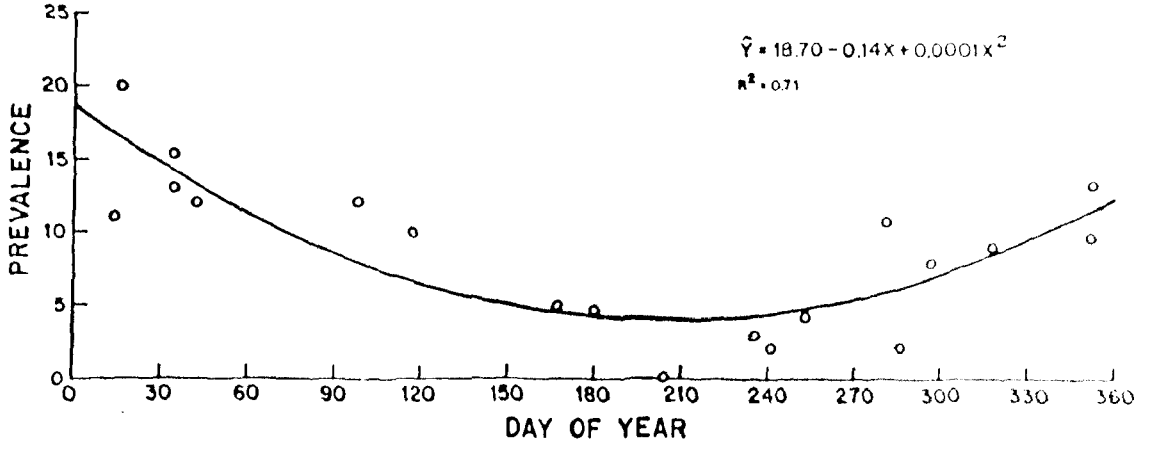

Figure 6. Prevalence of cellular proliferative disorders in *M. edulis*. The individual prevalence points represent values determined by histological examinations of mussels collected at various times from 1976-1981. The fitted line was determined by regression analysis.

The significance of the prevalence pattern is not known. It has been observed that the seasonal occurrence of the disorders correlates with the concentrations of PNAH (Mix et al., 1979b; this report) and the suggestion has been made that the appearance of atypical cells may constitute some sort of cellular response to toxic substances in the environment (Mix et al., 1979a,b). The importance of determining the metabolic capabilities, if any,

of *M. edulis* in modifying PNAH will be necessary to further clarify seasonal relationships. The recent discovery that a virus may be a causal agent responsible for similar conditions in *M. arenaria* from the east coast (Dr. P. Chang, Univ. Rhode Island, personal communication) dictates that future research efforts be directed towards determining whether or not viruses are associated with these conditions in *M. edulis*.

RELATIONSHIPS INVOLVING PNAH IN SHELLFISH MONITORS

The data on PNAH concentrations in bivalve molluscs reported here are more detailed than those from any other similar study. There have been several reports of PNAH in tissues of aquatic organisms, but most available data are restricted to BAP concentrations (see review by Neff, 1979). Only a limited amount of information is available on the concentrations of other unsubstituted PNAH. Also, the data included in some of those reports are compromised by limitations related to the analytical methods used and/or small sample sizes.

It has been commonly reported, without much justification, that quantitative and qualitative measurements of BAP can be used as an index of contamination for other PNAH. Information relative to the predictive use of such a BAP index has been generally absent or predictive models have lacked the precision necessary for use in environmental studies. Dunn (1980) found that BAP concentrations in his samples were correlated with the levels of PNAH containing 3 or more rings in *Fucus*. He concluded that BAP levels could be used as an index of the contamination of marine samples by carcinogenic PNAH. No details about the index nature or quantitative specifications were presented. Others have cautioned that BAP may serve as only a very rough index of PNAH contamination (Baum, 1978) and that, while it is important and one of the most ubiquitous PNAH carcinogens, it generally constitutes only between 1-20% of the total carcinogenic PNAH (Suess, 1976). Nevertheless, BAP has been used by the EPA and others as an indicator or marker compound for total PNAH content (refer to 2 citations in Brown et al., 1980; "Atmospheric Polycyclic Organic Matter (POM): Sources and Population Exposure." Draft EPA Report, by Energy and Environmental Analyses, Inc., Arlington, VA; and, "Preferred Standards Path Report by Polycyclic Organic Matter," U. S. EPA, Durham, NC, Oct. 1974).

Data on PNAH concentrations in *M. edulis* from Y1M, Y2M and Y140 were used in two statistical approaches to identify significant relationships between individual PNAH and total PNAH concentrations. Multiple regression and multiple correlation techniques were used in considerations of interrelationships between variables (individual PNAH). In these calculations, Y, the dependent variable, was considered to be the total PNAH concentration for one sample period and the X_i were independent variables. The general formula for multiple regression is

$$Y = a + b_1X_1i + b_2X_1i \cdots\cdots bnXni,$$

implying that one variable, Y (total PNAH), is linearly dependent upon multiple variables, X_1, X_2, etc. (individual PNAH). b_1, b_2, etc., are partial

regression coefficients; b_1 expresses how much Y would change for a unit change in X_1 if X_2, X_3, etc., were held constant. The Y intercept, a, is the value of Y when all X values are zero. Analyses of variance and t-tests were calculated during each analysis in order to determine the Xi's that contributed significantly to R^2, the coefficient of determination used to indicate the proportion of total variability in Y attributable to the dependence of Y on all Xi (Zar, 1974). Table 15 summarizes the results of the statistical analyses for PNAH data from each site.

TABLE 15. MULTIPLE REGRESSION AND CORRELATION ANALYSES.

SITE	R^2	VARIABLE (PNAH)[1,2]	REGRESSION COEFFICIENT(b)	t-test of b
Y1M	.99	BAA	1.19	33.1
		PHEN	1.05	26.3
		PYR	1.07	20.5
		FLUOR	0.93	18.4
		TRI	1.09	14.0
		BCP	0.87	8.2
		BGHIP	9.00	3.6
Y2M	.99	FLUOR	3.67	8.8
		DBACA	15.42	4.2
Y140	.99	PYR	3.24	7.2
		DBACA	8.32	6.6
		IP	23.16	3.4

[1] only those variables that were significant in determining the multiple correlation coefficient (R^2) are included.

[2] the listed order of variables indicates the relative contribution to R^2 for each site; the first listed PNAH contributed most, the last listed, least.

Examination of Table 15 indicates that for two sites, Y2M and Y140, only two or three PNAH, or independent variables, were necessary to complete the regression formula. For Y2M, the formula is

$$\text{TOTAL PNAH (Y)} = 986.23\ (a) + 3.67\ (\text{FLUOR}) + 15.42\ (\text{DBACA})$$

where X_1 and X_2 are the quantities of fluoranthene and dibenz(a,h)anthracene, respectively. Quantities of seven PNAH yield partial regression coefficients that contributed significantly to the multiple correlation coefficient for Y1M.

The primary purpose of these analyses was not to generate multiple regression formulas to be used for predictive purposes, although that was done. Such an approach may or may not be particularly useful for making general predictive statements about quantitative relationships for environmental PNAH. Further research will be necessary to determine the accuracy of such formulas.

For the present studies, the formulas were limited to describing the relationships of individual PNAH and total PNAH for a particular site during a definite period of intermittent sampling. Certain conclusions, summarized below, can be formed after examining the results of these analyses.

1. For each site, different independent variables (individual PNAH) were used to predict Y (total PNAH).
2. No single PNAH was a significant variable for predicting total PNAH at all three sites.
3. BAP was not a significant variable for predicting total PNAH at any site. Thus, the concept that BAP can be used as an index of PNAH contamination is not supported by the results of this study.
4. In monitoring programs, it may be possible to identify the significant variables (PNAH) after a suitable period of sampling and to subsequently measure only those variables for an adequate assessment of total PNAH. Complete analyses could be made periodically to confirm the continuing validity of the established regression. Such an approach may result in considerable cost reduction for long-term monitoring programs.

To further evaluate the interrelationships between PNAH, additional statistical analyses were conducted. Multiple comparison tests were used to compare pairs of samples. Student-Newman-Keuls tests were used for Y1M results and Friedman's test for Y2M results. The latter test was used because Levene's statistic showed that the variances were not homogenous at $P < 0.01$ for Y2M. The results of these analyses are summarized in Table 16.

There are two tentative conclusions that are supported by the information in Table 16. In general, the concentrations of individual 4-ring PNAH did not differ from each other; fluoranthene was a major exception since it was concentrated to higher levels in Yaquina Bay mussels. Except for BAP and coronene in Y2M mussels, there were no significant differences in PNAH concentrations between all 5-, 6- and 7-ring PNAH. Thus, while it was established that quantitative predictions about total PNAH cannot be made on the basis of individual PNAH measurements, the results from Table 16 indicate that general qualitative relationships existed in the present study. For example, detection of a certain quantity of PYR suggested that a similar quantity of BCP, TRI and BAA were present. Measurement of an individual PNAH concentration for any 5-, 6- or 7-ring PNAH indicated that approximately the same concentration would be found for any other unsubstituted PNAH with 5-7 rings. It may be productive to conduct these kinds of analyses for PNAH data collected from other established biological monitoring programs. Confirmation of the relationships identified during the present study may eventually lead to a simplified monitoring approach. Eventually, analysis of only a small number of PNAH may be necessary to produce acceptable results suitable for predictive purposes.

In these studies where PNAH measurements have been made in bivalve molluscs, it was evident that those with lower molecular weights (MW) were generally present in greater concentration than those with higher MW (Table 17). Such a pattern has also been evident in most other studies (Cahnmann and Kuratsune, 1957; Fario, 1971; Neff et al., 1976; Pancirov and Brown, 1977; Joe et al., 1979). However, one group (Risebrough et al., 1980) re-

TABLE 16. AN EVALUATION OF QUANTITATIVE RELATIONSHIPS BETWEEN INDIVIDUAL PNAH IN TISSUES OF *M. EDULIS* FROM YAQUINA BAY, OREGON.

	^{3}PHEN	^{4}FLUOR	^{4}PYR	^{4}BCP	^{4}TRI	^{4}BAA	^{5}BBF	^{5}BKF	^{5}DBACA	^{5}BAP	^{5}DBAHA	^{6}BGHIP	^{6}IP	^{7}COR
PHEN		*	*	*	*	*	*+	*+	*+	*+	*+	*+	*+	*+
FLUOR	*		*	*	*	*	*	*	*	*	*	*	*	*
PYR	*	*					*	*	*+	*	*+	*+	*+	*+
BCP	*	*					*	*	*	*	*+	*+	*+	*+
TRI	*	*					*	*	*+	*	*+	*+	*+	*+
BAA	*	*					*	*	*	*	*	*+	*+	*+
BBF	*+	*+	*	*	*	*								
BKF	*+	*+	*	*	*	*								
DBACA	*+	*+	*+	*	*+	*								
BAP	*+	*	*	*	*	*								+
DBAHA	*+	*+	*+	*+	*+	*								
BGHIP	*+	*+	*+	*+	*+	*+								
IP	*+	*+	*+	*+	*+	*+								
COR	*+	*+	*+	*+	*+	*+				+				

*indicates that means are not equal at site Y1M (e.g. reject H_o: $\bar{X}_{PHEN} = \bar{X}_{FLUOR}$ AT $P<.01$).

+indicates that means are not equal at site Y2M.

$^{3-7}$indicate the number of rings in the PNAH.

TABLE 17. THE MEAN PERCENTAGE INDIVIDUAL PNAH CONTRIBUTED TO THE TOTAL PNAH FOR ALL SAMPLING DATES (± S.D.).

BAY	SITE	SPECIES	n	PHEN	FLUOR	PYR	BCP	TRI
YAQUINA	Y1M	1	17	39.1±5..	17.0±3.0	9.3±3.3	9.7±2.8	10.0±1.9
YAQUINA	Y2M	2	12	22.2±2.0	18.3±0.4	11.1±0.3	9.7±0.7	10.7±0.7
YAQUINA	Y1YO	3	14	16.5±1.0	15.3±2.1	9.8±0.9	11.3±1.4	6.5±0.8
COOS	CSS	4	6	28.2±2.0	20.3±3.3	11.0±2.2	10.1±1.3	7.9±0.3
COOS	C3S	5	5	16.6±3.3	13.4±1.5	8.3±1.1	4.2±0.8	7.7±0.7
COOS	C11G	6	10	25.6±4.3	17.6±1.6	8.3±3.0	8.4±1.7	7.5±1.5
TILLAMOOK	T1M	7	2	28.8±0.7	19.6±0.8	15.2±1.3	10.0±0.4	6.8±0.5
TILLAMOOK	TSS	8	4	28.7±1.2	18.8±4.0	12.7±5.0	6.8±1.3	12.6±2.4
TILLAMOOK	TBC	9	3	18.9±9.1	15.4±2.3	8.9±0.9	11.0±2.2	11.2±1.6

BAY	SITE	SPECIES	n	BAA	CHRY	BBF	BKF	DBACA
YAQUINA	Y1M	1	17	11.1±5.1	----	0.9±0.4	0.9±0.1	0.7±0.1
YAQUINA	Y2M	2	12	9.4±1.2	8.6±1.1	1.8±0.2	1.7±0.3	1.1±0.1
YAQUINA	Y1YO	3	14	13.8±1.9	10.2±1.2	3.0±0.4	3.2±0.4	3.0±0.4
COOS	CSS	4	6	7.4±3.3	4.9±0.6	2.2±0.3	1.8±0.2	1.6±0.3
COOS	C3S	5	5	3.6±0.2	10.9±0.5	1.7±0.4	4.0±0.4	2.7±0.6
COOS	C11G	6	10	12.8±3.4	7.4±2.0	3.2±1.1	2.0±1.0	1.8±0.7
TILLAMOOK	T1M	7	2	5.2±0.7	7.6±0.5	2.4±0.4	1.6±0.3	1.0±0.1
TILLAMOOK	TSS	8	4	5.1±1.3	3.3±0.8	2.6±0.7	0.2±0.2	3.4±1.4
TILLAMOOK	TBC	9	3	5.9±0.6	9.2±1.0	2.8±0.7	3.6±0.6	1.1±0.4

TABLE 17. (continued)

BAY	SITE	SPECIES	n	BAP	DBAHA	BGHIP	IP	COR
YAQUINA	Y1M	1	17	0.5±0.2	0.8±0.4	0.2±0.0	0.2±0.0	0.2±0.1
YAQUINA	Y2M	2	12	2.7±0.2	1.0±0.2	0.8±0.2	0.5±0.3	0.4±0.2
YAQUINA	Y140	3	14	3.6±0.3	1.1±0.2	0.9±0.1	0.8±0.1	1.0±0.2
COOS	CSS	4	6	1.6±0.2	1.2±0.2	0.8±0.4	0.7±0.5	0.4±0.4
COOS	C3S	5	5	4.2±0.3	5.7±1.7	6.9±0.8	8.1±1.4	1.9±0.2
COOS	C11G	6	10	2.0±1.0	1.7±0.9	0.8±0.6	0.6±0.4	0.4±0.1
TILLAMOOK	T1M	7	2	0.6±0.1	0.6±0.1	0.4±0.1	0.3±0.1	0.3±0.1
TILLAMOOK	TSS	8	4	1.3±0.4	1.9±0.6	2.4±0.6	0.2±0.2	0.0±0.1
TILLAMOOK	TBC	9	3	2.6±0.2	2.0±0.6	2.7±0.3	1.7±0.9	1.4±0.2

SPECIES

[1] *M. edulis*, [2] *M. edulis*, [3] *C. gigas*, [4] *M. arenaria*, [5] *M. arenaria*, [6] *T. capax*, [7] *M. edulis*, [8] *M. arenaria*, [9] *C. gigas*.

ported highest levels of fluoranthene with approximately equal concentrations of phenanthrene and BAP; only those three PNAH were measured.

It seems apparent that some mechanisms may exist to account for the commonly observed pattern of uptake. These may include differential rates of uptake and/or elimination or greater biological availability of lower MW PNAH because of their higher water solubilities (Fazio, 1971). To identify significant relationships between PNAH concentrations and their water solubility, the data in Table 17 were converted to log values and regressed against their respective solubilities (Log S values; Table 4). Figure 7 illustrates the results of those analyses. Linear regression analyses were conducted for each of the 9 sites used in this study and the results are presented in Table 18.

Separate linear regression analyses were performed using solubility values from MacKay and Shiu (1977) and from May (1980) for phenanthrene, fluoranthene, pyrene, triphenylene, benzanthracene and chrysene with the common values for dibenz(a,c)anthracene, benzo(a)pyrene, benzo(g,h,i)perylene, and coronene from MacKay and Shiu (Table 4). For MacKay and Shiu's values for all 10 PNAH, calculated $F = 235.4 > F_{.05,1,87} = 3.95$; $R^2 = 0.73$. The regression formula used to calculate Y from X where Y = log mean PNAH concentration and X = -log S is

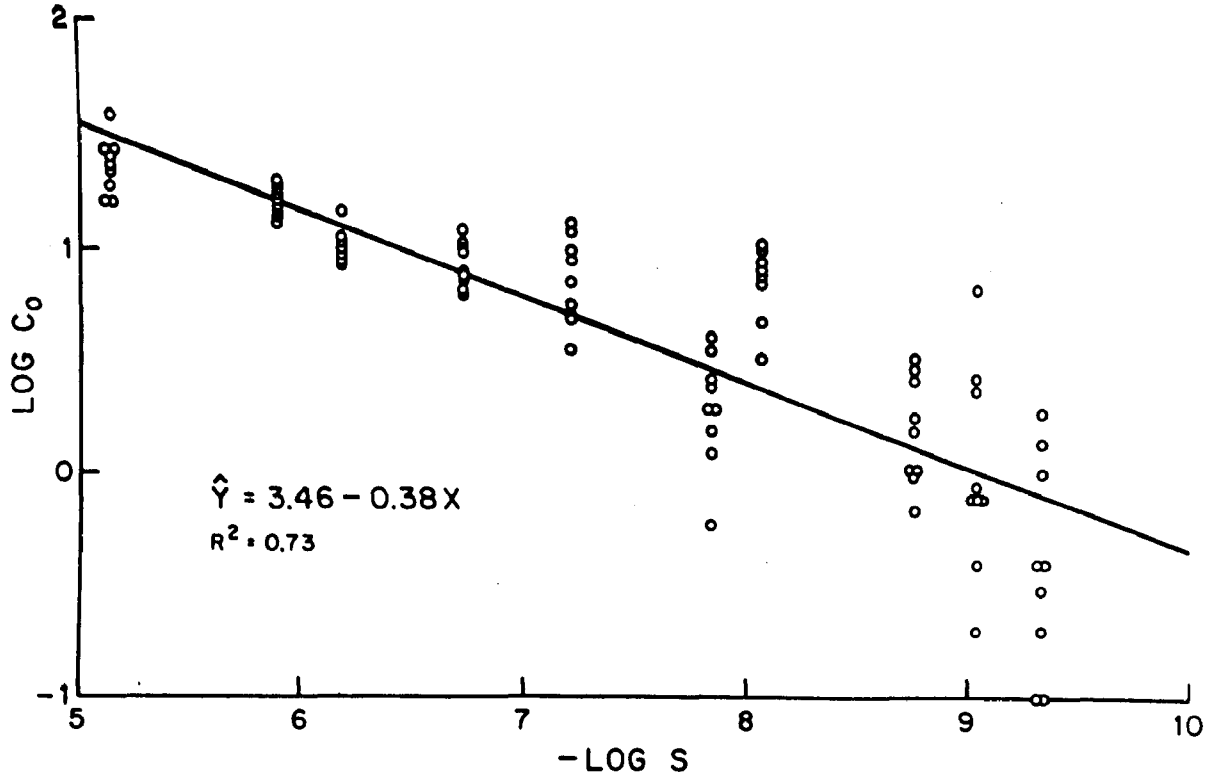

Figure 7. PNAH-water solubility regression relationship. See text for a complete explanation.

$$\hat{Y} = 3.46 - 0.38X.$$

For May's values, calculated $F = 205.0 > F_{.05,1,87} = 3.95$; $R^2 = 0.70$ and

$$\hat{Y} = 3.48 - 0.38X.$$

Thus, an empirical relation between log C_o (log % concentration in shellfish) and log S is observed for PNAH, as shown in Figure 7. It is interesting to note that the concentrations in shellfish were greater for the PNAH isomer which had the higher solubility in water. This is in contrast to the observation that the organic/water (e.g. octanol/water) partition coefficient shows an inverse relation to water solubility (Chiou et al., 1977). Because the concentration in the organic phase (in this case, shellfish), C_o is equal to the product of the partition coefficient (k) and concentration in water (C_w), the data suggest that the ratio of the concentrations of these PNAH in water would have to be generally greater than the ratio of their reciprocal partition coefficients or their water solubilities. Direct measurements of the PNAH concentrations in seawater will be necessary to confirm whether the uptake of PNAH's by shellfish can be represented by a simple partition process.

TABLE 18. LINEAR RELATIONSHIPS BETWEEN LOG S (X), WATER SOLUBILITY, AND LOG C_o (Y) MEAN CONCENTRATION (%), FOR 10 PNAH[1] IN SHELLFISH FROM YAQUINA (Y), COOS (C), AND TILLAMOOK (T) BAYS.

SITE	SPECIES	CALCULATED LINEAR REGRESSION FORMULA	F^2	R^2
Y1M	*M. edulis*	$\hat{Y} = 4.58 - 0.55X$	93.6	.93
Y2M	*M. edulis*	$\hat{Y} = 3.83 - 0.43X$	36.5	.82
Y1Y0	*C. gigas*	$\hat{Y} = 2.83 - 0.28X$	21.5	.73
CSS	*M. arenaria*	$\hat{Y} = 3.68 - 0.41X$	82.5	.91
C3S	*M. arenaria*	$\hat{Y} = 2.00 - 0.16X$	10.1	.56
C11G	*T. capax*	$\hat{Y} = 3.90 - 0.45X$	23.8	.75
T1M	*M. edulis*	$\hat{Y} = 4.05 - 0.48X$	41.2	.84
TSS	*M. arenaria*	$\hat{Y} = 3.77 - 0.43X$	20.6	.72
TBC	*C. gigas*	$\hat{Y} = 2.74 - 0.27X$	28.7	.78

[1] PNAH include phenanthrene, fluroanthene, pyrene, triphenylene, benzo(a)anthracene, chrysene, dibenz(a,c)anthracene, benzo(a)pyrene, benzo(g,h,i)perylene and coronene.

[2] $F_{.05,1,9} = 5.12$; $F_{.01,1,9} = 10.56$. All regressions, except C3S, were significant at P = .01; C3S was significant at P = .05.

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