Bull. Environ. Contam. Toxicol. (1985) 35:835–844

Bulletin of Environmental Contamination and Toxicology

Polynuclear Aromatic Hydrocarbons in Oyster Tissue Around Three Coastal Marinas

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Increasing pressure for construction of marinas in heretofore relatively pristine estuarine waters along the coastline of South Carolina has raised much concern over the potential and real impact of such development on the bountiful oyster resource located there. Approximately one-third of the State's 223,000 acres of shellfish are unconditionally closed to harvesting due mainly to bacterial pollution while the loss of productive shellfish area due to all types of pollution is about 0.6 percent annually.

Marinas present the potential for introduction of various pollutants into the surrounding waters such as coliform bacteria, primary pathogens, heavy metals, and petroleum hydrocarbons. Several researchers have explored the increase in petroleum hydrocarbons in oysters due to industrial sources and commercial shipping activities (Erhardt 1972, Farrington et al. 1983, Murray et al. 1980) but little data have been presented specifically addressing the effects of recreational marinas on petroleum hydrocarbon levels or, for that matter, other constituent levels in oysters near those marinas.

In order to obtain such data, a comprehensive assessment of water and oyster quality around three coastal marinas was conducted by the South Carolina Department of Health and Environmental Control (SCDHEC) during 1983. A data base was constructed of physiochemical analyses from water, chemical analyses from sediment and chemical/bacteriological/biological analyses from the American oyster, Crassostrea virginica (Gmelin).

Polynuclear aromatic hydrocarbons (PAH) were selected as the petroleum hydrocarbon fraction of interest since they are mainly of pyrogenic origin; have been shown to be the most toxic/carcinogenic fraction of oil (Anderson et al. 1974); have been shown to affect the respiration and heart rates of mussels (Sabourin and Tullis 1981); and have been shown to be linked to neoplasia in clams (Brown et al. 1971) and proliferative disorders in mussels (Mix et al. 1979). C. virginica was chosen

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as the mollusc of interest because of its widespread distribution in the estuaries of South Carolina, its importance as an economic and recreational resource, and its suitability as a sentinel organism for monitoring coastal pollution (Couch et al. 1979, Farrington 1983).

MATERIALS AND METHODS

Three existing marinas in coastal South Carolina (Beaufort County) were chosen for this project. A grid of ten stations was placed around each marina for intertidal oyster and sediment collections as depicted schematically in Figure 1. Sediment was also collected from a station in the center channel between each right (R) and left (L) bank station. Analyses were conducted for the following PAH compounds with the lower detection limits (ug/kg) noted in parentheses:

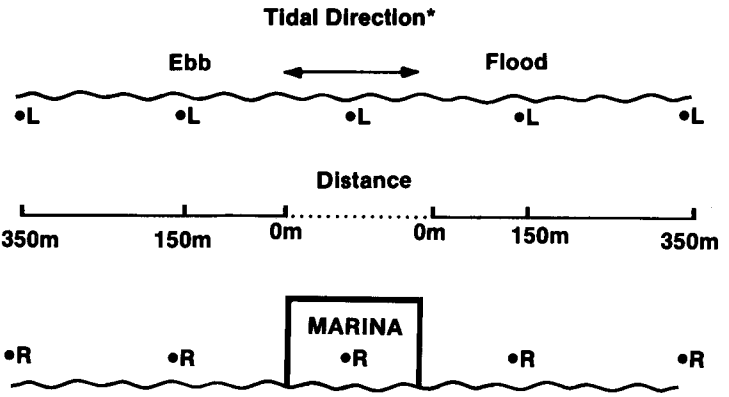
Acenaphthene-Anp-(10.0)	Chrysene-Chy-(8.0)
Acenaphthylene-Ayp-(160.0)	Dibenzo(ah)anthracene-DaA-(1.3)
Anthracene-Ant-(38.0)	Fluoranthene-Fla-(3.0)
Benzo(a)anthracene-BaA-(0.80)	Fluorene-Fle-(32.0)
Benzo(a)pyrene-BaP-(1.3)	Indeno(1,2,3-cd)pyrene-IP-(1.3)
Benzo(b)fluoranthene-BbF-(1.5)	Naphthalene-Nap-(32.0)
Benzo(k)fluoranthene-BkF-(0.65)	Phenathrene-Phn-(8.0)
Benzo(ghi)perylene-BgP-(1.5)	Pyrene-Pyr-(8.0)

Live oysters were manually collected from the mid-intertidal portion of the reefs at all stations. Each sample consisted of 15 adult oysters of marketable size (>7.5 cm in height). Sampling was conducted once per week per marina for three consecutive weeks during March 29 - April 14, 1983, and again during July 26 - August 11, 1983. Upon collection, the oysters were transported immediately to the laboratory where they were cleaned, shucked, packed in prepared containers and then refrigerated at 4°C until PAH analyses were conducted.

The top 3 cm of sediment within each reef was collected once per sampling period using a stainless steel spatula. Samples from the mid-channel stations were collected from a boat using a stainless steel Peterson dredge. Upon collection, all samples were dispensed into prepared containers and stored at 4°C until the PAH analyses were conducted.

Fifteen additional oysters were collected from each R and L station once per sampling period for condition index (CI) analyses. All fouling and commensal organisms were removed from the oysters in the field after which they were thoroughly cleaned. Analyses for CI followed the method of Lawrence and Scott (1982).

Extraction procedures for PAH in tissue samples followed those developed specifically for this project. The samples were homogenized using dry ice in a Waring blender after which the frozen homogenate was allowed to sublime for 24 hours at 0°C. The tissue was then extracted with an ether solvent solution in a chromatography column. The concentrate was purified using an automated



PB:	MD-700	-701	-702	-703	-704
OR:	MD-705	-706	-707	-708	-709
FI:	MD-710	-711	-712	-713	-714

*Tidal flow direction reversed from this schematic at Fripp Island marina

Figure 1: Schematic illustration of grid placed around each marina for oyster sampling.

Gel Permeation Chromatograph (GPC) and the final concentrate was analyzed by High Performance Liquid Chromatography (HPLC) using a reverse-phase Supelco LC-PAH column.

Extraction procedures of PAH in sediment were similar to those found in EPA Methodology (USEPA 1980) using a Heat Systems Ultrasonics, Inc. Sonicator as the mechanical dispersion device to extract the base-neutral fraction. The concentrate was then placed on a micro-florisil column to partially clean up the samples before injection on the HPLC. Sediment samples were then analyzed by HPLC using the same method that was followed for the tissue samples.

RESULTS AND DISCUSSION

Tables 1, 2 and 3 present the results of the tissue analyses from Palmetto Bay (PB), Outdoor Resorts (OR), and Fripp Island (FI) marinas, respectively. An total number of nine PAH compounds were detected at Palmetto Bay and Outdoor Resorts while only three were detected at Fripp Island. This was not surprising since the first two facilities are rather large by South Carolina standards with slips to accomodate approximately 100 boats. Fripp Island marina can only berth about 50 boats.

As can be seen from Tables 1-3, there was a large amount of variation between the discrete sample concentrations that comprised

Table 1. Mean PAH concentrations in Crassostrea virginica from Palmetto Bay Marina, South Carolina.

PAH	Mean wet weight concentration in ug/kg (a)									
	MD-700L	MD-700R	MD-701L	MD-701R	MD-702L	MD-702R	MD-703L	MD-703R	MD-704L	MD-704R
BaA:Sp[b]	*	*	*	8.7(6.5)	*	2.0(3.5)	*	6.9(7.2)	*	*
Su	1.5(1.4)	0.3(0.5)	1.3(1.1)	1.8(2.0)	*	12.0(2.9)	*	3.5(0.2)	*	*
BfF:Sp	*	*	*	1.8(1.8)	*	*	*	2.6(2.5)	*	*
Su	*	0.5(0.9)	0.5(0.9)	*	*	5.2(2.2)	0.5(0.9)	1.2(1.1)	*	*
BkF:Sp	*	*	*	0.1(0.2)	*	*	*	*	*	*
Su	*	*	*	*	*	0.9(1.6)	*	*	*	*
BgP:Sp	*	*	*	*	*	*	*	*	*	*
Su	0.4(0.8)	1.2(2.1)	*	*	*	*	*	*	*	*
BaP:Sp	*	*	*	*	*	*	*	*	*	*
Su	*	*	*	*	*	0.5(0.9)	*	*	*	*
Chy:Sp	*	*	*	14.2(16.6)	*	*	*	11.5(10.4)	*	*
Su	*	1.3(2.2)	*	*	*	8.3(14.4)	*	*	*	*
Fla:Sp	4.7(8.1)	13.2(11.7)	*	58.8(28.5)	11.0(10.6)	39.1(12.1)	12.3(11.1)	94.1(58.7)	12.4(11.0)	13.0(5.2)
Su	11.3(2.6)	1.4(2.4)	6.7(1.0)	8.1(3.4)	0.9(1.6)	40.7(2.0)	6.2(2.0)	15.2(5.4)	1.1(1.9)	2.5(4.4)
Phn:Sp	*	*	*	16.4(17.8)	*	6.0(10.3)	*	76.5(13.0)	*	*
Su	*	*	*	*	*	9.7(9.0)	*	*	*	*
Pyr:Sp	2.3(4.0)	*	*	36.2(37.1)	16.5(28.5)	27.1(25.9)	6.4(11.1)	10.0(17.4)	4.6(7.9)	11.3(19.5)
Su	15.8(15.8)	43.0(74.5)	3.1(5.4)	*	*	22.8(20.2)	13.4(15.8)	26.5(25.1)	*	*
Total:Sp	7.0	13.2	*	136.2	27.5	74.2	18.7	201.6	17.0	24.3
Su	29.0	47.7	11.6	9.9	0.9	100.1	20.1	46.4	1.1	2.5

a. figures in parentheses represent one standard deviation (n=3)
b. Sp=Spring; Su=Summer
* non-detectable

Table 2. Mean PAH concentrations in Crassostrea virginica from Outdoor Resorts Marina, South Carolina.

PAH	Mean wet weight concentrations in ug/kg (a,b)							
	MD-705L	MD-705R	MD-706L	MD-707L	MD-707R	MD-708R	MD-709L	MD-709R
BaA:Sp[c]	1.6(2.8)	3.4(5.8)	*	0.7(1.3)	*	*	*	*
Su	1.4(0.2)	5.0(2.7)	0.9(0.8)	1.7(0.8)	1.5(1.6)	1.8(0.8)	1.0(1.0)	0.9(0.8)
BaP:Sp	*	*	*	*	*	*	*	*
Su	1.0(1.7)	1.6(2.8)	*	*	*	*	*	*
BbF:Sp	*	*	*	*	*	*	*	*
Su	0.4(0.8)	3.2(2.4)	*	*	0.4(0.6)	0.6(1.0)	*	0.3 (0.6)
BkF:Sp	*	*	*	*	*	*	*	*
Su	*	0.2(0.4)	*	*	*	*	*	*
Chy:Sp	*	4.3(7.4)	*	*	*	*	*	*
Su	*	*	*	*	*	*	*	*
Fla:Sp	11.9(10.4)	16.5(6.0)	4.8(8.3)	4.3(7.4)	9.9(9.1)	20.7(6.6)	17.8(1.5)	5.8(10.0)
Su	5.9(0.9)	16.1(17.9)	5.5(1.3)	4.7(2.0)	6.0(5.8)	7.5(3.7)	4.8(4.6)	8.8(10.4)
IP: Sp	*	*	*	*	*	*	*	*
Su	*	1.7(2.9)	*	*	*	*	*	*
Phn:Sp	*	16.0(27.6)	*	*	*	*	*	4.9(8.5)
Su	*	5.1(8.8)	*	*	*	*	*	*
Pyr:Sp	*	50.1(43.6)	*	10.6(8.4)	*	46.9(41.6)	7.5(13.0)	10.2(17.7)
Su	3.8(6.6)	9.9(17.2)	1.6(2.7)	*	1.6(2.7)	*	1.9(3.2)	27.5(47.6)
Total:Sp	13.5	90.3	4.8	15.6	9.9	67.6	25.3	20.9
Su	12.5	42.8	8.0	6.4	9.5	9.9	7.7	37.5

a. oysters not available at MD-706R and MD-708L
b. figures in parentheses represent one standard deviation (n=3)
c. Sp=Spring; Su=Summer
* non-detectable

Table 3. Mean PAH concentrations in Crassostrea virginica from Fripp Island Marina, South Carolina.

Station		Mean wet weight concentration in ug/kg(a,b)			
		BaA	Fla	Pyr	Total
MD-710L :	Sp[c]	*	*	*	*
	Su	*	*	*	*
MD-710R :	Sp	*	*	*	*
	Su	*	*	*	*
MD-711L :	Sp	*	*	*	*
	Su	*	*	*	*
MD-711R :	Sp	*	*	*	*
	Su	*	0.7(1.2)	2.2(3.8)	2.9
MD-712L :	Sp	*	*	*	*
	Su	*	1.4(2.4)	5.8(10.0)	7.2
MD-712R :	Sp	*	6.3(10.9)	16.7(30.0)	23.0
	Su	1.2(1.2)	6.0(2.4)	*	7.2
MD-713R :	Sp	*	*	10.3(17.8)	10.3
	Su	*	*	*	*
MD-714L :	Sp	*	*	*	8
	Su	*	0.6(1.1)	3.1(5.4)	3.7
MD-714R :	Sp	*	*	21.7(37.5)	21.7
	Su	*	*	*	*

a. oysters not available at MD-713L
b. figures in parentheses represent one standard deviation (n=3)
c. Sp=spring; Su=summer
* non-detectable

each mean value. Because of this variability, there were no significant spatial or temporal differences ($p>.05$) between the means of any stations within each respective marina complex. Nevertheless, the data from each marina did indicate the accumulation of various PAH compounds in tissue. In general, the highest mean concentrations observed were for fluoranthene and pyrene.

Of the nine total PAH compounds detected in tissue from Palmetto Bay, only seven were observed in the spring sampling period, while all nine were seen in the summer period. Likewise, at Outdoor Resorts, only five of the total nine compounds were detected in the spring period. Two of three total compounds were found at the Fripp Island facility in the spring with all three found in the summer. Benzo(a)anthracene, fluoranthene and pyrene were identified in tissue from all three marinas while benzo(a)pyrene, benzo-(b)fluoranthene, benzo(k)fluoranthene, chrysene and phenanthrene were common to both Palmetto Bay and Outdoor Resorts.

The seasonal increase in the empirical number of compounds found in tissue suggested an amplification of PAH input to the respective systems due to increased boating activity associated with warmer weather. However, an examination of the seasonal total PAH

levels by station appeared to refute this observation. Fourteen of the stations demonstrated higher total PAH levels in tissue during the spring period, ten stations demonstrated higher tissue levels in the summer and three showed no PAH present in tissue during either period. Sediment PAH data and condition index data were used to resolve this apparent contradiction.

Table 4 contains a summary of PAH data from sediment collected during each sampling period. These data clearly indicated a temporal increase in PAH levels in sediment at Palmetto Bay (68 percent) and at Outdoor Resorts (266 percent). Fripp Island showed a decrease from spring to summer (15 percent) but this value was heavily influenced by the unexplained occurrence of fluorene at only one station during the spring period. These increases in total sediment PAH confirmed the original observation of amplified PAH input to the systems as a result of boating activity during the warmer months. Since these sixteen PAH compounds are principally of pyrogenic origin, the association with powerboating activites is certainly plausible. A recent report by Smith et al. (1984) affirmed this association between powerboating activity and tissue PAH levels in clams (Tridacna maxima) from Australia.

Upon uptake of PAH compounds, the oyster preferentially partitions these compounds in its lipid fraction where they are held by hydrophobic bonds (Neff et al. 1976). The neutral lipid content at the time of exposure and uptake appears to be the major factor in the extent of accumulation (Stegeman 1974) such that the higher the lipid content, the greater the expected PAH accumulation. Condition index is a macroscopic measurement of the fatness of oysters (Galtsoff 1964). Since the oyster stores glycogen and lipid prior to spawning, the CI would be higher for a pre-spawn period than for an immediate post-spawn period. Lee and Pepper (1956) found that total solids (glycogen and lipid) in southern oysters decreased from 13.5% in March to 9.2% in the summer due to spawning. This represents a 31.8% decrease that is similar to the 39.8-45.5% loss in CI observed in this study.

In this assessment, the spring sampling was conducted prior to the annual spawning of oysters and, as such, the higher CI values from that period were significantly greater ($p<.05$) than the CI values of the summer period. The pooled mean CI values from each marina showed decreases of 39.8 percent at Palmetto Bay, 41.1 percent at Outdoor Resorts and 45.5 percent at Fripp Island (Table 5). This table also contains the pooled total mean PAH tissue concentrations. These values exhibited a seasonal decrease concomitant with the decrease in CI values. Except for Fripp Island, the percent decrease in mean total PAH tissue concentration was very comparable to the percent decrease in CI values. This indicated that the PAH compounds were associated with the lipid portion of the tissue and were released upon spawning. In addition, the indicated diminution in lipid content presented decreased PAH

Table 4. Total PAH concentrations in sediment from three South Carolina coastal marinas.

PAH	Total dry weight concentration in ug/kg (a)		
	Palmetto Bay	Outdoor Resorts	Fripp Island
Ant	*;*	*;142.0	*;*
BaA	162.0;230.8	254.6;587.3	31.0;28.5
BaP	125.4;170.6	168.0;374.2	35.8;53.7
BbF	28.5;174.1	186.6;412.4	49.9;100.6
BkF	85.3;144.1	117.3;207.1	17.8;39.4
BgP	13.6;*	35.6;58.9	7.8;*
Chy	62.7;328.6	309.8;1203.7	*;*
Fla	555.0;690.2	507.2;1963.9	88.2;129.0
Fle	*;*	*;*	273.0;*
IP	*;41.4	75.8;176.9	34.4;*
Phn	131.0;195.2	26.9;1620.8	*;54.5
Pyr	396.1;645.1	599.8;1612.4	46.4;90.2
Total	1559.6;2620.1	2281.6;8359.6	584.3;495.9

a.spring value;summer value
*non-detectable

storage capacity in the oyster during the period of amplified PAH input to the water systems.

These data have shown that PAH compounds were inputted to the estuarine system around three marinas in South Carolina and were taken up and accumulated by oysters. Two different factors were responsible for PAH occurence in sediment (level of boating activity) and in tissue (level of lipid). Apparently, the maximum period for uptake/accumulation by oysters is during the cooler months when lipid and glycogen are being stored for spawning. This is also the period when oysters are harvested for human consumption in South Carolina. Even though boating activity is less during the cooler months around coastal marinas in this State, the normal seasonal increase in lipid content of the oyster augments what may otherwise be a lower tissue PAH burden.

These data represent, to our knowledge, the first report of PAH concentrations in oyster tissue specifically related to recreational marinas. The concentrations found in oyster tissue from the marina areas were generally higher than levels reported elsewhere from other locations (Farrington et al. 1983, Pancirov and Brown 1977). Considering that some PAH compounds are strongly suspected of being carcinogenic and/or mutagenic (USPHS 1983), the possible implications for public health upon consumption of oysters gathered from around recreational marinas could be significant. Likewise, the health of the organisms themselves may be impacted by chronic exposure to PAH by virtue of marina proximity as has been suggested by Mahoney and Noyes (1982) for other petroleum hydrocarbons. More detailed investigations will completely

Table 5. Seasonal changes in total mean PAH concentrations in tissue and mean pooled CI values.

Marina	Sampling Period	Seasonal Change in Parameter	
		Total Mean PAH[a]	Mean Pooled CI[b]
Palmetto Bay	Spring	519.7	10.3
	Summer	269.3	6.2
	% Loss	48.2	39.8
Outdoor Resorts	Spring	247.9	11.2
	Summer	134.3	6.6
	% Loss	45.8	41.1
Fripp Island	Spring	55.0	11.2
	Summer	21.0	6.1
	% Loss	61.8	45.5

a. PAH reported in ug/kg, wet weight
b. CI reported in dimensionless CI units

elucidate the true level of significance of these documented marina-associated PAH inputs to the surrounding water systems.

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Received December 11, 1984; accepted January 11, 1985