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Benzo(a)pyrene Concentrations in Mussels *(Mytilus edulis)* from Yaquina Bay, Oregon During June 1976 - May 1978

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Benzo(a)pyrene (BAP) and several other polynuclear aromatic hydrocarbons (PNAH), many which are carcinogenic in vertebrates, are found in low levels in crude oil, particularly refined oils, and thousands of kg of these compounds enter the sea each year (NAS 1975). Weathered and partially degraded oils may also contain additional oxidation products of potential carcinogenicity (FELDMAN 1973). More recently, several reports indicate that, in general, PNAHs found at ppb levels in marine animal tissues are derived from common combustion sources and not directly from petroleum contamination (BLUMER & YOUNGBLOOD 1975, HITES 1976, BROWN & WEISS 1978).

The use of bivalve mollusks for monitoring marine environments in order to detect and quantitate various pollutants, including chemical carcinogens, has been advocated by many investigators (e.g. GOLDBERG 1975). Indigenous populations of shellfish seem to be ideal subjects for evaluating carcinogenic PNAH loads in the marine environment (LEE 1977, MIX et al. 1977).

The purposes of this study were to measure BAP concentrations in indigenous populations of mussels for a two-year period, determine seasonal fluctuations in BAP body burdens, and analyze factors that may influence temporal concentration patterns.

MATERIALS AND METHODS

Collection and Preparation of Mussels. *M. edulis* were collected bimonthly from 13 sites in Yaquina Bay (Fig. 1). Immediately after collection, mussels from a single site were placed in labeled plastic bags, put on ice contained in coolers and transported back to our laboratory in Corvallis. Individual animals were removed from the shell and the pooled sample from each site was weighed and then stored in a plastic bag at -20°C until it was analyzed.

Sampling Sites. A brief description of each collecting site follows. Y1M: weathered, heavily creosoted pilings that formerly supported an old railroad trestle. Y2M, Y3M, Y4M: creosoted pilings that support cold storage facilities and fish processing plants; several nearby marinas. Y5M: creosoted pilings. Y6M: creosoted pilings supporting a small boat dock. Y7M: creosoted floating dock supporting a gas pump; near a large boat basin; Y8M: creosoted

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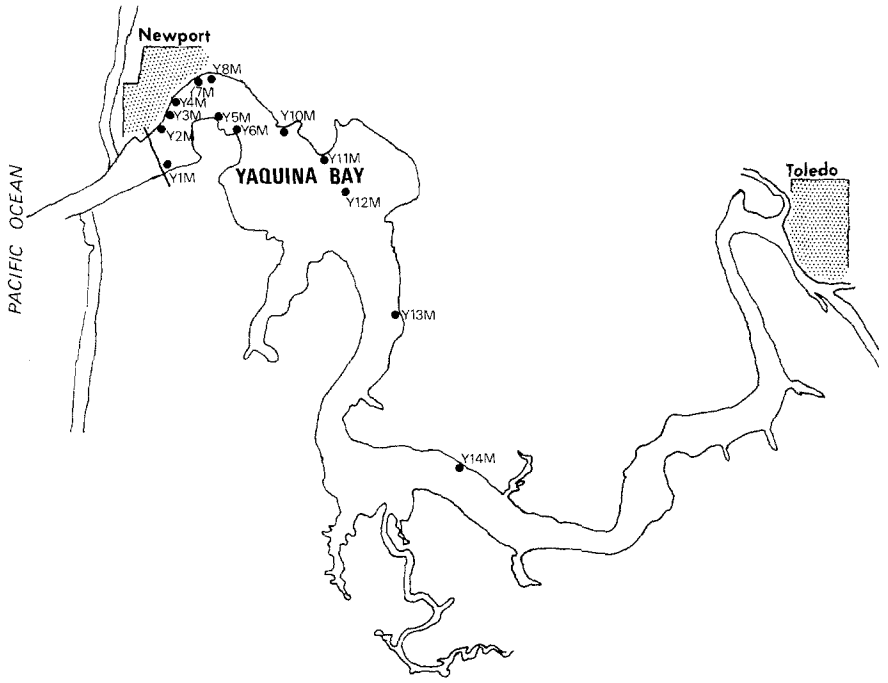

Fig. 1. Sites in Yaquina Bay, Oregon, where *M. edulis* were sampled from June 1976 - May 1978.

floating dock. Y10M: creosoted piling supporting a large ship dock. Y11M: rocks near liquid natural gas storage tanks. Y12M: creosoted pilings supporting a marker buoy in the main channel. Y13M: iron pilings near a marina. Y14M: creosoted piling.

BAP Analysis. An aliquot of 20-25 mussels per site was analyzed according to the method of Dunn (DUNN & STICH 1975, DUNN 1976). Each 30-40 g sample was digested by refluxing in an ethanol-KOH solution. Following digestion, the ethanol-KOH supernatant was extracted with 2,2,4-trimethylpentane (TMP) and the organic phase passed through a column of partially deactivated Florisil. The PNAH were eluted with benzene and, after removal of the benzene, the eluate was cleaned up by DMSO extraction in TMP.

BAP was isolated by preparative thin layer chromatography on 20% acetylated cellulose, made to volume in hexadecane and the concentration of BAP determined by spectrophotofluorimetry. Recovery of BAP by the extraction procedure was determined by fortifying the original digestion with an aliquot of G-^{3}HBAP (Amersham/Searle: SA = 79.3 ci/mg; 98% purity) and counting an aliquot of the final hexadecane solution; typical recoveries ranged from 60-80%. Replicate analyses of pooled samples were done during preliminary studies and for samples with unusually high BAP concentrations (e.g. Y4M

on 2/03/77). All replicate values were within 5% of each other.

RESULTS AND DISCUSSION

Benzo(a)pyrene concentrations varied considerably in mussels from different geographical sites and during different times of the year (Table 1). Mussels collected along the Oldtown bayfront (Y2M, Y3M, Y4M) generally had the highest BAP concentrations throughout the two year period. The reasons for this are not known nor are the sources of BAP. It is assumed that these mussels inhabit a more contaminated environment, possibly because of their proximity to marinas, boats and fish processing plants. Local sources of BAP may include fuel from boats and marinas, waste water from fish processing plants, and/or runoff after periods of heavy rainfall. Some BAP in bayfront mussels may have also come from creosoted pilings. However, mussels from other sites (Y1M, Y5M, Y6M, Y7M, Y8M, Y10M, Y12M, Y14M) also came from creosoted pilings and had substantially lower body burdens. Perhaps other factors, such as the age of the piling and proximity to strong tidal currents, were involved.

There were occasional periods when mussels from certain sites that generally had low BAP concentrations, appeared with unusually high body burdens (e.g. Y6M on 6/29/77). Although analytical error or inadvertent contamination during processing cannot be ruled out, this seems unlikely since other samples, handled in precisely the same manner, did not show such deviations. Small gas or oil spills may have been associated with the sporadic high concentrations.

The data on BAP body burdens were subjected to extensive statistical analyses. To determine if there were significant differences in BAP concentrations in mussels from Y1M-Y14M over the two year study period, the data were analyzed using a one-way ANOVA. It was found that there were significant differences between sites ($F = 8.15 > F.01\ [13,137] = 1.80$). A Student Newman-Keuls Multiple Range Test showed that only site Y2M differed significantly from the others ($F = 10.55 > F.01\ [13,137] = 2.26$. Mussels from Y2M had significantly higher BAP body burdens than mussels from other sites during the two year sampling period.

Each piece of data from Table 1 was then transformed into a "percentage of the mean" in order to obtain a universal unit that could be used in analyzing the data. For example, the average body burden of BAP in mussels from Y1M and Y2M during the two years was 1.99 and 25.57 ng/g, respectively. The fact that on 4.8.77, mussels from Y1M contained 1.72 ng/g BAP while those from Y2M had 21.89 ng/g means only that those from Y2M were more contaminated; such data alone could not be used to measure seasonal differences. However, 1.72 ng/g is 86.43% of the mean (1.99 ng/g) body burden in mussels from Y1M or -13.57% of the mean; similarly, mussels from Y2M had a -14.39% of the mean body burden. These percentages of the mean were then used to evaluate seasonal differences.

Table 1. BAP concentrations (μg/kg) in *M. edulis* from Yaquina Bay, Oregon (rounded to two figures).

	SITE												
Date	Y1M	Y2M	Y3M	Y4M	Y5M	Y6M	Y7M	Y8M	Y10M	Y11M	Y12M	Y13M	Y14M
6/15/76	0.1	30	8.1	15	0.9	3.0	4.1	0.4	5.2	0.5	0.4	0.4	4.3*
7/22/76	4.7	67	4.5	6.7	4.4	2.3	2.4	0.8	10	0.6	0.7	0.3	0.5
9/24/76	0.7	34	16	6.9	1.2	1.9	14	0.9	6.3	NS	0.8	0.8	0.3
11/16/76	0.6	40	8.4	8.9	2.7	17	19	NS	3.6	0.4	0.4	0.9	0.6
12/16/76	8.4*	12	7.2	7.5	0.6	6.1	3.8	NS	2.8	NS	0.3	0.1	0.4
2/03/77	3.8	33	71*	170*	0.9	8.1	1.7	2.0	3.0	0.7	0.5	0.2	0.2
4/08/77	1.7	22	7.9	12	1.5	4.4	NS	NS	3.8	0.7	0.5	NS	0.4
6/29/77	6.3	15	3.5	5.4	2.1	50*	3.5	1.5	NS	2.0	0.4	0.0	0.4
8/29/77	1.2	5.1	2.8	2.2	5.6	4.4	5.8	NS	NS	0.4	0.0	2.6	0.3
10/13/77	0.8	5.0	5.3	1.9	1.2	3.2	4.2	0.5	4.2	0.1	0.2	0.4	0.34
12/08/77	1.2	15	NS+	6.4	4.7	3.1	36	8.1*	9.4	NS	0.0	NS	NS
2/03/78	3.1	27	NS	13	7.7	32*	NS	2.3	10	3.0	1.2	NS	NS
4/28/78	0.7	20	NS	5.5	1.2	4.0	27	2.7	NS	NS	0.1	NS	NS
6/24/78	0.8	29	NS	17	NS	NS	NS	NS	NS	NS	NS	NS	NS
Average Conc.	2.0	26	6.5	8.5	2.7	7.5	11	1.4	6.0	1.0	0.4	0.7	0.4

* Data not included in statistical analyses because of large variation (>4X) from the mean.

\+ NS, Not sampled or not yet analyzed.

Figures 2 and 3 portray graphically the transformed data. The two years were considered separately for reasons discussed below. The percent mean body burdens were calculated using only the body burden data for the respective year.

There were dramatic differences in weather during the two year study period; the five month period from October, 1976 - February, 1977, was the driest ever recorded, while the same period for 1977 - 1978 was generally normal. The most significant difference between the two years was the virtual absence of rain during the winter (November - February) of 1976-77. The amount of rainfall during the winter of 1977-78 was rather typical for the Oregon coast. The influx of freshwater resulted in depressed temperatures and salinity in the estuary during this period.

As a result of the fluctuations in seasonal and environmental parameters during the two years, a two way ANOVA was used to determine if there were differences in seasonal-year correlation for BAP body burdens between 1976-77 and 1977-78. It was determined that there were significant differences between the two years ($F.01 = 7.06 > [5,80] = 2.33$). As a result of the statistical test, further analyses were conducted separately for 1976-77 and 1977-78.

A one way ANOVA was used to determine if there were seasonal fluctuations in BAP body burdens for each of the two years. There were no seasonal effects during 1976-77 ($F = 0.26 > F.01\ [4,54] = 2.54$) but there were seasonal effects during 1977-78 ($F = 9.36 > F.01\ [5,40] = 2.54$). A Student Newman-Keuls multiple comparison test was used to identify which "seasons" were significantly different. Each of the six sampling dates (i.e., June 1977; December 1977; etc.) was considered to be a season. Only the February 1978 season differed significantly from the other seasons at the 0.01 level. It should be noted, however, that the decline in BAP concentrations during the fall (Fig. 3), while not statistically significant at the 0.01 level (it was significant at the 0.06 level), was characteristic of all the different mussel populations as indicated by the tight clustering of data points.

There are numerous physical and/or chemical factors that could account for the general seasonal trends of 1977-78. Decreases in environmental availability may have occurred as a result of increased BAP photooxidation, during August and September when temperature and hours of sunlight are maximal. A similar explanation may partially account for increases during the winter months when there are prolonged periods with little or no direct sunlight.

Heavy winter rainfall and freshwater runoff from watersheds could contribute to increases in environmental BAP in several ways; rainout of increased levels of atmospheric BAP produced by combustion of fuel products to provide heat; increase runoff through urban sewage treatment facilities which have been shown to be sources of environmental BAP (HARRISON et al. 1975, DUNN & STICH 1975); increase storm sewer runoff from urban areas, introducing crank case oil, residual oils from roads, and related products which

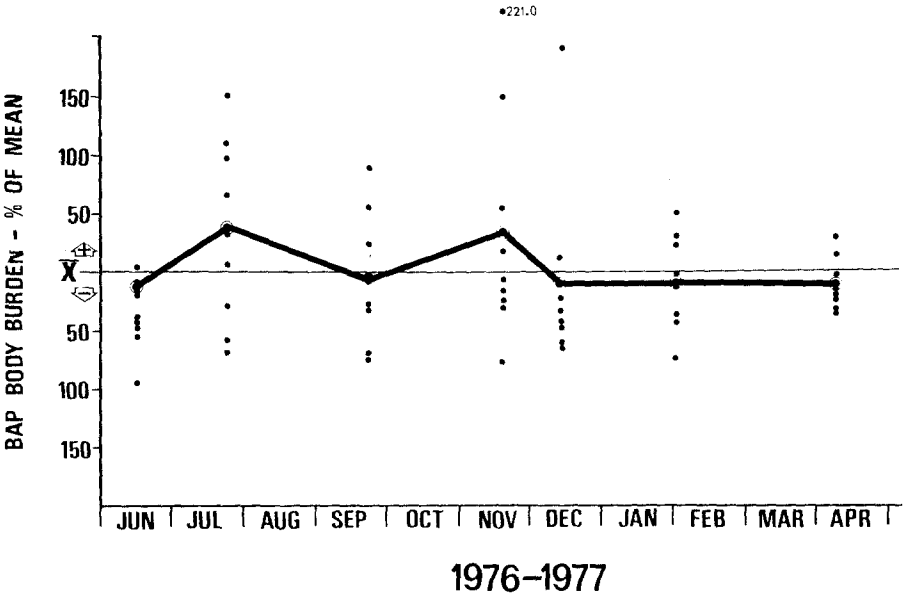

Fig. 2. Seasonal differences in BAP body burdens, expressed as a percentage of the mean, in *M. edulis* during 1976-77. Each point represents a single site, the circled dot is the overall mean.

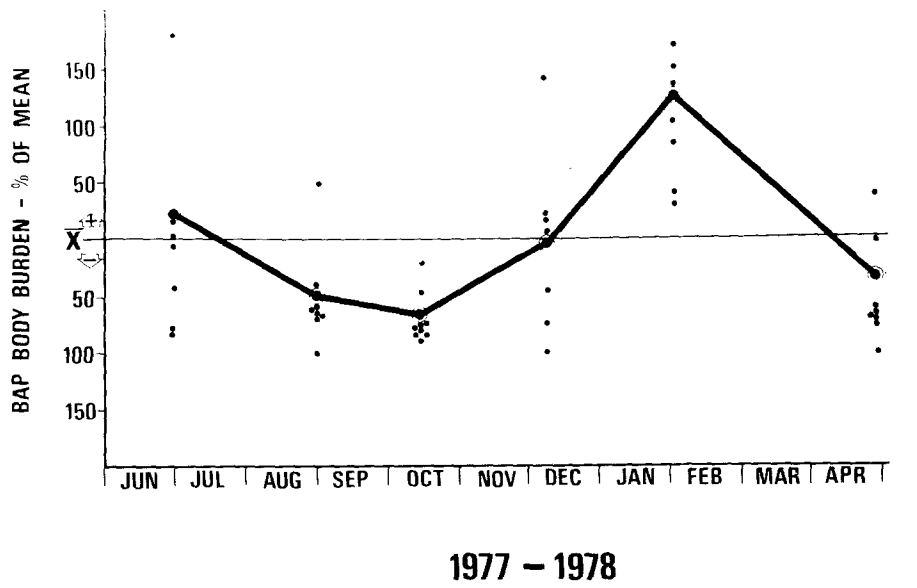

Fig. 3. Seasonal differences in BAP body burdens, expressed as a percentage of the mean, in *M. edulis* during 1977-78.

originate from the operation of motor vehicles (MACKENZIE & HUNTER 1979); transport residual BAP from slashburned areas of the watershed (BLUMER & YOUNGBLOOD 1975); and cause the resuspension of sediments in receiving bodies of water. Sediments serve as sinks for BAP in estuaries; however, sediment-absorbed PNAH may not be readily assimilated by shellfish (NEFF in press).

It is also possible that certain intrinsic biological processes may have accounted for the seasonal differences. It was noted that there was a direct relationship between BAP body burden and the degree of gonadal maturation in *M. edulis* in 1977-78 (MIX 1979). That apparent relationship may have been artefactual since there was no similar relationship in 1976-77. Nevertheless, 1977-78 was a typical year with respect to environmental parameters while 1976-77 was not. If temperature and salinity are involved in synchronizing the spring spawning cycle, then 1977-78 would be expected to be normal with respect to the sexual maturation of mussels.

Finally, temperature and salinity of the immediate environment affect many physiological functions in marine organisms. These factors also affect solubility, adsorption-desorption kinetics, octanol/water partition coefficients, etc. of PNAH in water (NEFF in press). In general, PNAH uptake is greater at reduced temperature, while changes in salinity have little or no effect (e.g. FUCIK & NEFF 1977). Thus, increased BAP concentrations in mussels during the winter may simply be a passive process related to lower water temperature and not necessarily to an increase in environmental BAP or incorporation via lipid synthesizing pathways utilized during gametogenesis.

In summary, we found a dependence of BAP accumulation upon geographical location of mussels in Yaquina Bay. Although the sources of BAP are not yet known, it is felt that point sources such as creosoted pilings, marinas, fish processing factories and boat traffic, contributed. The potential contributions from watersheds will be the subject of future research. There were general seasonal differences in BAP body burdens that may have been associated with increases in environmental BAP, intrinsic physiological factors and/or the effects of exogenous factors on PNAH uptake and incorporation.

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