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CHANGES IN THE LEVELS OF PCBs in *Mytilus edulis* ASSOCIATED WITH DREDGE SPOIL DISPOSAL

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Richard Arimoto
S. Y. Feng

Marine Sciences Institute
University of Connecticut
Groton, Connecticut 06340

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Summary

Blue mussels *Mytilus edulis* L. were used to monitor the levels of polychlorinated biphenyls (PCBs) before, during and after the disposal of dredged material in eastern Long Island Sound. Tissue concentrations of PCBs increased during the disposal operations, but decreased after their cessation. Temporal changes in PCB levels also were correlated with riverine discharge.

Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants described by the formula: $C_{12}Cl_nH_{10-n}$, with n = 1,2,...10. These industrially useful compounds were first manufactured in the United States in 1929, and the highest rates of production were between 1950 and 1970. PCBs were first identified in wildlife samples in 1966 (Jensen, 1966), and their toxicity for humans, birds and other animals in now well documented (Ahmed, 1976).

We have monitored some effects of the dredging operations in the Thames River (Connecticut, U.S.A.) because of the concern that the ocean dumping of dredged material could result in the transfer of PCBs to the commercially important marine fishery resources in the area. As a result of the monitoring program, we can characterize the effects of the dredging and dumping activities on the PCB body burdens of mussels *Mytilus edulis* deployed in the vicinity of the disposal area.

Materials and Methods

To study the possible deleterious effects of the dredging operations, PCB body burdens of *Mytilus edulis* from experimental field populations established on or near the New London CT. disposal area were compared with the PCB levels in mussels from two naturally occuring reference populations (Seaside, CT. and North Dumpling, NY. Fig. 1). Experimental populations were established in March 1977 using mussels collected from the subtidal reference population at North Dumpling. Between 25 and 30 polyethylene mesh bags, each containing 50 M. edulis, were attached to 1 meter high polyvinyl chloride (PVC) platforms (Fig. 2). The platforms with the attached bags of mussels were lowered to the sea floor from the research vessel UCONN at the appropriate dumpsite stations. Samples from all stations were collected by divers.

The mussels were prepared for gas chromatographic analysis by extracting lyophilized tissue for three hours with nanograde petroleum ether (Mallinckrodt Inc. St. Louis, MO.) in soxhlet apparatus. The crude extract was concentrated with Kuderna-Danish apparatus, and interfering substances were removed by liquid chromatography with a Florosil (Fisher Scientific Co. Fair Lawn, NJ.) packed column. The extract was eluted with nanograde hexane (Mallinckrodt) and concentrated before injection into the gas chromatograph.

The samples were analyzed with a Hewlett Packard 7620A gas chromatograph equipped with a Ni^{63} electron capture detector and a 6 foot by 4 mm (i.d.) glass column packed with 3% OV-1 on 100/120 mesh Gas Chrom Q (Applied Science Labs, State College PA). The analyses were done isothermally (injection port = 190°C, detector = 300°C) and required about 45 minutes for the final compounds to elute. The carrier gas was a mixture of 95% argon and 5% methane. The signal from the chromatograph was digitized with a Varian CDS-111C chromatography data system.

We developed a procedure to discriminate PCBs from interfering substances because preliminary analyses of sample chromatograms indicated that not all of the sample peaks were PCBs. For this procedure tolerance limits were calculated to include 99.9% of the PCB peaks at the 95% confidence level (Snedecor and Cochran, 1967). The tolerance limits that were calculated from 5 injections of a composite standard (equal parts of Aroclors 1242, 1254 and 1260; Monsanto Co., dissolved in hexane) linearly increased with retention time (r = 0.987). Thus a least squares regression equation was used to compute the tolerance limits for the peaks in each composite standard used for the daily calibration of the gas chromatograph.

In an attempt to identify the compounds responsible for the spurious peaks, a sample with a particularly large contamination peak, with a retention time of 30 minutes, was submitted to Dr. George Frame of the U.S. Coast Guard Research and Development Center for analysis by gas chromatography coupled with mass spectrometry (GC-MS).

The concentrations of PCBs in the mussel samples were determined by the method of Webb and McCall (1973). For this procedure, the sample chromatograms were divided into three separate areas based on the retention times of the peaks relative to DDE. The response factors that were calculated for three Aroclor standards (Aroclors 1242, 1254 and 1260) were then applied to the appropriate sample peaks. Through the use of this procedure, it was possible to estimate the amounts of the three Aroclors in the samples. We used a one-way analysis of variance to test for differences among the mean levels of the three Aroclors in the samples, and compared the means by Scheffe's procedure. For these analyses we used a Hewlett Packard 9845A desk top computer and a software program for basic statistics (Hewlett Packard, Ft. Collins, CO.).

Further statistical analyses were performed with the use of an

IBM S-370 computer and BMDP programs for two-way analysis of variance (two-way ANOVA) and stepwise regression (BMDP2V and BMDP2R, respectively, Copyrights 1977, The Regents of the University of California). For the two-way ANOVAs, the mussel samples were classified by station and sampling period (during or after dredging). The data for the pre-dredging period were not included in the two-way ANOVAs because no samples were available for Dumpsite 2 and Dumpsite 3. We used the two-way ANOVAs to determine whether the mean PCB levels in the mussels from the five monitoring stations were equal both during and after dredging.

Least squares regression analyses were used to study the influences of two independent variables on the mussels' total PCB and Aroclor body burdens. The independent variables examined were: (1) the volume of material dumped and (2) the rate of discharge of the Thames River. We used the measurements of these two variables that were made for the month prior to that in which the mussel samples were collected because we assumed that the response of the mussels to the influences of river inflow and dredge spoil disposal would not be immediate.

We used stepwise multiple regression to examine the joint influence of river inflow and dredge spoil disposal on the PCB levels in the mussels. The procedure uses a forward stepping algorithm to enter the independent variable that has the highest absolute correlation with the dependent variable into a regression equation. It then sequentially adds variables based on their partial correlation coefficients, and at each step re-examines the variables that previously have been incorporated into the equation. If the contribution of any variable is rendered insignificant by the subsequent addition of other independent variables, it is removed from the model. The F's-to-remove, or partial F-tests (Draper and Smith, 1966), computed by the stepwise regression procedure are useful for estimating the relative importance of the independent variables and also reflect the statistical accuracy with which the regression coefficients are estimated (Dixon and Brown, 1979).

Data for the volume of material dredged and dumped (10^3 yds^3 $\pm$ 10%) were obtained from the U.S. Army Corp of Engineers, Waltham, MA. Information on the rate of Thames River discharge (ft^3/sec) was obtained from the U.S. Department of the Interior, Geological Survey, Hartford, CT.

Results

The GC-MS analysis of the contaminated sample revealed the presence of phthalate esters (plasticizers), silicones and several unidentified compounds. Phthalate esters previously have been detected in marine organisms (Giam et al., 1978); thus the mussels we analyzed may have accumulated these compounds from the environment. However the plasticizers and other compounds may have been contaminants introduced during the preparation of the samples. Unidentified gas chromatographic peaks also have been reported in shellfish analyzed by the Mussel Watch Programme (Goldberg et al., 1978).

The levels of PCBs in the mussels from the 5 monitoring populations are presented in Table 1. The mean levels of the peaks classified as Aroclors 1242, 1254 and 1260 differed significantly ($F_{(2,138)} = 121.3$, $p < 0.0001$), and a comparison of the means using Scheffe's test indicated that the concentration of 1254 was highest, with 1242 intermediate and

1260 lowest (Fig. 3). It should be noted, however, that analyses of composite standards showed that the Webb and McCall (1973) procedure consistently overestimated the amount of 1254 relative to the two other Arclors.

The total PCB and Aroclor concentrations were higher during the disposal operations than after the completion of the project (Table 1). Of the five monitoring populations, only Dumpsite 3 did not have higher PCB levels during dumping. For the other stations, the pattern of elevated levels of PCBs during the dredging period was consistent for the three Aroclors as well as for the total PCB body burdens. The differences in the levels of 1254 and total PCBs during the two sampling periods were significant at $p = 0.07$, but the differences in the levels of 1242 and 1260 were not significant (Table 2). In addition, the two-way ANOVAs clearly showed that there were no significant differences among the five monitoring populations' total PCB or Aroclor concentrations.

Linear regression analyses showed that the total PCB levels in the mussels linearly increased with both the volume of spoil dumped (Fig. 4), and the inflow from the Thames River (Fig. 5). Stepwise multiple regression (Table 3) showed that both of the independent variables significantly contributed to the changes in the body burdens of Aroclors 1242, 1254 and total PCBs. Further analyses of the regression models indicated that the magnitudes of the contributions of the two independent variables were equal. Conversely the body burdens of Aroclor 1260 evidently were related neither to river discharge nor the dredging/disposal operations.

Discussion

The main objective of our monitoring program was to determine whether the disposal of dredge spoils would increase the levels of PCBs in experimental field populations of mussels that were deployed on or near the New London dumpsite. We found that the PCB concentrations in the mussels from the dumpsite did not differ significantly from those from the reference sites. Thus the factors that were responsible for the temporal variability in the mussels' PCB body burdens affected not only the dumpsite populations, but also the reference populations.

Our data indicate that the dumping of the dredged material influenced the PCB levels in the mussels, but that other factors which we either did not measure or did not identify were more important for controlling the levels of PCBs. Three lines of evidence support this contention. First the mean PCB levels in four of the five monitoring populations were higher during the disposal period than after dumping. Second regression analyses indicated that the temporal changes in the PCB body burdens were equally influenced by the volume of material dumped and by river discharge. Third even though the regression coefficients for the two independent variables were clearly significant, the models could only account for about one-third of the variance in the PCB levels; thus most of the variance must have been caused by factors not included in the models.

The PCB levels in marine sediments, as well as in mussels, appear to be primarily controlled by factors other than the dumping of dredged material. Chytalo (1979) analyzed sediments from three dumpsites and three reference areas in Long Island Sound, and reported that in general the sediments from the dumpsites were more highly contaminated with PCBs than those from the control sites. However the differences were not statistically significant. She also observed a gradient in the levels of Aroclor 1254 which increased from east to west. Samples from the New London dumpsite had a mean concentration of 0.14 ng Aroclor 1254/g dry sediment.

Our analyses indicated that the rate of discharge from the Thames River had an influence on the PCB levels in the mussels that was equal to that of dredging. As riverine discharge has been suggested to be a major source of PCBs for other estuarine ecosystems (Dexter and Pavlou, 1979), it is likely that PCBs associated with fluvial material were eventually incorporated in the tissues of the mussels.

PCBs are known to be strongly sorbed to suspended material (Dexter and Pavlou, 1979), phytoplankton (Harding and Phillips, 1978) and clay mineral particles (Chytalo, 1979). Mussels are filter feeders that non-selectively ingest particles greater than 2 μm in diameter (Foster-Grant, 1975). There also is evidence that organochlorines accumulate in mussels through the ingestion of contaminated particles (Roberts, 1972). Based on this information, we propose a probable mechanism for PCB uptake by mussels. PCBs are associated with particulate material that is ingested by the animals. As the particles are processed in the digestive system, the PCBs eventually reach equilibrium with the tissues. From there the PCBs may be transported to other tissues in which other processes, including direct partitioning, may affect the levels. Preliminary results from our laboratory (Arimoto, unpublished) support this uptake mechanism. PCBs have been found to be most highly concentrated in the digestive tissues of the animals, and fecal material has been shown to have levels of PCBs that are equal to those of the digestive tissues.

Suspended material concentrations in Long Island Sound normally range from 2 to 7 mg/liter (Riley, 1959). At these concentrations, mussels ingest most of the particles larger than 2 μm, although some may be rejected as pseudofeces in the upper part of the range (Widdows et al., 1978). Thus the changes in the character of the suspended material field caused by disposal operations and river discharge may have been responsible in part for the variability in the PCB concentrations in the mussels. As the suspended material field in the Sound in strongly influenced by windstress and storms (Bohlen, 1975), these meteorological factors also may have contributed to the temporal changes in PCB body burdens.

Although the PCB levels in the mussels increased during the disposal operations, the increase was transient, and the levels remained well below those established by the U.S. Food and Drug Administration. Clearly more information on the response of the suspended material field to dumping, river discharge and storms, and on the mechanisms of PCB uptake are required to fully assess the effects of dredge spoil disposal on the pollutant levels in mussels. Even without this information, our monitoring program provides a practical means to measure changes in pollutant levels that result from dredging operations, and easily could be adapted for other marine monitoring programs.

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Table 1

Total PCB and Aroclor concentrations in *Mytilus edulis*.
Concentrations are expressed in ng PCB/g dry tissue.

Type of PCB	Dumpsite 1		Dumpsite 2		Dumpsite 3		Seaside		North Dumpling	
	During	After	During	After	During	After	During	After	During	After
Total PCB	804	587	687	505	515	589	714	513	695	482
Aroclor 1242	360	216	226	195	208	296	283	177	250	138
Aroclor 1254	410	360	428	297	293	256	403	321	416	321
Aroclor 1260	34	11	34	13	13	37	28	14	28	24

During dredging: Jul. 77-Jun. 78.
After dredging: Jul. 78-Apr. 79.

Table 2

Summary of the two-way analyses of variance for 4 dependent variables. Data for each dependent variable were grouped by: (1) station and (2) sampling period.

Grouping Variable	Dependent Variable			
	Total PCB	Aroclor 1242	Aroclor 1254	Aroclor 1260
Station[1]	$F = 0.37$, n.s.[3]	$F = 0.58$, n.s.	$F = 0.92$, n.s.	$F = 0.12$, n.s.
Sampling Period[2] (During or After)	$F = 3.51$, $p = 0.07$	$F = 1.94$, n.s.	$F = 3.60$, p 0.07	$F = 1.75$, n.s.

1. Degrees of freedom for station means = 4,34.
2. Degrees of freedom for sampling period means = 1, 34.
3. n.s. = not significant.

Table 3

Stepwise regression analyses. The response of total PCB and Aroclor levels in Mytilus edulis to river discharge and the volume of spoils dumped.

Dependent Variable	Independent Variables				Total R^2
	River Discharge		Volume Dumped		
	F-to-remove	R^2	F-to-remove	R^2	
Total PCB	12.21	.19	11.42	.17	.36
Aroclor 1242	10.84	.18	8.99	.11	.29
Aroclor 1254	6.02	.11	6.52	.12	.23
Aroclor 1260	not entered*		not entered*		---

*The computer did not enter either river discharge or the volume of spoils dumped into a regression equation for Aroclor 1260 because there was no significant relationship between the variables.

Figure 1. Station locations. Polychlorinated biphenyl (PCB) concentrations were determined for *Mytilus edulis* from experimental dumpsite populations D1, D2 and D3 and for mussels from reference populations at North Dumpling, NY and Seaside, CT, U.S.A. The Thames River separates the cities of New London, CT and Groton, CT.

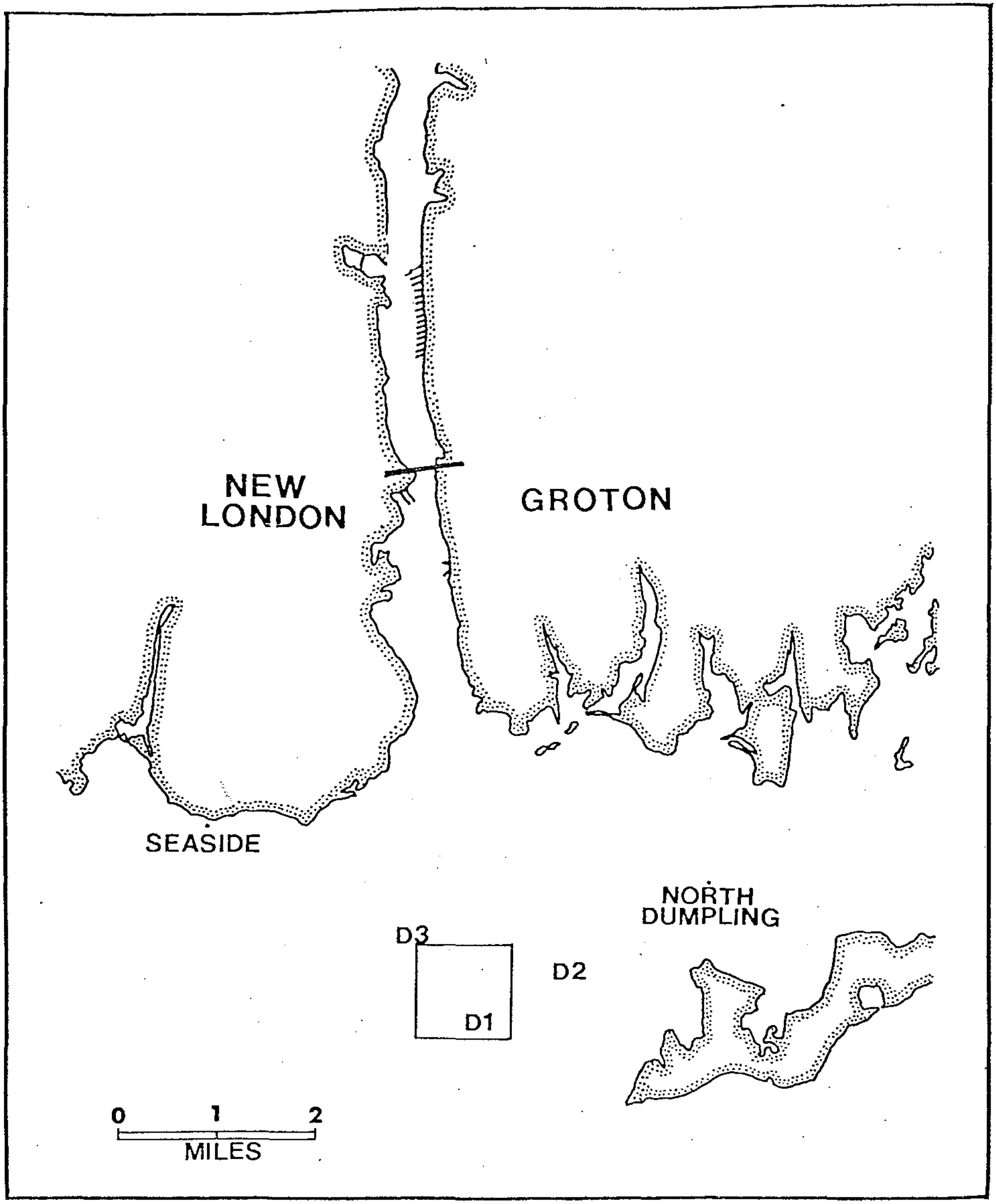

NEW LONDON
GROTON
SEASIDE
NORTH DUMPLING
D3
D2
D1
0
1
2
MILES

Figure 2. Schematic representation of the mussel platforms. The drawing is not to scale. The platforms are one meter high and are in approximately 20 meters of water.

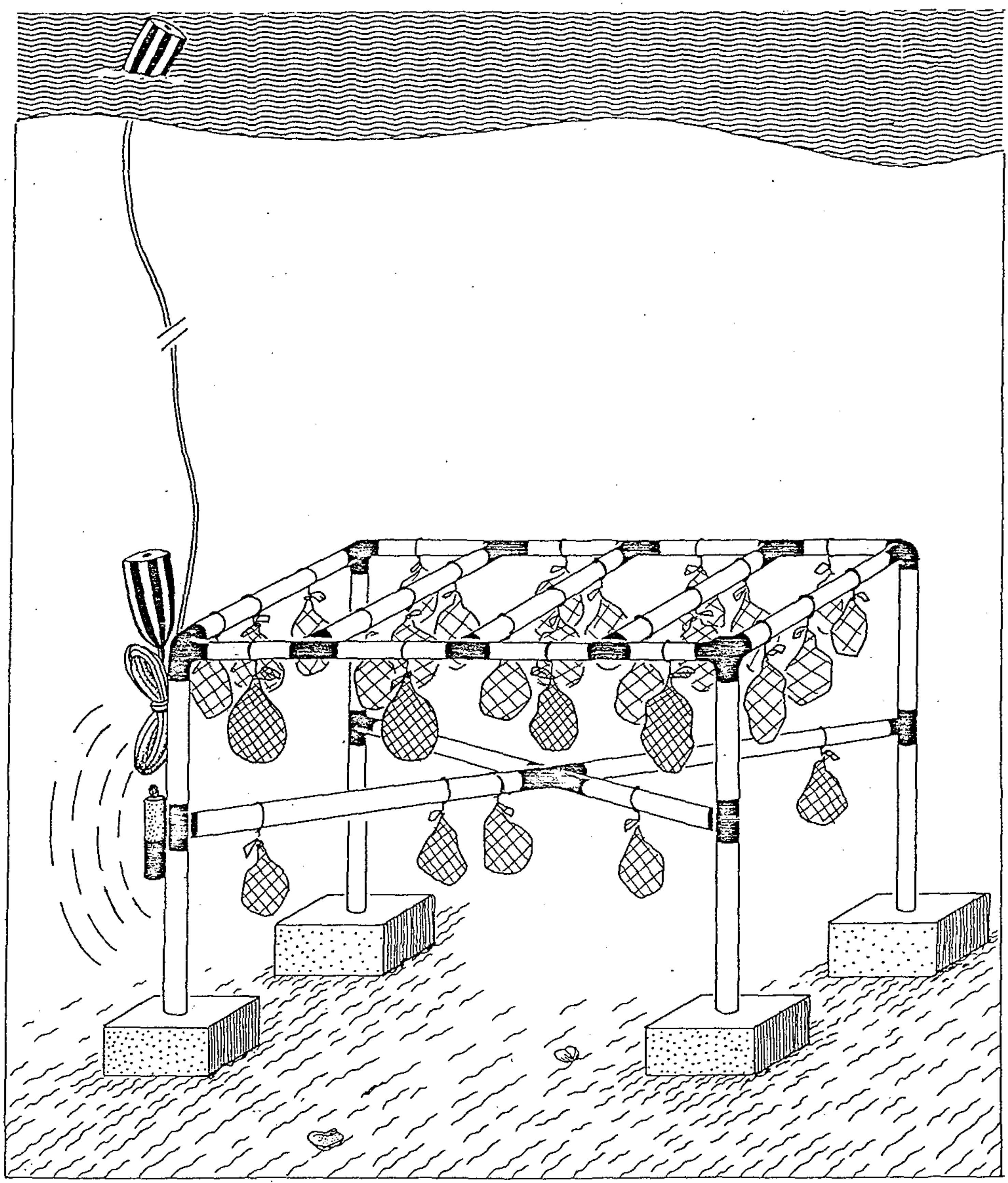


Figure 3. The mean levels of Aroclors 1242, 1254 and 1260 in the mussel samples. The vertical error bars represent $\pm$ one standard deviation.

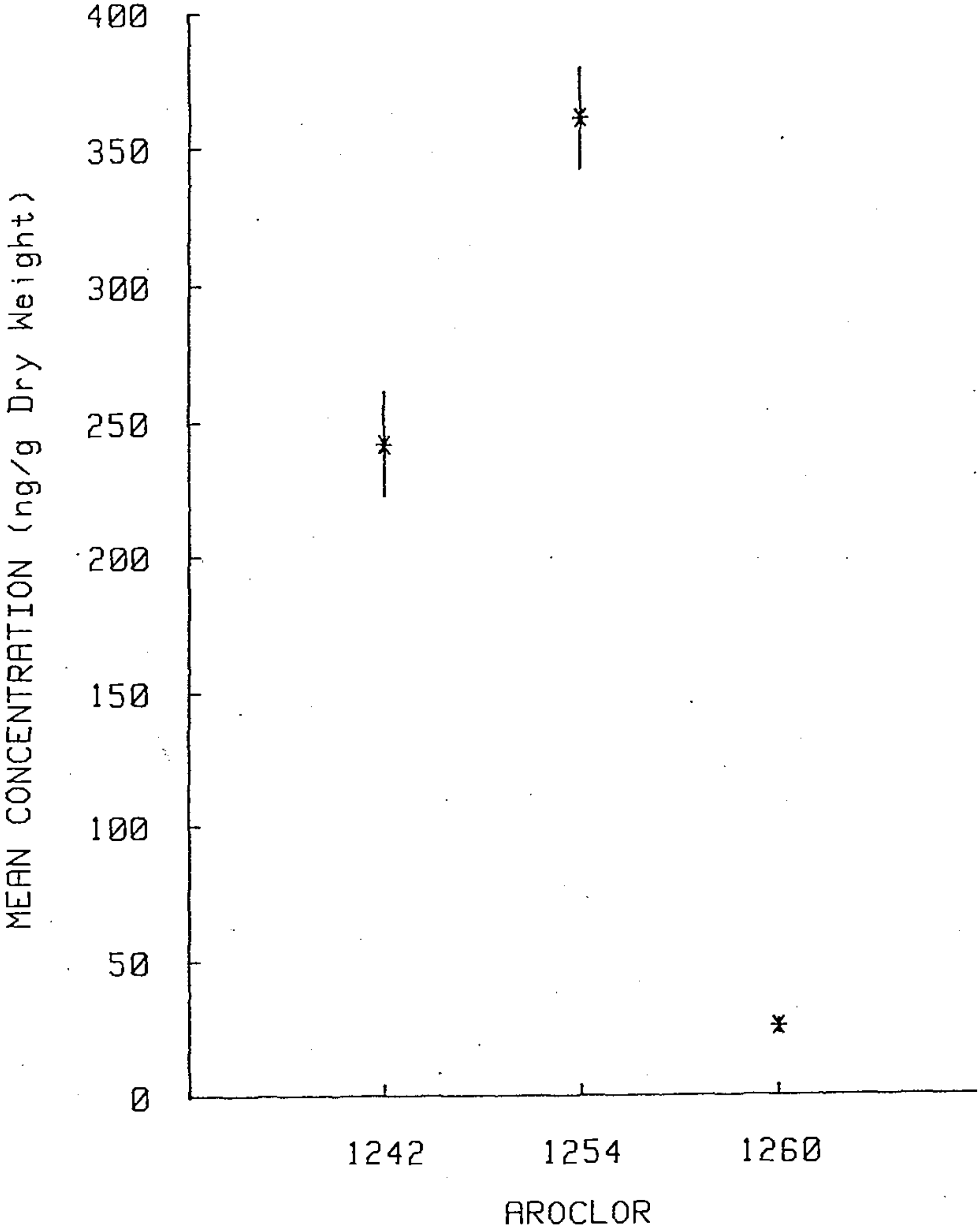
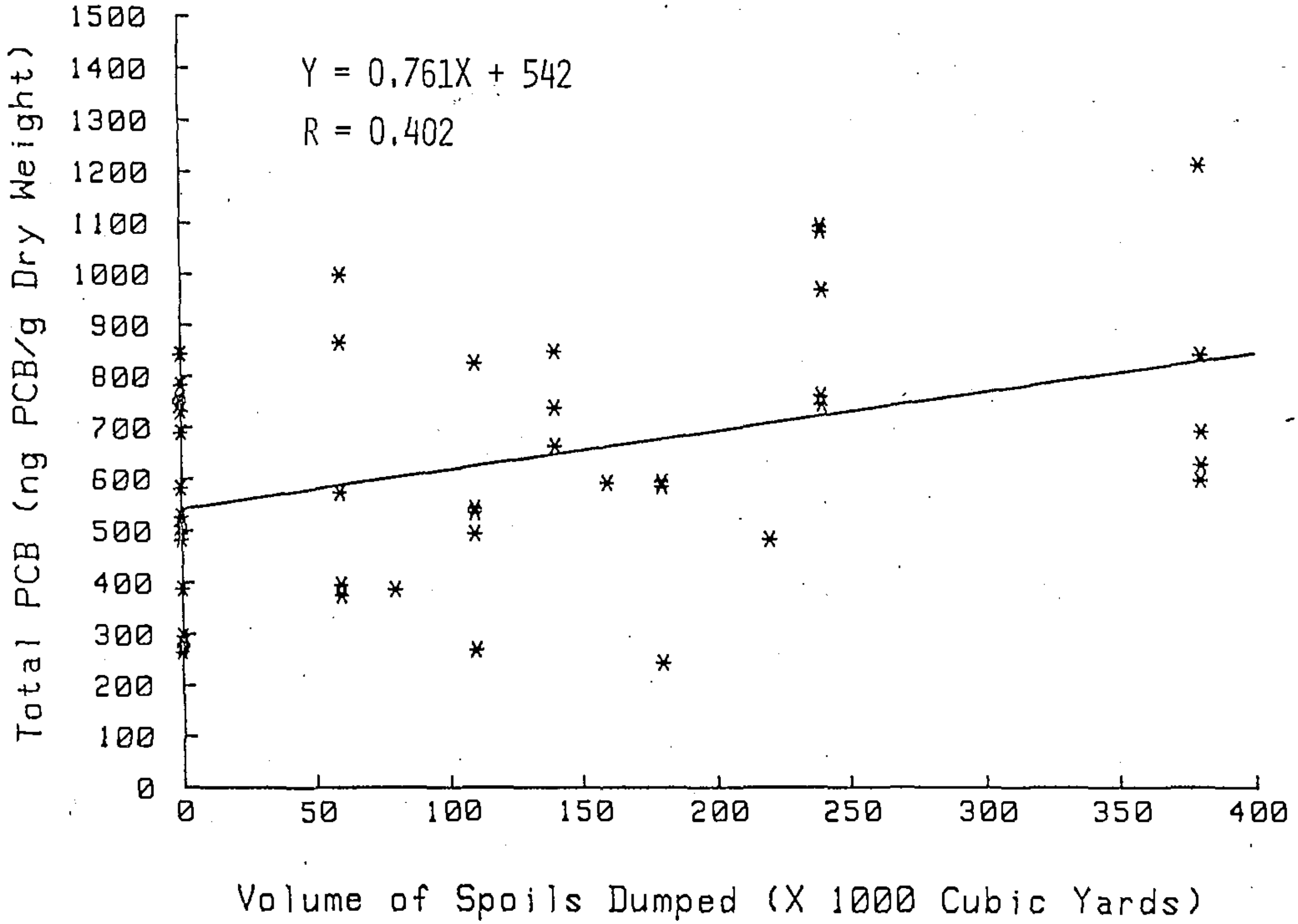
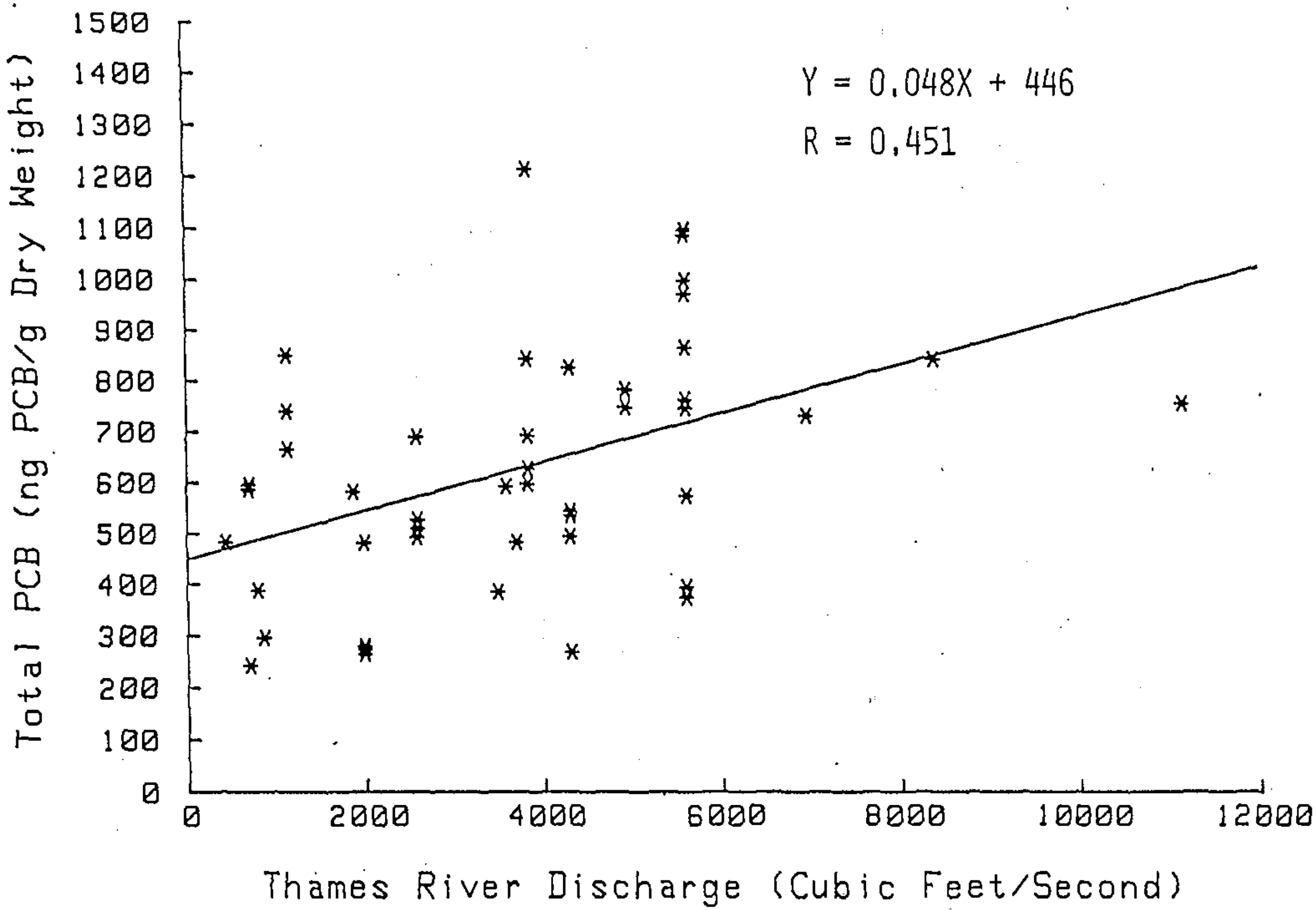

MEAN CONCENTRATION (ng/g Dry Weight)
400
350
300
250
200
150
100
50
0
1242
1254
1260
AROCLOR

Figure 4. Least squares regression of total PCB concentrations in *Mytilus edulis* on the volume of dredge spoils dumped.



Y = 0.761X + 542
R = 0.402
Total PCB (ng PCB/g Dry Weight)
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
0
50
100
150
200
250
300
350
400
Volume of Spoils Dumped (X 1000 Cubic Yards)

Figure 5. Least squares regression of total PCB concentrations in *Mytilus edulis* on the rate of Thames River discharge.


Y = 0.048X + 446
R = 0.451
Total PCB (ng PCB/g Dry Weight)
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
2000
4000
6000
8000
10000
12000
Thames River Discharge (Cubic Feet/Second)