1997

Body burden of polycyclic aromatic hydrocarbons and tributyltin in hard clams, Mercenaria mercenaria from the Elizabeth River, Va

Morris H. Roberts
Virginia Institute of Marine Science

Michael A. Unger
Virginia Institute of Marine Science

A Report to the
Virginia Marine Resources Commission

BODY BURDEN OF POLYCYCLIC AROMATIC HYDROCARBONS AND TRIBUTYL TIN IN HARD CLAMS, *MERCENARIA MERCENARIA* FROM THE ELIZABETH RIVER, VA

Morris H. Roberts, Jr. and Michael A. Unger
Virginia Institute of Marine Science

in collaboration with

Robert E. Croonenberghs
Division of Shellfish Sanitation

[1997]

The Elizabeth River, contaminated with PAH, TBT, and heavy metals, is potentially home to a variety of estuarine invertebrates of commercial importance, notably oysters, hard clams, and crabs. Harvestable oysters have virtually disappeared from the system, but it has been rumored among commercial fishermen that a population of small (little neck to cherrystone) hard clams (*Mercenaria mercenaria*) exists within the Elizabeth River system. These clams are of serious interest to commercial clammers in the area as a previously unharvested source of market-size clams. Crabs of a harvestable size are also reasonably abundant throughout this river according to local fishermen.

The Elizabeth River is presently closed to any type of commercial bivalve harvest because of bacterial and chemical contamination. Chemical contaminants of concern include heavy metals (including organotin compounds), pesticides, and PAHs. If these factors, bacterial or chemical, were shown to pose no human health risk after suitable depuration, the river could conceivably be opened to the taking of bivalve species.

Presently, local residents are believed to engage in subsistence harvest of blue crabs and potentially clams, raising questions about the human health risk associated with such consumption. Residents engaging is such a practice are at some risk of developing various water-borne diseases (from bacterial contamination) or toxic effects, including cancer, from exposure to chemical contaminants. The risk of developing cancer from exposure to chemical contaminants is a function of body burdens accumulated by the animals, the probable exposure from eating the animals (which requires information on consumption rates), and the risk of cancer associated with that level of exposure. A key piece of information is the body burden of chemicals in the edible portions of food animals.

We focused our study described herein on two chemical contaminants of concern, polycyclic aromatic hydrocarbons (PAH, derived largely from creosote spills into the system) and tributyltin (TBT, used in antifoulant paints) in hard clams. At present, there is data regarding the body burden of PAH in blue crabs within the system (Hale, 1988; Mothershead *et al.* 1991), and possibly enough to provide a preliminary estimate of the human risk of crab consumption. Totally lacking are data regarding contaminants in hard clams from the Elizabeth. Data available regarding PAH in oysters transplanted into the Elizabeth River (Bender *et al.*, 1986; Bender *et al.*, 1987) are not directly transferrable to hard clams.

The present study was originally conceived by Robert E. Croonenberghs in 1992 and subsequently implemented after a conversation between M.H. Roberts, R.E. Croonenberghs, and Dr. Venita Newby-Owens concerning the risk of subsistence harvest from the Elizabeth River by residents of Portsmouth. The objective was to estimate body burdens of PAH and TBT in clams from stations throughout the Elizabeth River including at least a preliminary estimate of contaminant variability in meal-sized portions. Secondarily, the data for areal distribution of clams with high body burdens was to be mapped against the known distribution of aqueous or sedimentary contamination for these compounds available from the literature.

Methods:

On 21 and 22 June 1994, one of us (Dr. Robert Croonenberghs, Director of the Division of Shellfish Sanitation) attempted to collect clams from each of 20 stations. These collections were made aboard the F/V Kimberly Dashield captained by Thomas Leggett using typical commercial harvesting gear and techniques. In addition, two reference stations were located in the lower James River, one just east of Fort Wool, and the other southwest of Newport News Point. A total of 16 stations were actually sampled in the Elizabeth River, 6 in the main stem, 1 in the Lafayette River mouth, 4 in the Eastern Branch, 3 in the Western Branch and 2 in the Southern Branch of the Elizabeth River. Four additional stations were planned for sampling in the Southern Branch, but were dropped when no significant numbers of clams were collected in the two most downstream stations in this branch. Sampling depth at each station was generally constant and in the range 8 to 17 ft except at the reference station off Newport News where the depth was 54 ft (Table 1, Fig. 1). Of the clams collected at each station, approximately 40 (only 22 from station ER4) were frozen and submitted to VIMS for analysis. Sufficient clams from which to produce composite samples were provided from the two reference sites plus 12 Elizabeth River stations.

During the collection, careful records were kept of the vessel location at the start and end of each trawl using Loran C. On shipboard, clams were washed with ambient river water to remove contaminated mud before being placed into labeled plastic bags on ice for transport. The harvested clams were taken to the Division's lab in Norfolk, rewashed with fresh water to remove any remaining external contaminants, placed into clean labeled plastic bags, and frozen (unshucked) for submission to VIMS for analysis. Other clams from each site were submitted to DCLS for heavy metal and pesticide analysis (these data are included herein by permission of Dr. Croonenberghs).

There were sufficient clams provided from each station (save one) to produce 3 composite samples of 12 clams each. Since funding was limited, we analyzed one composite sample from each station for PAH and all three composites from each station for TBT.

At VIMS, samples of clams from each station were shucked, homogenized and desiccated in preparation for extraction. An aliquot of one homogenized tissue sample from each station was extracted and analyzed for PAH by gas chromatography (Bieri, *et al.*, 1986; Huggett *et al.*, 1986). Another aliquot was taken from each composite from each station, extracted and analyzed for TBT by gas chromatography (Unger *et al.*, 1986; Rice *et al.* 1987). The remainder from all composite samples has been stored for future analysis should there be a need to examine these or other possible analytes from clam tissues from the Elizabeth River. A separate synoptic sample of clams was analyzed for heavy metals. The clams were digested with nitric acid in sealed vials at low temperature. Lead, copper, chromium, nickel and zinc were analyzed by ICP (EPA Method 200.7); arsenic was measured using a hydride generation method. The detection limits were 1 μg/g tissue for all metals except arsenic. The detection limit for arsenic was 0.25 μg/g tissue.

Huggett, Bender, and Unger (1984) described the distribution of PAH in sediment from the Elizabeth River at a series of stations extending from the mouth to a distance of 25 miles upstream into the Southern Branch. These data represent analyses of samples collected over the period from 1981 to 1983 by grab sampling with a Ponar grab and gravity core. Samples were analyzed by chemists at VIMS using similar protocols to those used for the tissue samples that form the basis for this report. Since the same GC protocols and data analyses were used, the compound identifications are made with a similar degree of confidence. The sediment concentrations are considered to be reasonable surrogates for the exposure concentrations because the PAHs, though little soluble in water, are in equilibrium between sediment and water. The low solubility and high variability of PAHs in water precludes reliable measurement in this matrix.

The distribution of TBT in the water in the Elizabeth River is provided from two primary sources: two sets of samples collected in 1986 and 1995 respectively by one of us (MAU), and a comprehensive set of survey data from the US Navy collected quarterly between 1988 and 1992. Other data sets have been examined, and excluded from further consideration because of high detection limits. Data regarding TBT in the sediment are sparse and might not be interpretable as a surrogate of exposure if contaminated with

paint chips or other materials containing an excess of TBT equilibrium (Unger *et al.* 1988). The water column data is considered to be a reasonable representation of the spatial trend for TBT exposure to clams.

Results and Discussion

Polycyclic Aromatic Hydrocarbons

A single replicate composite sample of clams from each station was analyzed for PAH. The concentration of total resolvable PAH ranged from 150 ng/g tissue for clams from Station EB2 to 8500-8700 in clams from stations ER6 and EB1 (Fig. 2). Clams at both reference stations contained approximately 2000 ng/g tissue. The tissue concentrations in clams from the lower portion of the main stem and the mouths of the Lafayette River and the Western Branch were little different from that in clams from the reference locations. From Station ER4 (off Lovett Pt.) upstream to ER6 there was a progressive increase in concentration from 3500 to 4800 to 7100 to 8700 ng/g. The highest concentration represents a four-fold increase over the concentration in clams from the reference stations.

Stations located within the region potentially subject to subsistence harvest include ER1, LR1, ER3, ER4, WB1, and WB2. The mean total resolvable PAH for these stations is 2589 $\pm$ 900 ng/g which is barely higher than the mean for the reference stations (1975 $\pm$ 175 ng/g).

Based on the assumptions that sediment PAH concentrations are an appropriate surrogate for exposure concentration and that sediment PAH concentrations have remained approximately the same for the past decade, we compared the clam PAH data to available sediment PAH data. The most comprehensive single data set relates to a series of stations tracing the main stem of the Elizabeth River and the Southern Branch at one mile intervals (from Huggett, *et al.*, 1984). That paper lists concentrations for 11 abundant PAHs. For the present comparison, these values were summed, and plotted against distance upstream (in miles) from the mouth to a station in Southern Branch (Fig. 3). The stations that most closely correspond to those in the present study are stations 7 and 8 with ER3, station 9 with ER2, and station 13 with ER5, ER5A and ER6. There is a consistent upward trend in sediment PAH concentration and in clam body burden, the clam generally containing less than the equivalent amount of sediment on a ng/g dry weight basis.

Body burdens in oysters transplanted into the Elizabeth River and exposed for 9 wk at selected stations along the length of the river were included in the same paper (Huggett *et al.*, 1984; Bender *et al.*, 1986; Bender *et al.*, 1987) (Fig 3). These oysters had accumulated approximately 3 to 10 times as much PAH as clams from similar locations in the present study. However, the oyster data is from another bivalve species (*Crassostrea virginica*) collected eight years earlier. Nevertheless, the spatial trends are similar with the highest PAH concentration being observed at the most upstream stations near the confluence of the Eastern and Southern Branches.

Tributyltin

Tributyltin concentrations ranged from a low of 213 μg/kg at station WB2 in the Western Branch of the Elizabeth River to a high of 1633 μg/kg at Station ER6 at the confluence of the Eastern and Southern Branches of the Elizabeth River (Figure 4). Analysis of three replicate samples (twelve animals each) from each location showed that there was little variation between replicate samples and station differences can be compared with confidence.

The general trend in body burden was for the highest concentrations to occur in clams from stations near the confluence of the Eastern and Southern Branches. Concentrations decreased progressively with distance downstream towards the mouth of the main stem and at stations upstream in the major tributaries.

This spatial trend for TBT shown in clam tissue has been shown previously for water column concentrations by researchers at VIMS (Figure 5) and during a long-term monitoring program conducted by the US Navy (Figure 6). To compare results from the Navy's four year monitoring effort, average concentrations for stations within the Elizabeth River were plotted with results from this clam study (Figure 7). Not all stations coincide for both studies but trends can be compared on the basis of distance from the mouth of the river (some station overlap). Similarities between data sets illustrates that homogenized clam tissues can serve as monitors of environmental concentrations and provides evidence that the system is at steady state.

There is little available data on TBT concentrations in shellfish from the Elizabeth River. A previous 1987 VIMS study (Espourteille *et al.*, 1993) analyzed oyster and sediments from the Elizabeth River as well as other locations in Chesapeake Bay. Animals were collected during the 1987 study from several locations

in southern Chesapeake Bay including stations in the Elizabeth River. Feral oysters collected from stations in the Elizabeth River contained the highest TBT concentrations and ranged from 1300 to 5600 μg/kg dry weight. These concentrations are higher than those measured during the present study by a factor of five but are from another species (*Crassostrea virginica*) and are from samples collected eight years earlier before any possible effect of TBT regulations. However, spatial trends seen during this previous study are similar to those observed in the present study, with the highest TBT concentrations in tissues of oysters collected near the confluence of the Southern and Eastern Branches at Hospital Point.

In a recent study designed to evaluate changes in TBT concentrations in Chesapeake Bay oysters since 1987 legislation restricting TBT application, Unger *et al.* (1995) collected oysters from several locations sampled during the 1987 VIMS study (Espourteille *et al.*, 1993). TBT concentrations for all stations ranged from a low of 19 μg/kg dry weight at Chincoteague, Virginia to a high of 3200 μg/kg at Hospital Point. Overall, TBT concentrations were found to be lower than those measured by Espourteille *et al.* in 1987 indicating that legislation has been successful at reducing TBT exposure to these animals. Samples were collected from the Elizabeth River at Lambert Point and Hospital Point in October of 1994. The TBT concentration measured at Hospital Point (3200 μg/kg dry weight) was once again the highest measured during the survey. The sample collected at Lambert's Point contained the next highest concentration at 1600 μg/kg dry weight.

During a 1986 sampling of the Elizabeth River, the US Navy reported TBT concentrations for oysters (*Crassostrea virginica*) that ranged from 550 to 5200 μg/kg as TBTCl wet weight. Since these concentrations are reported on a wet weight basis, they are somewhat higher than those measured by Espourteille *et al.* in 1987. The highest concentration measured 5200 μg/kg (US Navy station 13a) was once again near the confluence of the Southern and Eastern Branches of the river.

Metals in Clam Tissue

While not a part of the present study, a set of clams collected from the same stations and at the same time were analyzed for 6 heavy metals (lead, cadmium, arsenic, chromium, copper, and zinc) by DCLS at the direction of one of us (REC). These data are included here with permission (REC) to show in one place all body burden data derived from this sampling effort.

At all stations, cadmium and chromium in the clam tissues were less than the detection limit (1 μg/g). Lead was at or slightly above the detection limit (1 μg/g) in clams from 7 of 11 stations, reaching a maximum of 2 μg/g in clams from Station EB1 in the Eastern Branch. Arsenic, copper, and zinc were well above the detection limit (0.25, 1.0 and 1.0 μg/g respectively) in clams from all stations. The maximum concentrations of arsenic was 3.5 μg/g observed in clams from Stations ER6 and WB2 and 4.7 μg/g in clams from Station WB1. The maximum concentration of copper was 7 μg/g, observed in clams from Stations ER6 and EB1, though these were not significantly higher values than at many other stations in the system (Copper concentrations were $\geq$5 μg/g in clams from 8 of 11 stations). Zinc was the most abundant metal with concentrations ranging from 24 μg/g in clams from Newport News and Fort Wool to 71 μg/g in clams from Station WB2 in the Western Branch and 68 μg/g in clams from Station ER6 in the main stem (Fig. 8). As with PAH and TBT, the maximum concentrations were observed most often at the confluence of the Eastern and Southern Branches.

Zinc was the most abundant of the metals at every station by approximately an order of magnitude. Zinc was clearly more abundant in clams from the most industrialized reaches of the river. Lead was near detection limits in clams throughout the system, while arsenic and copper showed trends that may be related to specific industry types.

Significance of body burdens for human consumption of clams

There are no federal action levels established for any of the constituents of PAH or for TBT. Therefore one cannot comment on the significance of the concentrations observed if food portions of 8 clams were eaten. The PAH concentrations observed in clams from the downstream Elizabeth River stations are comparable to those observed in clams from Newport News flats and Fort Wool, two areas considered to have a low level of PAH contamination. At the upstream stations of the Elizabeth River, concentrations in the clams were 3 to 4 times those at the most downstream station, but there are no data to indicate what level of risk might be associated with consuming clams contaminated to this level. Similarly for TBT, clams at the upstream stations contained 3-8 times more TBT than clams from the reference stations. As with PAH, there are no data to indicate the potential risk to humans from consumption.

There is guidance from the FDA relating to levels of concern for several heavy metals in foods (Table 2). A comparison of the data for clams collected in this study to FDA levels of concern suggest that there

would be minimal hazard from metals associated with consuming these clams, except for a potential concern for lead exposure for young children and pregnant women eating clams from the upstream end of the Eastern Branch of the Elizabeth River.

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Table 1 List of stations with longitude, latitude and depth for start and end of trawls at station. All samples were collected on 12 or 22 June 1994.

Station Designation	Start of Trawl			End of Trawl		
	Latitude	Longitude	Depth (ft)	Latitude	Longitude	Depth (ft)
NN	36°57.88'	76°27.30'	54	36°57.90'	76°27.30'	54.5
FW	36°59.34'	76°19.60'	17.5	36°59.44	76°19.69'	NA
ER1	36°54.26'	76°21.70'	16	36°54.29'	76°21.66'	15
ER2	36°53.88'	76°21.30'	10-12	36°53.87'	76°21.31'	10-12
ER3	36°53.6'	76°22.54'	NA	36°52.95'	76°22.56'	5-7
ER4	36°52.5'	76°21.57'	16-17	nd		
ER5	36°50.86'	76°17.84'	17	36°50.86'	76°17.77'	NA
ER6	36°50.19'	76°17.84'	22	36°50.30'	76°16.5'	NA
LR1	36°54.51'	76°20.68'	9	36°54.49'	76°20.65'	10
WB1	36°51.58'	76°22.57'	21-22	nd		
WB2	36°50.69'	76°23.33'	8-9	36°50.68'	76°23.31'	NA
WB3	No clams found					
EB1	36°50.53'	76°18.28'	-2	nd		
EB2	36°50.66'	76°17.53'	8	36°50.54'	76°18.24'	NA
EB3	No clams found					
EBFP	Small oysters observed at RR Bridge above Campostella Bridge					
SB17	Small oysters observed at the Jordan Bridge, no clams found					
SB18	No clams found					

Table 2 Levels of Concern for Metals in Shellfish (in $\mu g/g$) from FDA Guidance.

Population Category	Lead[1]	Cadmium[2]	Arsenic[3]	Chromium[4]
Children, 2-5 yr	1.5	6	130	20
Pregnant Women	2.1			
Adults, 18-44	6.3	5	110	17

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1 Data from *Guidance Document for Lead in Shellfish*, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 1993.

2 Data from *Guidance Document for Cadmium in Shellfish*, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 1993.

3 Data from *Guidance Document for Arsenic in Shellfish*, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 1993.

4 Data from *Guidance Document for Chromium in Shellfish*, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 1993.

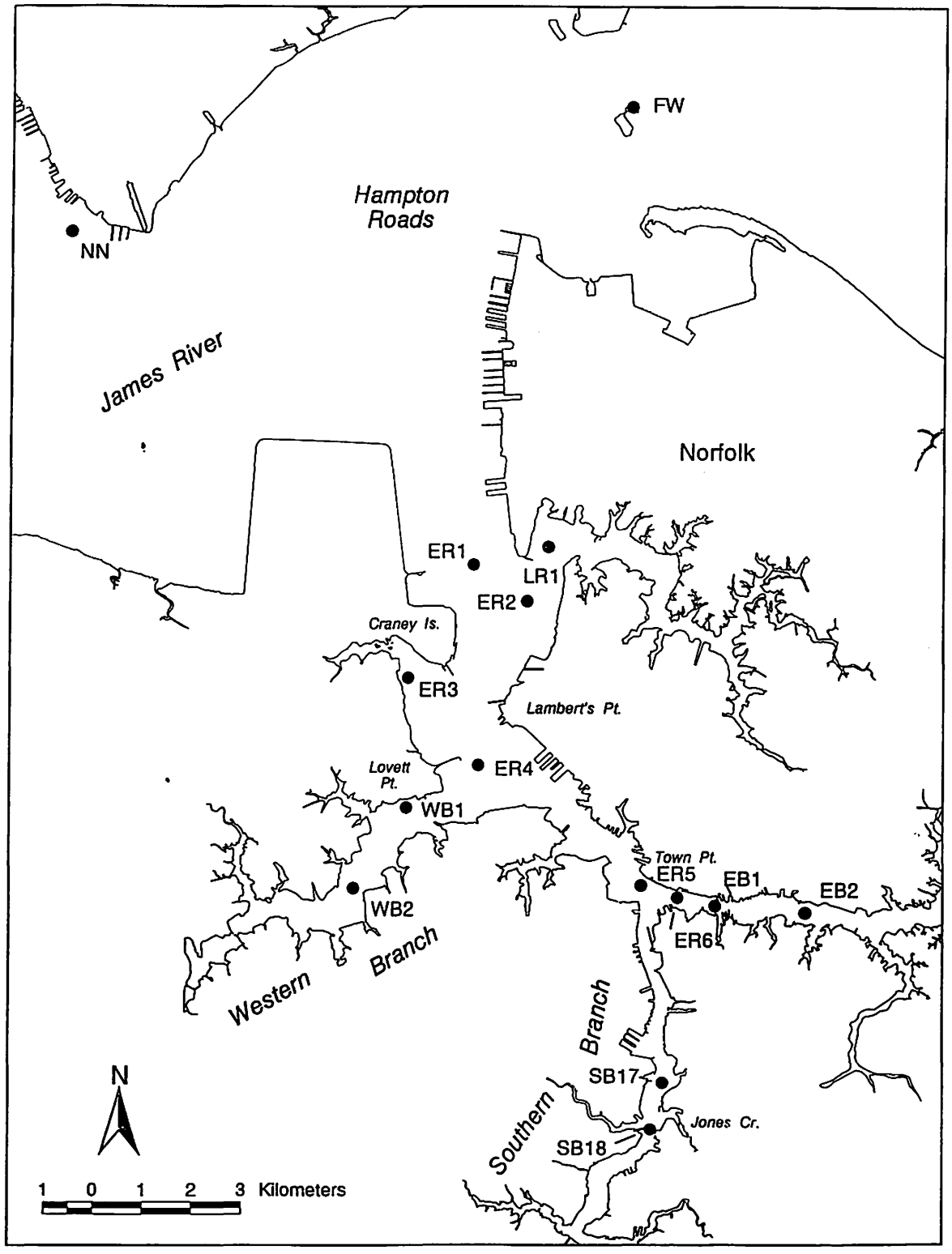

Figure 1 Map of the Elizabeth River and mouth of the James River. Clams were collected at all stations except those in the Southern Branch (SB17 and SB18). The stations in the lower James River serve as reference points for the Elizabeth River stations.

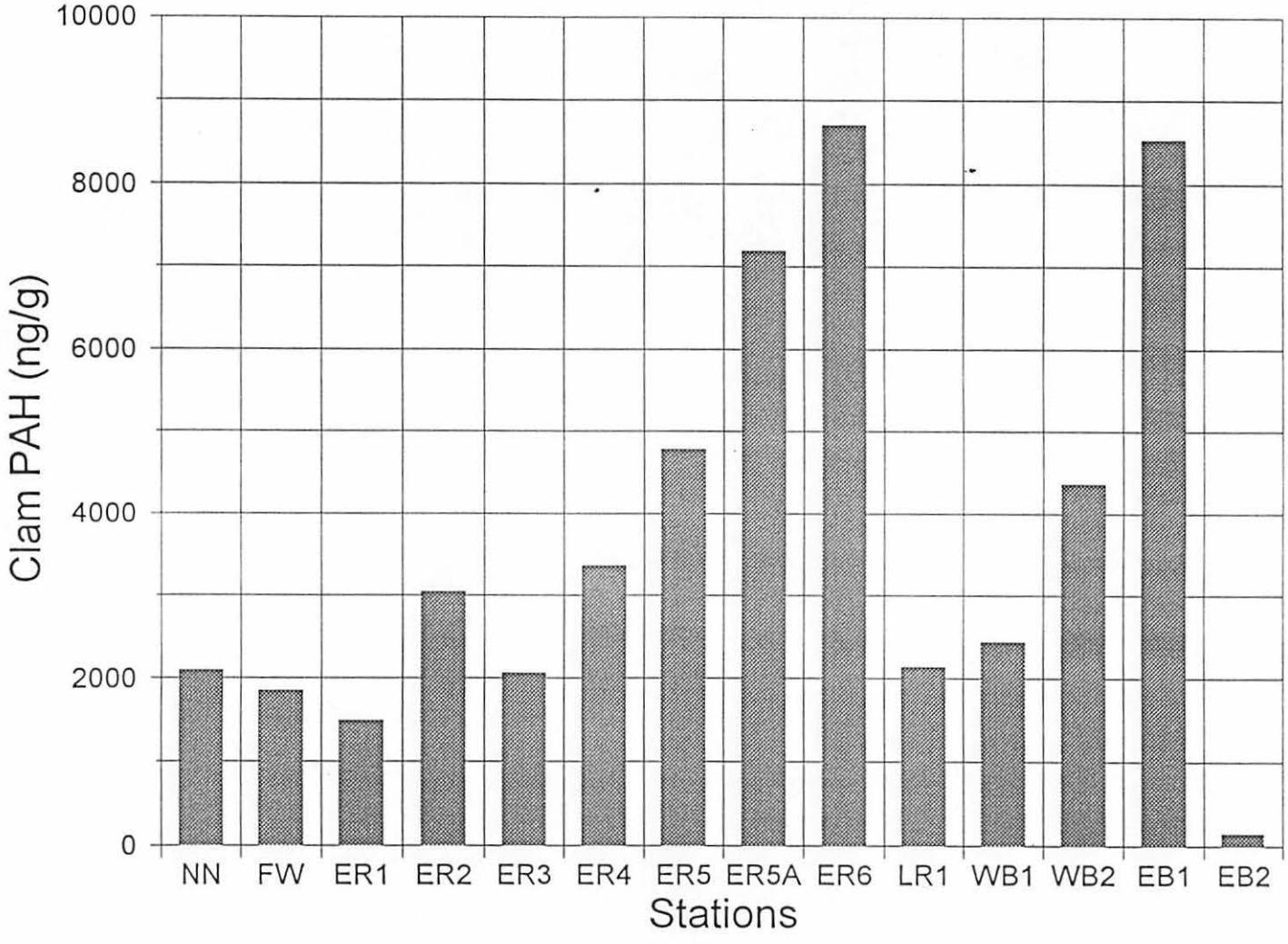

Figure 2 The concentration of total resolvable PAH in composite samples (8 clams per sample).

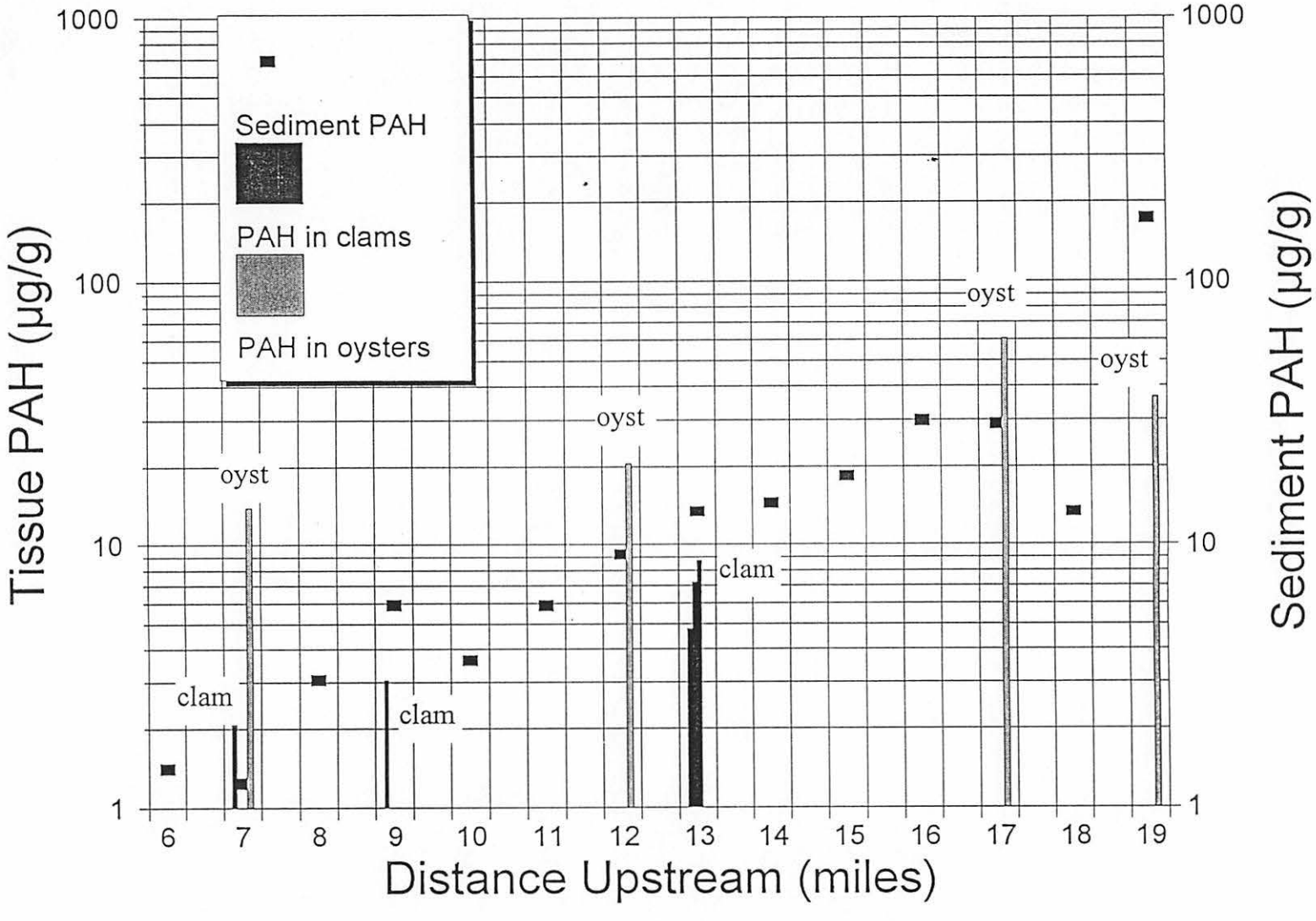

Figure 3 A comparison of PAH concentrations in clam tissue, oyster tissue (data from Huggett *et al.*, 1985; Bender *et al.*, 1986; Bender *et al.*, 1987), and sediment (data from Huggett *et al.*, 1984) as a function of distance upstream in the Elizabeth River.

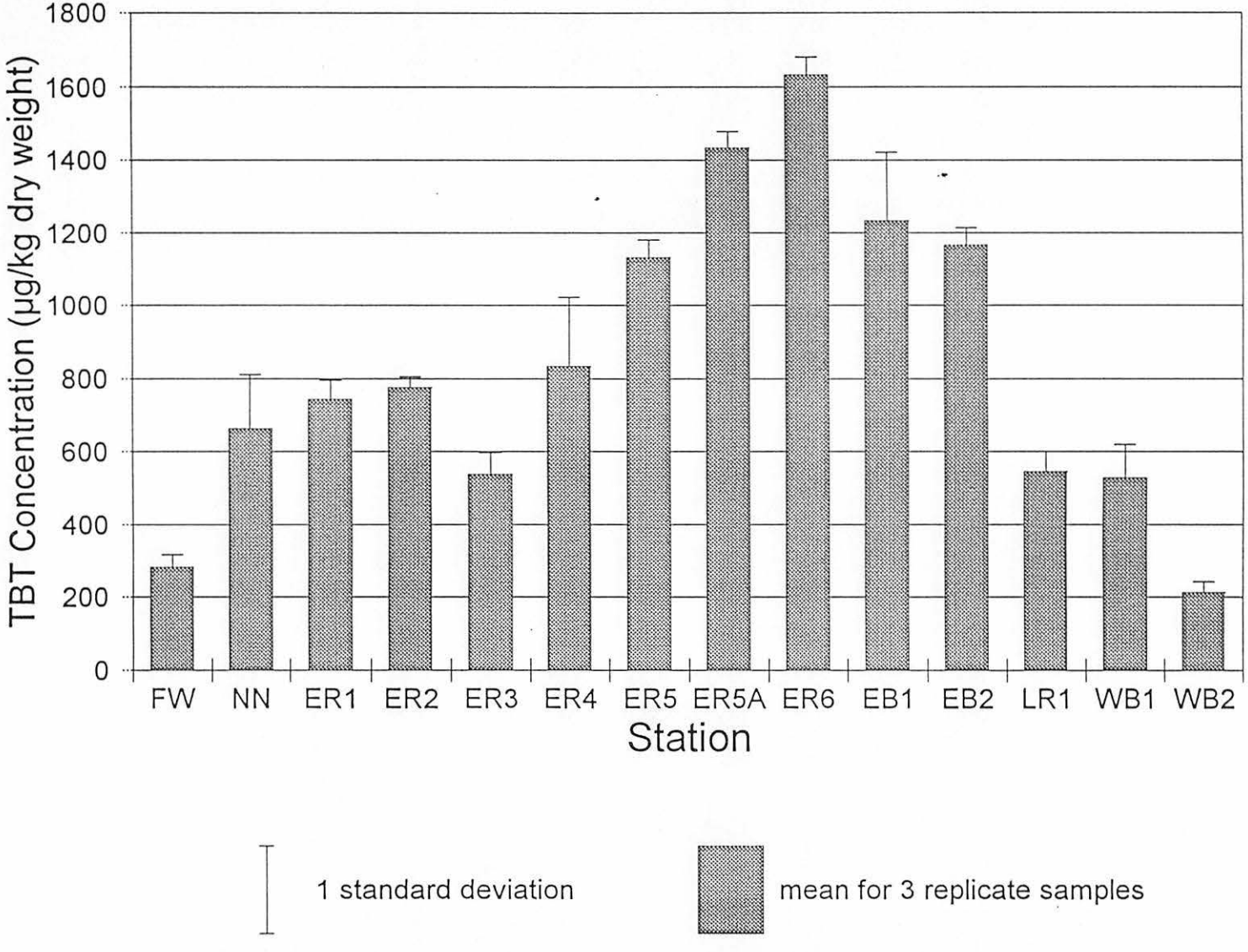

Figure 4 The concentration of TBT in clam tissue composite samples arrayed against station. The bar represents the mean of measurements of three replicate composite samples. The vertical line represents 1 standard deviation.

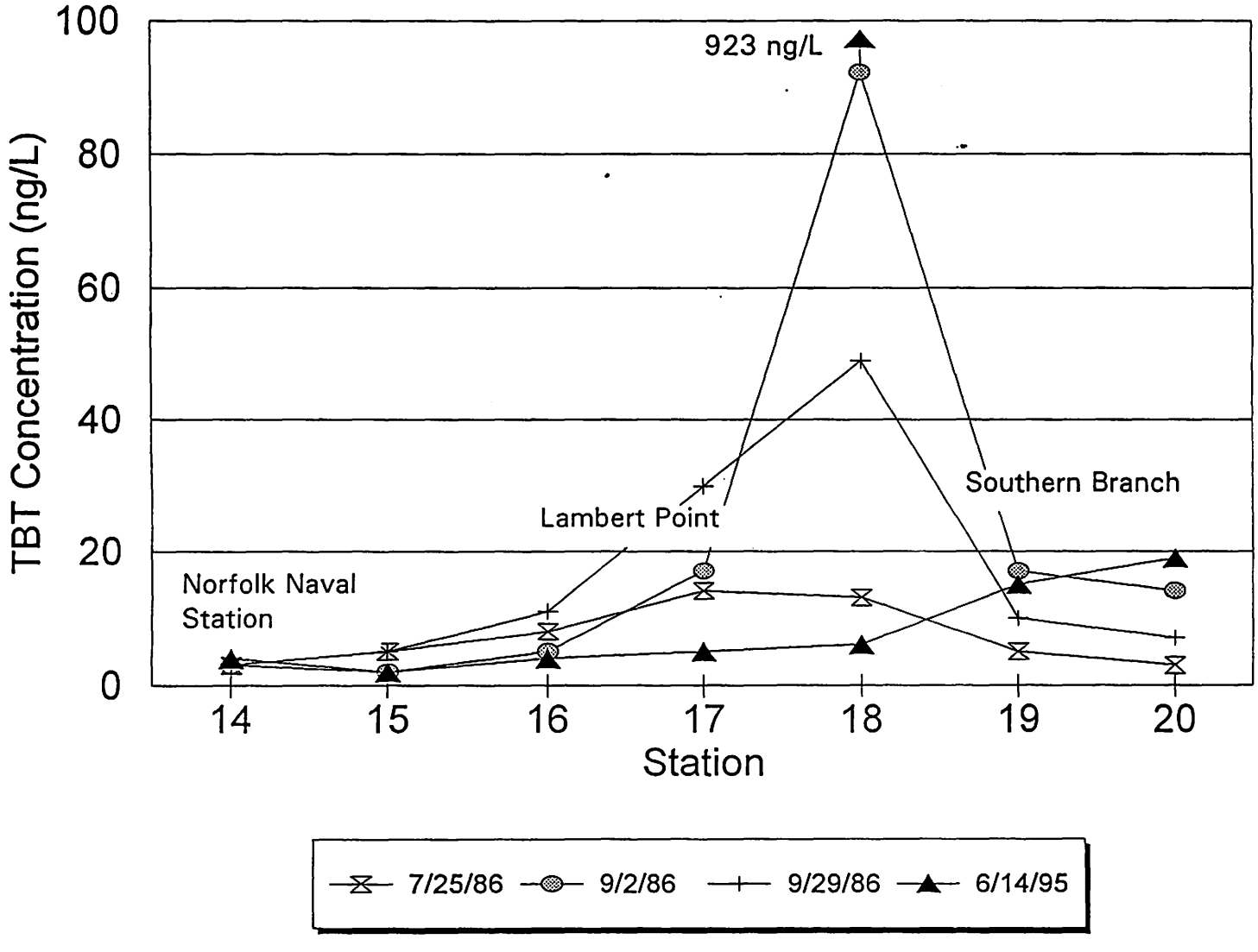

Figure 5 Monitoring data for TBT in water collected by VIMS in 1986 and 1995.

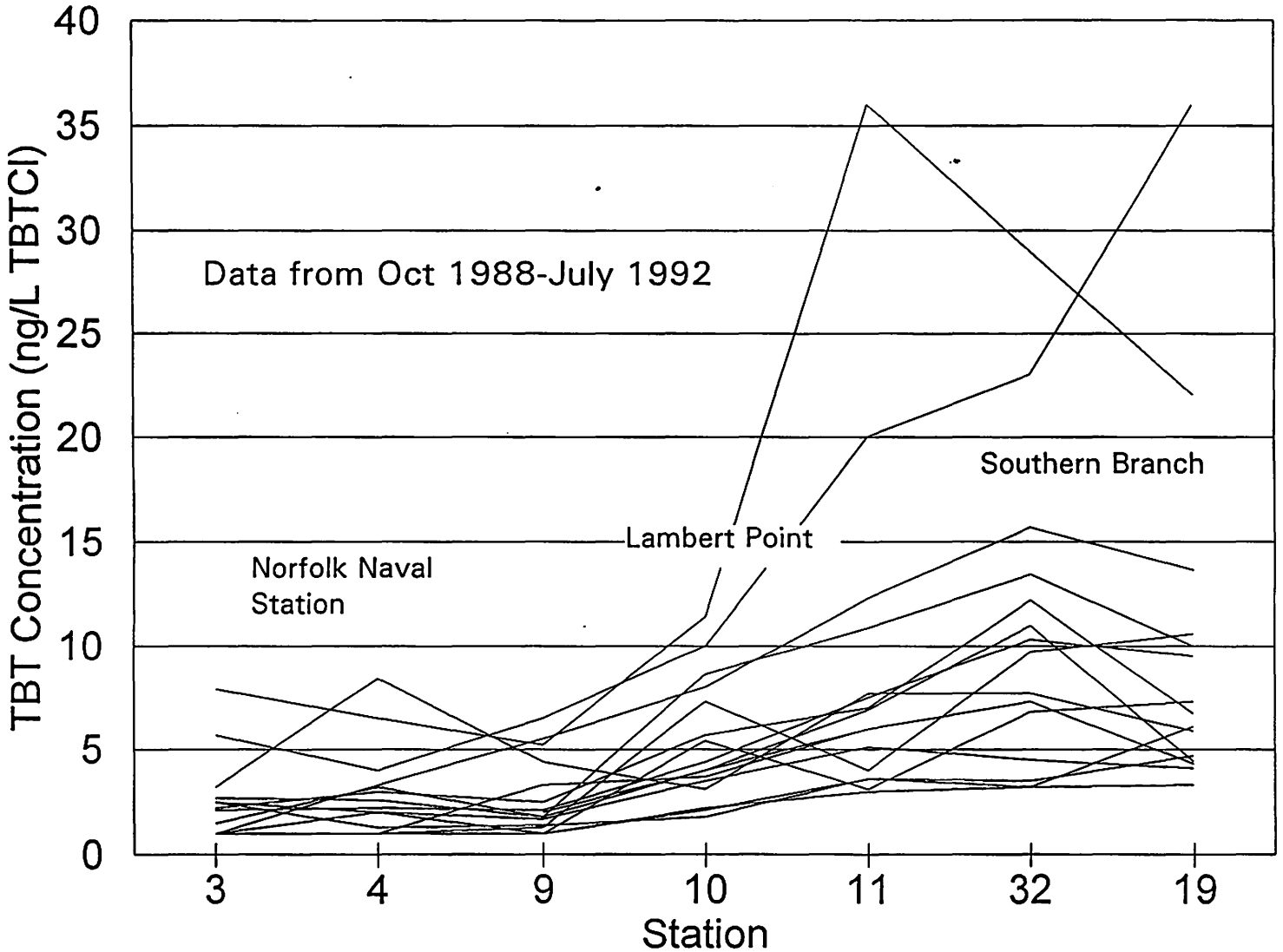

Figure 6 Monitoring data for TBT in water collected by the U.S. Navy between October 1988 and July 1992.

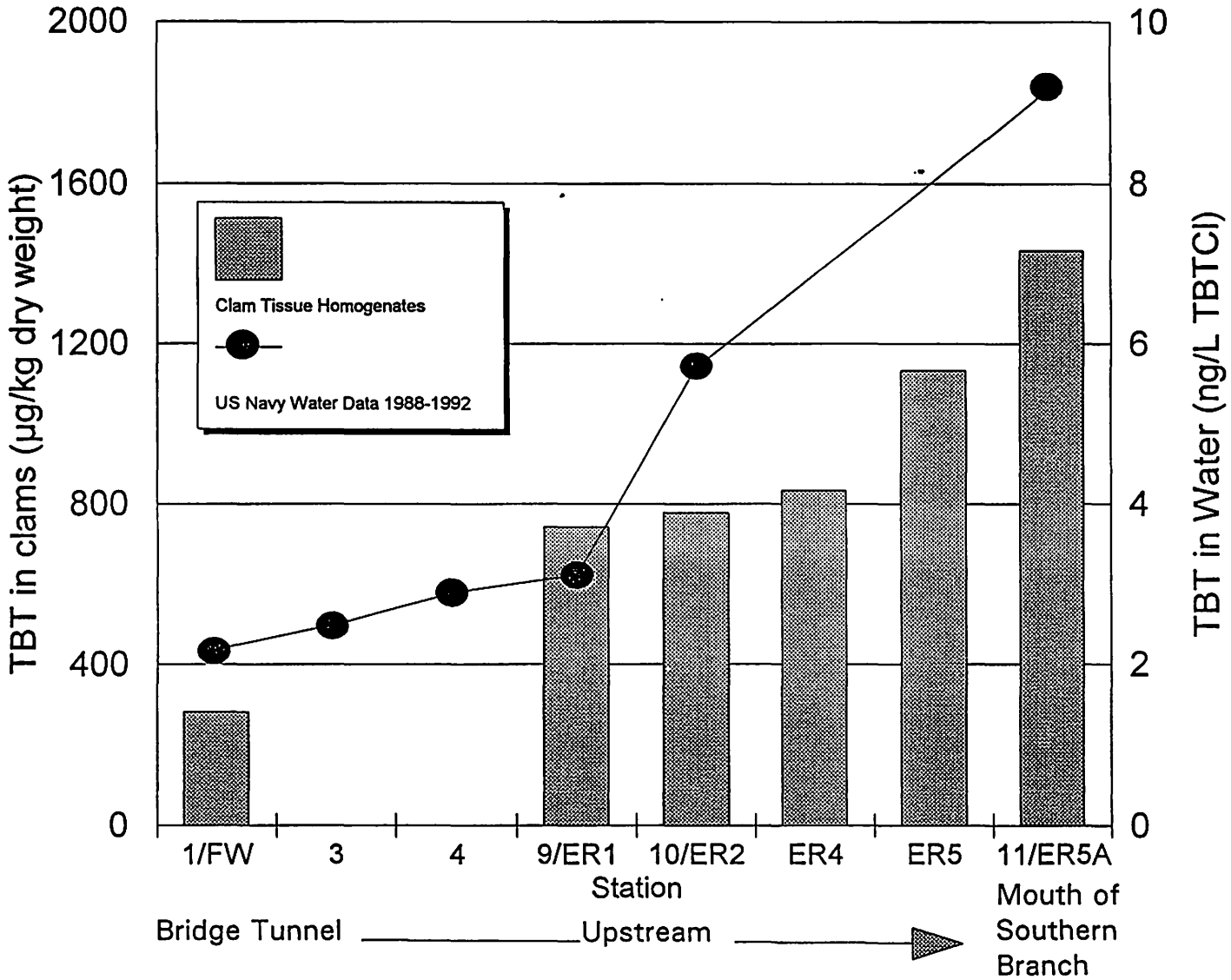

Figure 7 A comparison of TBT in clam tissue and in water from the Elizabeth River.

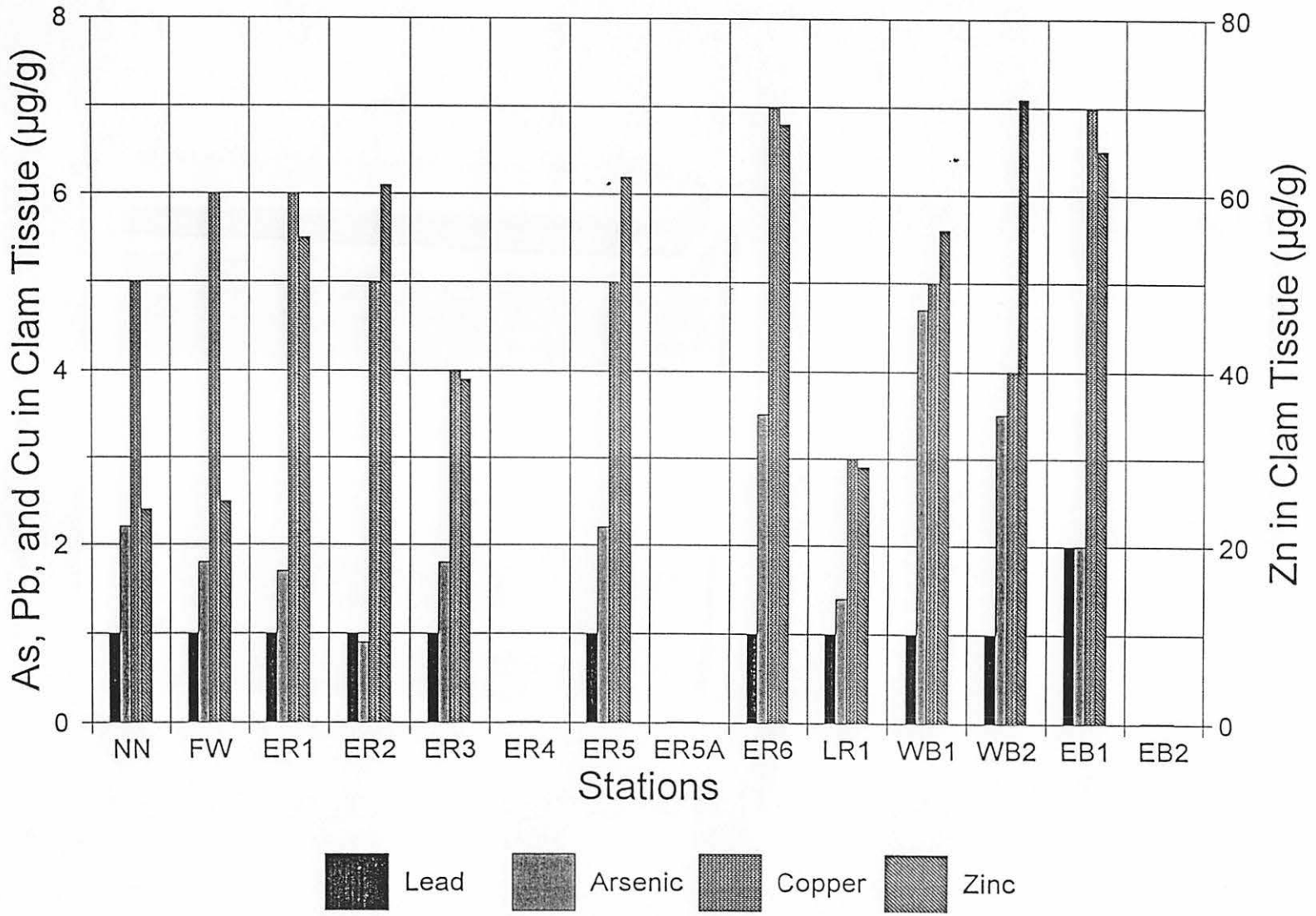

Figure 8 Concentrations of Lead, Arsenic, Copper and Zinc in clam tissue at each station.