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HEAVY METAL CONCENTRATIONS IN THE CLAM RANGIA CUNEATA FROM THE RAPPAHANNOCK AND JAMES RIVERS

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ABSTRACT

This project investigated the feasibility of using the brackish water clam Rangia cuneata as a heavy metal pollution indicator, and further investigated the state of heavy metal pollution in the James River. Rangia cuneata were sampled in the fall of 1972 from the Rappahannock and James Rivers, and meats were analyzed by atomic absorption spectrophotometry for wet weight concentrations of copper, zinc, cadmium and lead.

Levels of copper and zinc in Rangia cuneata were shown to be unaffected by clam size, spawning differences, salinity or distance upriver, and substrate grain size. Heavy metal concentrations in the oxidized channel sediments, determined from other studies conducted at the same time, were compared to levels in Rangia cuneata, but no relationships were found.

Lead was below detection limits (0.2 ppm) in Rangia cuneata at all stations, and cadmium levels appeared to be consistent in both rivers. Copper was found to increase upriver in the James, indicating an upstream source, and zinc concentrations were found to peak at the mouth of the Chickahominy River. A number of possible causes were cited.

HEAVY METAL CONCENTRATIONS IN THE CLAM RANGIA CUNEATA FROM THE RAPPAHANNOCK AND JAMES RIVERS

INTRODUCTION

The presence of heavy metals in estuaries is a complex problem posing many questions. How do the metals reach the estuary? What happens to them once there? What do the levels mean to the environment and man? Also, how can we determine where large concentrations or unnatural inputs occur? These are some basic questions, the latter of which this project investigated.

Heavy metals reach estuaries through many routes: in precipitation (Gorham 1961; Zitko and Carson 1971), with inflowing seawater (Wolfe and Rice 1972), in runoff leaching the surrounding drainage basin (Sprague et al. 1965; Rolfe and Edgington 1973) and from industrial effluents (Schneider 1971).

Estuaries act as nutrient sinks (Odum 1970), where metals are held for varying lengths of time by both physical-chemical and biological processes. Physical-chemical processes include: incorporation of metals in organic detritus (Gutknecht 1963; Lowman et al. 1966; Williams and Murdoch 1969); flocs and precipitation with iron and manganese (Lowman 1963); sorption by suspended and bottom sediments which retain the largest percentage of metals in the estuary at any one time (Duke, Willis and Price, 1966; Pomeroy et al. 1969; Lowman, Rice and Richards 1971); and circulation patterns which may concentrate metals in various portions of the estuary (Pritchard 1958; Redfield et al. 1963; Postma 1967; Pritchard et al. 1971). Biological concentration in the estuary occurs within organisms, by the diel migration of zooplankton and subsequent sorption of metals on their surfaces (Polikapov 1966; Small and Fowler 1973), and through deposition of metals in feces and pseudofeces by filter feeding organisms (Osterberg et al. 1963; Booth and Knauer 1972).

The metals exist in the estuary in both the solution and solid fractions. In the solution fraction they may exist as soluble ion hydroxides, sulphates, etc. (Krauskopf 1956; Goldberg 1957; Bachman 1963), as complexes with inorganic elements and with organic ligands such as humic acids and metabolic products (Barber and Ryther, 1969; Bender et al. 1970; Neilson and Wium-Anderson 1970; Stevenson 1972). In the solid fraction they may exist as inorganic precipitates (Piro 1970); as exchangeable ions held by organic and inorganic exchange complexes like detritus, mucous, and the surfaces of biota and clays (Korringa 1952; Nacci et al. 1970; Huggett and Bender 1972); as specifically sorbed units within the crystal lattice of clays (Johnson et al. 1967); and within the biota.

The levels to which metals are concentrated by organisms in the estuary are affected by numerous variables. The species, relative concentrations, and fractions of a metal along with various environmental factors such as pH, temperature, salinity and DO have been shown to affect levels attained by organisms (Chipman et al. 1958; Brooks and Rumsby 1965; Cross et al. 1969; Duke et al. 1969; Lunz 1972). Although species differences often affect levels concentrated, it may be more informative to classify organisms by feeding types (specific and non-specific deposit feeders, filter feeders, etc.) since there is often little variance within these types from one locale (Berner et al. 1962; Lowman et al. 1966; Phelps et al. 1969); a notable exception being the oyster, which concentrates some metals to considerably higher levels than do other filter feeding mollusks. The metabolic rate of organisms has also been shown to significantly affect levels concentrated (Odum 1961; Mishima and Odum 1965; Seymour 1966).

Understanding the relationship between the ecosystem and a metallic concentration in one of its "compartments" is basic to understanding the metal's impact on the ecosystem. A number of researchers have published background levels of metals in organisms useful in reference to pollution studies (Parker 1962; Frazier 1972; Graham 1972). Some of these metals, e.g. copper and zinc, are essential in low levels to organisms, yet others, e.g. cadmium and lead, are considered to exist in organisms as contaminants, since no need has been shown for them (Williams 1953; Schroeder 1960). Bryan (1971) summarized the literature concerning toxic effects of some heavy metals on organisms and indicated a concern for the marine environment when levels of Cu, Ag, or Zn reach an order of magnitude higher than the normal value for seawater. The poisoning of people in Japan due to high levels of mercury in shellfish (Irukayama 1967), and the recent concern over high mercury concentrations in tuna, swordfish, and blue marlin illuminate a potentially toxic pathway to man for heavy metals in the environment.

Since heavy metals, due to man's activities, pose a public health as well as environmental threat, an indicator to detect polluting sources is desirable. Oysters have been shown to be effective indicators of metal pollution (Schuster and Pringle 1969; Huggett, Bender and Slone 1973); however, oysters only exist in water of 7 ppt salinity or higher, therefore, another indicator extending farther up the estuary is needed.

The brackish water clam *Rangia cuneata* Gray, appeared to be a promising organism, for its range extends from the upper reaches of the oyster's population well up into fresh water.

Rangia cuneata, a pelecypod mollusk of the family Mactridae, is a common inhabitant of low salinity estuaries along the Eastern and Gulf Coasts of North America from the Potomac River in Maryland (Pfitzenmeyer and Drobeck 1964) to Campeche, Mexico (Gooch 1971). *Rangia* have apparently extended their distribution again into the estuaries along the Atlantic Coast from Florida to Maryland over the past 20 years, filling a previously open "ecological niche" and undergoing a "population explosion" (Hopkins et al. 1972). This may represent a resurgence of survivors from the Pleistocene Atlantic Coast population (Hopkins and Andrews 1970). These clams are extremely abundant, seemingly ubiquitous within their range, require a salinity below 15 ppt most of the time, extend into freshwater, and need a saline water intrusion for the larvae to set (Cain 1972). They are ecologically important, converting detritus into clam meat (Darnell 1958; Tenore et al. 1968; Odum and Copeland 1969), and constitute 99% of the benthic biomass in the oligohaline section of the James River estuary (Cain 1972). Two studies in Lake Pontchartrain, Louisiana (Suttkus et al. 1954; Darnell 1958) indicated 15 species of fishes, three crustaceans, and many wild ducks fed on young *Rangia*. McIntire (1958) believed these clams formed a basic portion of the diet of Indians living along the Gulf Coast and presently an underdeveloped market exists for both the shells and meat.

In this study, natural levels of heavy metals in *Rangia* from the Rappahannock River provided a control for comparison with levels in *Rangia* from the polluted James River. Since the natural concentrations of heavy metals in rivers is a function of the weathering of rocks, and since the strata of the Rappahannock and James River basins are basically the same (Virginia Division of Water Resources 1970a,b), it is logical to assume the natural levels of metals in the two rivers to be nearly equal; a belief further substantiated by Huggett, Bender and Slone (1973). They also found unnatural levels of heavy metals in the James River.

The purpose of the experiment was twofold: First, to determine the effect of various parameters (e.g. clam size, substrate grain size, sediment metal concentrations, and distance upriver) on the levels of metals concentrated by Rangia in reference to its future use as a pollution indicator. Second, to further assess the state of heavy metal pollution in the James River.

MATERIALS AND METHODS

A preliminary study was undertaken to determine whether size, as an indication of age, affects the levels of metals concentrated by Rangia cuneata. Clams were sampled by hand from a sandy flat in the James River in July, 1972. They were shucked, being careful not to cut the meat; the often muddy, periostrical tissue on the gills was removed, and the meats were allowed to drain for 5 minutes on a plastic screen. The clams were then digested in concentrated nitric acid (Reagent ACS, Fisher Scientific), 5 ml per 5 g increment, diluted to a known "sample" volume, filtered with acid-washed glass wool, and analyzed on a Varian Techtron AA-5 atomic absorption spectrophotometer for copper, zinc, cadmium and lead.

Calibration curves were constructed by plotting concentrations in ppm. of standards against the resulting peak heights of the standards. The apparent concentration of the sample was then read in ppm. from the reference curve, and the actual concentration of metal in the clam was determined by the following formula:

$$\text{PPM. Metal} = \frac{(\text{apparent concentration ppm.})\ (\text{sample vol. ml})}{(\text{clam weight g})}$$

The sensitivity of the AA for each element is as follows: Cu, .040 ug/ml; Zn, .009 ug/ml; Cd, .011 ug/ml; Pb, .11 ug/ml. The precision of analysis was determined by following analytical procedure on 10 replicate solutions of mixed standards; interval estimates were: Cu ± 0.1 ppm.; Zn ± 0.2 ppm.; Cd ± 0.05 ppm.

Sampling for the basic study was undertaken in the James River from September 19-26, 1972, and in the Rappahannock River from November 10-19, 1972. Stations were sampled on the north and south sides of the rivers at 2 nautical mile intervals throughout the range of the clams; these stations are shown in Figures 1 and 2. Twenty (+) clams were taken from each station, paired according to equal shell lengths (± 1 mm), shucked, and frozen in plastic bags for later digestion and analysis. To determine whether freezing samples in plastic bags introduced error, 2 samples were frozen for 2 weeks and analyzed; one sample contained deionized water and the other a known mixture of standards. After freezing, the deionized water revealed no trace metals leached from the bag and the standards were statistically equal, indicating no significant adsorption onto the bag.

To determine whether sediment size influences the levels of metals concentrated by _Rangia_, sediment samples were taken by a modified Van Veen grab at each station. Subsamples of the upper 3 inches were removed, frozen in plastic bags, and later analyzed for sand, silt and clay fractions by screening and settling time according to Folk (1968).

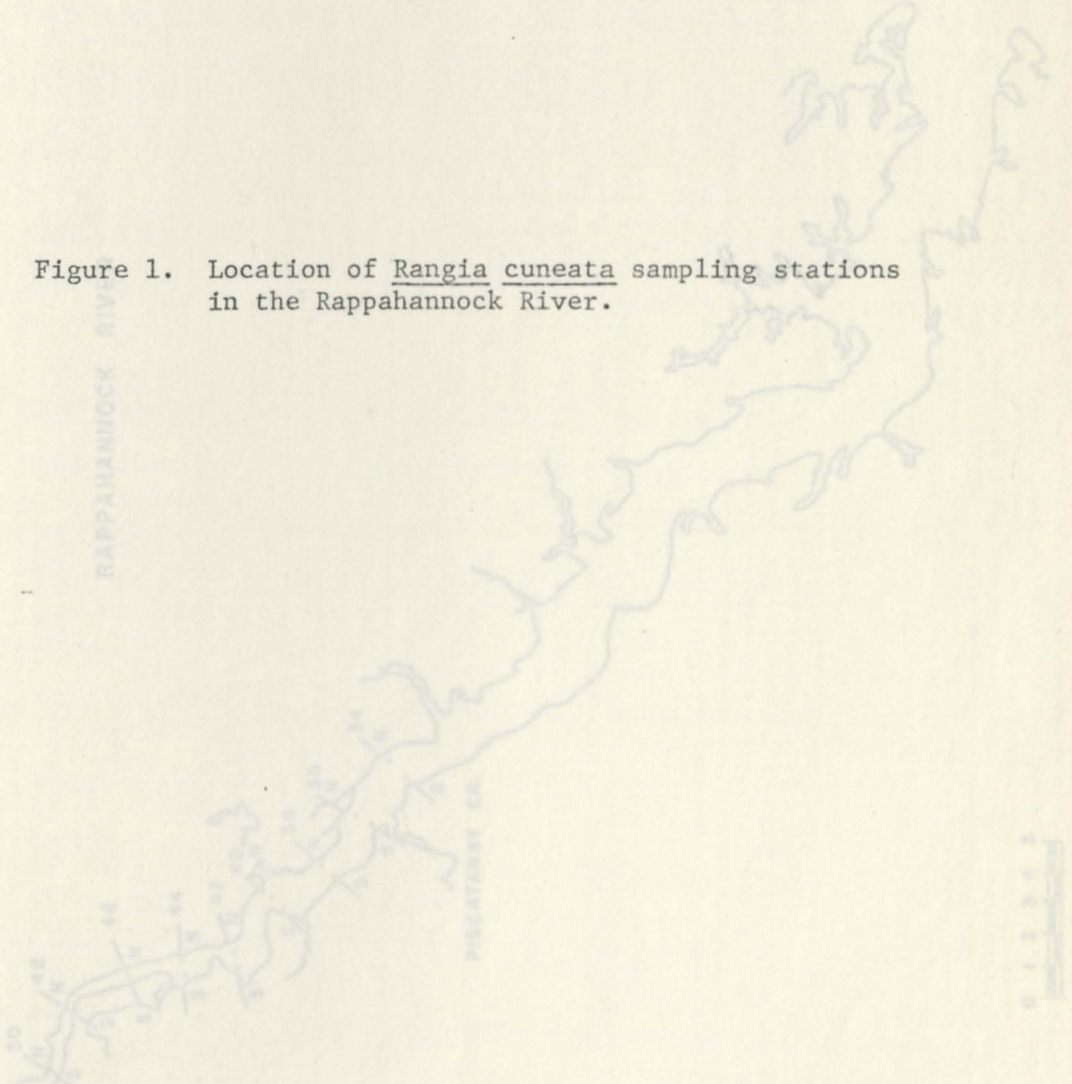


Figure 1. Location of *Rangia cuneata* sampling stations in the Rappahannock River.

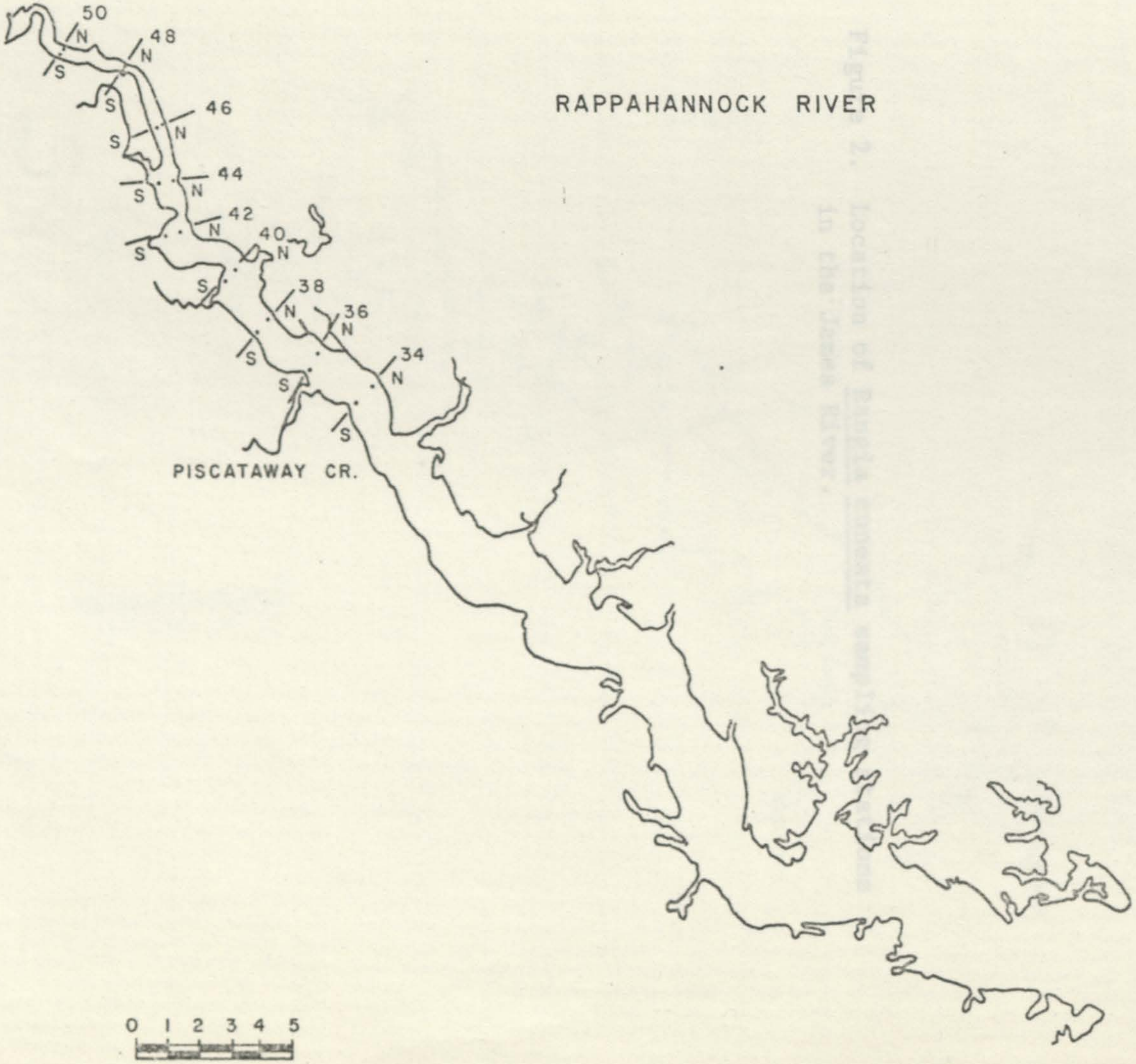
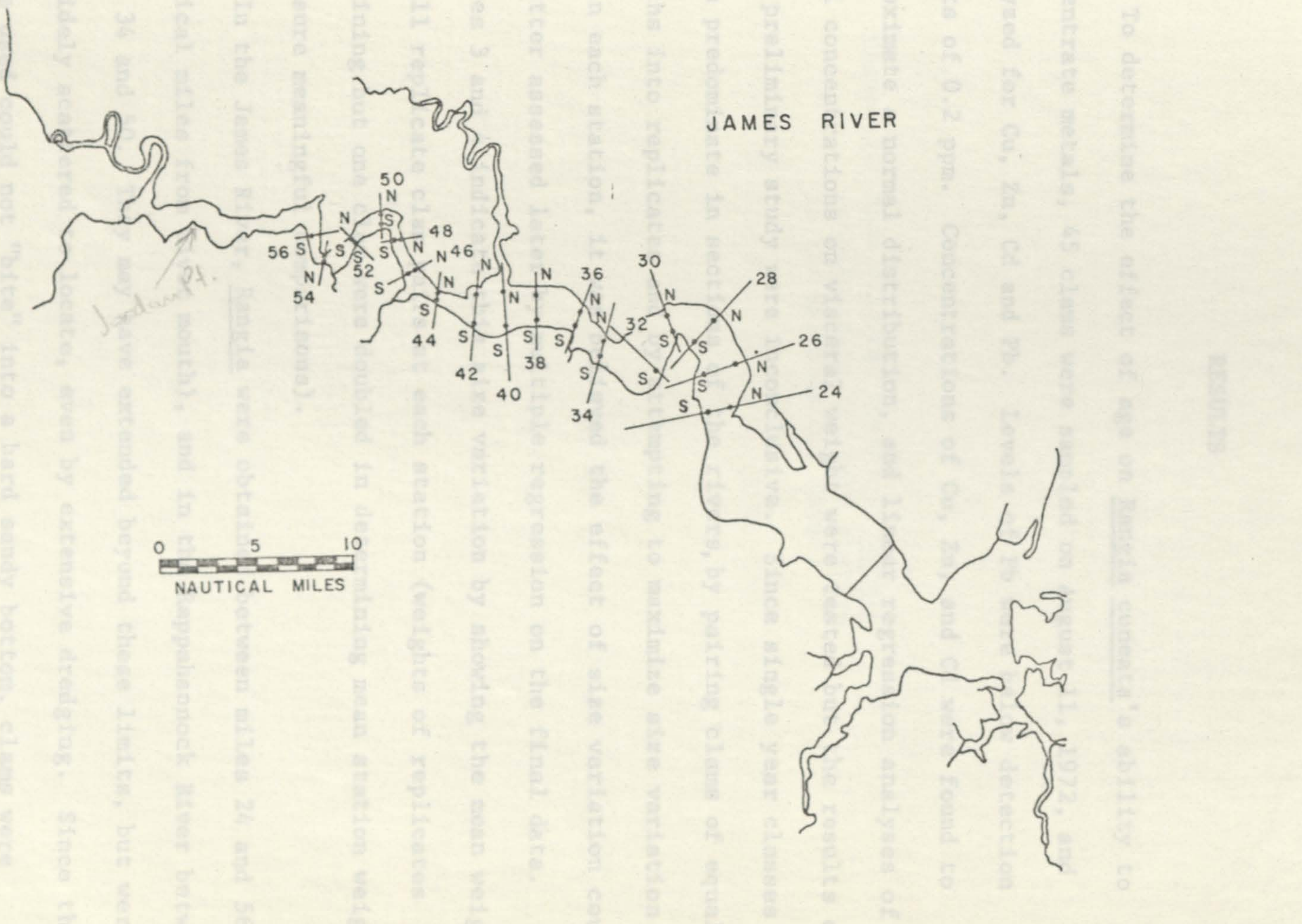

RAPPAHANNOCK RIVER
50
48
46
44
42
40
38
36
34
N
S
PISCATAWAY CR.
0 1 2 3 4 5

Figure 2. Location of Rangia cuneata sampling stations in the James River.

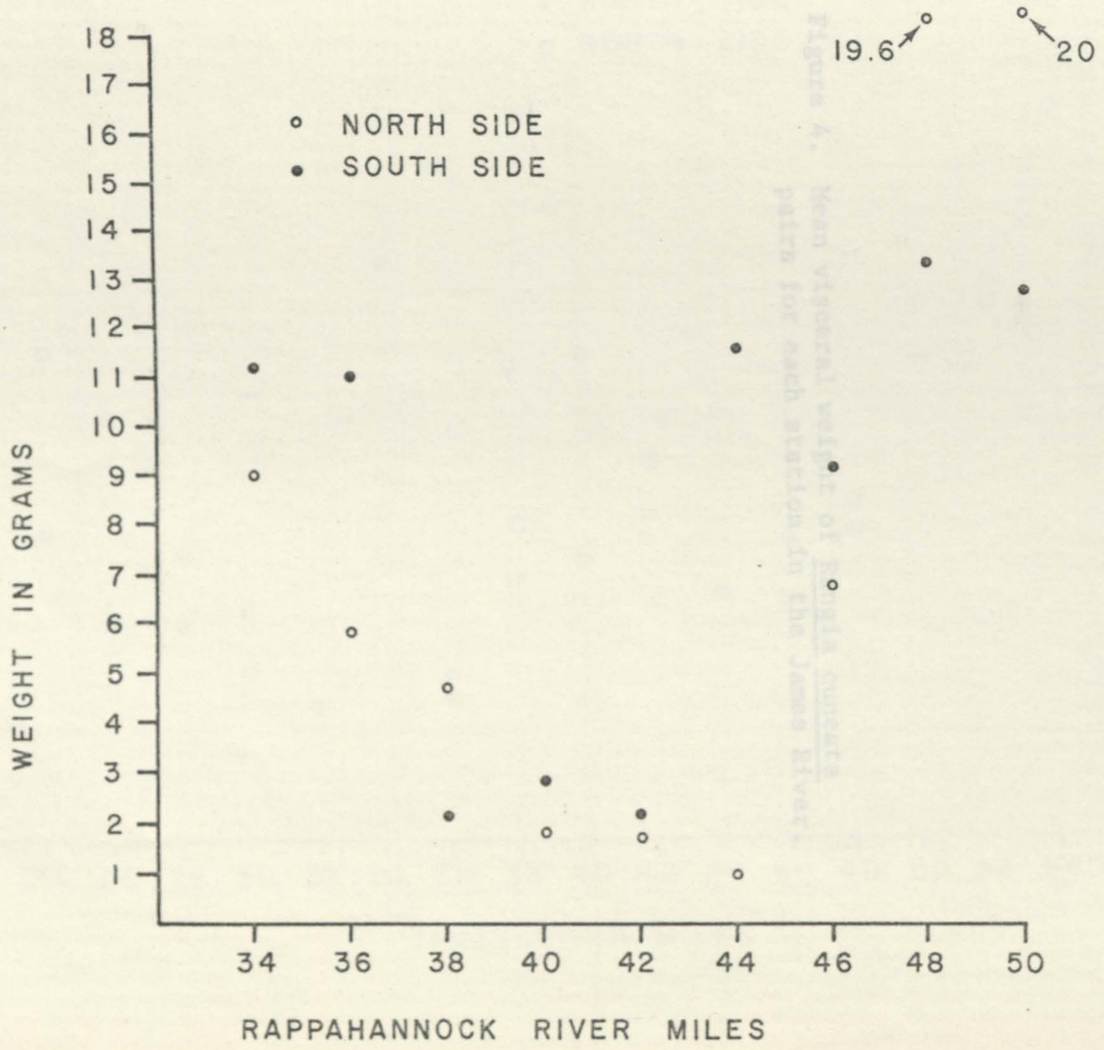

JAMES RIVER
Jordan Pt.
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
54
56
N
S
0
5
10
NAUTICAL MILES

RESULTS

To determine the effect of age on *Rangia cuneata*'s ability to concentrate metals, 45 clams were sampled on August 11, 1972, and analyzed for Cu, Zn, Cd and Pb. Levels of Pb were below detection limits of 0.2 ppm. Concentrations of Cu, Zn, and Cd were found to approximate a normal distribution, and linear regression analyses of metal concentrations on visceral weight were tested but the results of this preliminary study were inconclusive. Since single year classes often predominate in sections of the rivers, by pairing clams of equal lengths into replicates and by attempting to maximize size variation within each station, it was believed the effect of size variation could be better assessed later by multiple regression on the final data. Figures 3 and 4 indicate this size variation by showing the mean weight for all replicate clam pairs at each station (weights of replicates containing but one clam were doubled in determining mean station weights to insure meaningful comparisons).

In the James River, *Rangia* were obtained between miles 24 and 56 (nautical miles from river mouth), and in the Rappahannock River between miles 34 and 50. They may have extended beyond these limits, but were too widely scattered to locate, even by extensive dredging. Since the dredge used could not "bite" into a hard sandy bottom, clams were generally sampled from a muddier substrate; though in some instances where populations were dense, the grab could be employed on a sandy bottom. When less than 20 clams were taken, 10 replicates (if possible)

Figure 3. Mean visceral weight of *Rangia cuneata* pairs for each station in the Rappahannock River.



○ NORTH SIDE
● SOUTH SIDE
19.6
20
WEIGHT IN GRAMS
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
34
36
38
40
42
44
46
48
50
RAPPAHANNOCK RIVER MILES

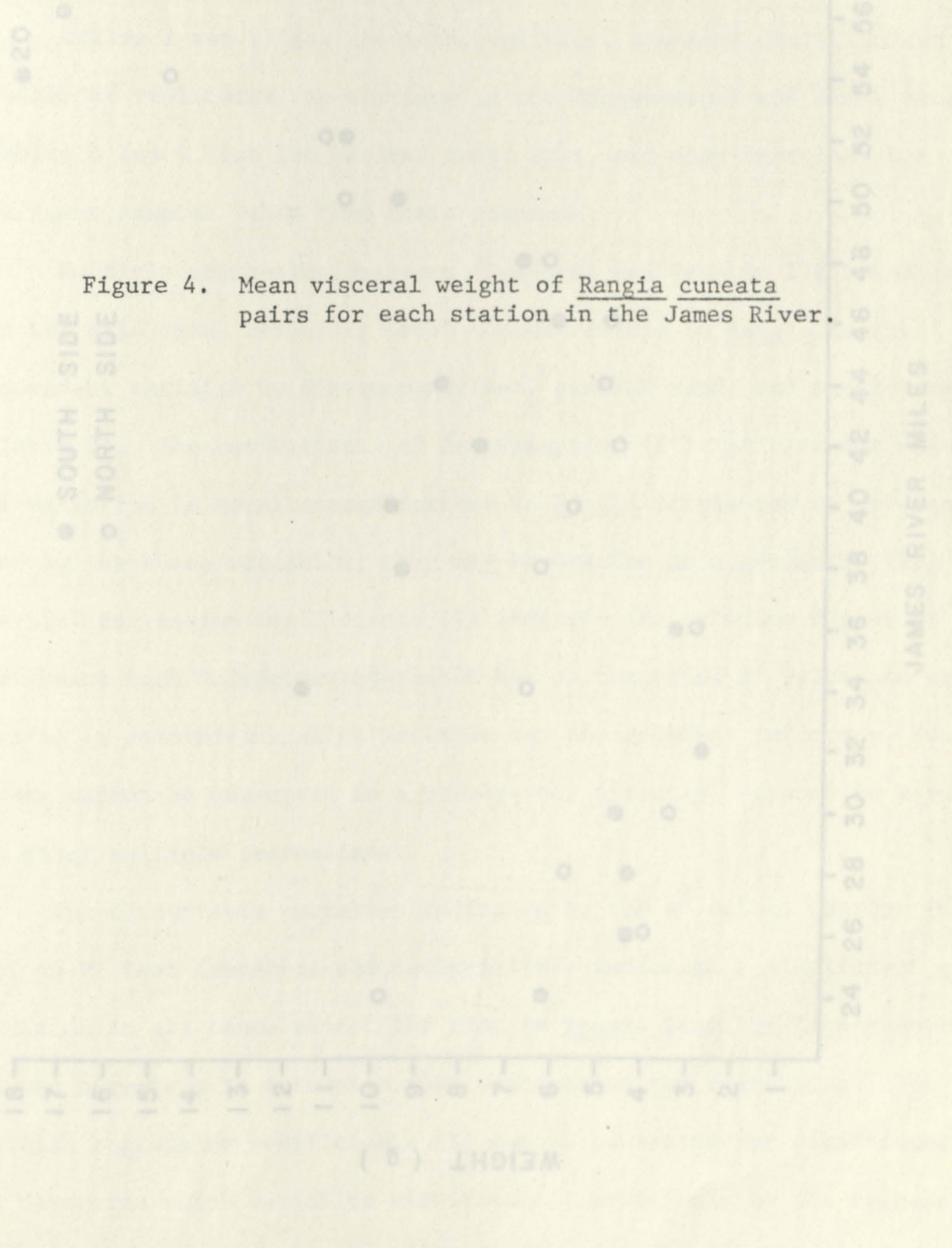
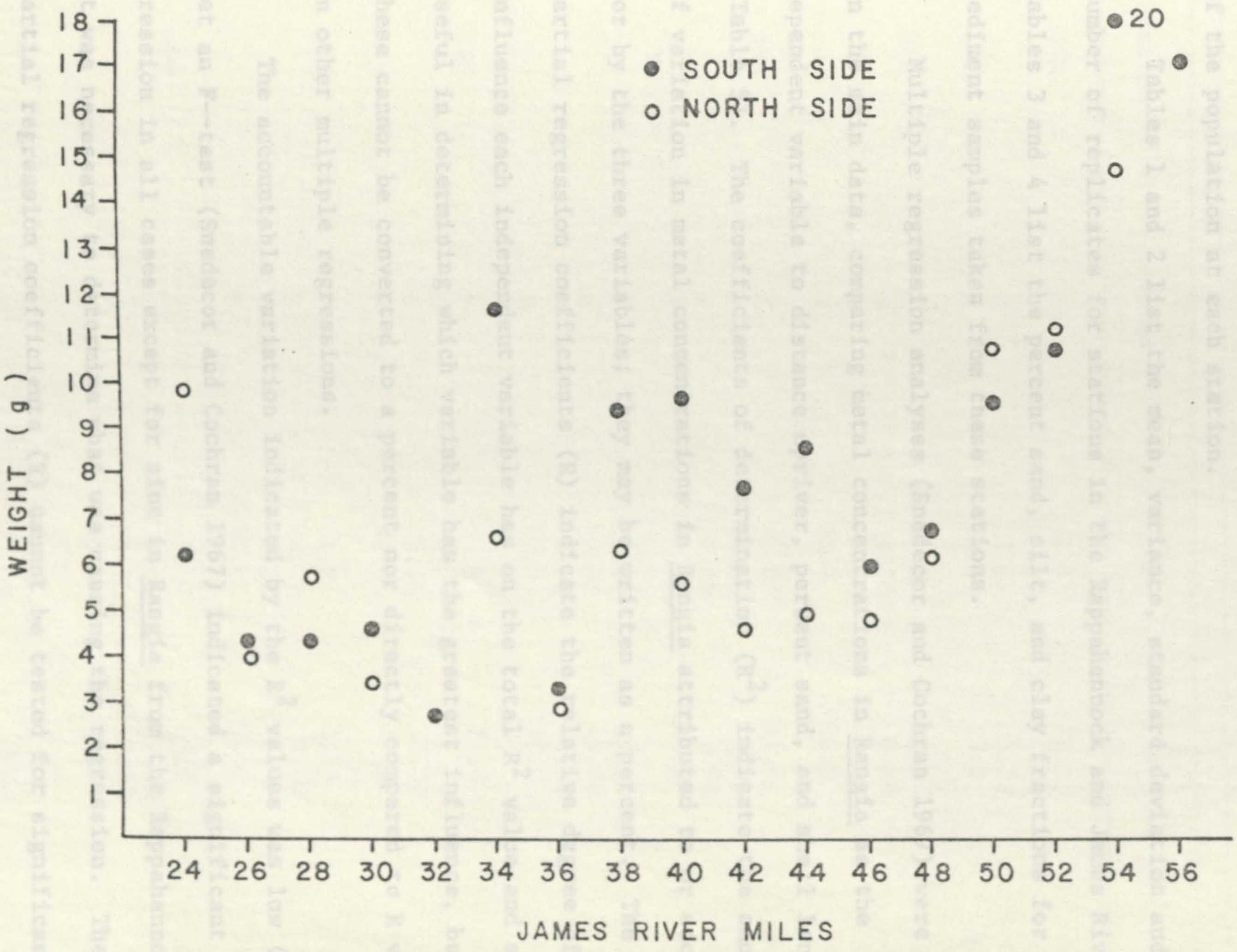


Figure 4. Mean visceral weight of <u>Rangia</u> <u>cuneata</u> pairs for each station in the James River.



SOUTH SIDE
NORTH SIDE
20
WEIGHT (g)
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
54
56
JAMES RIVER MILES

were made, some paired and some not. This pairing of the clams does not statistically improve the data since each pair is treated as one observation; yet, due to the larger number of clams tested, the data are probably closer than 10 single clam replicates to the true mean of the population at each station.

Tables 1 and 2 list the mean, variance, standard deviation and number of replicates for stations in the Rappahannock and James Rivers. Tables 3 and 4 list the percent sand, silt, and clay fractions for sediment samples taken from these stations.

Multiple regression analyses (Snedecor and Cochran 1967) were run on the main data, comparing metal concentrations in Rangia as the dependent variable to distance upriver, percent sand, and shell length (Table 5). The coefficients of determination (R^2) indicate the amount of variation in metal concentrations in Rangia attributed to or accounted for by the three variables; they may be written as a percent. The partial regression coefficients (R) indicate the relative degree of influence each independent variable has on the total R^2 value and are useful in determining which variable has the greatest influence, but these cannot be converted to a percent nor directly compared to R values in other multiple regressions.

The accountable variation indicated by the R^2 values was low ($<50\%$), yet an F--test (Snedecor and Cochran 1967) indicated a significant regression in all cases except for zinc in Rangia from the Rappahannock. It was necessary to determine what was causing the regression. The partial regression coefficients (R) cannot be tested for significance to determine which variables significantly contribute to the regression. Various regressions were run using different combinations of independent

Table 1. The mean in ppm. ($\bar{X}$), variance (S^2), standard deviation (S), and number of replicates tested (N) for heavy metal concentrations in *Rangia cuneata* at each of the 18 sampling stations in the Rappahannock River. N and S represent north and south sides of the river.

Station	Indices	Cu	Zn	Cd
34N	$\bar{X}$	2.0	12.3	0.05
	S^2	0.07	1.75	0.00
	S	0.26	1.32	0.02
	N	10.0	10.0	10.0
34S	$\bar{X}$	2.0	12.8	0.05
	S^2	0.08	0.29	0.00
	S	0.28	0.54	0.00
	N	10.0	10.0	10.0
36N	$\bar{X}$	1.8	11.1	0.1
	S^2	0.08	0.75	0.00
	S	0.28	0.86	0.05
	N	9.0	10.0	10.0
36S	$\bar{X}$	1.8	12.6	0.06
	S^2	0.37	5.40	0.00
	S	0.61	2.32	0.02
	N	10.0	10.0	10.0
38N	$\bar{X}$	1.8	11.9	0.1
	S^2	0.04	1.10	0.00
	S	0.20	1.05	0.04
	N	10.0	10.0	10.0
38S	$\bar{X}$	1.8	11.6	0.2
	S^2	0.07	1.09	0.00
	S	0.26	1.04	0.05
	N	10.0	10.0	10.0
40N	$\bar{X}$	1.7	15.2	0.3
	S^2	0.05	7.12	0.01
	S	0.21	2.67	0.09
	N	10.0	10.0	10.0
40S	$\bar{X}$	1.8	15.0	0.3
	S^2	0.05	2.46	0.00
	S	0.22	1.57	0.06
	N	10.0	9.0	10.0

Table 1. (Cont.)

Station	Indices	Cu	Zn	Cd
42N	$\bar{X}$	1.4	12.0	0.4
	S^2	0.02	1.46	0.09
	S	0.13	1.21	0.31
	N	10.0	10.0	10.0
42S	$\bar{X}$	1.8	11.6	0.2
	S^2	0.09	0.98	0.00
	S	0.29	0.99	0.03
	N	10.0	10.0	10.0
44N	$\bar{X}$	1.5	12.3	0.4
	S^2	0.04	0.82	0.01
	S	0.20	0.91	0.11
	N	10.0	9.0	10.0
44S	$\bar{X}$	1.9	12.5	0.2
	S^2	0.06	0.54	0.00
	S	0.24	0.74	0.06
	N	10.0	10.0	10.0
46N	$\bar{X}$	1.8	11.9	0.2
	S^2	0.06	0.52	0.01
	S	0.23	0.72	0.09
	N	10.0	10.0	10.0
46S	$\bar{X}$	2.0	11.4	0.2
	S^2	0.10	2.26	0.00
	S	0.31	1.50	0.05
	N	10.0	10.0	10.0
48N	$\bar{X}$	1.2	9.3	0.1
	S^2	0.01	0.18	0.01
	S	0.07	0.42	0.11
	N	2.0	2.0	2.0
48S	$\bar{X}$	1.7	11.0	0.1
	S^2	0.09	3.0	0.00
	S	0.31	1.7	0.05
	N	10.0	10.0	10.0
50N	$\bar{X}$	2.0	12.0	0.2
	S^2	0.41	2.0	0.01
	S	0.64	1.41	0.07
	N	2.0	2.0	2.0
50S	$\bar{X}$	1.9	14.1	0.1
	S^2	0.13	2.57	0.00
	S	0.36	1.60	0.02
	N	10.0	10.0	9.0

Table 2. The mean in ppm. ($\bar{X}$), variance (S^2), standard deviation (S), and number of replicates tested (N) for heavy metal concentrations in *Rangia* at each of the 32 sampling stations in the James River. N and S represent north and south sides of the river.

Station	Indices	Cu	Zn	Cd
24N	$\bar{X}$	2.7	13.9	0.1
	S^2	0.22	1.82	0.00
	S	0.47	1.35	0.03
	N	10.0	9.0	10.0
24S	$\bar{X}$	2.2	11.4	0.1
	S^2	0.43	3.75	0.00
	S	0.66	1.94	0.05
	N	10.0	10.0	10.0
26N	$\bar{X}$	2.4	14.2	0.2
	S^2	0.27	1.48	0.00
	S	0.52	1.22	0.05
	N	10.0	10.0	9.0
26S	$\bar{X}$	2.1	12.6	0.2
	S^2	0.32	1.63	0.00
	S	0.56	1.28	0.05
	N	10.0	10.0	10.0
28N	$\bar{X}$	2.8	13.6	0.1
	S^2	0.19	1.75	0.00
	S	0.43	1.32	0.06
	N	10.0	8.0	10.0
28S	$\bar{X}$	2.6	13.5	0.1
	S^2	0.32	1.12	0.00
	S	0.57	1.06	0.05
	N	10.0	9.0	10.0
30N	$\bar{X}$	2.4	14.6	0.2
	S^2	1.05	18.69	0.01
	S	1.03	4.32	0.09
	N	10.0	10.0	9.0
30S	$\bar{X}$	3.4	13.1	0.1
	S^2	0.27	2.45	0.00
	S	0.52	1.57	0.04
	N	10.0	10.0	10.0

Table 2. (Cont.)

Station	Indices	Cu	Zn	Cd
32S	$\bar{X}$	2.6	13.9	0.2
	S^2	0.15	5.86	0.00
	S	0.39	2.42	0.03
	N	10.0	10.0	10.0
34N	$\bar{X}$	2.5	11.7	0.13
	S^2	0.37	9.66	0.00
	S	0.61	3.11	0.06
	N	3.0	3.0	3.0
34S	$\bar{X}$	2.5	13.5	0.1
	S^2	0.12	1.04	0.00
	S	0.34	1.02	0.02
	N	10.0	10.0	10.0
36N	$\bar{X}$	2.6	14.7	0.2
	S^2	0.43	1.34	0.00
	S	0.66	1.16	0.06
	N	10.0	10.0	10.0
36S	$\bar{X}$	3.1	16.3	0.1
	S^2	0.09	1.71	0.00
	S	0.31	1.31	0.05
	N	10.0	10.0	10.0
38N	$\bar{X}$	2.7	14.2	0.2
	S^2	0.39	1.67	0.00
	S	0.63	1.29	0.04
	N	9.0	9.0	10.0
38S	$\bar{X}$	3.8	19.6	0.2
	S^2	0.41	9.61	0.00
	S	0.64	3.10	0.05
	N	10.0	10.0	10.0
40N	$\bar{X}$	3.5	17.6	0.2
	S^2	0.32	9.03	0.00
	S	0.57	3.0	0.05
	N	10.0	10.0	10.0
40S	$\bar{X}$	3.7	19.5	0.2
	S^2	0.17	7.42	0.00
	S	0.42	2.72	0.05
	N	10.0	10.0	10.0

Table 2. (Cont.)

Station	Indices	Cu	Zn	Cd
42N	$\bar{X}$	3.3	15.8	0.2
	S^2	0.29	4.72	0.00
	S	0.54	2.17	0.06
	N	10.0	10.0	10.0
42S	$\bar{X}$	3.5	16.6	0.2
	S^2	0.92	2.36	0.01
	S	0.96	1.53	0.09
	N	10.0	10.0	10.0
44N	$\bar{X}$	3.2	14.3	0.2
	S^2	0.32	2.03	0.00
	S	0.57	1.42	0.04
	N	10.0	10.0	10.0
44S	$\bar{X}$	4.3	15.7	0.2
	S^2	0.09	1.52	0.00
	S	0.30	1.23	0.04
	N	10.0	10.0	10.0
46N	$\bar{X}$	4.8	15.4	0.2
	S^2	0.62	1.41	0.00
	S	0.79	1.19	0.03
	N	10.0	10.0	10.0
46S	$\bar{X}$	4.5	16.2	0.1
	S^2	0.29	2.09	0.00
	S	0.54	1.45	0.05
	N	10.0	10.0	10.0
48N	$\bar{X}$	4.2	14.5	0.2
	S^2	0.29	2.61	0.00
	S	0.54	1.62	0.05
	N	10.0	10.0	10.0
48S	$\bar{X}$	4.6	15.8	0.1
	S^2	0.40	0.50	0.00
	S	0.63	0.70	0.03
	N	10.0	10.0	10.0
50N	$\bar{X}$	4.3	14.6	0.1
	S^2	0.28	2.40	0.00
	S	0.53	1.55	0.06
	N	10.0	10.0	10.0

Table 2. (Cont.)

Station	Indices	Cu	Zn	Cd
50S	$\bar{X}$	4.4	13.8	0.1
	S^2	0.02	0.53	0.00
	S	0.16	0.73	0.02
	N	10.0	10.0	10.0
52N	$\bar{X}$	4.6	13.6	0.1
	S^2	0.21	1.43	0.00
	S	0.46	1.19	0.03
	N	10.0	10.0	10.0
52S	$\bar{X}$	3.9	13.4	0.1
	S^2	0.10	0.83	0.00
	S	0.31	0.91	0.02
	N	10.0	10.0	10.0
54N	$\bar{X}$	4.0	14.0	0.1
	S^2	1.40	8.20	0.00
	S	1.18	2.86	0.03
	N	8.0	7.0	8.0
54S	$\bar{X}$	4.0	11.4	0.05
	S^2	4.81	7.61	0.00
	S	2.19	2.76	0.04
	N	2.0	2.0	2.0
56S	$\bar{X}$	3.2	12.0	0.05
	S^2	0.26	3.22	0.00
	S	0.51	1.80	0.03
	N	10.0	9.0	10.0

Table 3. Percentage composition by weight of the sediment at each of the 18 sampling stations in the Rappahannock River. N and S represent north and south sides of the river.

Station	% Sand >62.5u	% Silt 62.5u-3.9u	% Clay <3.9u
34N	10.7	62.2	27.1
34S	1.6	71.6	26.8
36N	1.1	74.2	24.7
36S	1.2	73.8	25.0
38N	70.2	16.8	13.0
38S	5.6	69.1	25.3
40N	63.6	17.5	18.8
40S	3.7	71.6	24.7
42N	74.6	12.5	12.9
42S	1.7	74.3	24.0
44N	3.5	63.6	32.9
44S	.8	76.2	23.0
46N	22.7	55.8	21.5
46S	1.1	81.6	17.3
48N	62.1	19.2	18.7
48S	3.1	25.6	71.3
50N	7.9	59.7	32.4
50S	1.6	49.6	48.8

Table 4. Percentage composition by weight of the sediment at each of the 32 sampling stations in the James River. N and S represent north and south sides of the river.

Station	% Sand > 62.5u	% Silt 62.5u-3.9u	% Clay < 3.9u
24N	2.0	32.0	66.0
24S	5.7	58.1	36.2
26N	14.0	60.8	25.2
26S	2.4	71.4	26.2
28N	18.0	56.1	25.9
28S	30.6	49.5	19.9
30N	38.1	41.7	20.2
30S	1.3	75.4	23.3
32S	2.6	82.8	14.6
34N	35.0	31.9	33.1
34S	8.7	66.6	24.7
36N	.8	75.5	23.7
36S	3.0	61.3	35.7
38N	1.0	69.4	29.6
38S	.8	64.0	35.2
40N	3.4	58.9	37.7
40S	.3	69.3	36.4
42N	1.9	63.9	34.2
42S	1.5	40.9	57.6
44N	1.1	77.0	21.9
44S	6.1	64.9	29.0

Table 4. (Cont.)

Station	% Sand >62.5u	% Silt 62.5u-3.9u	% Clay <3.9u
46N	53.8	24.3	21.9
46S	15.7	26.6	57.7
48N	8.2	58.7	33.1
48S	.8	45.5	53.7
50N	37.6	32.2	30.2
50S	.9	60.3	38.8
52N	3.0	43.8	53.1
52S	12.7	66.4	20.9
54N	81.7	5.3	13.0
54S	13.1	46.8	40.1
56S	1.2	70.5	28.3

Table 5. Multiple regression statistics testing the influence of distance upriver, percent sand, and shell length on heavy metal concentrations in <u>Rangia</u> <u>cuneata</u>. **Indicates significance at the 99% level.

Metal	R Values for			R^2	F
	Distance Upriver	% Sand	Shell Length		
		<u>Rappahannock River</u>			
Cu	-0.010	-0.002	0.007	.105	6.208**
Zn	-0.007	0.006	-0.018	.014	.775
Cd	0.009	0.001	-0.008	.444	43.060**
		<u>James River</u>			
Cu	0.070	0.009	-0.001	.442	76.969**
Zn	0.043	-0.025	-0.066	.047	4.783**
Cd	-0.000	0.000	-0.003	.105	18.066**

variables in an attempt to raise the R^2 values in cases where "antagonistic" effects between variables might be occurring, but the R^2 values were lowered in all instances.

Multiple regressions were run substituting visceral weight for shell length. Replicates containing one clam had to be withheld from the analyses (14 replicates withheld from the James, 24 from the Rappahannock). These results (Table 6) indicate slightly higher R^2 values apparently due to a slightly better fit for weight, but these do not clarify previous results.

Partial correlation coefficients (r) which indicate the amount of covariance between two variables both considered as occurring independently of each other can be tested for significance. No conversion to correlation coefficients could be found for a regression of three independent variables (Snedecor and Cochran 1967). Therefore, conversions had to be made considering a total of only three variables (one dependent and two independent) at a time (Tables 7 and 8). These partial correlation coefficients (e.g. $r_{Y3.2}$) test the covariance of metal levels in <u>Rangia</u> (Y) with one variable (X_3 = shell length) while a second variable (X_2 = % sand) is considered as being held constant throughout the river, the other independent variable (X_1 = distance upriver) is not considered in the test.

Most partial correlation coefficients were insignificant. Some multiple regressions (Cu in the Rappahannock, Zn in the James) were indicated as significant because the variables together accounted for enough variation to indicate regression, even though no individual variable significantly affected metal levels in <u>Rangia</u>. Other multiple

Table 6. Multiple regression statistics testing the influence of distance upriver, percent sand, and visceral weight on heavy metal concentrations in Rangia cuneata. **Indicates significance at the 99% level.

Metal	R Values for Distance Upriver	R Values for % Sand	R Values for Shell Length	R^2	F
		Rappahannock River			
Cu	-0.014	-0.001	0.023	.160	8.365**
Zn	-0.009	0.008	-0.014	.009	.488
Cd	0.011	-0.000	-0.017	.523	48.308**
		James River			
Cu	0.073	0.009	-0.013	.464	80.269**
Zn	0.063	-0.031	-0.118	.059	5.857**
Cd	0.001	0.000	-0.009	.225	26.867**

Table 7. Partial correlation coefficients testing the influence of distance upriver, percent sand, and shell length on heavy metal concentrations in *Rangia cuneata*. *Indicates significance at the 95% level; **at the 99% level.

X_1 = Distance Upriver

X_2 = % Sand

X_3 = Shell Length

Rappahannock River

Partial Correlation	Heavy Metals Y = Cu	 Y = Zn	 Y = Cd
$r_{Y1 \cdot 2}$	0.012	0.001	0.043
$r_{Y1 \cdot 3}$	0.011	0.001	0.036
$r_{Y2 \cdot 1}$	0.048	0.007	0.059
$r_{Y2 \cdot 3}$	0.042	0.006	0.069
$r_{Y3 \cdot 1}$	0.059	0.011	0.329**
$r_{Y3 \cdot 2}$	0.054	0.010	0.338**
	James River		
$r_{Y1 \cdot 2}$	0.431**	0.013	0.024
$r_{Y1 \cdot 3}$	0.430**	0.012	0.011
$r_{Y2 \cdot 1}$	0.003	0.010	0.010
$r_{Y2 \cdot 3}$	0.004	0.010	0.006
$r_{Y3 \cdot 1}$	-0.009	0.010	0.174*
$r_{Y3 \cdot 2}$	0.029	0.011	0.172*

Table 8. Partial correlation coefficients testing the influence of distance upriver, percent sand, and visceral weight on heavy metal concentrations in *Rangia cuneata*. **Indicates significance at the 99% level.

X_1 = Distance Upriver

X_2 = % Sand

X_3 = Visceral Weight

Partial Correlation	Heavy Metals Y = Cu	Y = Zn	Y = Cd
	Rappahannock River		
$r_{Y1 \cdot 2}$	0.010	0.001	0.065
$r_{Y1 \cdot 3}$	0.000	0.001	0.037
$r_{Y2 \cdot 1}$	0.017	0.007	0.017
$r_{Y2 \cdot 3}$	0.006	0.007	0.018
$r_{Y3 \cdot 1}$	0.110	0.004	0.317**
$r_{Y3 \cdot 2}$	0.109	0.003	0.321**
	James River		
$r_{Y1 \cdot 2}$	0.449**	0.021	0.012
$r_{Y1 \cdot 3}$	0.446**	0.021	-0.032
$r_{Y2 \cdot 1}$	0.015	0.015	0.004
$r_{Y2 \cdot 3}$	0.013	0.016	0.002
$r_{Y3 \cdot 1}$	-0.032	-0.002	0.224**
$r_{Y3 \cdot 2}$	0.062	0.003	0.220**

regressions (Cd in the Rappahannock and James, Cu in the James) contained one variable which significantly affected metal concentrations in <u>Rangia</u>, resulting in significant regression.

DISCUSSION

Size

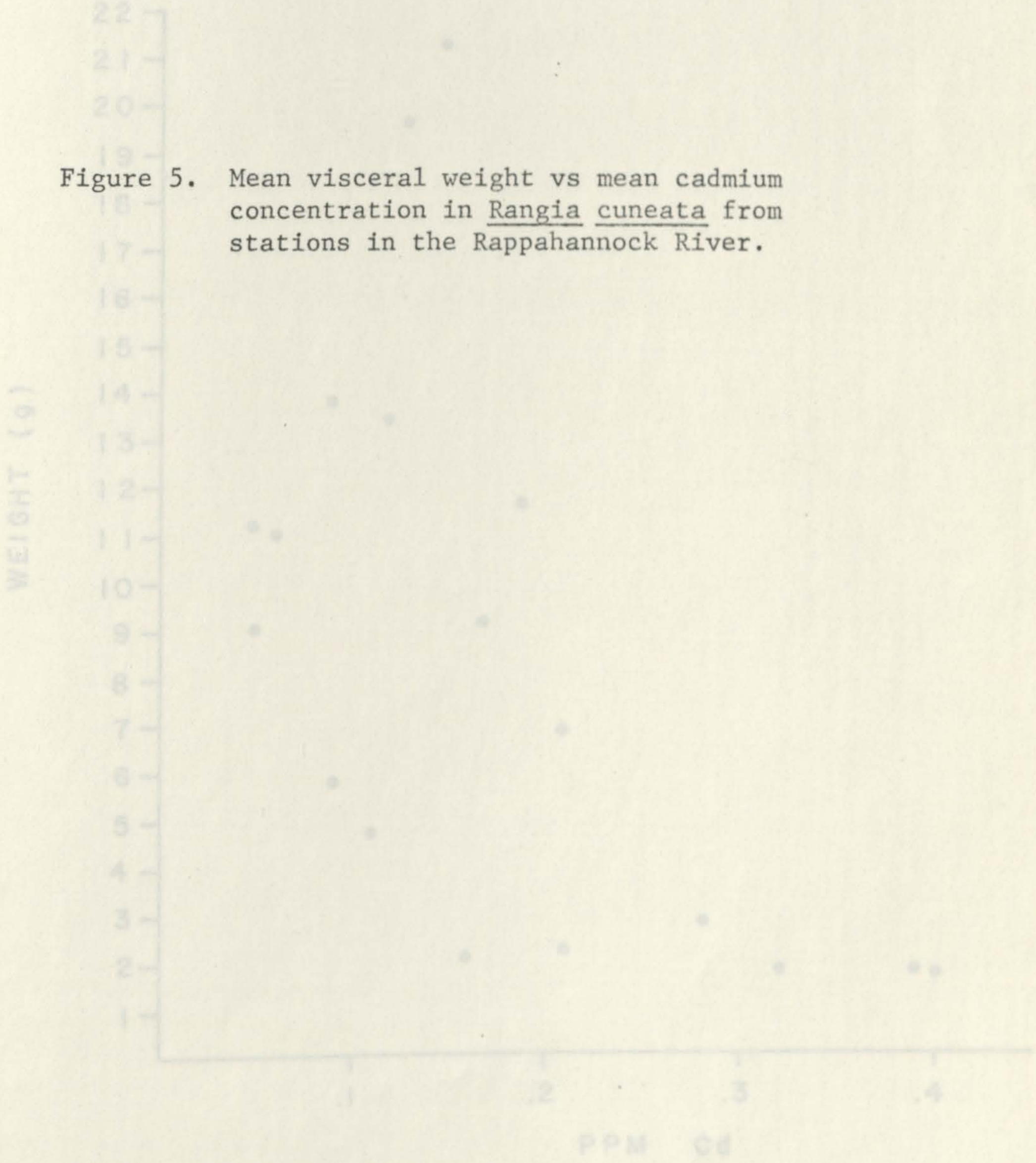
The size of Rangia cuneata appears to have various effects on the levels of metals concentrated, depending on the element observed. As shown in Tables 7 and 8, the partial correlation coefficients of shell length with zinc and copper and weight with zinc and copper were not significant, indicating size does not significantly affect the levels of these two metals in the clams and thus can be ignored as a variable.

The clams concentrate cadmium inversely with size, smaller clams concentrating to higher levels (Figures 5 and 6). The partial correlation coefficients of Cd levels with shell length were significant at the 99% level in the Rappahannock, but only significant at the 95% level in the James. Partial correlation coefficients of Cd levels and visceral weight, due to a better fit, were significant at the 99% confidence level in both rivers.

Sediment Composition

Percent sand was used as an indicator of sediment type, for this effectively divides grain size into fractions larger and smaller than 62.5μ, and renders the data to one "manageable" number. These percentages were converted by the arc sine transformation (Snedecor and Cochran 1967) to normalize the data, then regressed with various combinations of other independent variables to assess substrate effect on levels of metals concentrated. None of these analyses nor the computed partial

Figure 5. Mean visceral weight vs mean cadmium concentration in <u>Rangia</u> <u>cuneata</u> from stations in the Rappahannock River.



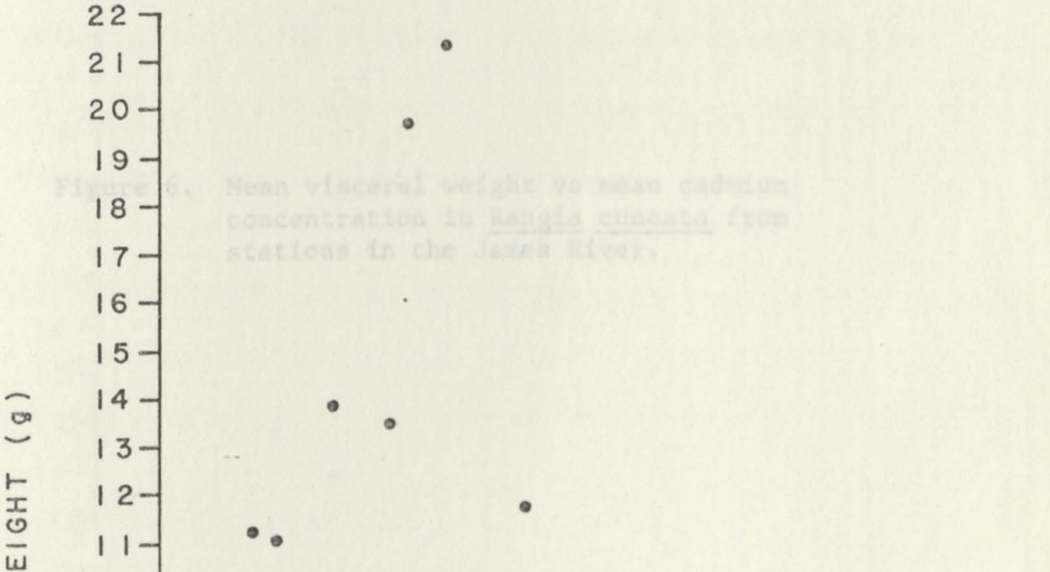
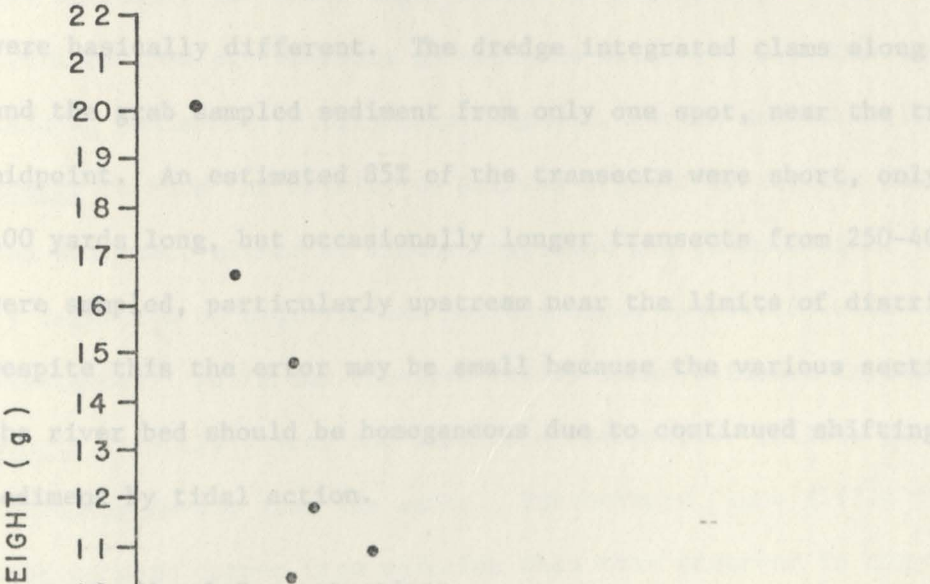


Figure 6. Mean visceral weight vs mean cadmium concentration in *Rangia cuneata* from stations in the James River.

Figure 6. Mean visceral weight vs mean cadmium concentration in *Rangia cuneata* from stations in the James River.

correlation coefficients in Tables 7 and 8 showed sediment size to affect levels of metals concentrated by Rangia. Percent sand, silt and clay were plotted and compared to metal concentrations in the clams, but no indication of a correlation could be found.

Some error was undoubtedly introduced into this sediment comparison study as the methods of sampling the clams and sediment were basically different. The dredge integrated clams along a transect and the grab sampled sediment from only one spot, near the transect midpoint. An estimated 85% of the transects were short, only about 100 yards long, but occasionally longer transects from 250-400 yards were sampled, particularly upstream near the limits of distribution. Despite this the error may be small because the various sections of the river bed should be homogeneous due to continued shifting of the sediment by tidal action.



Substrate Metal Concentrations

The Ecology-Pollution Department of VIMS analyzed sediment samples from the Rappahannock and James Rivers (Huggett and Bender unpubl.). These samples were taken in the fall of 1972, from the oxidized layer in the center of the channel at half-mile intervals from the mouth to mile 60 in the Rappahannock and mile 54 in the James. The sediment was sieved and the <62.5μ fraction was analyzed for inorganic, organic, and total Cu and Zn fractions. In the fall, 1972, the Ecology-Pollution Department, in conjunction with the Army Corps of Engineers (Norfolk District), also studied oxidized sediment characteristics [Cu and Zn concentrations (total and <62.5μ), volatile solids, total solids, total nitrogen, etc.] from several portions of the James River, samples being taken in the center and immediately outside the channel. These data correlation coefficients in Tables 7 and 8 showed sediment size to affect levels of metals concentrated by Rangia. Percent sand, silt and clay were plotted and compared to metal concentrations in the clams, but no indication of a correlation could be found.

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It may be that Rangia are assimilating low levels of metals from the sediment, yet their major source of metals may be the water as hydrated ions or insoluble particulate matter, etc., thus effectively masking sedimentary influences. Tenore, Horton and Duke (1968) found Rangia to have the ability to utilize organic matter and phosphate from the sediment, either by direct ingestion and assimilation, or indirectly by ingestion of bacteria and benthic algae associated with these substances. Wolfe and Schelske (1969) found evidence to indicate Rangia directly filter out insoluble radioactive fallout particles, including Zn-65, from the water. Furthermore, Lunz (1972) found oysters took up more copper from solution than that adsorbed to clay. Drobeck and Carpenter (1970) indicated metals complexed with sediment were either not accumulated or had a smaller effect than ionic forms on the oyster.

Distance Upriver and Implications

Figures 7 and 8 show the mean copper concentrations in Rangia plotted against distance upriver in the Rappahannock and James Rivers. The mean copper concentrations in Rangia in the Rappahannock River range between 1.3-2.0 ppm. This consistency differs with Huggett, Bender and Slone's (1973) work on oysters, where position in the estuary did affect levels concentrated. Since there is a salinity gradient from mile 34 (5 ppt) to mile 40 (< 0.1 ppt), salinity, at least at these concentrations, would appear to have no effect on levels of Cu concentrated by

Figure 7. Mean copper concentrations in *Rangia cuneata* from stations in the Rappahannock River.

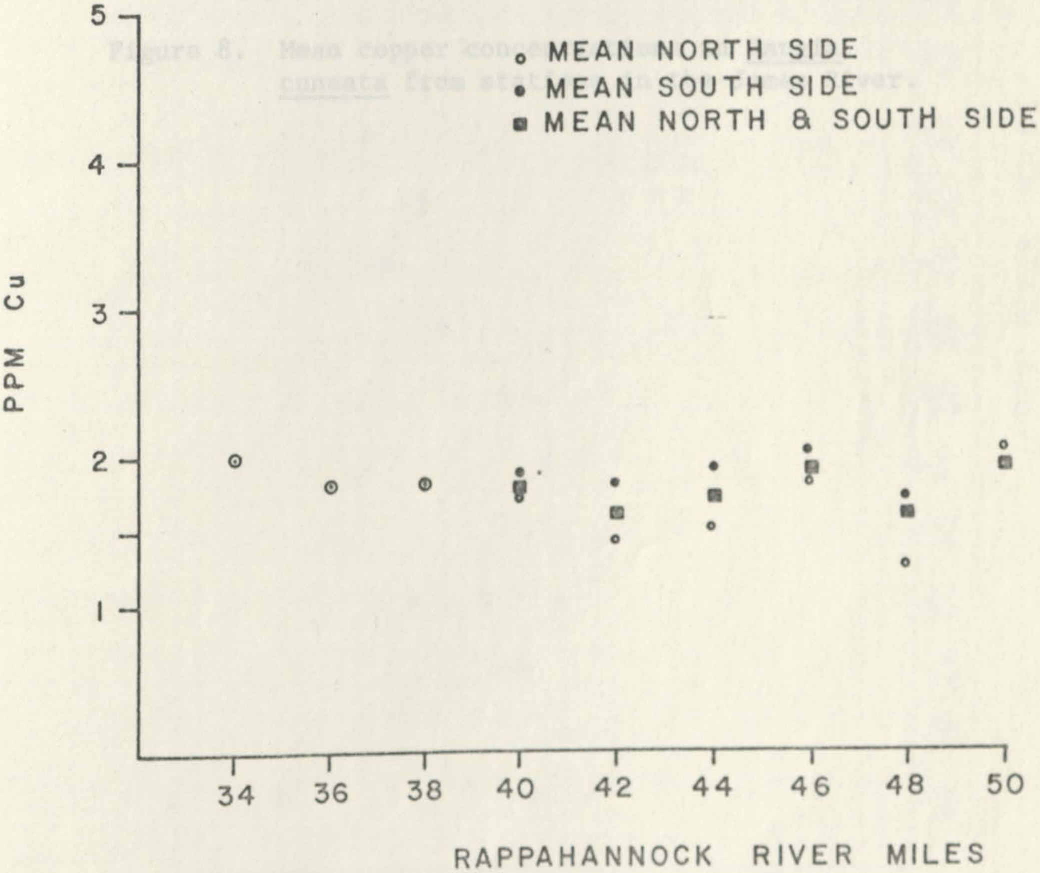
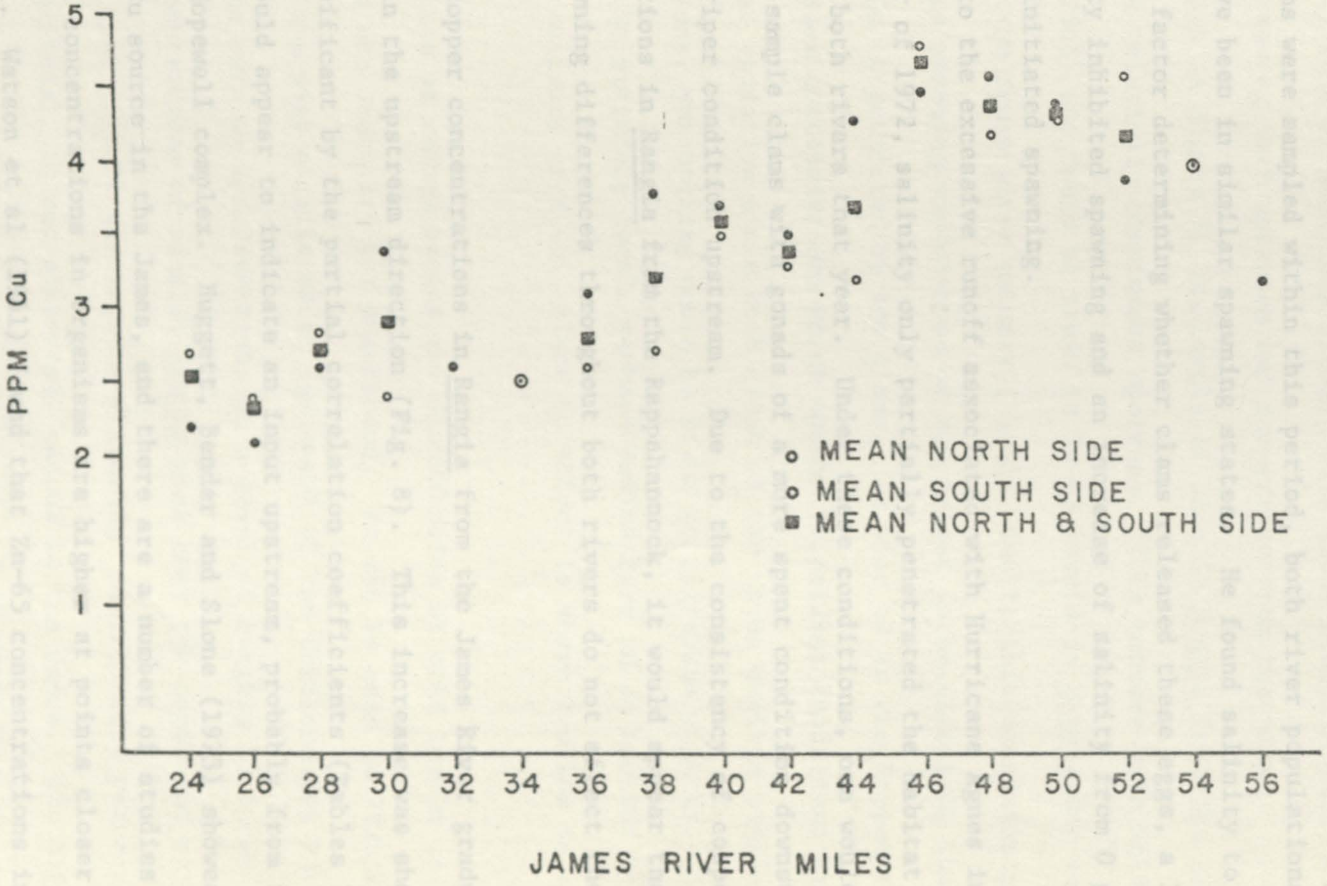

○ MEAN NORTH SIDE
● MEAN SOUTH SIDE
■ MEAN NORTH & SOUTH SIDE
PPM Cu
5
4
3
2
1
34
36
38
40
42
44
46
48
50
RAPPAHANNOCK RIVER MILES

Figure 8. Mean copper concentrations in <u>Rangia cuneata</u> from stations in the James River.


PPM Cu
5
4
3
2
1
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
54
56
JAMES RIVER MILES
○ MEAN NORTH SIDE
● MEAN SOUTH SIDE
■ MEAN NORTH & SOUTH SIDE

Rangia. Duke, Willis, Price and Fischler (1969) found similar results with Zn-65 in the clam Mercenaria mercenaria.

Cain (1972) indicated Rangia to be ripe with eggs from May through late November, at which time they begin to reabsorb the remaining eggs. Since clams were sampled within this period, both river populations should have been in similar spawning states. He found salinity to be the major factor determining whether clams released these eggs, a lack of salinity inhibited spawning and an increase of salinity from 0 ppt to 5 ppt initiated spawning.

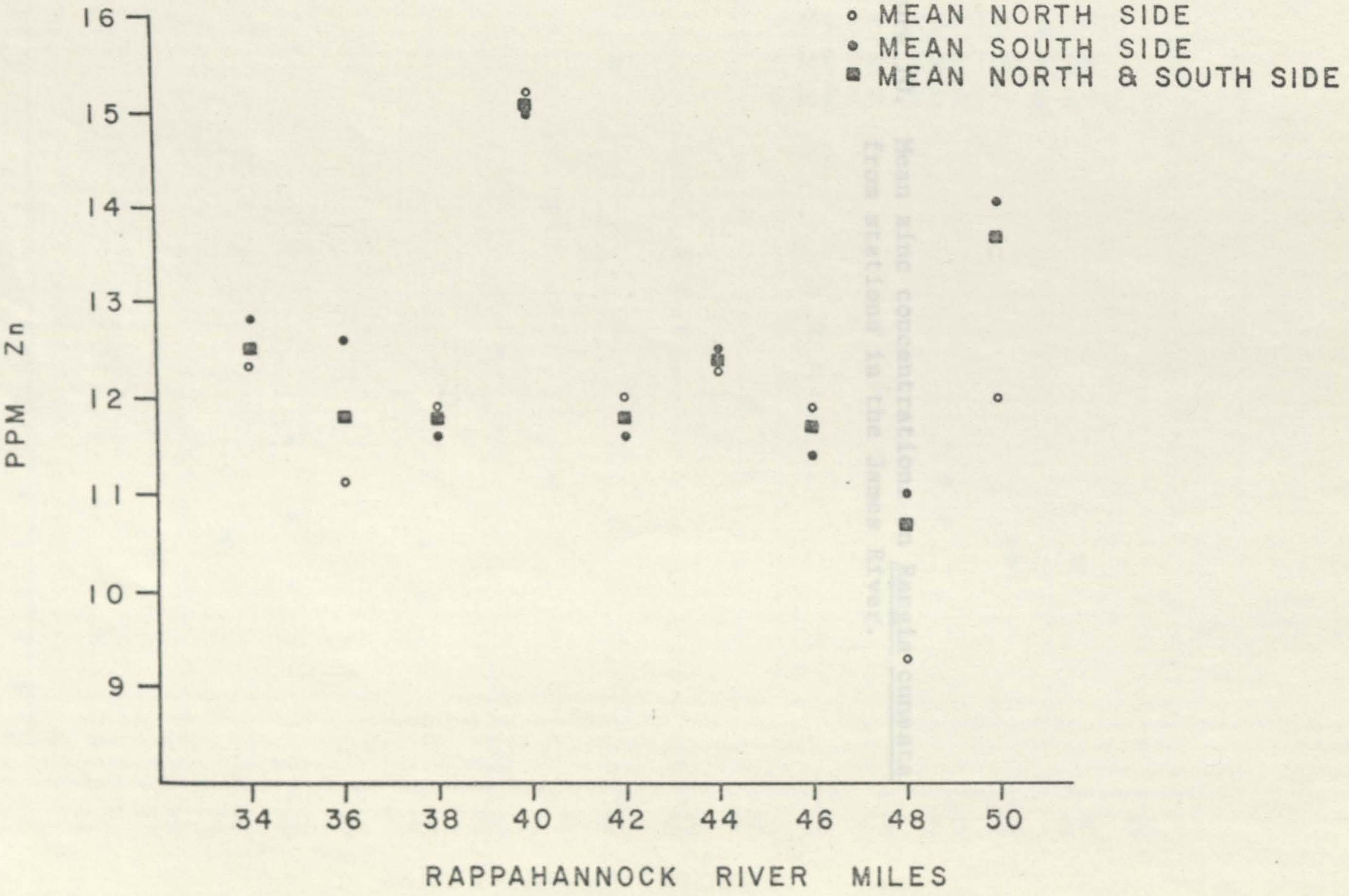
Due to the excessive runoff associated with Hurricane Agnes in the summer of 1972, salinity only partially penetrated the habitat of Rangia in both rivers that year. Under these conditions, one would expect to sample clams with gonads of a more spent condition downstream and of a riper condition upstream. Due to the consistency of copper concentrations in Rangia from the Rappahannock, it would appear that these spawning differences throughout both rivers do not affect the data.

The copper concentrations in Rangia from the James River gradually increase in the upstream direction (Fig. 8). This increase was shown to be significant by the partial correlation coefficients (Tables 7 and 8), and would appear to indicate an input upstream, probably from the Richmond-Hopewell complex. Huggett, Bender and Slone (1973) showed an upstream Cu source in the James, and there are a number of studies that show metal concentrations in organisms are higher at points closer to an outfall. Watson et al (1961) found that Zn-65 concentrations in organisms along the Oregon coast increased towards the mouth of the Columbia River, which was releasing Zn-65 from the Hanford reactor into the ocean. Preston (1967) found higher concentrations of Zn-65 in oysters closer to a power station in the Bradwell estuary, and Roosenburg (1969) showed oysters in the Patuxent River to have the highest Cu levels at positions closest to a steam electric plant with levels decreasing both upstream and downstream from the plant.

As shown in Figure 9, the levels of zinc in Rangia from the Rappahannock are relatively consistent upstream. This concurs with Wolfe and Schelske (1969) who found no trend in Zn-65 levels in Rangia due to fallout in the Neuse River estuary. Again, due to this consistency, salinity and spawning differences do not appear to be affecting the levels of Zn concentrated by the clams. At mile 40 in the Rappahannock, there are peak concentrations which appear to reflect conditions other than natural variation since both sides of the river exhibit similar concentrations, yet nothing could be found to correlate with it. The cause must be extremely local, perhaps sedimentary pH as opposed to merely the salinity gradient, since no effect is shown 2 miles to either side.

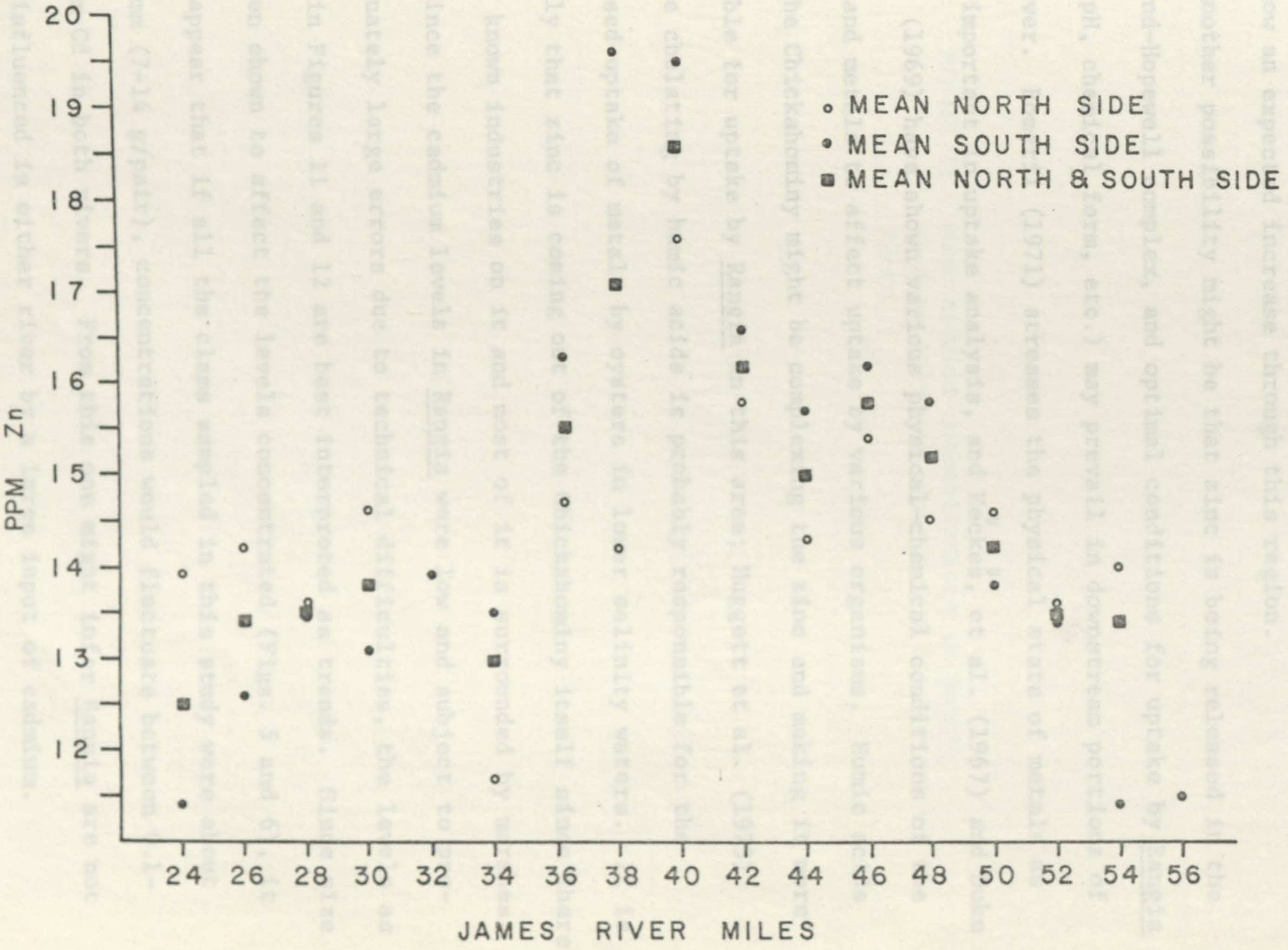
The cause or causes behind the distribution of zinc levels in Rangia from the James are not understood. As can be seen in Figure 10, concentrations peak at mile 40 at the mouth of the Chickahominy River, and decrease both up and downstream to a level of roughly 13.5 ppm Zn. Though concentrations of zinc in Rangia peak near the upper end of the transition zone in both rivers, the differing distribution patterns suggest additional, if not different, causes in the James. The highest concentrations were from Rangia at miles 38 and 40 which were located in an old spoil bank on the south side of the river extending from mile 36.5 to 43. Spoil had not been dumped there for two years prior

Figure 9. Mean zinc concentrations in *Rangia cuneata* from stations in the Rappahannock River.



○ MEAN NORTH SIDE
● MEAN SOUTH SIDE
▨ MEAN NORTH & SOUTH SIDE
PPM Zn
16
15
14
13
12
11
10
9
34
36
38
40
42
44
46
48
50
RAPPAHANNOCK RIVER MILES

Figure 10. Mean zinc concentrations in <u>Rangia</u> <u>cuneata</u> from stations in the James River.



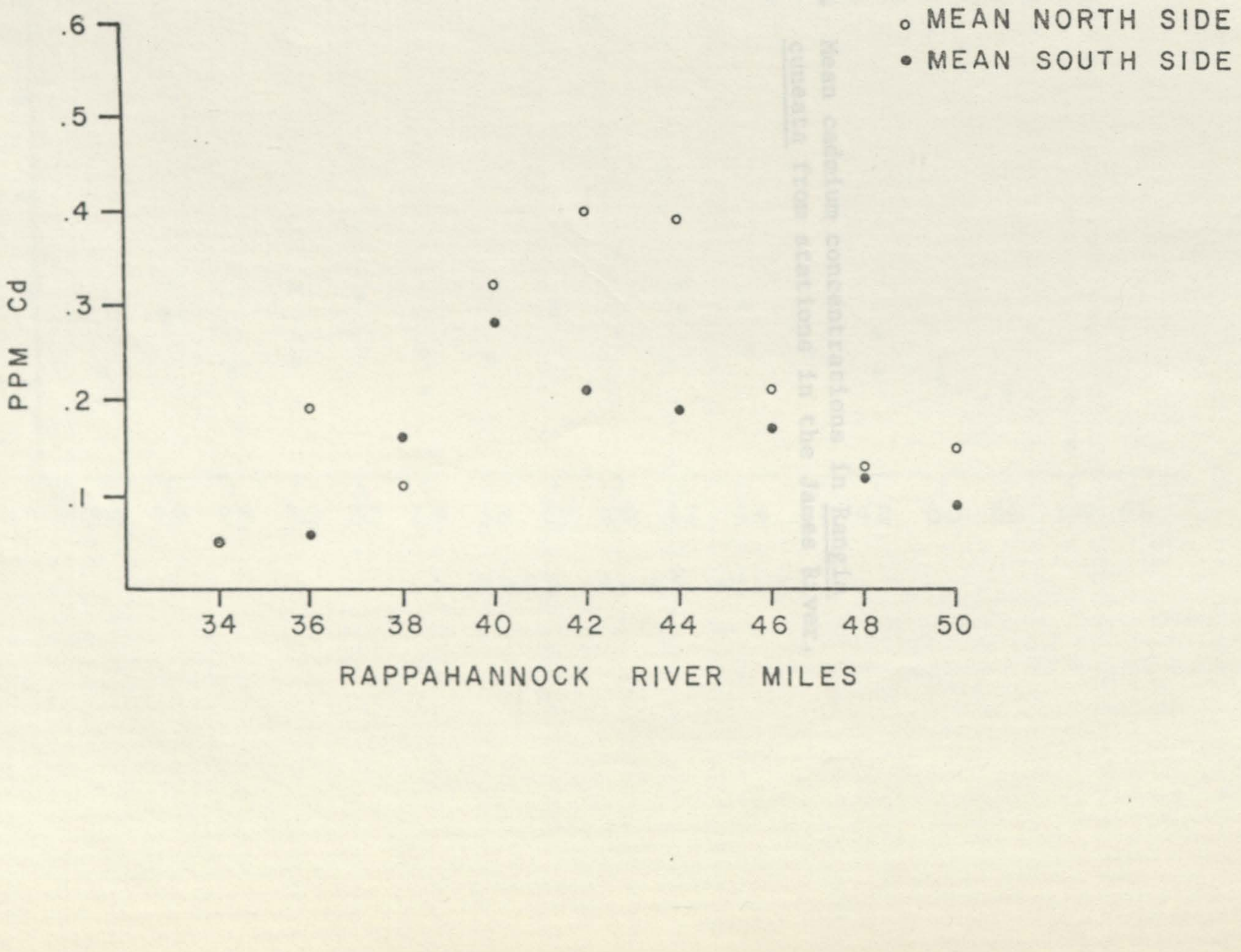
PPM Zn
20
19
18
17
16
15
14
13
12
○ MEAN NORTH SIDE
● MEAN SOUTH SIDE
■ MEAN NORTH & SOUTH SIDE
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
54
56
JAMES RIVER MILES

to sampling. The reducing spoil may be rich in interstitial ionic zinc (Phelps et al. 1969; Windom 1972) and being taken up through the clam's mantle. This hypothesis could not be tested since these sediments were not analyzed; however, the analyses of oxidized channel sediments did not show an expected increase through this region.

Another possibility might be that zinc is being released in the Richmond-Hopewell complex, and optimal conditions for uptake by *Rangia* (e.g. pH, chemical form, etc.) may prevail in downstream portions of the river. Romeril (1971) stresses the physical state of metals as being important in uptake analysis, and Kečkeš, et al. (1967) and Duke et al. (1969) have shown various physical-chemical conditions of the water and metals to affect uptake by various organisms. Humic acids from the Chickahominy might be complexing the zinc and making it more available for uptake by *Rangia* in this area; Huggett et al. (1973) believe chelating by humic acids is probably responsible for the increased uptake of metals by oysters in lower salinity waters. It is unlikely that zinc is coming out of the Chickahominy itself since there are no known industries on it and most of it is surrounded by marshes.

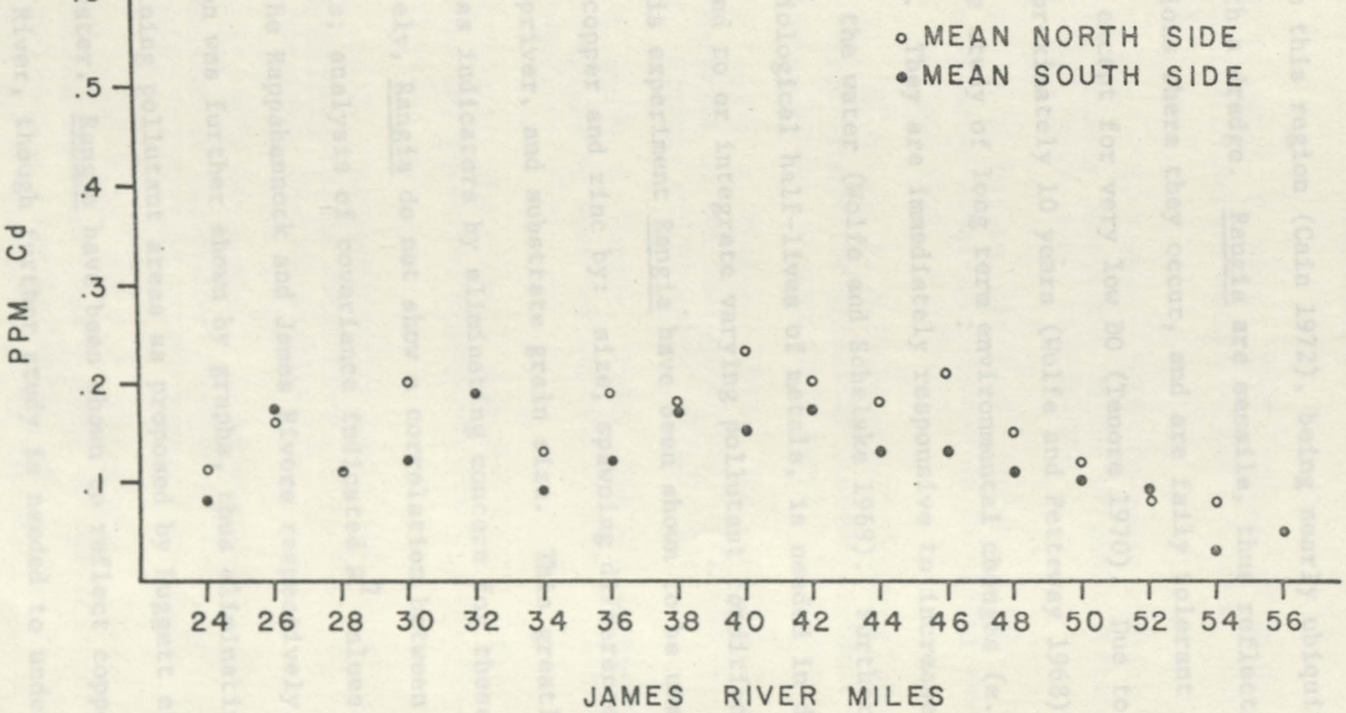
Since the cadmium levels in *Rangia* were low and subject to proportionately large errors due to technical difficulties, the levels as shown in Figures 11 and 12 are best interpreted as trends. Since size has been shown to affect the levels concentrated (Figs. 5 and 6), it would appear that if all the clams sampled in this study were about 40-50 mm (7-14 g/pair), concentrations would fluctuate between 0.1-0.2 ppm Cd in both rivers. From this one might infer *Rangia* are not being influenced in either river by a large input of cadmium.

Figure 11. Mean cadmium concentrations in *Rangia cuneata* from stations in the Rappahannock River.



○ MEAN NORTH SIDE
● MEAN SOUTH SIDE
PPM Cd
.6
.5
.4
.3
.2
.1
34
36
38
40
42
44
46
48
50
RAPPAHANNOCK RIVER MILES

Figure 12. Mean cadmium concentrations in <u>Rangia cuneata</u> from stations in the James River.


○ MEAN NORTH SIDE
● MEAN SOUTH SIDE
PPM Cd
.6
.5
.4
.3
.2
.1
24
26
28
30
32
34
36
38
40
42
44
46
48
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52
54
56
JAMES RIVER MILES

Rangia as an Indicator

Although more study is needed, there are a number of factors indicating that Rangia may be a suitable indicator of heavy metal pollution in the oligohaline portions of estuaries. It is the dominant organism in this region (Cain 1972), being nearly ubiquitous and easily sampled with a dredge. Rangia are sessile, thus reflecting environmental conditions where they occur, and are faily tolerant of pollution conditions except for very low DO (Tenore 1970). Due to their long life span of approximately 10 years (Wolfe and Petteway 1968), they can be used in the study of long term environmental changes (e.g. chronic metal pollution). They are immediately responsive to increases of radioactive fallout in the water (Wolfe and Schelske 1969). Further study, such as on their biological half-lives of metals, is needed in determining how they respond to or integrate varying pollutant conditions.

In this experiment Rangia have been shown to be unaffected in their levels of copper and zinc by: size, spawning differences, salinity or distance upriver, and substrate grain size. This greatly facilitates their use as indicators by eliminating concern for these variables. Unfortunately, Rangia do not show a correlation between copper and zinc levels; analysis of covariance indicated R^2 values of .096 and .129 for the Rappahannock and James Rivers respectively. This lack of correlation was further shown by graphs, thus eliminating the approach to determining pollutant areas as proposed by Huggett et al. (1973) for the oyster. Rangia have been shown to reflect copper pollution in the James River, though further study is needed to understand their response to zinc pollution there.

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